# Psychobiological Consequences of Stress in Sensitive Developmental Stages and Potential Protective Factors

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## Declaration of Independence

The submitted articles in partial fulfillment of the requirements for the degree of Doctor of Philosophy were written in collaboration with the mentioned co-authors. Three original articles were produced. Neither the author, co-authors nor any other persons published the articles elsewhere. All citations are indicated and only the tools cited were used.

For the purpose of the cumulative dissertation, the following papers have been submitted for publication in various journals. Copies of the articles are found in the appendix:

**Article 1:**

Voellmin, A., Winzeler, K., Hug, E., Wilhelm, F.H., Schaefer, V., Gaab, J., La Marca, R., Pruessner, J.C., & Bader, K. (submitted). Blunted endocrine and cardiovascular reactivity in young healthy women reporting a history of childhood adversity**.** *Psychoneuroendocrinology.*

**Article 2:**

Voellmin A., Entringer, S., Moog, N., Wadhwa, P.D.W., & Buss, C. (2013). Maternal positive affect over the course of pregnancy is associated with the length of gestation and reduced risk of preterm delivery. *Journal of Psychosomatic Research*, 75 (4), 336-340.

**Article 3:**

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## Abstract

The aim of the present dissertation is to contribute to the knowledge of the negative health consequences due to early stress experiences in humans and to investigate potential protective factors beneficial for health.

While a stress response to a threatening situation of limited duration is functional, it can become dysfunctional when stressors are chronic or perceived as uncontrollable. Stress during pregnancy, childhood and adolescence has been associated with the development of physical and mental disorders. However, most studies that investigated the association of adverse childhood experiences (ACE) and stress responses in adulthood were conducted on clinical samples and reported contradictive results regarding the alterations of the stress system. Studies on healthy participants are still scarce, however, a few studies observed blunted endocrine and cardiovascular responses to stress in relation to a history of ACEs.

Therefore, the first study reported in this dissertation aimed to replicate the attenuated endocrine and cardiovascular stress reactivity in healthy, female subjects reporting a history of ACEs and furthermore investigated the impact of duration and age of onset of ACEs. Results confirmed that a history of ACEs was associated with a dampened endocrine and cardiovascular stress response to a mental stress test and that especially long enduring, chronic ACEs seem to have the strongest impact on the attenuated stress reactivity (Article 1).

Article 2 investigated the impact of positive affect on biological outcomes in pregnancy. There is accumulative evidence that positive emotions are related to beneficial physical and psychological health outcomes. Furthermore, it is widely accepted that maternal psychosocial stress is a risk factor for obstetric birth outcomes like shorter length of gestation and preterm delivery. Therefore, in this study conducted on 169 women with singleton pregnancies, the hypothesis if maternal positive affect during pregnancy is associated with beneficial consequences in terms of increased length of gestation and reduced risk of preterm birth was tested. Results showed that higher maternal positive affect and a steeper increase in

maternal positive affect over pregnancy were positively associated with length of gestation and reduced risk of preterm delivery. It was argued that positive emotions may exert their beneficial effect by impacting maternal and fetal stress and immune systems.

Article 3 investigated the efficacy of the Montreal Imaging Stress Task (MIST) in healthy female subjects. The MIST is a standardized psychosocial stress test and has not been validated outside the MRT in a healthy female sample so far. Results confirmed that the MIST is a valid instrument for inducing a multidimensional stress response and that it can be classified as being a moderate stress test.

# 1. Theoretical Background

## 1.1 The Human Stress System

Stress can be defined as the interplay between internal or external adverse effects, which challenge or threaten the maintenance of an organism's complex dynamic equilibrium (homeostasis) (Chrousos, 2009). Under favorable conditions, the neuroendocrine system enforces growth, development, reproduction of the organism, and the survival of the self and the species. Under threatening conditions, the stress system is activated to support the organism's survival by adapting to the stressful situation. An activation of the stress system leads to behavioral and physiological changes in order to adjust homeostasis and increase survival odds (Chrousos, 2009). The term "allostasis" commonly refers to the biological responses that promote adaptation and reestablish homeostasis. Those include systematic mediators like sympathetic and parasympathetic activity, cortisol, pro- and anti-inflammatory cytokines, and metabolic hormones (McEwen, 2006).

Per se, the term "stress" refers to the coping with the internal or external adverse situations (stressors), that is, the physiological, psychological, and behavioral responses. Stressors include an intense body of potentially adverse forces, which can be emotional or physical (Chrousos, 2009). According to (Mason, 1968), in order to induce a stress response by the body, a situation has to be interpreted as being novel, and/or unpredictable, and/or the individual must have the feeling that he/she does not have control over the situation (Lupien, McEwen, Gunnar, & Heim, 2009). Also, the presence of a social evaluative threat to a situation has emerged as an important characteristic of a stressor (Dickerson & Kemeny, 2004). Furthermore, stressors can be absolute (a real threat like an earthquake) or relative (stressors that will cause a stress response in certain individuals). Another classic distinction is also made in terms of the quantity of the stressors, with stressors being time-limited or acute versus chronic and long enduring. Acute stressors can include daily hassles, for example

personal (critical) life events like marriage, birth of a child, failing exams, but also traumatic events like severe accidents, rape, or kidnapping (Ehlert, La Marca, E., & Kübler, 2013). However, per definition, the term trauma must include a threat of severe injury or death of one self or another person involved, accompanied by experiencing feelings like intense fear, helplessness, or horror (DSM-IV; Sass, Wittchen & Zaudig, 2003). Long-term or chronic stressors can involve being deprived as a child, an unsatisfying work situation, poverty, or chronically ill family members (Ehlert, La Marca, E., & Kübler, 2013).

### 1.1.1 Activation of the Stress System

Stressors are detected through a neurobiological network that includes the thalamus, the sensory cortex, and the amygdala (Danese & McEwen, 2012). The amygdala, which has evolved to identify environmental threats for survival, is moderated by the hippocampus and the prefrontal cortex. These three components form a network of brain areas involved in detecting environmental threats (Danese & McEwen, 2012). Based on learning processes and the memory of previous experiences, the hippocampus has inhibitory control over amygdala activity. The prefrontal cortex exerts inhibitory control over amygdala activity through executive functions (McEwen, 2007).

If a stressor activates the stress system, the amygdala triggers the firing in the locus coeruleus (LC), which activates the sympathetic nervous system, in order to promote a fight or flight response. Furthermore, the amygdala triggers firing in the paraventicular nucleus (PVN) of the hypothalamus, which induces the release of corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP), leading to a neuroendocrine stress response. The hypothalamic-pituitary-adrenal (HPA) axis, together with the efferent sympathetic/adrenomedullary system (SAM), represents the effector limbs, through which the brain influences all body organs when exposed to stress (Tsigos & Chrousos, 2002). Also, the

brain activates a subset of vagal and sacral parasympathetic efferents to mediate responses to stress.

### 1.1.2 The Sympathetic/Adrenomedullary System

The autonomic nervous system provides a rapid response mechanism to control a wide range of functions (Tsigos & Chrousos, 1994). The sympathetic nervous system and/or the parasympathetic nervous system regulate cardiovascular, respiratory, gastrointestinal, renal, and endocrine functioning (Gilbey & Spyer, 1993).

Once a stimulus is perceived as a threat, an intense and prolonged discharge from the LC activates the sympathetic part of the autonomic nervous system (Hellhammer & Hellhammer, 2008). The activation of the SAM leads to the release of catecholamines, predominantly epinephrine (EPI) but also some norepinephrine (NE), by the medulla of the adrenal gland. EPI and NE mainly increase blood supply to the brain and muscles, leading to fight/flight reactions. These catecholamines do not cross the blood–brain barrier, but their peripheral action is paralleled in the brain by NE produced by the LC. In response to stress, this brain locus supports vigilance and physical arousal, demands attention and alertness, and participates in processes that activate the HPA axis (Hellhammer & Hellhammer, 2008).

The parasympathetic system is believed to assist sympathetic functions by withdrawing its inhibition or it can antagonize them by increasing its activity (Tsigos & Chrousos, 2002).

### 1.1.3 The Hypothalamic–Pituitary–Adrenal (HPA) Axis

According to a circadian rhythm, neurons in the hypothalamic PVN secrete CRH and AVP, which then stimulate the production of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. ACTH acts on the adrenal cortex to initiate the release of cortisol

(Tsigos & Chrousos, 2002). Under resting conditions, CRH and AVP are secreted in a circadian, pulsatile manner, with about two to three secretory episodes per hour (Horrocks et al., 1990). CRH and AVP pulses increase in the early morning hours, resulting finally in ACTH and cortisol secretory bursts in the general circulation (Horrocks et al., 1990). These diurnal variations are influenced by changes in lighting, feeding schedules and activity and are disrupted by stress.

Upon exposure to stress, the amplitude and synchronization of the CRH and AVP pulsations increases, which in turn stimulates the secretion of ACTH and cortisol. ACTH plays the key role of the cortisol secretion regulation by the adrenal cortex. However, the HPA axis' activity can get potentiated also through other factors such as lipid mediators of inflammation or various cytokines, which are part of the autonomic innervation of the adrenal cortex or which originated from the adrenal medulla (Tsigos & Chrousos, 2002). ACTH initiates the release of cortisol in the adrenal cortex. Cortisol has a strong catabolic action and increases glucose levels, which increase the availability of energy substrates to assist the organism under stressful conditions (Tsigos & Chrousos, 2002). Glucocorticoids also improve cardiovascular tone and alter immune function. Through actions in the brain, they facilitate the formation of memories, thus shape behavior and physiological reactions to new stressors, and promote goal-directed behavior (McCormick & Mathews, 2007).

Cortisol is the final effector of the HPA axis. It regulates but also terminates the basal HPA activity, by exerting a negative feedback on the HPA axis via receptors in the hippocampus, the PVN, and the pituitary gland. The negative feedback cycle results in suppression of the CRH and AVP production. The inhibitory cortisol feedback on the ACTH secretory response leads to a limitation of the duration of the total tissue exposure to glucocorticoids, to reduce the catabolic, antireproductive, and immunosuppressive effects of these hormones (Tsigos & Chrousos, 2002).

## 1.2 Health Risks of Chronic Activation of the Stress System

While a short-term activation of the HPA axis is a basic adaptive mechanism to reestablish homeostasis (referred to as allostasis), a prolonged activation can present a health risk to the organism. According to Chrousus, (2009), chronic and prolonged activation of the stress system can lead to dysregulation in terms of growth, development and body composition, which might account for many health conditions like behavioral, endocrine, metabolic, cardiovascular, autoimmune, and allergic disorders (Chrousos, 2009). These prolonged responses are referred to as allostatic overload (McEwen, 2006).

Briefly, in the nervous system, chronic stress can lead to structural and functional abnormalities in the prefrontal cortex, the amygdala, and the hippocampus (McEwen, 2007; McEwen & Gianaros, 2011). These abnormalities are characterized by dendrite shortening in the prefrontal cortex, as well as impairment in attention. In the amygdala, chronic stress exposure can enhance dendrite growth, thus leading to enhanced response to unlearned fear and fear conditioning. In the hippocampus, repeated exposure to stress can lead to deficits in declarative, contextual, and spatial memory (McEwen & Gianaros, 2011).

In the endocrine system, chronic stress can result in inadequate stress system activity and responsiveness, which in turn, influences the functions of the homeostatic system (Miller, Chen, & Zhou, 2007). Glucocorticoids pass the blood-brain-barrier, access the brain where they bind to receptors. Three of the most important brain areas containing glucocorticoid receptors are the hippocampus, the amygdala, and the frontal lobes, which are brain structures known to be involved in learning and memory (Lupien et al., 2009). Furthermore, glucocorticoids antagonize insulin and the increase of blood pressure, thus increasing the risk for the metabolic syndrome. Also, according to McEwen (1998) activation of the HPA axis leads to immune suppression, which in a chronic state can lead to increased risk of infection (McEwen, 1998).

Hypersecretion of CRH has been observed and associated with anxiety, depression, post-traumatic stress disorder in children, eating disorders, chronic alcoholism, amenorrhea, and reduced fertility (Chrousos, 2009). Also, disruption of the HPA axis and the functions of the sympathetic systems have been reported in obesity, metabolic syndrome, and in hypertension. Furthermore, dysregulations of the stress system have been associated with gastrointestinal disorders like the irritable bowel syndrome (Chrousos, 2009).

Furthermore, chronic stress exposure can dysregluate the immune system, and lead to prolonged elevation of inflammation levels (Chrousos, 1995). Glucocorticoids have an immunosuppressive function, reducing the release of proinflammatory cytokines (e.g. Interleukin-6 and tumor necrosis factor (TNF-α)). Abnormal immune function has been associated with chronic inflammatory and/or autoimmune and allergic diseases. These abnormalities have been associated with decreased CRH activity, and have been linked to chronic pain disorders, fibromyalgia, and chronic pelvic pain (Heim, Ehlert, & Hellhammer, 2000).

The development and severity of these conditions depend on the genetic, epigenetic, and constitutional vulnerability or resilience of the individual to stress (Chrousos, 2009). Individual differences as well as hyper- and hypoactivation of the stress response may be explained through genetic but also environmental, and developmental factors (Charmandari, Tsigos, & Chrousos, 2005). Brain components involved in stress response show increased plasticity during prenatal life, childhood, and adolescence and seem to be particularly sensitive to stressors at that time (Charmandari et al., 2005).

### 1.3. Effects of Stress in Sensitive Developmental Stages

The exposure to stress during critical developmental periods like prenatal and postnatal life, childhood, and adolescence, as well as the presence of adverse or protective

environments, and the duration of the stressor can have an impact of the development of these stress related disorders (Chrousos, 2009). Because the nervous, endocrine, and immune systems go through profound changes during childhood and are not fully mature at birth (Giedd & Rapoport, 2010), they represent vulnerable periods to the influence of stress, and therefore, the sensitive periods of prenatal life and childhood will be the focus of the following chapters.

### 1.3.1 Stress in Pregnancy

In pregnancy, various empirical studies have identified maternal psychosocial stress as a significant risk factor for adverse fetal development and birth outcomes related to length of gestation (preterm birth) and fetal growth (low birthweight/small for gestational age birth (Rini, Dunkel-Schetter, Wadhwa, & Sandman, 1999; Alder, Fink, Bitzer, Hosli, & Holzgreve, 2007; Hobel, Goldstein, & Barrett, 2008). Furthermore, studies in young adults exposed to maternal psychosocial stress during intrauterine life showed that these participants exhibited a significant dysregulation of physiological parameters like body composition and glucose-insulin metabolism (insulin resistance, higher BMI), immune function (altered cytokine production), altered endocrine function (increased ACTH and reduced cortisol levels during pharmacological and psychosocial stimulation paradigms), and cognitive function (decreased gray matter density, altered working memory performance) (Entringer, Kumsta, et al., 2008; Entringer, Wust, et al., 2008; Entringer, Kumsta, Hellhammer, Wadhwa, & Wust, 2009). In these studies, the effects were independent of other established obstetric and childhood risk factors. Therefore, these findings suggest an increased risk of developing negative physiological and cognitive health outcomes after the intra-uterine exposure to maternal stress.

The UC Irvine Development, Health, and Disease Research Program has examined the

interface between biological, behavioral, and social processes in human pregnancy over the past several years. They have proposed a theoretical model that empathizes the role of CRH in mediating the effects of maternal stress in human parturition, providing a plausible biological link between known environmental risk factors and preterm birth. The authors suggest that external conditions lead to a stress reaction in the maternal brain, causing alterations in maternal physiology, which in turn may influence fetal development and birth outcomes. However, they suggest that this process is reciprocal, since the state of pregnancy itself causes biological changes in maternal physiology.

In pregnancy, the effects of psychosocial stress seem to be mediated, in part, by alterations in maternal-placental-fetal (MPF) endocrine and immune processes (Wadhwa, Entringer, Buss, & Lu, 2011). Fetal growth and maturation are known to be regulated by HPA hormones, in particular by cortisol. Cortisol influences fetal development directly by passing through the placenta or indirectly through stimulation of CRH in the placenta. There is, however, one crucial difference in the regulation of hypothalamic and placental CRH. In contrast to the negative feedback control on hypothalamic CRH, glucocorticoids stimulate the expression of CRH gene (hCRHmRNA) in the placenta, establishing a positive feedback loop that results in a progressive elevation of CRH, ACTH, and cortisol levels over the course of gestation (Robinson, Emanuel, Frim, & Majzoub, 1988).

Therefore, in pregnancy, maternal cortisol production increases 2- to 4-fold over the course of gestation, and autonomic responses (heart rate, blood pressure) rise in the later stages of gestation to support aspects of fetal growth and development (Mastorakos & Ilias, 2003; Murphy, Smith, Giles, & Clifton, 2006; Sandman et al., 2006). However, with advancing gestation, maternal endocrine, cardiovascular and psychological responses to stress become attenuated, and the degree of attenuation has been shown to be a significant predictor of reduced length of gestation and earlier delivery (Buss et al., 2009; Entringer et al., 2010).

Furthermore, in pregnancy, immune function is reduced and altered as a consequence of

the balance between tolerating the fetus and not suppressing maternal immune responses to an extent that increases maternal or fetal vulnerability to infection. Higher levels of inflammatory markers have been observed in pregnant women who reported increased psychosocial stress. For example, in pregnancy, maternal psychosocial stress has been associated with an increased risk for reproductive tract infection (Culhane et al., 2001; Culhane, Rauh, McCollum, Elo, & Hogan, 2002).

Therefore, as described above, maternal stress in pregnancy represents a risk factor for adverse fetal development and birth outcomes, and can lead to alterations of the stress system above this developmental stage (pregnancy) throughout adulthood.

### 1.3.2 Stress in Childhood

Stress in childhood, often referred to as adverse childhood experiences (ACEs) or early life stress (ELS), is widely prevalent and has been shown to be a risk factor for the development and persistence of mental disorders such as depression, anxiety disorders, substance use disorder, or attention-deficit/hyperactivity disorder (Famularo, Kinscherff, & Fenton, 1992; Heim & Nemeroff, 2001; De Bellis, 2002).

ACEs are characterized by stress associated with extreme fear and great emotional charge (La Prairie, 2010). The most widespread forms of ACEs in humans are sexual, physical and emotional maltreatment and neglect. According to La Prairie, Heim & Nemeroff (2010), other forms of ACEs include parental loss, natural disasters, poverty, and inadequate parental care as a result of mental or physical illness, accidents, war, physical illness, and maternal stress during pregnancy. Epidemiological studies in the USA have reported 3.6 million cases of child abuse and neglect annually (US Department of Health and Human Services, 2008), and have reported a considerable overlap between physical, emotional, and

sexual abuse in children, indicating that children who are affected by one form of abuse are at higher risk to suffer other forms of abuse.

ACEs are thought to be strong extrinsic stressors leading to changes in stress sensitivity and functioning of the HPA axis, resulting in alterations that persist throughout adulthood. Therefore, HPA axis alterations have been suggested to underlie the association between ACEs and mental/physical disorders (Heim & Nemeroff, 2001; Tarullo & Gunnar, 2006).

According to Tarullo and Gunnar (2006), healthy newborns exhibit increases in cortisol and ACTH in response to adverse stressors such as childhood inoculations or physical examinations (Gunnar, Larson, Hertsgaard, Harris, & Brodersen, 1992; Tarullo & Gunnar, 2006). By the end of the first year of life, a decrease of the HPA responsiveness has been observed, persisting throughout the toddler and preschool years. The authors state that this so called "stress hyporesponsive period" is believed to protect the vulnerable, developing brain from the potentially negative impact of elevated HPA hormones. During these stages of development, the HPA system is buffered by responsive, sensitive parental caregiving. For example, Gunnar et al. (1992) reported that if 9-month olds were provided with a caring, responsive babysitter during a separation paradigm, they showed no increases in cortisol, in contrast to those infants who were left with a cold, distant babysitter during the separation, who exhibited significant elevations in cortisol. Furthermore, preschoolers who attended day care only showed rise in cortisol over the day, if they were looked after by unresponsive staff, compared to no significant rise in cortisol in those preschoolers, who had responsive and stimulating care during the day (Dettling, Parker, Lane, Sebanc, & Gunnar, 2000). Not only does parenting seem to influence HPA axis responsiveness to adverse circumstances, also, associations of lower parental care during childhood and reduced hippocampal grey matter volume in adults have been reported (Engert et al., 2010).

Taken as a whole, studies that have explored the effects of maltreatment on the HPA axis in children, including basal functioning, or reactivity to psychosocial stress tasks, have hypothesized that maltreatment in childhood leads to a dysregulation of the HPA axis, associated with either hypo- or hyperresponsive phenotypes. Chronically high basal cortisol levels can lead to immune suppression and reduced synaptic plasticity. During childhood, when the brain is developing rapidly, chronically high cortisol levels appear to shape the way these brain circuits interpret environmental threat, as well as the magnitude and duration of stress responses in the future, which in turn increases vulnerability to stress and increased risk of developing mood or anxiety disorders (Heim & Nemeroff, 2001; Tarullo & Gunnar, 2006).

On the other hand, chronically low cortisol levels could compromise future and necessary psychobiological stress reactivity. After an initial hypersecretion of cortisol, the HPA axis may burn out leading to a blunted pattern, possibly due to a compensatory increase in the negative HPA axis feedback, or a diminished release of cortisol by the adrenal glands, possibly due to alterations in the number of glucocorticoid receptors (Heim, Ehlert, et al., 2000; Fries, Hesse, Hellhammer, & Hellhammer, 2005). In other words, the HPA axis becomes increasingly efficient in shutting down the stress response, which may be an adaptive response to minimize the potential damage done to body tissues by a chronically activated stress response.

### 1.3.3 Stress in Adolescence

Since the adolescent brain undergoes vigorous maturation, adolescence might be a sensitive period during which stress could have deleterious impact on the developing brain. The prevalence of psychiatric disorders increases in adolescence (e.g. anxiety, depression and drug use) (Spear, 2000; Costello, Mustillo, Erkanli, Keeler, & Angold, 2003; Patton & Viner, 2007; Spear, 2009). Since periods of heightened stress occur often before the development of

mental disorders, it could be hypothesized that increases of HPA axis reactivity during adolescence results in increased sensitivity to the onset of stress-related mental disorders. However, according to Romero (2010), relatively little is known about how stress reactivity changes from childhood to puberty to adulthood and what complex interaction between physiology, genes, and environment mediate pubertal increase in the development of psychopathology (Romeo, 2010).

Studies in typically developing children showed that basal cortisol levels increase from childhood to puberty (Halligan, Herbert, Goodyer, & Murray, 2004; Netherton, Goodyer, Tamplin, & Herbert, 2004). Tarullo et al. (2006) hypothesize that the beginning of puberty signals the end of the human stress hyporesponsive period. This could be related to a change in sex steroid levels with the beginning of puberty, which have been reported to influence HPA axis activity (McCormick & Mathews, 2007). In puberty, the neuroendocrine axis goes through multiple changes, resulting in altered hormonal output. Gonadal hormones increase, leading to substantial changes in secondary sexual characteristics, fertility, and reproductive behavior (Romeo, Richardson, & Sisk, 2002). Preliminary findings suggest that HPA axis responsiveness increases from childhood to adulthood (Gunnar, Frenn, Wewerka, & Van Ryzin, 2009; Stroud et al., 2009); however, studies, which investigated the impact of ACEs during adolescence, have not been conducted so far.

### 1.4 Protective Factors in the Context of Vulnerability

In his review on the understanding of the protective and damaging effects of stress, McEwen (2008) states the following: " *Protection and damage are the two contrasting sides of the physiology involved in defending the body against the challenges of daily life* [...]." He refers to the fact that the stress response functions in an adaptive way to reestablish homeostasis (McEwen, 2008). If prolonged, stress can lead to a series of negative health

consequences. Therefore, and especially during sensitive developmental stages, stress can enhance vulnerability. The investigation of factors related to influence HPA axis and SNS functioning in a protective way seems appropriate, considering the above outlined risk factors related to alterations in the human stress system. Per definition, protection results from the presence of factors that diminish negative outcomes and increase the odds of positive adaptation (McEwen, 2008).

There are some converging sources of evidence that suggest that the HPA axis might remain mutable over time and could be impacted by environmental changes. However, physiological reactivity is a complex, dynamic construct that, according to Obradovic (2012), emerges as a product of early genetic and environmental influences and can also be shaped by later contextual experiences (Obradovic, 2012). Theoretical models of genetic factors and environmental influences (e.g. sensitive parenting, social support, relaxation, coping), which provide an understanding of resilience to stress, are well beyond the scope of this framework. Even though studies have demonstrated that psychotherapeutic treatments in adults show improvement in HPA functioning (Gaab et al., 2003; Gaab, Sonderegger, Scherrer, & Ehlert, 2006; Hammerfald et al., 2006) or that parenting programs can be beneficial in normalizing HPA functioning after prolonged stress exposure (Fisher, Stoolmiller, Gunnar, & Burraston, 2007), the aim of the present chapter is to summarize findings of associations observed in different studies related to the importance and impact of positive affect on physical and psychological health and stress reactivity.

### 1.4.1 Positive Affect as a Protective Factor to Promote Health

Positive affect can be defined as the feelings that reflect a state of pleasurable engagement with the environment such as happiness, joy, excitement and contentment (Tomkins, 1963). According to Steptoe (2010), research literature relating positive affect to

physical health is growing. In psychiatry and behavioral medicine, negative psychological states such as depression, anxiety, and stress have been objective of many studies, and associations between these constructs are well established (Steptoe, 2010). However, Steptoe argues that positive psychological states and traits are more than the absence of emotional distress, and that positive psychological states and traits are in part independent of negative affect, therefore contributing to health independently (Steptoe, 2010).

There is cumulative evidence that positive emotions are related to beneficial physical and psychological health outcomes. For example, positive affect and psychological well-being have been linked to favorable health outcomes, reduced risk of physical illness and prolonged survival (Dockray & Steptoe, 2010). Chida and Steptoe (2008) report in their review of longitudinal studies on happiness and future mortality that happiness predicted a decreased risk of mortality in healthy and diseased populations, also after controlling for initial health and other factors, and they showed that positive affect predicted mortality over and above negative emotions, showing that positive affect had an impact beyond the absence of negative affect (Chida & Steptoe, 2008). Furthermore, in a large representative sample of elderly people in the UK, higher levels of positive affect were significantly associated with a higher survival rate in the five years after the survey, even after controlling for demographic factors, health behaviors, self-reported health, and other conditions (Steptoe & Wardle, 2011). The study reported a 35% decrease in mortality for the happiest group.

### 1.4.2 Possible Pathways of Positive Affect and Health

Behavioral and/or psychobiological processes are believed to underlie the association between positive affect and beneficial health outcomes. Different pathways linking positive wellbeing or positive affect with health have been discussed.

The first possible underlying mechanism is that there is a common genetic substrate, that is, that there might be genetic factors common to positive affect and health risks (Steptoe, 2010). Preliminary evidence regarding the link between genetics and well-being has been reported. De Neve et al. (2011) found in a large scaled study on 2574 adolescents in the US that those with the two long variations of the 5-HTT gene were approximately 17% more likely to be the most satisfied with their life compared to carriers of the two short variants (De Neve, 2011). However, their replication of the finding showed mixed results (De Neve, Christakis, Fowler, & Frey, 2012). Therefore, at the present, there is no direct evidence for such pathways linking genes to happiness and health (Steptoe, 2010).

The second pathway links lifestyle factors to positive emotional states. Emotions direct behavior, and therefore, can influence health habits and lifestyle behavior (e.g. nutrition, physical activity, social engagement) related to favorable health. Therefore, happier individuals could make healthier lifestyle choices. Also, positive affect might be a marker of psychosocial factors related to coping, social support, self-esteem, and optimism (Fredrickson, 2001). Different studies support the indirect route from positive affect to health. For example, studies showed that happier individuals tend to eat healthier (Grant, Wardle, & Steptoe, 2009) and engage more in sports (Schneider, Graham, Grant, King, & Cooper, 2009). Importantly, these findings raise the point that healthier people may be happier because of their good health, and not the other way around. Certainly, this may be true for some findings reported, however, there are studies that support the pathway going from happiness to health (De Neve, Diener, E., Tay, L., & Xuereb C., 2013), controlling for possible confounding variables like negative affect. For example, Diener and Chan (2011) concluded in their review of different types of evidence regarding the causal link between subjective well-being to health and longevity, that positive feelings seem overwhelmingly causally related to health (Diener, 2011).

Another plausible pathway for the effects of positive affect may be through

psychobiological activation in neuroendocrine, autonomic, immune, and inflammatory processes, however, the exact mechanisms, according to Bostock (2011), are not well understood (Bostock, Hamer, Wawrzyniak, Mitchell, & Steptoe, 2011). Positive affect could lead to weaker threat perceptions of daily stressful events, according to the theories of Lazarus and Folkman (1984), leading to lower activation of the stress system (Lazarus & Folkman, 1984). Also, according to Bostock and Steptoe (2011), positive affect might be linked to the sensitivity of central stress control mechanisms without cognitive awareness (Bostock et al., 2011). According to Davidson (2004), positive emotional states and traits have been linked to specific brain regions that are closely associated with the regulation of cardiovascular and endocrine systems (Davidson, 2004).

Studies have shown associations of positive affect and cardiovascular function. Specifically, they relate positive affect to accelerated recovery from cardiovascular reactivity (Tugade, Fredrickson, & Barrett, 2004; Dockray & Steptoe, 2010) to decreased blood pressure in ambulatory assessments (Ong & Allaire, 2005), and to elevated parasympathetic activation (Bhattacharyya, Whitehead, Rakhit, & Steptoe, 2008). Other studies reported that positive affect was linked to lower cortisol concentrations over the course of the day (Steptoe, Gibson, Hamer, & Wardle, 2007; Brummett, Boyle, Kuhn, Siegler, & Williams, 2009) and to higher antibody responses to hepatitis B vaccination (Marsland, Cohen, Rabin, & Manuck, 2006). A study by Ditzen et al. (2008) reported the protective role of intimacy in couples from stress (Ditzen, Hoppmann, & Klumb, 2008). Their results showed that couple intimacy was associated with reduced daily salivary cortisol levels and that this effect was mediated by experienced positive affective states. However, only a few studies have investigated the effect of positive affect and psychobiological parameters in laboratory settings so far. Bostock et al. (2011) showed, in a sample of young, healthy women, that higher positive affect was associated with lowered blood pressure and dampened salivary cortisol responses to a laboratory stress test, after adjusting for baseline measures, age, BMI, and negative affect.

Another study of pregnant women reported that higher psychological resources (self-efficacy and daily uplifts) were associated with dampened stress reactivity (Nierop, Wirtz, Bratsikas, Zimmermann, & Ehlert, 2008). After a mental stress test, higher resources and daily up-lifts were associated with a blunted alpha-amylase reactivity and lower psychological stress. Higher resources predicted lower cortisol stress reactivity of borderline significance (Nierop et al., 2008).

A limitation in the research of well-being is that positive affect is associated with a range of behavioral and psychosocial factors that may also contribute to health benefits (Steptoe, 2010). Large-scale studies that convincingly demonstrate that specifically improving positive affect is beneficial for physical health outcomes or biological responses are needed. This would support a scientifically-founded basis for development of intervention methods for enhancing well-being and resilience.

# 2. Research Questions

## 2.1 Research Question Article 1

As stated in the theoretical piece of this work, adverse childhood experiences (ACEs), including physical, emotional and sexual abuse, affect a significant portion of the population and have been shown to be risk factors for the development and persistence of mental disorders such as depression, anxiety disorders, substance use disorders, or attention-deficit/hyperactivity disorder. Changes in stress sensitivity and functioning of the hypothalamic-pituitary-adrenal (HPA) axis have been suggested as causal factors (De Bellis, 2002; Tarullo & Gunnar, 2006; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008).

Different reactivity phenotype patterns have emerged in different studies, with a number of studies showing exaggerated HPA axis and SNS levels in the context of ACEs and psychopathology (Heim et al., 2000b; Heim and Nemeroff, 2001; Bremner et al., 2003; Rao et al., 2008). In contrast to findings in clinical samples, a growing number of studies on healthy participants reported blunted endocrine (Carpenter et al., 2007; Elzinga et al., 2008; Carpenter et al., 2010; Lovallo et al., 2012) and cardiovascular (Lovallo et al., 2012) responses in association with ACEs.

To the best of our knowledge, the impact of age of onset and duration of ACEs has not been investigated in healthy samples so far. There is evidence that these factors could have a differential impact on stress reactivity in adulthood (Tarullo and Gunnar, 2006; Schoedl et al., 2010; Tottenham and Sheridan, 2010). From a developmental perspective, age at traumatization is believed to be an important factor. Brain components involved in stress response show large plasticity during pre- and postnatal periods and during early childhood, and some plasticity during later childhood and adolescence (Andersen et al., 2008). Also, the duration of adverse experiences could have an impact on psychobiological constructs. Particularly those environmental events that cause exceeding or prolonged stimulation of the

stress system during these critical developmental periods could lead to abnormal neurodevelopment and therefore to lasting alterations in stress reactivity of the HPA axis and the SNS (Schoedl et al., 2010).

Therefore, the aim of the first article was to replicate the attenuated endocrine and cardiovascular stress reactivity in association with a history of ACEs in a young, healthy, female sample. The impact of duration as well as age of onset of stressful life events in childhood and adolescence on endocrine and cardiovascular reactivity in adulthood was examined.

### 2.2 Research Question Article 2

The association between maternal psychological state during pregnancy and birth outcomes is well established. The focus of previous studies has been on the potentially detrimental consequences of maternal stress on pregnancy and birth outcomes, particularly shortened gestation and increased risk of preterm birth. Despite a growing literature linking positive affect with favorable health outcomes (e.g. Dockray & Steptoe, 2010), this construct has received little attention in the context of pregnancy. Therefore, in the current study, we tested the hypothesis that maternal positive affect during pregnancy is associated with beneficial consequences in terms of increased length of gestation and reduced risk of preterm birth, considering the effect of perceived maternal stress.

### 2.3 Research Question Article 3

The *Montreal Imaging Stress Task* (MIST) seems to be an effective tool to provoke physiological and emotional stress (Dedovic et al., 2005; La Marca et al., 2011). Until now, the MIST has been used and evaluated predominantly in MRT studies. Therefore, we aimed

to validate if the MIST also leads to an effective stress reaction in healthy women outside the MRT scan.

# 3. Methods

Three original articles were conducted. The following section *briefly* describes the study designs and the methods used.

## 3.1 Methods Article 1

### 3.1.1 Study Design

A quasi-experimental approach was used to investigate the relationship between ACEs and endocrine and cardiovascular reactivity after psychosocial stress induction. All participants eligible for study participation filled out the Early Trauma Inventory self-report (ETI-SR) (Bremner, Bolus, & Mayer, 2007), and engaged in the laboratory stress test while salivary cortisol and heart rate responses were conducted serially.

### 3.1.2 Study Sample

The sample included 104 young and healthy females between the ages of 18 and 25 years (M=21.7; SD=1.5), recruited at three schools for health care professions and social work in Basel, Switzerland. Exclusion criteria were physical or psychiatric illness, pregnancy, regular and heavy tobacco use (> 5 cigarettes a day), consumption of illegal drugs, and use of medication, which interferes with the nervous or the adrenocorticoid system. For women taking no oral contraceptives, the laboratory assessment was held in the luteal phase of the participant's menstrual cycle (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999).

### 3.1.3 Data Assessment

The German version of the "Structured Clinical Interview for DSM-IV/Axis I Disorders" (SCID-I) was conducted (Wittchen, Wunderlich, Gruschwitz, & Zaudig, 1997) to exclude participants with psychiatric illness. Relevant data including age, current medications, drug consumption, age of menarche, date of last menstruation, BMI, and intake of hormonal contraceptives were assessed during the interview. The Early Trauma Inventory self-report (ETI-SR) (Bremner et al., 2007), which includes general trauma (31 items), physical (9 items), emotional (7 items), and sexual abuse (15 items), was used for the assessment of ACEs. Participants were asked a series of yes or no questions regarding potential trauma and stress exposition.

For positively answered items, age of onset, frequency of trauma or abuse, and emotional impact (0=no negative impact, 1=slightly negative, 2=moderately negative, 3=strongly negative) were assessed. In total, five different ACE scores were built. First, all values were summed to a total score of all occurred events that had been rated with an emotional impact of at least 1 = slightly negative (*ACE total sum score*). Next, a sum score for ACEs lasting less than a year (*ACEs<one year*) and for ACEs lasting longer than a year (*ACEs>one year*) was built. Finally, events which occurred before or after a participants' menarche, were summed up to *ACEs before* and *after menarche,* respectively.

For the stress induction, the Montreal Imaging Stress Task (MIST, Dedovic et al., 2005) was used. Heart rate and salivary cortisol was measured before, during, and after stress induction. Emotional responses to the MIST were assessed using Visual Analogue Scales (VAS) for mood, tension, and stress.

### 3.1.4 Statistical Analyses

General linear models (GLM) for repeated measures served to determine the effects of ACEs on endocrine and cardiovascular responses. ACE scores were used as continuous variables to examine effects of time, ACE scores, and the interaction of time by ACE scores. ACE groups were then entered in the model in order to visualize the results. Covariates included use of oral contraceptives, BMI, and, in a second analysis, the VAS scores for mood, tension, and stress.

## 3.2 Methods Article 2

### 3.2.1 Study Design

A quasi-experimental approach was used to investigate the association between sum as well as rate of change of maternal positive affect, length of gestation and preterm birth. Maternal positive affect and perceived stress were serially assessed in first, second and third trimester. Pregnancy and birth outcomes were abstracted from the medical record.

### 3.2.2 Study Sample

Data for the present analysis were collected in the context of a longitudinal pregnancy and birth outcomes study conducted by the University of California, Irvine; Development, Health and Disease Research Program. The study sample was comprised of a population-based cohort of 169 pregnant women assessed serially over the course of gestation (at 15.2 $\pm$ 0.9 weeks (T1; mean $\pm$ SD), 19.7 $\pm$ 0.9 weeks (T2) and 30.7 $\pm$ 0.7 weeks (T3)) and followed through birth. Subjects were English-speaking, adult women with singleton, intrauterine pregnancies. Exclusion criteria included tobacco, alcohol, or other drug use in pregnancy, use of in vitro fertilization/reproductive technology, and uterine or cervical

abnormalities. Furthermore, women who had an elective cesarean section (n = 66) and women who had missing information about mode of delivery (n = 25) were excluded from the analyses. The final sample included 169 women.

### 3.2.3 Data Assessment

Positive affect was assessed using a questionnaire on attitudes towards pregnancy, adapted from prior research in pregnancy (Mancuso, Schetter, Rini, Roesch, & Hobel, 2004; Gurung, Dunkel-Schetter, Collins, Rini, & Hobel, 2005). This self-report questionnaire consists of 7 positive and 6 negative feelings towards pregnancy. At each assessment, a sum score for positive attitudes toward pregnancy, termed *positive affect*, was computed from the 13 items. Current levels of perceived stress were measured with the Perceived Stress Scale (PSS) (Cohen, Kamarck, & Mermelstein, 1983). The PSS consists of 12 items that are designed to measure how uncontrollable, unpredictable and overloaded participants find their lives. For each participant, an average score was computed over all time points of assessment and used as a covariate in the analyses.

Sociodemographic information (i.e., marital status, family income and maternal age at delivery) was assessed by interview. Length of gestation was abstracted from medical charts after delivery and assessed as a continuous variable by completed weeks gestation.

### 3.2.4 Statistical Analyses

Gestational age at the time of study visit was centered at the gestational week of the first study visit (T1). Specifically, time zero in the centered variable is equivalent to the mean gestational age at T1 (15-18 weeks). A linear regression model was fitted using the positive affect scores as outcomes and the centered gestational age as predictor. For each participant, the intercept of the regression line was used as level of positive affect at the first study visit

and the slope of the regression line as a measure of change in maternal positive affect. Both variables were used as predictors for gestational length in a linear regression model. Covariates included perceived stress, parity, maternal age, race/ethnicity, family income, marital status, and presence of obstetric risk.

Additionally, a logistic regression was conducted with the same predictors and covariates and preterm birth (< 37 completed weeks of gestation) as the outcome of interest.

## 3.3 Methods Article 3

### 3.3.1 Study Design

A quasi-experimental approach was used to assess the rate of change of endocrine and cardiovascular responses before and after stress induction.

### 3.3.2 Study Sample

The study sample is described in section 3.1.2, however, for this analysis, 97 participants went into the analyses, because the analyses were conducted before the recruitment process was completed.

### 3.3.3 Data Assessment

Inclusion and exclusion criteria were assessed as described in section 3.1.3.

Free salivary cortisol was collected at seven measurement points. Two took place before the stress test (-10 and -1 mins) and five after the stress test (+1, +10, + 25, +40 and +55 mins) using salivettes (Sarstedt, Sevelen, Switzerland).

Heart rate was recorded using Vitaport 3 data acquisition system (TEMEC Instruments B.V., Netherlands). Electrocardiogram (ECG) recordings were taken using Lead-II electrode

placement (RedDot™, 2248-50, 3F Health Care, Germany) on the thorax with three disposable electrodes. A sampling rate of 1024 Hz was used for ECG recordings with a low pass filter of 512 Hz and a high pass filter of 0.5 Hz.

Electrodermal activity (EDA) was measured with two Ag/AgCl Beckman electrodes filled with an isotonic paste. Electrodes were attached to the volar surfaces of the medial index and middle fingers of the subject's left hand. A constant-voltage device was used to pass 0.5V between electrodes.

An automatic blood pressure monitor was used to measure blood pressure and pulse with a cuff around the upper left arm (CRITIKON DINAMAP™ 1846 SX, USA).

A Visual Analog Scale (VAS) for stress served as measure of subjective emotional response of participants. The scale ranged from "not stressed" (0) to "very stressed" (100).

### 3.3.4 Statistical Analyses

Univariate repeated measure analyses were used to determine the rate of change for cortisol, blood pressure, heart rate, and electrodermal response before, during, and after the MIST. BMI and use of oral contraceptives were used as covariates. Furthermore, dependent t-tests were conducted to analyze the rate of change from baseline to peak, and from peak to relaxation, respectively.

## 4. Summary of Results

The following section gives a *brief* overview of the results from the three articles described. For more details, please see the articles contained in the appendix.

### 4.1 Blunted endocrine and cardiovascular reactivity in young healthy women reporting a history of childhood adversity

In general, results obtained by repeated measure analyses indicated that the stress task induced a significant increase in cortisol levels ($p<.001$; $\eta_p^2=.06$) and heart rate ($p<.001$; $\eta_p^2=.71$). Subjects experienced significant worsening of mood ($p<.001$; $\eta_p^2=.24$), increases in tension ($p<.001$; $\eta p2=.24$), and stress ($p<.001$; $\eta_p^2=.22$).

Repeated measures analysis of cortisol responses to stress showed a significant interaction of time x *ACE total sum score* ($p<.05$; $\eta_p^2=.03$) as well as a significant main effect of *ACE total sum score* ($p < .01$; $\eta_p^2 = .09$). *ACE total groups* differed significantly in their overall cortisol output in a dose-response manner ($p<.01$; $\eta_p^2=.12$).

Repeated measures of analysis of cortisol response to the stress task resulted in a significant main effect of duration of *ACEs > one year* ($p < .001$; $\eta_p^2 = .17$) and a significant interaction of time and duration of *ACEs > one year* ($p < .01$; $\eta_p^2 = .04$). However, these effects were not observed for the association between ACEs that lasted shorter in duration (*ACEs < one year*) and cortisol responses to the stress task (main effect, $p = .87$; interaction effect, $p = .81$).

For age of onset, a significant interaction effect was observed for both, occurrence of ACE before and after menarche, indicating that subjects with ACEs before ($p=.001$; $\eta_p^2=.05$) as well as after menarche ($p<.05$; $\eta_p^2=.03$) both had significantly attenuated cortisol responses to the stress task.

Repeated measures analysis of heart rate response to the stress task showed a significant main effect of *ACE total sum score* ($p<.01$; $\eta_p^2=.08$) as well as a significant

interaction effect of time x *ACE total sum score* ($p<.01$; $\eta_p^2=.07$). Results from the repeated measures analysis with the *ACE total groups* confirmed the impact of ACE by a significant main effect of group ($p<.01$; $\eta_p^2=.13$), indicating a dose-response relationship.

However, the further analyses with duration as well as age of onset of ACEs revealed no significant dose-response relationships with heart rate responses to the stress task (data not shown).

### 4.2 Maternal positive affect over the course of pregnancy is associated with the length of gestation and reduced risk of preterm delivery

In the sample, the mean length of gestation at birth was 38.9 ± 2.1 weeks (± SD), and ranged from 26.3 to 42.0 weeks. 20 of these deliveries (11.8%) were preterm (≤ 37 completed weeks gestation). Mean positive affect significantly increased over gestation ($F_{(1.7;249.8)} = 5.63$; $p = .006$), specifically values at the first assessment were significantly lower than at the second and third assessment. Perceived stress did not change over gestation ($p > .10$). As expected, maternal mean positive affect and mean perceived stress were inversely correlated ($r = -0.644$, $p < .001$).

Both the positive affect score at mean gestational age at T1 (intercept) and the rate of change over the course of gestation (slope) significantly and positively predicted length of gestation, indicating that more positive affect toward pregnancy in the early second trimester as well as a steeper increase in positive affect over gestation are associated with longer duration of pregnancy. Specifically, a 1SD (7.97 point) increase in the intercept (representing positive affect levels in the early second trimester) is associated with a 4.57 day increase in length of gestation. Furthermore, a 1SD (0.76 point) increase in the rate of change in positive affect over gestation is associated with 3.50 days increase in pregnancy duration.

Results for the logistic regression model showed that positive affect at T1 (intercept) was significantly and inversely related to preterm delivery (OR = 0.843; [95% CI = 0.757–0.939]; p = .002). This means that 1 point increase in positive affect intercept is associated with an 18.6% reduced risk of delivering preterm (for OR < 1: % = [(OR/1) − 1] ∗ 100). However, positive affect slope was not a significant predictor of preterm delivery (OR = 0.551; [95% CI = 0.210–1.445]; p = .226), indicating that the change in positive affect over pregnancy did not differ in mothers who delivered preterm as compared to mothers who delivered at term.

### 4.3 Die Effektivität des Montreal Imaging Stress Task bei jungen, gesunden Frauen

(The Efficacy of the Montreal Imaging Stress Task in Young Healthy Women)

Results obtained by repeated measure analyses indicated that the stress task induced a significant increase in cortisol levels $F(5, 464) = 3.86$, $p < .05$, $\eta_p^2 = .04$. On average, participants experienced a peak in cortisol responses 25 minutes after the stress task ($M = 21.58$, $SD = 9.33$). In comparison to baseline ($M = 17.73$, $SD = 6.74$), cortisol values differed significantly from baseline to peak, $t(95) = -4.05$, $p < .001$, $r = .38$, and showed a significant decrease 55 minutes after the MIST ($M = 18.63$, $SD = 7.63$), $t(95) = -4.14$, $p < .001$, $r = .39$.

Electrodermal responses significantly changed over time, $F(1.58, 129.69) = 65.93$, $p < .001$, $\eta_p^2 = .45$. In comparison to baseline ($M = 6.89$, $SD = 4.19$), participants showed a significant increase to peak ($M = 9.63$, $SD = 3.86$), $t(89) = -8.85$, $p < .001$, $r = .68$., and a significant decline after the MIST ($M = 7.75$, $SD = 3.56$), $t(84) = 15.34$, $p < .001$, $r = .82$.

Also, heart rate changed significantly over the course of the laboratory stress induction, $F(2.42, 218.02) = 190.21$, $p < .001$, $\eta_p^2 = .68$). Heart rate differed significantly from baseline ($M = 71.53$, $SD = 9.15$) to peak ($M = 101.03$, $SD = 20.81$; $t(94) = -16.18$, $p < .001$, $r = .86$).

As expected, heart rate responses significantly decreased after the MIST, ($M$ = 75.72, $SD$ = 10.11; $t(90) = 16.53$, $p < .001$, $r = .87$).

Furthermore, the MIST led to significant changes of systolic $F(9, 819) = 10.57$, $p < .001$, $\eta_p^2 = .12$ and diastolic blood pressure $F(3.57, 325.27) = 5.00$, $p < .001$, $\eta_p^2 = .05$

# 5. Discussion

The first aim of the present dissertation was to contribute to the understanding of the psychobiological consequences of stress experiences during sensitive developmental stages, specifically childhood and adolescence. The psychobiological consequences of adverse childhood experiences (ACE) were investigated in a sample of young and healthy females by examining their endocrine and cardiovascular stress reactivity to a psychosocial stress task. The second aim was to gain further knowledge of the beneficial effects of maternal positive affect in pregnancy on length of gestation and on risk of preterm birth. Pregnancy, itself, represents a sensitive developmental stage, involves major psychobiological transformations, and has been considered a stressful life event itself. The third aim of the present dissertation was to validate the Montreal Imaging Stress Task (MIST) in females outside the MRT, in order to provide an efficient experimental mode of inducing and measuring stress for the purpose of future studies examining the psychobiological consequences of stress in humans.

## 5.1 Article 1: "Blunted endocrine and cardiovascular reactivity in young healthy women reporting a history of childhood adversity"

Results of the first article are in line with previous reports of attenuated endocrine (Carpenter et al., 2007; Elzinga et al., 2008; Carpenter, Shattuck, Tyrka, Geracioti, & Price, 2010; Lovallo et al., 2012) as well as cardiovascular (Lovallo et al., 2012) stress responses to a psychosocial stress test in healthy adults with a history of adverse childhood experiences. Importantly, blunted cortisol and heart rate responses were independent of emotional responses, suggesting that the diminished endocrine and cardiovascular stress reactivity cannot be explained by a reduced emotional reaction to stress (which may be interpreted as flattened affect) after a history of childhood adversity.

To the best of our knowledge, the present study is the first to demonstrate that in healthy female subjects, especially long enduring, chronic ACEs seem to show the strongest association with blunted cortisol reactivity, adding valuable knowledge to the impact of chronic childhood adversity on alterations of the HPA axis in adult females. However, in this sample, timing of ACEs (before vs. after menarche) did not contribute compellingly to a further understanding of the relationship between ACEs and attenuated cortisol reactivity.

Per se, our results show a deviation from an expected endocrine and cardiovascular stress response in participants free of mental and physical illness in association with a history of ACEs. According to Obradovic (2012), taking together recent findings on stress reactivity in the context of early adversity, it is more accurate to state that exposure to early life stress may lead to dysregulated physiological phenotypes rather than to a particular pattern of hyper- or hyposensitivity (Obradovic, 2012). These different phenotypes might be mediated by the interaction of complex environmental and genetic factors.

As stated in the theoretical part of this framework, in childhood, stress is believed to be buffered through a sensitive environment. However, if a child experiences a chronically stressful environment, after an initial hypersecretion of cortisol, the HPA axis could counter-regulate its response and cortisol output might rebound to below normal. A plausible biological explanation could be an increased glucocorticoid negative feedback with a downregulation of CRF receptors, or a diminished release of cortisol by the adrenal glands (Heim, Ehlert, et al., 2000; Fries et al., 2005). Our finding that only chronic events, not acute, were associated with a blunted cortisol response supports this view.

In terms of genetic and epigenetic factors, there is some preliminary evidence for alterations in stress reactivity in association with ACEs. For example, a history of ACEs has been linked to an epigenetic regulation of the glucocorticoid receptor in the hippocampus (McGowan et al., 2009). Moreover, a recent study on healthy adults who experienced the loss of a parent during childhood, maltreatment, or low parental care showed epigenetic alterations

of a region of the human glucocorticoid receptor gene, which in turn was associated with a blunted cortisol reactivity after a neuroendocrine challenge test in these participants (Tyrka, Price, Marsit, Walters, & Carpenter, 2012). Another study linked prenatal maternal depression to increased methylation of the glucocorticoid receptor gene, and showed exaggerated salivary cortisol output to stress at 3 months of age (Oberlander et al., 2008).

However, our results raise the challenging question of whether the observed deviation can be interpreted as a potential risk for or as a sign of resilience to the development of later mental and physical disorders. Even if initially adaptive, blunted cortisol reactivity could compromise future and necessary psychobiological stress reactivity. For example, low cardiovascular and/or endocrine reactivity to acute psychological stress has been associated with depression, fibromyalgia, obesity, burn out, substance use disorders, and chronic pain syndromes (Griep et al., 1998; Heim, Ehlert, Hanker, & Hellhammer, 1998; Pruessner, Hellhammer, & Kirschbaum, 1999; Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000; Gold & Chrousos, 2002; Gur, Cevik, Nas, Colpan, & Sarac, 2004; Phillips, Hunt, Der, & Carroll, 2011; de Rooij, 2012; Jones et al., 2012; Phillips, Roseboom, Carroll, & de Rooij, 2012). Further studies are needed to investigate these changes in central regulation of the glucocorticoid receptor in brain regions involved in stress responses in association to ACEs.

### 5.1.1 Strengths and Limitations

The present study contributes to the knowledge of the psychobiological consequences of stress in sensitive developmental stages. Our replicated finding that ACEs represent a risk factor in the development of alterations of the human stress system in the absence of current physical and mental illness contributes to a further understanding of the adverse consequences of stress in sensitive developmental stages. Next to the replication of this finding, a strength of the current study is the further investigation of age of onset and duration of ACEs in the

context of a laboratory stress task. Our results showed that especially long enduring, chronic stress experiences were associated with a blunted cortisol response in this sample. This result supports the premise that the human stress system is at risk to become attenuated during stress-sensitive periods, especially if the stressful events are long enduring. Another strength of the present study is the rather high sample size for a study which included an experimental stress induction. Also, because of the age limitation, our sample represents an age group of healthy females, which has not been object to the investigation of ACEs and stress reactivity thus far.

A limitation of the present study is that only peripheral readouts of stress hormone activation were measured. The HPA axis and the cardiovascular system are complex and multilayer systems. Thus, identifying the exact mechanisms or location of the observed dysregulations poses a great challenge. Furthermore, that only women were recruited and tested has to be mentioned as another potentially limitating factor. Especially as some of the recent models on stress reactivity changes after chronic or traumatic stress make mention of sex differences, it would have been informative to assess the stress response in men as well (Bangasser & Valentino, 2012; Bangasser, 2013). Thus, due to the characteristics of the study sample, the presented findings can only be generalized to young women free of mental and physical illness.

Also, participants were attendees of schools for health care professions and social work, which could have led to a selection bias. For methodological reasons, the specification between ACEs shorter/longer than one year and pre-/postmenarche cannot be assumed to be completely independent from each other. Since some participants reported both, acute and chronic ACEs as well as pre- and postmenarche ACEs, with our sample size, it was not possible to have distinct groups for only ACEs >1 year/< 1 year and only pre-/postmenarche.

### 5.1.2 Clinical Implications and Future Research

Longitudinal research is needed to investigate if participants without present psychopathology, who had shown blunted stress responses after stress induction, are at higher risk to develop psychiatric disorders later in life or remain healthy. Furthermore, since our results did not reveal a significant impact of age of onset in association with a blunted stress response, future studies will be needed to further investigate this premise. Perhaps, our differentiation of age of onset was not specific enough, therefore studies which investigate more specific age groups are needed.

The present study revealed diminished heart rate responses in association with the total number of ACEs, but not with the subgroups regarding duration and age of onset. Recently, Bauer & Boyce (2002) have suggested the examination of the HPA axis and the SNS simultaneously for a better understanding of their coordination (additive or interactive; or opposing or complementary) (Bauer, Quas, & Boyce, 2002). Only few studies have examined the exact nature of their coordination in adult samples so far (Lovallo et al., 2000; Ali & Pruessner, 2012; Andrews & Pruessner, 2012; Lovallo et al., 2012; Andrews & Pruessner, 2013), and results are mixed. Methodological differences between the reported studies could account for the different findings. More empirical research is needed to investigate the exact nature of SNS alterations after ACEs, as well as the coordination and interactions between the two stress systems.

## 5.2 Article 2: "Maternal positive affect over the course of pregnancy is associated with the length of gestation and reduced risk of preterm delivery"

Results from the present study suggest that higher levels of positive affect in pregnancy are associated with longer length of gestation and with a reduced risk of delivering preterm. The level of positive affect in the early second trimester of pregnancy as well as the rate of

increase in positive affect over the course of pregnancy were positively associated with a longer length of gestation. The magnitude of this effect was such that every point increase in positive affect in the early second trimester was associated with an 18.6% reduced risk of delivering preterm. Moreover, the association between maternal positive affect and length of gestation was independent of maternal perceived stress, suggesting that it is not solely the absence of maternal stress that accounts for the beneficial effect of maternal positive affect on length of gestation.

Several empirical studies have identified high maternal psychosocial stress as a risk factor for adverse pregnancy and birth outcomes (Alder et al., 2007; Beydoun & Saftlas, 2008; Muglia & Katz, 2010; Wadhwa et al., 2011). The authors and others have reported that after accounting for the influence of established obstetric and socio-demographic risk factors, maternal psychosocial stress is significantly and independently associated with an increased risk of preterm birth and restricted fetal growth (Wadhwa, Sandman, Porto, Dunkel-Schetter, & Garite, 1993; Rini et al., 1999; Glynn, Wadhwa, Dunkel-Schetter, Chicz-Demet, & Sandman, 2001; Hobel et al., 2008). Alterations in stress-related maternal-placental-fetal endocrine and immune physiology have been suggested as pathways that may underlie this association (Wadhwa et al., 2011). In support of this suggested biological transmission pathway, elevated levels of maternal cortisol, pro-inflammatory cytokines and placental corticotrophin-releasing hormone (CRH) have been shown to be associated with shorter length of gestation (Romero, Avila, Santhanam, & Sehgal, 1990; Santhanam et al., 1991; Hobel, Dunkel-Schetter, Roesch, Castro, & Arora, 1999; Wadhwa et al., 2004; Murtha et al., 2007; Buss et al., 2009; Entringer, Buss, Andersen, Chicz-DeMet, & Wadhwa, 2011) and reduced fetal growth (Wadhwa et al., 1993; Wadhwa et al., 2004; Bolten et al., 2011).

It is possible that maternal positive affect may exert its beneficial effects on length of gestation by also impacting maternal-placental-fetal endocrine and immune physiology. In non-pregnant individuals associations have been reported between positive affect or optimism

and lower cortisol concentrations, attenuated cortisol awakening responses, lower stress reactivity, and a lower inflammatory milieu (Polk, Cohen, Doyle, Skoner, & Kirschbaum, 2005; Steptoe et al., 2007; Steptoe, O'Donnell, Badrick, Kumari, & Marmot, 2008; Brydon, Walker, Wawrzyniak, Chart, & Steptoe, 2009; Bostock et al., 2011). Also, in the very small number of studies that addressed the association between maternal positive affect or positive life events and stress in pregnancy, preliminary evidence suggests that positive affect may buffer the effect of stress, and therefore, positive affect may dampen endocrine and psychological responses to stress in pregnancy, which may be protective for the developing fetus.

### 5.2.1 Strengths and Limitations

A strength of the current study is the innovative approach to investigate potential protective factors to the crucial issue of preterm birth. The investigation of positive affect in association with health is a newer topic in psychology and behavioral medicine, however, the belief that a mother's psychological state has an impact on the developing fetus has persisted through time and culture. The longitudinal assessed results demonstrate that maternal positive affect in pregnancy has a beneficial impact on the child. Through the control of important covariates, our results show the impact of maternal positive affect over and above the absence of negative affect.

The study has some limitations. Because our assessment of positive affect was based on a self-report questionnaire, and was not assessed on a daily basis, it is possible that participants were inaccurate in their recall of positive affect over the past week. Future studies should use ecological momentary assessment of positive affect. Another limitation is the absence of additional possible confounding or mediating variables that were not assessed; such as social support and health behaviors related to diet and physical activity. However, a

meta-analysis of studies of positive affect and mortality has found that the resilient effects of positive affect persist after accounting for the effects of behavioral factors (Chida & Steptoe, 2008).

### 5.2.2 Clinical Implications and Future Research

To the best of our knowledge, this is the first study to show, in a prospective, longitudinal design, that positive affect is associated with length of gestation and decreased risk of preterm delivery. In this study population, maternal positive affect in pregnancy was a better predictor of gestational length than perceived stress. Therefore, our data support the premise that interventions aimed to increase maternal positive affect may be beneficial for fetal development. More empirical research is needed to examine the role of maternal positive affect as a resilience factor and its effect on pregnancy physiology, and maternal health promoting behavior and fetal development. Also, we admit that a limitation of our study is the absence of additional possible confounding or mediating variables, such as social support and health behaviors related to diet and physical activity, which were not assessed in this study. Future studies will be needed to assess the indirect effect of health habits and positive affect in association with obstetric outcomes.

## 5.3 Article 3: "Die Effektivität des Montreal Imaging Stress Task bei jungen, gesunden Frauen"

(The Efficacy of the Montreal Imaging Stress Task in Young Healthy Women)

This study investigated the efficacy of the Montreal Imaging Stress Task (MIST). Until now, the MIST has been used and evaluated predominantly in MRT studies. In a sample of 97 young healthy females, physiological and subjective measures of stress were obtained under controlled laboratory conditions in a baseline, experimental and relaxation phase. The

MIST led to significantly heightened cortisol, cardiovascular, and electrodermal responses. Therefore, our results show that the MIST leads to a valid stress response outside the MRT scan and therefore can be used as an adequate tool to induce stress in healthy, young women. Our findings replicate the results of prior studies (Pruessner, Champagne, Meaney, & Dagher, 2004; Dedovic et al., 2005; Pruessner et al., 2008; Soliman et al., 2008; La Marca et al., 2011), which showed that the MIST leads to an significant increase of cortisol. Furthermore, our data replicate the results of prior studies that also demonstrated an increase in psychophysiological parameters (Jones et al., 2011; La Marca et al., 2011).

### 5.3.1 Strengths and Limitations

The strength of the current study is that the MIST was evaluated as a reliable stress test for the first time outside an MRT setting in a healthy female population. The MIST can be considered a moderate stress test.

The MIST offers a promising alternative to conventional psychosocial stress tests. Advantages of the MIST are that participants sit still during the stress protocol, therefore causing less artifacts measuring psychobiological parameters like heart rate or electrodermal activity, compared to the Trier Social Stress Test where participants give a speech in front of a audience while standing. Furthermore, since the MIST is considered to evoke a moderate endocrine stress response, the observed findings represent HPA activity closer to naturalistic settings (Smyth et al., 1998).

A limitation of the current study is that it is not evident how the setting outside the MRT could have influenced the stress response of the participants. It could be speculated that in the original setting, the distance to the laboratory staff would reduce the social-evaluative threat. Therefore, our setting could have led to a stronger stress response and that this would explain our higher responder rate compared to other studies.

### 5.3.2 Clinical Implications and Future Research

Future research could compare the "gold standard" stress test TSST with the MIST and the cortisol reactivity in a naturalistic setting in association to psychological outcomes. It would be of interest to test whether the findings, like the results reported in Article 1, follow the same pattern and if other hormonal readouts would be tested, potential differences in CRH or ACTH would be observed.

# Conclusion

Stress in sensitive developmental stages can alter the human stress system. The negative health consequences related to a hyperresponsive/hyporesponsive stress system have been reported in several studies (e.g. Chrousos, 2009; Ehlert, 2013). Congruent with prior studies, the results of our study confirm that in a healthy population, ACEs are associated with blunted cortisol responses to a mental stress test. The MIST was used and proofed to be a valid and reliable method for stress induction. We were able to detect that the most powerful association was between chronic, long enduring events and blunted cortisol responses. This finding supports the assumption, that after an initial phase of heightened reactivity of the stress system, an adaptation to the stressful environment might occur in the individual, resulting in a hyporesponsive HPA axis.

According to Slopen, McLaughin, and Shonkoff (2014), the increased understanding of the underlying pathophysiology that explains the association between ACEs and adult disease can inform the development of more effective preventive or early therapeutic interventions (Slopen, McLaughlin, & Shonkoff, 2014). Therefore, to prevent the HPA axis from a primary hypersensitivity and then from burning out, interventions to buffer the HPA axis are needed. For ACEs, family support or parenting programs, foster placing, or family psychotherapy could be a way to reduce the impact of ACEs on the stress system. Slopen et al. (2014) report in their systematic review results of intervention programs, which aimed to improve cortisol regulation in children who were exposed to adverse environments (foster care, institutional care, maltreatment, parental death, high risk families with drug use, depression or low socio-economic status). Their reviewed literature shows that cortisol regulation in children can be altered by psychosocial interventions targeting children and their caregivers. However, as they state, there is inconsistency with regard to how the interventions influenced cortisol activity, and they also address methodological issues of some studies as a limitation (Slopen et al., 2014).

Therefore, further studies are needed to investigate what specific protective factors can buffer HPA axis reactivity and/or improve cortisol regulation. Even though we have not investigated the direct effect of a protective factor on the HPA axis, we have found positive affect to be an important protective factor in pregnant women. In pregnancy, positive affect significantly reduced the odds of delivering preterm. Maternal stress has been associated with preterm birth in various studies (e.g. (Wadhwa et al., 2011), and positive affect has been related to reduced stress responses. Therefore, future studies are needed to investigate *how* positive affect is related to beneficial health outcomes and if it might be a potential protective factor to buffer HPA axis reactivity also in children affected by adverse and stressful environments.

# References


Alder, J., Fink, N., Bitzer, J., Hosli, I., & Holzgreve, W. (2007). Depression and anxiety during pregnancy: a risk factor for obstetric, fetal and neonatal outcome? A critical review of the literature. *J Matern Fetal Neonatal Med, 20*(3), 189-209.

Ali, N., & Pruessner, J. C. (2012). The salivary alpha amylase over cortisol ratio as a marker to assess dysregulation of the stress system. *Physiol Behav, 106*(1), 65-72.

Andrews, J., & Pruessner, J. (2012). The combined dexamethasone/TSST paradigm: A new method for psychoneuroendocrinology. *PLoS One, 7*(6).

Andrews, J., & Pruessner, J. (2013). The combined propanol/TSST paradigm - A new method for psychoneuroendocrinology. *PLoS One, 8*(2).

Bangasser, D. A. (2013). Sex differences in stress-related receptors: "micro" differences with "macro" implications for mood and anxiety disorders. *Biol Sex Differ, 4*(1), 2.

Bangasser, D. A., & Valentino, R. J. (2012). Sex differences in molecular and cellular substrates of stress. *Cell Mol Neurobiol, 32*(5), 709-23.

Bauer, A. M., Quas, J. A., & Boyce, W. T. (2002a). Associations between physiological reactivity and children's behavior: advantages of a multisystem approach. *J Dev Behav Pediatr, 23*(2), 102-13.

Beydoun, H., & Saftlas, A. F. (2008). Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. *Paediatr Perinat Epidemiol, 22*(5), 438-66.

Bhattacharyya, M. R., Whitehead, D. L., Rakhit, R., & Steptoe, A. (2008). Depressed mood, positive affect, and heart rate variability in patients with suspected coronary artery disease. *Psychosom Med, 70*(9), 1020-7.

Bolten, M. I., Wurmser, H., Buske-Kirschbaum, A., Papousek, M., Pirke, K. M., & Hellhammer, D. (2011). Cortisol levels in pregnancy as a psychobiological predictor for birth weight. *Arch Womens Ment Health, 14*(1), 33-41.

Bostock, S., Hamer, M., Wawrzyniak, A. J., Mitchell, E. S., & Steptoe, A. (2011). Positive emotional style and subjective, cardiovascular and cortisol responses to acute laboratory stress. *Psychoneuroendocrinology, 36*(8), 1175-83.

Bremner, J. D., Bolus, R., & Mayer, E. A. (2007). Psychometric properties of the Early Trauma Inventory-Self Report. *J Nerv Ment Dis, 195*(3), 211-8.

Bremner, J. D., Vythilingam, M., Vermetten, E., Adil, J., Khan, S., Nazeer, A., et al. (2003). Cortisol response to a cognitive stress challenge in posttraumatic stress disorder (PTSD) related to childhood abuse. *Psychoneuroendocrinology, 28*(6), 733-50.

Brummett, B. H., Boyle, S. H., Kuhn, C. M., Siegler, I. C., & Williams, R. B. (2009). Positive affect is associated with cardiovascular reactivity, norepinephrine level, and morning rise in salivary cortisol. *Psychophysiology, 46*(4), 862-9.

Brydon, L., Walker, C., Wawrzyniak, A. J., Chart, H., & Steptoe, A. (2009). Dispositional optimism and stress-induced changes in immunity and negative mood. *Brain Behav Immun, 23*(6), 810-6.

Buss, C., Entringer, S., Reyes, J. F., Chicz-DeMet, A., Sandman, C. A., Waffarn, F., et al. (2009). The maternal cortisol awakening response in human pregnancy is associated with the length of gestation. *Am J Obstet Gynecol, 201*(4), 398 e1-8.

Carpenter, L. L., Carvalho, J. P., Tyrka, A. R., Wier, L. M., Mello, A. F., Mello, M. F., et al. (2007). Decreased adrenocorticotropic hormone and cortisol responses to stress in healthy adults reporting significant childhood maltreatment. *Biol Psychiatry, 62*(10), 1080-7.

Carpenter, L. L., Shattuck, T. T., Tyrka, A. R., Geracioti, T. D., & Price, L. H. (2010). Effect of childhood physical abuse on cortisol stress response. *Psychopharmacology (Berl)*. *214*(1), 367-75.

Charmandari, E., Achermann, J. C., Carel, J. C., Soder, O., & Chrousos, G. P. (2012). Stress response and child health. *Sci Signal, 5*(248), mr1.

Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of the stress response. *Annu Rev Physiol, 67*, 259-84.

Chida, Y., & Steptoe, A. (2008). Positive psychological well-being and mortality: a quantitative review of prospective observational studies. *Psychosom Med, 70*(7), 741-56.

Chrousos, G. P. (1995). The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med, 332*(20), 1351-62.

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nat Rev Endocrinol, 5*(7), 374-81.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *J Health Soc Behav, 24*(4), 385-96.

Costello, E. J., Mustillo, S., Erkanli, A., Keeler, G., & Angold, A. (2003). Prevalence and development of psychiatric disorders in childhood and adolescence. *Arch Gen Psychiatry, 60*(8), 837-44.

Culhane, J. F., Rauh, V., McCollum, K. F., Elo, I. T., & Hogan, V. (2002). Exposure to chronic stress and ethnic differences in rates of bacterial vaginosis among pregnant women. *Am J Obstet Gynecol, 187*(5), 1272-6.

Culhane, J. F., Rauh, V., McCollum, K. F., Hogan, V. K., Agnew, K., & Wadhwa, P. D. (2001). Maternal stress is associated with bacterial vaginosis in human pregnancy. *Matern Child Health J, 5*(2), 127-34.

Danese, A., & McEwen, B. S. (2012). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiol Behav, 106*(1), 29-39.

Davidson, R. J. (2004). Well-being and affective style: neural substrates and biobehavioural correlates. *Philos Trans R Soc Lond B Biol Sci, 359*(1449), 1395-411.

De Bellis, M. D. (2002). Developmental traumatology: a contributory mechanism for alcohol and substance use disorders. *Psychoneuroendocrinology, 27*(1-2), 155-70.

De Neve, J. E. (2011). Functional polymorphism (5-HTTLPR) in the serotonin transporter gene is associated with subjective well-being: evidence from a US nationally representative sample. *J Hum Genet, 56*(6), 456-9.

De Neve, J. E., Christakis, N. A., Fowler, J. H., & Frey, B. S. (2012). Genes, Economics, and Happiness. *J Neurosci Psychol* Econ, 5(4) 10.1037/a0030292..

De Neve, J. E., Diener, E., Tay, L., & Xuereb C. (2013). The objective benefits of subjective well-being. In J. F. Helliwell, Layard, R., & Sachs, J (Ed.), *World happiness report.* (pp. 54 - 79). New York: Earth Institute.

de Rooij, S. R. (2012). Blunted cardiovascular and cortisol reactivity to acute psychological stress: A summary of results from the Dutch Famine Birth Cohort Study. *Int J Psychophysi*ol. *90*(1), 21-7.

Dedovic, K., Renwick, R., Mahani, N. K., Engert, V., Lupien, S. J., & Pruessner, J. C. (2005). The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. *J Psychiatry Neurosci, 30*(5), 319-25.

Dettling, A. C., Parker, S. W., Lane, S., Sebanc, A., & Gunnar, M. R. (2000). Quality of care and temperament determine changes in cortisol concentrations over the day for young children in childcare. *Psychoneuroendocrinology, 25*(8), 819-36.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull, 130*(3), 355-91.

Diener, E., Chan, M. (2011). Happy people live longer: subjective well-being contributes to health and longevity. *Applied Psychology: Health and Well-being, 3*, 1 - 43.

Ditzen, B., Hoppmann, C., & Klumb, P. (2008). Positive couple interactions and daily cortisol: on the stress-protecting role of intimacy. *Psychosom Med, 70*(8), 883-9.

Dockray, S., & Steptoe, A. (2010). Positive affect and psychobiological processes. *Neurosci Biobehav Rev, 35*(1), 69-75.

Ehlert, U. (2013). Enduring psychobiological effects of childhood adversity. *Psychoneuroendocrinology, 38*(9), 1850-7.

Ehlert, U., La Marca, R., E., A., & Kübler, U. (2013). *Biopsychologie*. Stuttgart: Kohlhammer.

Elzinga, B. M., Roelofs, K., Tollenaar, M. S., Bakvis, P., van Pelt, J., & Spinhoven, P. (2008). Diminished cortisol responses to psychosocial stress associated with lifetime adverse events a study among healthy young subjects. *Psychoneuroendocrinology, 33*(2), 227-37.

Engert, V., Buss, C., Khalili-Mahani, N., Wadiwalla, M., Dedovic, K., & Pruessner, J. C. (2010). Investigating the association between early life parental care and stress responsivity in adulthood. *Dev Neuropsychol, 35*(5), 570-81.

Entringer, S., Buss, C., Andersen, J., Chicz-DeMet, A., & Wadhwa, P. D. (2011). Ecological momentary assessment of maternal cortisol profiles over a multiple-day period predicts the length of human gestation. *Psychosom Med, 73*(6), 469-74.

Entringer, S., Buss, C., Shirtcliff, E. A., Cammack, A. L., Yim, I. S., Chicz-DeMet, A., et al. (2010). Attenuation of maternal psychophysiological stress responses and the maternal cortisol awakening response over the course of human pregnancy. *Stress, 13*(3), 258-68.

Entringer, S., Kumsta, R., Hellhammer, D. H., Wadhwa, P. D., & Wust, S. (2009). Prenatal exposure to maternal psychosocial stress and HPA axis regulation in young adults. *Horm Behav, 55*(2), 292-8.

Entringer, S., Kumsta, R., Nelson, E. L., Hellhammer, D. H., Wadhwa, P. D., & Wust, S. (2008). Influence of prenatal psychosocial stress on cytokine production in adult women. *Dev Psychobiol, 50*(6), 579-87.

Entringer, S., Wust, S., Kumsta, R., Layes, I. M., Nelson, E. L., Hellhammer, D. H., et al. (2008). Prenatal psychosocial stress exposure is associated with insulin resistance in young adults. *Am J Obstet Gynecol, 199*(5), 498.e1-498.e7.

Famularo, R., Kinscherff, R., & Fenton, T. (1992). Psychiatric diagnoses of maltreated children: preliminary findings. *J Am Acad Child Adolesc Psychiatry, 31*(5), 863-7.

Fisher, P. A., Stoolmiller, M., Gunnar, M. R., & Burraston, B. O. (2007). Effects of a therapeutic intervention for foster preschoolers on diurnal cortisol activity. *Psychoneuroendocrinology, 32*(8-10), 892-905.

Fredrickson, B. L. (2001). The role of positive emotions in positive psychology. The broaden-and-build theory of positive emotions. *Am Psychol, 56*(3), 218-26.

Fries, E., Hesse, J., Hellhammer, J., & Hellhammer, D. H. (2005). A new view on hypocortisolism. *Psychoneuroendocrinology, 30*(10), 1010-6.

Gaab, J., Blattler, N., Menzi, T., Pabst, B., Stoyer, S., & Ehlert, U. (2003). Randomized controlled evaluation of the effects of cognitive-behavioral stress management on cortisol responses to acute stress in healthy subjects. *Psychoneuroendocrinology, 28*(6), 767-79.

Gaab, J., Sonderegger, L., Scherrer, S., & Ehlert, U. (2006). Psychoneuroendocrine effects of cognitive-behavioral stress management in a naturalistic setting--a randomized controlled trial. *Psychoneuroendocrinology, 31*(4), 428-38.

Giedd, J. N., & Rapoport, J. L. (2010). Structural MRI of pediatric brain development: what have we learned and where are we going? *Neuron, 67*(5), 728-34.

Gilbey, M. P., & Spyer, K. M. (1993). Essential organization of the sympathetic nervous system. *Baillieres Clin Endocrinol Metab, 7*(2), 259-78.

Glynn, L. M., Wadhwa, P. D., Dunkel-Schetter, C., Chicz-Demet, A., & Sandman, C. A. (2001). When stress happens matters: effects of earthquake timing on stress responsivity in pregnancy. *Am J Obstet Gynecol, 184*(4), 637-42.

Gold, P. W., & Chrousos, G. P. (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol Psychiatry, 7*(3), 254-75.

Grant, N., Wardle, J., & Steptoe, A. (2009). The relationship between life satisfaction and health behavior: a cross-cultural analysis of young adults. *Int J Behav Med, 16*(3), 259-68.

Griep, E. N., Boersma, J. W., Lentjes, E. G., Prins, A. P., van der Korst, J. K., & de Kloet, E. R. (1998). Function of the hypothalamic-pituitary-adrenal axis in patients with fibromyalgia and low back pain. *J Rheumatol, 25*(7), 1374-81.

Gunnar, M. R., Frenn, K., Wewerka, S. S., & Van Ryzin, M. J. (2009). Moderate versus severe early life stress: associations with stress reactivity and regulation in 10-12-year-old children. *Psychoneuroendocrinology, 34*(1), 62-75.

Gunnar, M. R., Larson, M. C., Hertsgaard, L., Harris, M. L., & Brodersen, L. (1992). The stressfulness of separation among nine-month-old infants: effects of social context variables and infant temperament. *Child Dev, 63*(2), 290-303.

Gur, A., Cevik, R., Nas, K., Colpan, L., & Sarac, S. (2004). Cortisol and hypothalamic-pituitary-gonadal axis hormones in follicular-phase women with fibromyalgia and chronic fatigue syndrome and effect of depressive symptoms on these hormones. *Arthritis Res Ther, 6*(3), R232-8.

Gurung, R. A. R., Dunkel-Schetter, C., Collins, N., Rini, C. M., & Hobel, C. J. (2005). Psychosocial predictors of prenatal anxiety. . *J Soc Clin Psychol, 24*(4), 497-519.

Halligan, S. L., Herbert, J., Goodyer, I. M., & Murray, L. (2004). Exposure to postnatal depression predicts elevated cortisol in adolescent offspring. *Biol Psychiatry, 55*(4), 376-81.

Hammerfald, K., Eberle, C., Grau, M., Kinsperger, A., Zimmermann, A., Ehlert, U., et al. (2006). Persistent effects of cognitive-behavioral stress management on cortisol responses to acute stress in healthy subjects--a randomized controlled trial. *Psychoneuroendocrinology, 31*(3), 333-9.

Heim, C., Ehlert, U., Hanker, J. P., & Hellhammer, D. H. (1998). Abuse-related posttraumatic stress disorder and alterations of the hypothalamic-pituitary-adrenal axis in women with chronic pelvic pain. *Psychosom Med, 60*(3), 309-18.

Heim, C., Ehlert, U., & Hellhammer, D. H. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology, 25*(1), 1-35.

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry, 49*(12), 1023-39.

Heim, C., Newport, D. J., Heit, S., Graham, Y. P., Wilcox, M., Bonsall, R., et al. (2000). Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA, 284*(5), 592-7.

Heim, C., Newport, D. J., Mletzko, T., Miller, A. H., & Nemeroff, C. B. (2008). The link between childhood trauma and depression: Insights from HPA axis studies in humans. *Psychoneuroendocrinology, 33*(6), 693-710.

Hellhammer, D. H., & Hellhammer, J. (Eds.). (2008). *Stress: the brain-body connection* (Vol. 174). Basel: Karger.

Hobel, C. J., Dunkel-Schetter, C., Roesch, S. C., Castro, L. C., & Arora, C. P. (1999). Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. *Am J Obstet Gynecol, 180*(1 Pt 3), S257-63.

Hobel, C. J., Goldstein, A., & Barrett, E. S. (2008). Psychosocial stress and pregnancy outcome. *Clin Obstet Gynecol, 51*(2), 333-48.

Horrocks, P. M., Jones, A. F., Ratcliffe, W. A., Holder, G., White, A., Holder, R., et al. (1990). Patterns of ACTH and cortisol pulsatility over twenty-four hours in normal males and females. *Clin Endocrinol (Oxf), 32*(1), 127-34.

Jones, A., McMillan, M. R., Jones, R. W., Kowalik, G. T., Steeden, J. A., Deanfield, J. E., et al. (2012). Adiposity is associated with blunted cardiovascular, neuroendocrine and cognitive responses to acute mental stress. *PLoS One, 7*(6), e39143.

Jones, A., Steeden, J. A., Pruessner, J. C., Deanfield, J. E., Taylor, A. M., & Muthurangu, V. (2011). Detailed assessment of the hemodynamic response to psychosocial stress using real-time MRI. *J Magn Reson Imaging, 33*(2), 448-54.

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosom Med, 61*(2), 154-62.

La Marca, R., Waldvogel, P., Thorn, H., Tripod, M., Wirtz, P. H., Pruessner, J. C., et al. (2011). Association between Cold Face Test-induced vagal inhibition and cortisol response to acute stress. *Psychophysiology, 48*(3), 420-9.

La Prairie, J. L., Heim, C.M., & Nemeroff, C.B. . (2010). The neroendocrine effects of early life trauma. In E. V. C. P. R.A. Lanius (Ed.), *The Impact of Early Life Trauma on Health and Disease: The Hidden Epidemic*. Cambridge: Cambridge University Press.

Lazarus, R. S., & Folkman, S. (1984). *Stress, Appraisal and Coping*. New York: Springer.

Lovallo, W. R., Dickensheets, S. L., Myers, D. A., Thomas, T. L., & Nixon, S. J. (2000). Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. *Alcohol Clin Exp Res, 24*(5), 651-8.

Lovallo, W. R., Farag, N. H., Sorocco, K. H., Cohoon, A. J., & Vincent, A. S. (2012). Lifetime adversity leads to blunted stress axis reactivity: studies from the Oklahoma Family Health Patterns Project. *Biol Psychiatry, 71*(4),

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci, 10*(6), 434-45.

Mancuso, R. A., Schetter, C. D., Rini, C. M., Roesch, S. C., & Hobel, C. J. (2004). Maternal prenatal anxiety and corticotropin-releasing hormone associated with timing of delivery. *Psychosom Med, 66*(5), 762-9.

Marsland, A. L., Cohen, S., Rabin, B. S., & Manuck, S. B. (2006). Trait positive affect and antibody response to hepatitis B vaccination. *Brain Behav Immun, 20*(3), 261-9.

Mason, J. W. (1968). A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosom Med, 30*(5), Suppl:576-607.

Mastorakos, G., & Ilias, I. (2003). Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Ann N Y Acad Sci, 997*, 136-49.

McCormick, C. M., & Mathews, I. Z. (2007). HPA function in adolescence: role of sex hormones in its regulation and the enduring consequences of exposure to stressors. *Pharmacol Biochem Behav, 86*(2), 220-33.

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *N Engl J Med, 338*(3), 171-9.

McEwen, B. S. (2006). Protective and damaging effects of stress mediators: central role of the brain. *Dialogues Clin Neurosci, 8*(4), 367-81.

McEwen, B. S. (2007). Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev, 87*(3), 873-904.

McEwen, B. S. (2008). Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol, 583*(2-3), 174-85.

McEwen, B. S., & Gianaros, P. J. (2011). Stress- and allostasis-induced brain plasticity. *Annu Rev Med, 62*, 431-45.

McGowan, P. O., Sasaki, A., D'Alessio, A. C., Dymov, S., Labonte, B., Szyf, M., et al. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci, 12*(3), 342-8.

Miller, G. E., Chen, E., & Zhou, E. S. (2007). If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychol Bull, 133*(1), 25-45.

Muglia, L. J., & Katz, M. (2010). The enigma of spontaneous preterm birth. *N Engl J Med, 362*(6), 529-35.

Murphy, V. E., Smith, R., Giles, W. B., & Clifton, V. L. (2006). Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev, 27*(2), 141-69.

Murtha, A. P., Sinclair, T., Hauser, E. R., Swamy, G. K., Herbert, W. N., & Heine, R. P. (2007). Maternal serum cytokines in preterm premature rupture of membranes. *Obstet Gynecol, 109*(1), 121-7.

Netherton, C., Goodyer, I., Tamplin, A., & Herbert, J. (2004). Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender. *Psychoneuroendocrinology, 29*(2), 125-40.

Nierop, A., Wirtz, P. H., Bratsikas, A., Zimmermann, R., & Ehlert, U. (2008). Stress-buffering effects of psychosocial resources on physiological and psychological stress response in pregnant women. *Biol Psychol, 78*(3), 261-8.

Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., & Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics, 3*(2), 97-106.

Obradovic, J. (2012). How can the study of physiological reactivity contribute to our understanding of adversity and resilience processes in development? *Dev Psychopathol, 24*(2), 371-87.

Ong, A. D., & Allaire, J. C. (2005). Cardiovascular intraindividual variability in later life: the influence of social connectedness and positive emotions. *Psychol Aging, 20*(3), 476-85.

Patton, G. C., & Viner, R. (2007). Pubertal transitions in health. *Lancet, 369*(9567), 1130-9.

Phillips, A. C., Hunt, K., Der, G., & Carroll, D. (2011). Blunted cardiac reactions to acute psychological stress predict symptoms of depression five years later: evidence from a large community study. *Psychophysiology, 48*(1), 142-8.

Phillips, A. C., Roseboom, T. J., Carroll, D., & de Rooij, S. R. (2012). Cardiovascular and cortisol reactions to acute psychological stress and adiposity: cross-sectional and

prospective associations in the Dutch Famine Birth Cohort Study. *Psychosom Med, 74*(7), 699-710.

Polk, D. E., Cohen, S., Doyle, W. J., Skoner, D. P., & Kirschbaum, C. (2005). State and trait affect as predictors of salivary cortisol in healthy adults. *Psychoneuroendocrinology, 30*(3), 261-72.

Pruessner, J. C., Champagne, F., Meaney, M. J., & Dagher, A. (2004). Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. *J Neurosci, 24*(11), 2825-31.

Pruessner, J. C., Dedovic, K., Khalili-Mahani, N., Engert, V., Pruessner, M., Buss, C., et al. (2008). Deactivation of the limbic system during acute psychosocial stress: evidence from positron emission tomography and functional magnetic resonance imaging studies. *Biol Psychiatry, 63*(2), 234-40.

Pruessner, J. C., Hellhammer, D. H., & Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosom Med, 61*(2), 197-204.

Rao, U., Hammen, C., Ortiz, L. R., Chen, L. A., & Poland, R. E. (2008). Effects of early and recent adverse experiences on adrenal response to psychosocial stress in depressed adolescents. *Biol Psychiatry, 64*(6), 521-6.

Rini, C. K., Dunkel-Schetter, C., Wadhwa, P. D., & Sandman, C. A. (1999). Psychological adaptation and birth outcomes: the role of personal resources, stress, and sociocultural context in pregnancy. *Health Psychol, 18*(4), 333-45.

Robinson, B. G., Emanuel, R. L., Frim, D. M., & Majzoub, J. A. (1988). Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. *Proc Natl Acad Sci U S A, 85*(14), 5244-8.

Romeo, R. D. (2010). Adolescence: a central event in shaping stress reactivity. *Dev Psychobiol, 52*(3), 244-53.

Romeo, R. D., Richardson, H. N., & Sisk, C. L. (2002). Puberty and the maturation of the male brain and sexual behavior: recasting a behavioral potential. *Neurosci Biobehav Rev, 26*(3), 381-91.

Romero, R., Avila, C., Santhanam, U., & Sehgal, P. B. (1990). Amniotic fluid interleukin 6 in preterm labor. Association with infection. *J Clin Invest, 85*(5), 1392-400.

Sandman, C. A., Glynn, L., Schetter, C. D., Wadhwa, P., Garite, T., Chicz-DeMet, A., et al. (2006). Elevated maternal cortisol early in pregnancy predicts third trimester levels of

placental corticotropin releasing hormone (CRH): priming the placental clock. *Peptides, 27*(6), 1457-63.

Santhanam, U., Avila, C., Romero, R., Viguet, H., Ida, N., Sakurai, S., et al. (1991). Cytokines in normal and abnormal parturition: elevated amniotic fluid interleukin-6 levels in women with premature rupture of membranes associated with intrauterine infection. *Cytokine, 3*(2), 155-63.

Sass, H., Wittchen, H.-U. & Zaudig, M. (2003). *Diagnostisches und Statistisches Manual Psychischer Störungen: Textrevision (DSM-IV-TR).* . Göttingen: Hogrefe.

Schneider, M., Graham, D., Grant, A., King, P., & Cooper, D. (2009). Regional brain activation and affective response to physical activity among healthy adolescents. *Biol Psychol, 82*(3), 246-52.

Schoedl, A. F., Costa, M. C., Mari, J. J., Mello, M. F., Tyrka, A. R., Carpenter, L. L., et al. (2010). The clinical correlates of reported childhood sexual abuse: an association between age at trauma onset and severity of depression and PTSD in adults. *J Child Sex Abus, 19*(2), 156-70.

Slopen, N., McLaughlin, K. A., & Shonkoff, J. P. (2014). Interventions to Improve Cortisol Regulation in Children: A Systematic Review. *Pedi*atrics, *133*(2), 312-26.

Smyth, J., Ockenfels, M. C., Porter, L., Kirschbaum, C., Hellhammer, D. H., & Stone, A. A. (1998). Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. *Psychoneuroendocrinology, 23*(4), 353-70.

Soliman, A., O'Driscoll, G. A., Pruessner, J., Holahan, A. L., Boileau, I., Gagnon, D., et al. (2008). Stress-induced dopamine release in humans at risk of psychosis: a [11C]raclopride PET study. *Neuropsychopharmacology, 33*(8), 2033-41.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev, 24*(4), 417-63.

Spear, L. P. (2009). Heightened stress responsivity and emotional reactivity during pubertal maturation: Implications for psychopathology. *Dev Psychopathol, 21*(1), 87-97.

Steptoe, A. (2010). Positive Well-Being and Health. In A. Steptoe (Ed.), *Handbook of Behavioral Medicine* (pp. 185 - 95). London: Springer.

Steptoe, A., Gibson, E. L., Hamer, M., & Wardle, J. (2007). Neuroendocrine and cardiovascular correlates of positive affect measured by ecological momentary assessment and by questionnaire. *Psychoneuroendocrinology, 32*(1), 56-64.

Steptoe, A., O'Donnell, K., Badrick, E., Kumari, M., & Marmot, M. (2008). Neuroendocrine and inflammatory factors associated with positive affect in healthy men and women: the Whitehall II study. *Am J Epidemiol, 167*(1), 96-102.

Steptoe, A., & Wardle, J. (2011). Positive affect measured using ecological momentary assessment and survival in older men and women. *Proc Natl Acad Sci U S A, 108*(45), 18244-8.

Stroud, L. R., Foster, E., Papandonatos, G. D., Handwerger, K., Granger, D. A., Kivlighan, K. T., et al. (2009). Stress response and the adolescent transition: performance versus peer rejection stressors. *Dev Psychopathol, 21*(1), 47-68.

Tarullo, A. R., & Gunnar, M. R. (2006). Child maltreatment and the developing HPA axis. *Horm Behav, 50*(4), 632-9.

Tomkins, S. S. (1963). *Affect Imagery Consciousness: The Positive Affects*. New York: Springer.

Tottenham, N., & Sheridan, M. A. (2010). A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. *Front Hum Neurosci, 3*, 68.

Tsigos, C., & Chrousos, G. P. (1994). Physiology of the hypothalamic-pituitary-adrenal axis in health and dysregulation in psychiatric and autoimmune disorders. *Endocrinol Metab Clin North Am, 23*(3), 451-66.

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res, 53*(4), 865-71.

Tugade, M. M., Fredrickson, B. L., & Barrett, L. F. (2004). Psychological resilience and positive emotional granularity: examining the benefits of positive emotions on coping and health. *J Pers, 72*(6), 1161-90.

Tyrka, A. R., Price, L. H., Marsit, C., Walters, O. C., & Carpenter, L. L. (2012). Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults. *PLoS One, 7*(1), e30148.

US Department of Health and Human Services (2008). *Child maltreatment 2006*. Washington, DC: US Government Prinitng Agency.

Wadhwa, P. D., Entringer, S., Buss, C., & Lu, M. C. (2011). The contribution of maternal stress to preterm birth: issues and considerations. *Clin Perinatol, 38*(3), 351-84.

Wadhwa, P. D., Garite, T. J., Porto, M., Glynn, L., Chicz-DeMet, A., Dunkel-Schetter, C., et al. (2004). Placental corticotropin-releasing hormone (CRH), spontaneous preterm birth, and fetal growth restriction: a prospective investigation. *Am J Obstet Gynecol, 191*(4), 1063-9.

Wadhwa, P. D., Sandman, C. A., Porto, M., Dunkel-Schetter, C., & Garite, T. J. (1993). The association between prenatal stress and infant birth weight and gestational age at birth: a prospective investigation. *Am J Obstet Gynecol, 169*(4), 858-65.

Wittchen, H.-U., Wunderlich, U., Gruschwitz, S., & Zaudig, M. (1997). *SKID-I: Strukturiertes Klinisches Interview für DSM-IV - Achse I: Psychische Störungen*. Göttingen: Hogrefe.

Appendix A

Voellmin, A., Winzeler, K., Hug, E., Wilhelm, F.H., Schaefer, V., Gaab, J., La Marca, R., Pruessner, J.C., & Bader, K. (submitted).

Blunted endocrine and cardiovascular reactivity in young healthy women reporting a history of childhood adversity. *Psychoneuroendocrinology.*

# Blunted endocrine and cardiovascular reactivity in young healthy women reporting a history of childhood adversity

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## Abstract

**Background**: Chronic or prolonged stress exposure in childhood can alter structural and functional brain development, leading to mental and physical illness and alterations of psychobiological stress systems in adulthood. Recently, attenuation in stress reactivity of the hypothalamic-puituitary-adrenal (HPA) axis and cardiovascular system have been related to the number of adverse childhood experiences (ACEs). We set out to investigate the effects of ACE duration and age of ACE onset as predictors of stress reactivity. **Methods**: 104 women in the age range 18-25 years (mean=21.7) free of mental and physical illness underwent psychosocial stress testing with the Montreal Imaging Stress Task (MIST). Free saliva cortisol and heart rate were assessed repeatedly before and after the MIST. **Results:** Number of ACEs was associated with attenuated cortisol and heart rate responses to stress in a dose-response relationship. Whereas overall duration of ACEs significantly influenced cortisol response, age of first ACE onset did not contribute further to the dampened stress response. **Conclusions**: ACEs are associated with blunted endocrine and cardiovascular stress reactivity in young and healthy women. Adverse life events in childhood, particularly if they occur repeatedly and chronically, have a strong impact on stress reactivity in adulthood, potentially predisposing for later mental or physical disorders.


***Keywords*: hypothalamus-pituitary-adrenal axis, sympathetic nervous system, adverse childhood experiences, female, stress reactivity, resilience, trauma**

## 1. Introduction

Adverse childhood experiences (ACEs), including physical, emotional and sexual abuse, affect a significant portion of the population and have been shown to be risk factors for the development and persistence of mental disorders such as depression, anxiety disorders, substance use disorders, or attention-deficit/hyperactivity disorder. Changes in stress sensitivity and functioning of the hypothalamic-pituitary-adrenal (HPA) axis have been suggested as causal factors (De Bellis, 2002; Tarullo and Gunnar, 2006; Heim et al., 2008). The HPA axis, together with the sympathetic nervous system (SNS), are key players in the formation of the stress response (Chrousos, 2009). Models of stress reactivity and health outcomes have therefore been a main focus in research towards understanding adversity and resilience processes. Different reactivity phenotype patterns have emerged in different studies, with a number of studies showing exaggerated HPA axis and SNS levels in the context of ACEs and psychopathology (Heim et al., 2000b; Heim and Nemeroff, 2001; Bremner et al., 2003; Rao et al., 2008).

In contrast to findings in clinical samples, a growing number of studies on healthy participants reported blunted endocrine (Carpenter et al., 2007; Elzinga et al., 2008; Carpenter et al., 2010; Lovallo et al., 2012) and cardiovascular (Lovallo et al., 2012) responses in association with ACEs. For example, Carpenter et al. (2007) reported blunted plasma cortisol responses to a psychosocial stress test in a healthy sample with a history of ACEs compared to participants without a history of childhood maltreatment (Carpenter et al., 2007). Also, a recent study by Lovallo et al. (2012) showed in a large sample of healthy participants diminished cortisol as well as heart rate responses with an increasing number of adverse life events, indicating an inverse dose-response relationship of ACEs and reactivity to a mental stress test (Lovallo et al., 2012).

To the best of our knowledge, the impact of age of onset and duration of ACEs has not been investigated in healthy samples so far. There is evidence that these factors could have a differential impact on stress reactivity in adulthood (Tarullo and Gunnar, 2006; Schoedl et al., 2010; Tottenham and Sheridan, 2010). Since most studies used brief self-report questionnaires or life events checklists to assess ACEs (e.g. (Carpenter et al., 2010; Lovallo et al., 2012)), questions of duration of the events or the respective age when they happened widely remain unanswered. From a developmental perspective, age at traumatization is believed to be an important factor. Brain components involved in stress response show large plasticity during pre- and postnatal periods and during early childhood, and some plasticity during later childhood and adolescence (Andersen et al., 2008). Also, the duration of adverse experiences could have an impact on psychobiological constructs. Particularly those environmental events that cause exceeding or prolonged stimulation of the stress system during these critical developmental periods could lead to abnormal neurodevelopment and therefore to lasting alterations in stress reactivity of the HPA axis and the SNS (Schoedl et al., 2010).

Therefore, the aim of the present study was to replicate the findings of attenuated endocrine and cardiovascular stress reactivity in association with a history of ACEs in a young, healthy, female adult sample. Furthermore, we aimed to investigate the impact of duration as well as age of onset of adverse life events in childhood and adolescence on the stress reactivity in adulthood. To elucidate the impact of age of onset and duration, in this study, the Early Trauma Inventory-Self Report (ETI-SR) served as a more detailed method in measuring ACEs (Bremner et al., 2007). The questionnaire retrospectively assesses a wide range of stress and trauma exposure before the age of 18 and considers age of onset as well as duration of the events. In contrast to Lovallo et al. (2012) who employed standard public speaking and mental arithmetic stressors, we used a novel

stress protocol, the Montreal Imaging Stress Task (MIST), that has been developed to be compatible with functional magnetic resonance brain imaging (Dedovic et al., 2005), but can be used in laboratory stress studies as well (La Marca et al., 2011).

## 2. Methods and Materials

### 2.1 Participants

The sample included 104 young and healthy females in the age of 18 to 25 years (M=21.7; SD=1.5), recruited at three schools for health care professions and social work in Basel, Switzerland. The sample was part of an ongoing project which included only female participants. Exclusion criteria were physical or psychiatric illness, pregnancy, regular and heavy tobacco use (> 5 cigarettes a day), the consumption of illegal drugs, and the use of medication that interferes with the central nervous or the adrenocorticoid system. Furthermore, participants were requested to minimize physical exercise during the hour preceding the laboratory examination and not to take large meals, coffee, or cigarettes. For participants taking no oral contraceptives, the laboratory assessment was held in the luteal phase of the participant's menstrual cycle (Kirschbaum et al., 1999).

Participants received monetary compensation for their participation and provided written informed consent prior to participation. The ethical principles of the Declaration of Helsinki were followed and the study was accepted by the local Ethics Committee. All appointments took place in a laboratory of the Psychiatric Clinics of the University of Basel, Switzerland.

### 2.2 Procedure

After a preliminary screening assessment, participants reported to the laboratory for the stress examination, which took place between 3:30 pm and 6:00 pm to control for

circadian variation and lasted for approximately 2.5 hours. Participants were told that the laboratory assessment would include a test on cognitive performance.

Upon arrival, participants were seated in a comfortable chair in front of a table with a computer screen and several magazines. After the heart rate sensors were attached, a ten minute resting period followed to customize participants with the laboratory. Then, a baseline measurement was conducted for five minutes. Immediately before the baseline measurement, participants provided the first saliva sample. Another saliva sample was collected immediately before participants engaged in the MIST. Following the stress exposure, a recovery period was conducted during which five additional salivary cortisol samples were collected together with self-report measures of the participants' emotional response to the stress task. At the end of the laboratory testing, participants were debriefed and signed a second written informed consent to approve the further use of their data.

**2.3 Stress induction**

The MIST (La Marca et al., 2011) was used to induce a multidimensional stress response. The MIST (Dedovic et al., 2005) is a standardized psychosocial stress test during which participants have to solve arithmetic tasks displayed on a computer screen under time pressure and social evaluation. The software adapts the difficulty of the tasks to the individual performance level of each participant, so that it is not possible to correctly answer more than 45-50 % of the arithmetic tasks in the experimental condition. Participants had to complete three experimental runs, each lasting four minutes. After each of the first two runs, to further enhance social evaluative threat, participants were informed by the investigator that their performance was poor and that they were expected to do better in the subsequent run.

## 2.4 Measures

### 2.4.1 Biological measures

Free salivary cortisol was collected at seven measurement points, whereof two took place before the stress test (-10 and -1 mins) and five after the stress test (+1, +10, + 25, +40 and +55 mins) using salivettes (Sarstedt, Sevelen, Switzerland). All saliva samples were first stored at -22 °C, then thawed and centrifuged at 3000rpm, before cortisol concentration in saliva was determined by enzyme immunoassay (Alpco Diagnostics, 2012). The average intra-assay variability of the enzyme immunoassay test is 8.27 %, whereas the average inter-assay variability was determined at 8.33 % (Alpco Diagnostics, 2012).

Heart rate was recorded using Vitaport 3 data acquisition system (TEMEC Instruments B.V., Netherlands). Electrocardiogram (ECG) recordings were taken using Lead-II electrode placement (RedDot$^{TM}$, 2248-50, 3F Health Care, Germany) on the thorax with three disposable electrodes. A sampling rate of 1024 Hz was used for ECG recordings with a low pass filter of 512 Hz and a high pass filter of 0.5 Hz. Anslab, a software for scientific analysis of physiological data, was used to analyze detected consecutive R-waves and calculate R-R intervals, which were transformed to heart rate (Autonomic Nervous System Laboratory, Wilhelm & Peyk, 2005). Heart rate was averaged for *Baseline*, *Stress 1, Stress 2, Stress 3,* and *Recovery* periods in reference to time markers manually set in accordance with the various sections of the experiment.

### 2.4.2 Psychological measures

A diagnostic screening including the German version of the “Structured Clinical Interview for DSM-IV/Axis I Disorders” (SCID-I) was conducted in order to detect and

exclude participants suffering from a mental disorder (Wittchen et al., 1997). Relevant data including age, medication, drug consumption, age of menarche, BMI, date of last menstruation, and intake of hormonal contraceptives were also assessed during the interview.

ACEs before the age of 18 years were assessed retrospectively using a German translation of the "Early Trauma Inventory-Self Report" (ETI-SR) (Bremner et al., 2007), which includes general trauma (31 items), physical (9 items), emotional (7 items), and sexual abuse (15 items). The ETI-SR has been demonstrated to be a valid measure of early trauma, and has shown high internal consistency in all trauma domains (Cronbach $\alpha > 0.7$) (Bremner et al., 2007).

Participants were asked a series of questions concerning potential trauma and stress exposure, which they answered with yes or no. Next, on positively answered items, age of onset, frequency of trauma or abuse, and emotional impact (0=no negative impact, 1=slightly negative, 2=moderately negative, 3=strongly negative) were assessed. In total, five different ACE scores were built. First, a sum score was computed from all events rated with an emotional impact of at least 1 (*ACE total sum score*). Furthermore, a sum score for ACEs lasting less than a year (*ACEs<one year*) and for ACEs lasting more than a year (*ACEs>one year*) was computed. In a next step, events which occurred before or after a participants' menarche were summed up to *ACEs before* and *after menarche,* respectively. ACE mean scores of the study sample are depicted in Table 1.

Because the ETI-SR does not provide cut-off scores for grouping, evenly distributed quartile groups (*ACE total groups*) were built via rank function of SPSS for the *ACE total sum score*. The grouping via rank function resulted in the following group distributions: group 1 = 0 ACE, group 2 = 1 ACE, group 3 = 2-3 ACEs, and group 4 = 4

or more ACEs. Groups with regard to duration and age of onset were then built according to the same group distribution as for the *ACE total groups*.

Depressive symptomatology was assessed via the German version of the Center for Epidemiological Studies Depression Scale (CES-D; German version: ADS-K; Hautzinger and Bailer, 1993).

Visual Analog Scales (VAS) for mood, tension, and stress served as measures of subjective emotional response of participants during the psychosocial stress test. The scales ranged from "not stressed" (0) to "very stressed" (100), experiencing "no tension" (0) to "extreme tension" (100), and "having a good mood" (0) to "having a bad mood" (100), respectively.

Please insert Table 1 here

**2.5 Data analysis**

Statistical analyses were performed using IBM SPSS Statistics, version 20 (SPSS Inc., Chicago, IL). Descriptive statistics were conducted for all variables. Skewed data were logarithmically transformed where appropriate.

First, repeated measures General Linear Model (GLM) was used to assess if the stress task led to a significant stress response for the dependent variables salivary cortisol, heart rate, as well as for the subjective emotional responses to the MIST. Next, GLMs for repeated measures served to determine the effects of ACEs on endocrine and cardiovascular responses. In these models, the *ACE total sum score* as well as the different scores for duration and age of onset were used as continuous variables to examine effects of time, ACE scores, and the interaction of time by ACE scores. In a

second step, in order to visualize the results, the different ACE groups were then used as fixed factors for the GLMs, respectively.

To protect against violation of sphericity, Greenhouse-Geisser corrections were applied where appropriate. Effect sizes were determined by partial eta-square, reflecting small (.01), medium (.06), or large (.14) effect sizes (Green et al., 2000).

To account for their potential confounding influence on cortisol concentration (Kirschbaum et al., 1999), BMI and use of oral contraceptives were included as covariates in all statistical models. In this sample, depressive symptoms were overall low and were neither related to cortisol, heart rate, nor ACEs (data not shown) and therefore not controlled for in the analyses. Emotional responses to the MIST (mood, tension, stress) were entered as covariates in post-hoc analyses.

Technical difficulties with Vitaport 3 data acquisition system led to data loss in heart rate measurements (missing completely at random). Eventually, heart rate measures of 88 participants were available and went into the analyses. Cortisol data of three subjects had to be excluded because of unlikely high and fluctuating values, or because of acute illness, and therefore, cortisol measures of 101 participants went into the analyses.

## 3. Results

Demographic and trauma characteristics of the sample are displayed in Table 1. According to univariate analyses of variance, the ACE groups did not differ significantly in terms of demographic characteristics (e.g. age, age of onset of menarche, oral contraceptive use, and depressive symptoms). However, for the *ACE total groups*, BMI was significantly higher [p=.02] in women reporting 4 or more ACEs (M=23.38, SD=3.55) compared to women reporting no ACEs (M=21.16, SD=2.07).

Results obtained by GLM repeated measure analyses indicated that the stress task induced a robust and significant increase in cortisol levels [p<.001; $\eta_p^2$=.06] and heart rate [p<.001; $\eta_p^2$=.71]. Subjects experienced significant worsening of mood [p<.001; $\eta_p^2$=.24], as well as increases in tension [p<.001; $\eta_p^2$=.24], and stress [p<.001; $\eta_p^2$=.22]. Regarding their emotional reaction to the MIST, participants did not show differences in their baseline and peak levels in relation to the total number of ACEs, as indicated by univariate analyses of variances (data not shown).

### 3.1 Impact of ACE on cortisol responses to stress

Repeated measures analysis of cortisol responses to stress showed a significant interaction of time x *ACE total sum score* [p<.05; $\eta_p^2$=.03] as well as a significant main effect of *ACE total sum score* [p < .01; $\eta_p^2$ = .09]. Results remained significant when the emotional responses to the stress task were entered additionally as covariates. *ACE total groups* differed significantly in their overall cortisol output in a dose-response manner [p<.01; $\eta_p^2$=.12, Figure 1].

Please insert Figure 1 here

Next, it was tested whether the duration of ACEs had an impact on cortisol responses to the stress task. Repeated measures analysis of cortisol response to the stress task resulted in a significant main effect of duration of *ACEs > one year* [$p < .001$; $\eta_p^2 = .17$] and a significant interaction of time and duration of *ACEs > one year* [$p < .01$; $\eta_p^2 = .04$]. Results remained significant when the emotional responses to the stress task were entered additionally as covariates. Results from the repeated measures analysis including only the *ACE > one year group* confirmed the impact of number of ACEs by a significant main effect of number of ACEs [$p<.01$; $\eta_p^2=.17$], indicating a dose-response relationship (Figure 2). However, these effects were not observed for the association between ACEs that lasted shorter in duration (*ACEs < one year*) and cortisol responses to the stress task [main effect, $p = .87$; interaction effect, $p = .81$].

Please insert Figure 2 here

For age of onset, a significant interaction effect was observed for both, occurence of ACE before and after menarche, indicating that subjects with ACEs before [$p=.001$; $\eta_p^2=.05$] as well as after menarche [$p<.05$; $\eta_p^2=.03$] both showed significantly attenuated cortisol responses to the stress task.

### 3.2 Impact of ACE on heart rate reactivity to stress

Repeated measures analysis of heart rate response to the stress task showed a significant main effect of *ACE total sum score* [$p<.01$; $\eta_p^2=.08$] as well as a significant interaction effect of time x *ACE total sum score* [$p<.01$; $\eta_p^2=.07$]. Results from the repeated measures analysis with the *ACE total groups* confirmed the impact of ACE by a significant main effect of group [$p<.01$; $\eta_p^2=.13$], indicating a dose-response relationship

(Figure 3). Results remained significant, when the emotional responses to the stress task were entered as covariates.

However, the further analyses with duration as well as age of onset of ACEs revealed no significant dose-response relationships with heart rate responses to the stress task (data not shown).

Please insert Figure 3 here

## 4. Discussion

We set out to assess the impact of ACEs on psychobiological stress reactivity and its modulation by the number, duration and age of onset of ACEs in healthy young women. Our results are in line with previous reports of attenuated endocrine (Carpenter et al., 2007; Elzinga et al., 2008; Carpenter et al., 2010; Lovallo et al., 2012) as well as cardiovascular (Lovallo et al., 2012) stress responses to a psychosocial stress test in healthy adults with a history of adverse childhood experiences. Furthermore, our results substantiate the importance of the mean number of ACEs on endocrine and cardiovascular response to psychosocial stress. Importantly, blunted cortisol and heart rate responses were independent of emotional responses, suggesting that the diminished endocrine and cardiovascular stress reactivity cannot be explained by a reduced emotional reaction to stress (which may be interpreted as flattened affect) after a history of childhood adversity.

To the best of our knowledge, the present study is the first to demonstrate that in healthy young women, especially long enduring, chronic ACEs show the strongest association with a blunted cortisol reactivity, adding valuable knowledge to the impact of chronic childhood adversity on alterations of the HPA axis. However, in this sample, timing of ACEs (before vs. after menarche) did not contribute compellingly to a further understanding of the relationship between ACEs and attenuated cortisol reactivity.

Per se, our results show a deviation from an expected endocrine and cardiovascular stress response in participants free of mental and physical illness in association with a history of ACEs. According to Obradovic (2012), taking together recent findings on stress reactivity in the context of early adversity, it is more accurate to state that exposure to early life stress may lead to dysregulated physiological phenotypes rather than to a particular pattern of hyper- or hyporesponsivity (Obradovic, 2012). The

recently proposed adaptive calibration model (Del Giudice et al., 2011) offers an evolutionary-developmental theory of individual differences in physiological reactivity processes. The authors hypothesize that, at a very general level, a nonlinear relation between adverse life event exposure and stress response exists. However, in the context of high adversity, the model predicts an either vigilant profile, characterized by high biological stress responsiveness, or an unemotional, underresponsive profile, characterized by generally low HPA axis and SNS activity. Thus, these opposite phenotypes might be mediated by other factors and their interactions, e.g. the interaction of complex environmental and genetic factors.

In terms of environmental factors, studies have demonstrated that the HPA axis in early human development is under strong social regulation (Tarullo and Gunnar, 2006). Therefore, several studies suggested parental caregiving as a moderator of the HPA reactivity (Gunnar et al., 1992; Nachmias et al., 1996; Tarullo and Gunnar, 2006). Thus, sensitive parenting appears to buffer cortisol responses in fearful situations, whereas being deprived of an evolutionarily expectable level of care (e.g. institutional rearing) has been associated with blunted cortisol production (Carlson and Earls, 1997; Gunnar et al., 2001). However, studies with institutionalized children were also able to show that improved caregiving environments had an effect on normalizing dampened HPA axis diurnal rhythms (Dozier et al., 2008; Cicchetti et al., 2011; Fisher et al., 2011). These findings provide support for an adaptive response of the stress system to its environment, probably in order to enhance survival odds. It could be speculated that after an initial hypersecretion of cortisol due to chronic stressful environments, the HPA axis could counter-regulate its response and cortisol output might rebound to below normal. A plausible biological explanation could be an increased glucocorticoid negative feedback with a downregulation of CRF receptors, or a diminished release of cortisol by the

adrenal glands (Heim et al., 2000a; Fries et al., 2005). This view is supported by our finding that only chronic events, not acute events, were associated with a blunted cortisol response.

Recent studies link genetic and epigenetic alterations to stress reactivity in association with ACEs as well. A history of ACEs has been associated with an epigenetic regulation of the glucocorticoid receptor in the hippocampus (McGowan et al., 2009). Moreover, a recent study on healthy adults who experienced the loss of a parent during childhood, maltreatment, or low parental care showed epigenetic alterations of a region of the human glucocorticoid receptor gene, which in turn was associated with a blunted cortisol reactivity after a neuroendocrine challenge test in these participants (Tyrka et al., 2012). Another study linked prenatal maternal depression to increased methylation of the glucocorticoid receptor gene, and showed exaggerated salivary cortisol output to stress at 3 months of age (Oberlander et al., 2008). Further studies are needed to investigate these changes in central regulation of the glucocorticoid receptor in brain regions involved in stress responses in association to ACEs.

Our results raise the challenging question of whether the observed alterations in stress responsivity can be interpreted as a potential risk factor for or as a sign of resilience to the development of later mental and physical disorders. Even if initially adaptive, blunted cortisol reactivity could compromise future and necessary psychobiological stress reactivity. For example, low cardiovascular and/or endocrine reactivity to acute psychological stress has been associated with depression, fibromyalgia, obesity, burn out, substance use disorders, and chronic pain syndromes (Griep et al., 1998; Heim et al., 1998; Pruessner et al., 1999; Lovallo et al., 2000; Gold and Chrousos, 2002; Gur et al., 2004; Phillips et al., 2011; Jones et al., 2012).

Considering that participants in the present study were recruited from a school of higher education and were free of psychopathology in adulthood, they may have been selected in a way that the blunted stress reactivity pattern may stand for resilience to the development of mental illness in the aftermath of childhood adverse experiences. Longitudinal, population-based research is needed to investigate if participants without present psychopathology, who had shown blunted stress responses after stress induction, are at higher risk to develop psychiatric disorders later in life or remain healthy.

The present study revealed diminished heart rate responses in association with the total number of ACEs, but not with the subgroups regarding duration and age of onset. Recently, Bauer & Boyce have suggested the examination of the HPA axis and the SNS simultaneously for a better understanding of their coordination (additive or interactive; or opposing or complementary) (Bauer et al., 2002). Only few studies have examined the exact nature of their coordination in adult samples so far (Ali and Pruessner, 2012; Lovallo et al., 2012; Andrews and Pruessner, 2013), and results are mixed. Methodological differences between the reported studies could account for the different findings. More empirical research is needed to investigate the exact nature of SNS alterations after ACEs, as well as the coordination and interactions between the two stress systems.

In the present study, we sucessfully replicated findings from prior research (Carpenter et al., 2007; Carpenter et al., 2010; Lovallo et al., 2012) using the novel Montreal Imaging Stress Task (MIST) outside the MRT. The MIST offers a promising alternative to conventional psychosocial stress tests. Advantages of the MIST are that participants sit still during the stress protocol, therefore causing less artefacts measuring psychobiological parameters like heart rate or electrodermal activity. Furthermore, since

the MIST is considered to evoke a moderate endocrine stress response, the observed findings represent HPA activity close to naturalistic settings (Smyth et al., 1998).

A limitation of the present study is that only peripheral readouts of stress hormone activation were measured. The HPA axis and the cardiovascular sytem are complex and multilayer systems and therefore we are not able to identify the exact mechanisms or location of the observed dysregulations. Furthermore, that only women were recruited and tested has to be mentioned as another potentially limitating factor. Especially as some of the recent models on stress reactivity changes after chronic or traumatic stress make mention of sex differences, it would have been informative to assess the stress response in men as well (Bangasser, 2013). Thus, due to the characteristics of the study sample, the presented findings can only be generalized to young women free of mental and physical illness. Also, participants were attendees of schools for health care professions and social work, which could have led to a selection bias as outlined above. Because of methodological reasons, the specification between ACEs shorter/longer than one year and pre-/postmenarche cannot be assumed to be completely independent from each other. Since some participants reported both, acute and chronic ACEs as well as pre- and postmenarche ACEs, with our sample size, it was not possible to have fully distinct and statistically orthogonal groups.

Despite these limitations, the present study strengthens the assumption that adverse childhood experiences give rise to a blunted stress reactivity of the HPA axis and the SNS in young healthy women. In this study population, number and duration of adverse events in childhood showed the strongest association with an attenuated stress response in adulthood. These findings suggest that the reactivity of the human stress system is indeed shaped by the experience of extrinsic chronic stressors in childhood and adolescence.

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## References

Ali, N., Pruessner, J.C., 2012. The salivary alpha amylase over cortisol ratio as a marker to assess dysregulation of the stress system. Physiol Behav 106, 65-72.

Alpco Diagnostics (2011, February 14). Cortisol (Saliva) ELISA - For the quantitative determination of cortisol in human saliva. Retrieved August 2, 2012, from http://www.alpco.com/pdfs/11/11-CORHU-E01-SLV.pdf

Andersen, S.L., Tomada, A., Vincow, E.S., Valente, E., Polcari, A., Teicher, M.H., 2008. Preliminary evidence for sensitive periods in the effect of childhood sexual abuse on regional brain development. The Journal of neuropsychiatry and clinical neurosciences 20, 292-301.

Andrews, J., Pruessner, J., 2013. The combined propanol/TSST paradigm - A new method for psychoneuroendocrinology. PLoS One 8.

Bangasser, D.A., 2013. Sex differences in stress-related receptors: "micro" differences with "macro" implications for mood and anxiety disorders. Biology of sex differences 4, 2.

Bauer, A.M., Quas, J.A., Boyce, W.T., 2002. Associations between physiological reactivity and children's behavior: Advantages of a multisystem approach. Journal of Developmental & Behavioral Pediatrics 23, 102-113.

Bremner, J.D., Bolus, R., Mayer, E.A., 2007. Psychometric properties of the Early Trauma Inventory-Self Report. J Nerv Ment Dis 195, 211-218.

Bremner, J.D., Vythilingam, M., Vermetten, E., Adil, J., Khan, S., Nazeer, A., Afzal, N., McGlashan, T., Elzinga, B., Anderson, G.M., Heninger, G., Southwick, S.M., Charney, D.S., 2003. Cortisol response to a cognitive stress challenge in posttraumatic stress disorder (PTSD) related to childhood abuse. Psychoneuroendocrinology 28, 733-750.

Carlson, M., Earls, F., 1997. Psychological and neuroendocrinological sequelae of early social deprivation in institutionalized children in Romania. Ann N Y Acad Sci 807, 419-428.

Carpenter, L.L., Carvalho, J.P., Tyrka, A.R., Wier, L.M., Mello, A.F., Mello, M.F., Anderson, G.M., Wilkinson, C.W., Price, L.H., 2007. Decreased adrenocorticotropic hormone and cortisol responses to stress in healthy adults reporting significant childhood maltreatment. Biol Psychiatry 62, 1080-1087.

Carpenter, L.L., Shattuck, T.T., Tyrka, A.R., Geracioti, T.D., Price, L.H., 2010. Effect of childhood physical abuse on cortisol stress response. Psychopharmacology (Berl).

Chrousos, G.P., 2009. Stress and disorders of the stress system. Nat Rev Endocrinol 5, 374-381.

Cicchetti, D., Rogosch, F.A., Toth, S.L., Sturge-Apple, M.L., 2011. Normalizing the development of cortisol regulation in maltreated infants through preventive interventions. Dev Psychopathol 23, 789-800.

De Bellis, M.D., 2002. Developmental traumatology: a contributory mechanism for alcohol and substance use disorders. Psychoneuroendocrinology 27, 155-170.

Dedovic, K., Renwick, R., Mahani, N.K., Engert, V., Lupien, S.J., Pruessner, J.C., 2005. The Montreal Imaging Stress Task: using functional imaging to investigate the effects of perceiving and processing psychosocial stress in the human brain. J Psychiatry Neurosci 30, 319-325.

Del Giudice, M., Ellis, B.J., Shirtcliff, E.A., 2011. The Adaptive Calibration Model of stress responsivity. Neurosci Biobehav Rev 35, 1562-1592.

Dozier, M., Peloso, E., Lewis, E., Laurenceau, J.P., Levine, S., 2008. Effects of an attachment-based intervention on the cortisol production of infants and toddlers in foster care. Dev Psychopathol 20, 845-859.

Elzinga, B.M., Roelofs, K., Tollenaar, M.S., Bakvis, P., van Pelt, J., Spinhoven, P., 2008. Diminished cortisol responses to psychosocial stress associated with lifetime adverse events a study among healthy young subjects. Psychoneuroendocrinology 33, 227-237.

Fisher, P.A., Van Ryzin, M.J., Gunnar, M.R., 2011. Mitigating HPA axis dysregulation associated with placement changes in foster care. Psychoneuroendocrinology 36, 531-539.

Fries, E., Hesse, J., Hellhammer, J., Hellhammer, D.H., 2005. A new view on hypocortisolism. Psychoneuroendocrinology 30, 1010-1016.

Gold, P.W., Chrousos, G.P., 2002. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Mol Psychiatry 7, 254-275.

Green, S.B., Salkind, N.J., Akey, T.M., 2000. Using SPSS for Windows, 2nd ed. Prentice Hall, Englewood Cliffs.

Griep, E.N., Boersma, J.W., Lentjes, E.G., Prins, A.P., van der Korst, J.K., de Kloet, E.R., 1998. Function of the hypothalamic-pituitary-adrenal axis in patients with fibromyalgia and low back pain. J Rheumatol 25, 1374-1381.

Gunnar, M.R., Larson, M.C., Hertsgaard, L., Harris, M.L., Brodersen, L., 1992. The stressfulness of separation among nine-month-old infants: effects of social context variables and infant temperament. Child Dev 63, 290-303.

Gunnar, M.R., Morison, S.J., Chisholm, K., Schuder, M., 2001. Salivary cortisol levels in children adopted from romanian orphanages. Dev Psychopathol 13, 611-628.

Gur, A., Cevik, R., Nas, K., Colpan, L., Sarac, S., 2004. Cortisol and hypothalamic-pituitary-gonadal axis hormones in follicular-phase women with fibromyalgia and

chronic fatigue syndrome and effect of depressive symptoms on these hormones. Arthritis Res Ther 6, R232-238.

Hautzinger, M., Bailer, M., 1993. Allgemeine Depressions-Skala (ADS): Manual. Beltz Test, Weinheim.

Heim, C., Ehlert, U., Hanker, J.P., Hellhammer, D.H., 1998. Abuse-related posttraumatic stress disorder and alterations of the hypothalamic-pituitary-adrenal axis in women with chronic pelvic pain. Psychosom Med 60, 309-318.

Heim, C., Ehlert, U., Hellhammer, D.H., 2000a. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 25, 1-35.

Heim, C., Nemeroff, C.B., 2001. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol Psychiatry 49, 1023-1039.

Heim, C., Newport, D.J., Heit, S., Graham, Y.P., Wilcox, M., Bonsall, R., Miller, A.H., Nemeroff, C.B., 2000b. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. Jama 284, 592-597.

Heim, C., Newport, D.J., Mletzko, T., Miller, A.H., Nemeroff, C.B., 2008. The link between childhood trauma and depression: Insights from HPA axis studies in humans. Psychoneuroendocrinology 33, 693-710.

Jones, A., McMillan, M.R., Jones, R.W., Kowalik, G.T., Steeden, J.A., Deanfield, J.E., Pruessner, J.C., Taylor, A.M., Muthurangu, V., 2012. Adiposity is associated with blunted cardiovascular, neuroendocrine and cognitive responses to acute mental stress. PLoS One 7, e39143.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom Med 61, 154-162.

La Marca, R., Waldvogel, P., Thorn, H., Tripod, M., Wirtz, P.H., Pruessner, J.C., Ehlert, U., 2011. Association between Cold Face Test-induced vagal inhibition and cortisol response to acute stress. Psychophysiology 48, 420-429.

Lovallo, W.R., Dickensheets, S.L., Myers, D.A., Thomas, T.L., Nixon, S.J., 2000. Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. Alcohol Clin Exp Res 24, 651-658.

Lovallo, W.R., Farag, N.H., Sorocco, K.H., Cohoon, A.J., Vincent, A.S., 2012. Lifetime adversity leads to blunted stress axis reactivity: studies from the Oklahoma Family Health Patterns Project. Biol Psychiatry 71, 344-349.

McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonte, B., Szyf, M., Turecki, G., Meaney, M.J., 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 12, 342-348.

Nachmias, M., Gunnar, M., Mangelsdorf, S., Parritz, R.H., Buss, K., 1996. Behavioral inhibition and stress reactivity: the moderating role of attachment security. Child Dev 67, 508-522.

Oberlander, T.F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., Devlin, A.M., 2008. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics 3, 97-106.

Obradovic, J., 2012. How can the study of physiological reactivity contribute to our understanding of adversity and resilience processes in development? Dev Psychopathol 24, 371-387.

Phillips, A.C., Hunt, K., Der, G., Carroll, D., 2011. Blunted cardiac reactions to acute psychological stress predict symptoms of depression five years later: evidence from a large community study. Psychophysiology 48, 142-148.

Pruessner, J.C., Hellhammer, D.H., Kirschbaum, C., 1999. Burnout, perceived stress, and cortisol responses to awakening. Psychosom Med 61, 197-204.

Rao, U., Hammen, C., Ortiz, L.R., Chen, L.A., Poland, R.E., 2008. Effects of early and recent adverse experiences on adrenal response to psychosocial stress in depressed adolescents. Biol Psychiatry 64, 521-526.

Schoedl, A.F., Costa, M.C., Mari, J.J., Mello, M.F., Tyrka, A.R., Carpenter, L.L., Price, L.H., 2010. The clinical correlates of reported childhood sexual abuse: an association between age at trauma onset and severity of depression and PTSD in adults. J Child Sex Abus 19, 156-170.

Smyth, J., Ockenfels, M.C., Porter, L., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1998. Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. Psychoneuroendocrinology 23, 353-370.

Tarullo, A.R., Gunnar, M.R., 2006. Child maltreatment and the developing HPA axis. Horm Behav 50, 632-639.

Tottenham, N., Sheridan, M.A., 2010. A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. Front Hum Neurosci 3, 68.

Tyrka, A.R., Price, L.H., Marsit, C., Walters, O.C., Carpenter, L.L., 2012. Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults. PLoS One 7, e30148.

Wittchen, H.-U., Wunderlich, U., Gruschwitz, S., Zaudig, M., 1997. SKID-I: Strukturiertes Klinisches Interview für DSM-IV - Achse I: Psychische Störungen. Hogrefe, Göttingen.

**Table 1**

Participant characteristics and ACE scores of the study sample (N = 104)

| Variable | M | SD | Range/% |
|---|---|---|---|
| Age [yrs] | 21.66 | 1.54 | 18 – 25 |
| Age of onset menarche[a] [yrs] | 12.95 | 1.28 | 10 – 16 |
| Oral contraceptive use, n (%) | | | 59 (56.7) |
| Body Mass Index [kg/m$^2$] | 21.81 | 2.53 | 18.37 – 31.14 |
| Depressive symptoms (ADS-K) | 7.08 | 4.93 | 0 – 24 |
| ACE total sum score | 2.76 | 3.17 | 0 – 15 |
| ACEs ≥ 1 year | 1.27 | 2.40 | 0 – 12 |
| ACEs < 1 year | 1.44 | 1.55 | 0 – 6 |
| ACEs before menarche | 1.96 | 2.99 | 0 – 15 |
| ACEs after menarche | 0.76 | 1.05 | 0 – 6 |

[a]*N* = 103

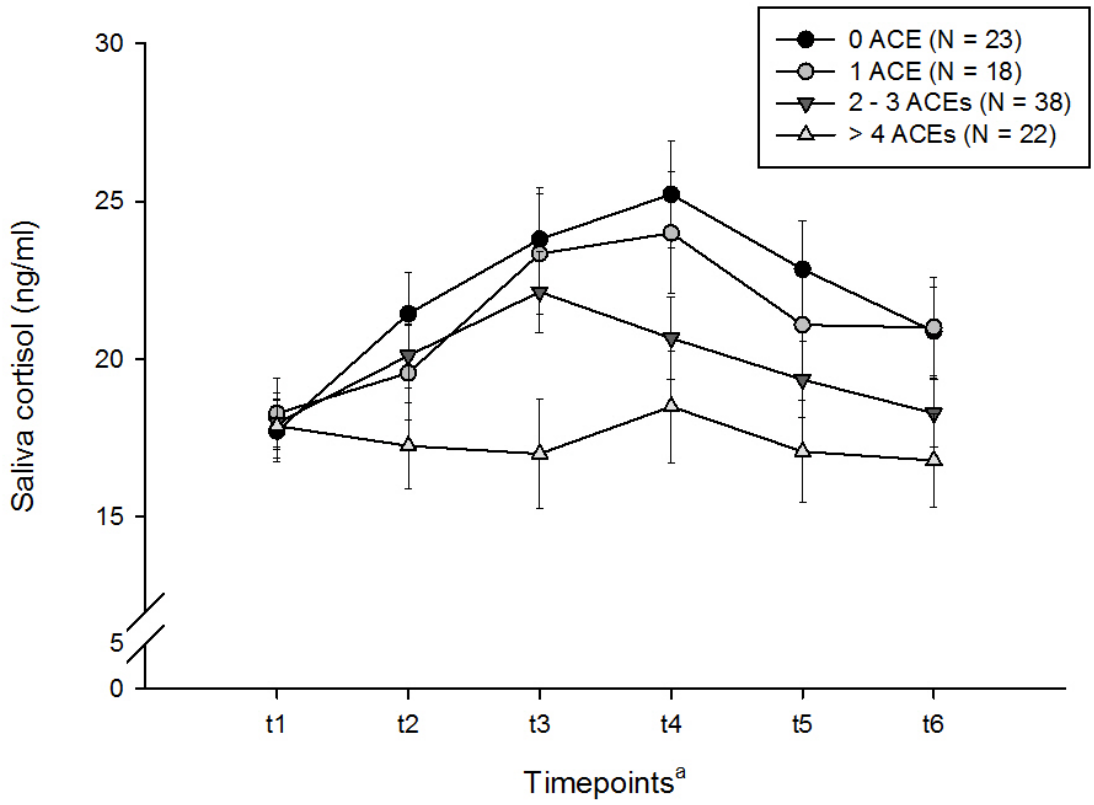


**Fig. 1.** Cortisol responses to the MIST are depicted for women who experienced 0, 1, 2-3 or 4 or more ACEs. Values represent average cortisol ± standard error of the mean for the *ACE total groups* controlled for oral contraceptive use and BMI. Higher ACE sum scores are associated with blunted cortisol responses.

[a]Timepoints relative to the MIST: t1 = -1min, t2 = +1min, t3 = +10min, t4 = +25min, t5 = +40min, t6 = +55min.

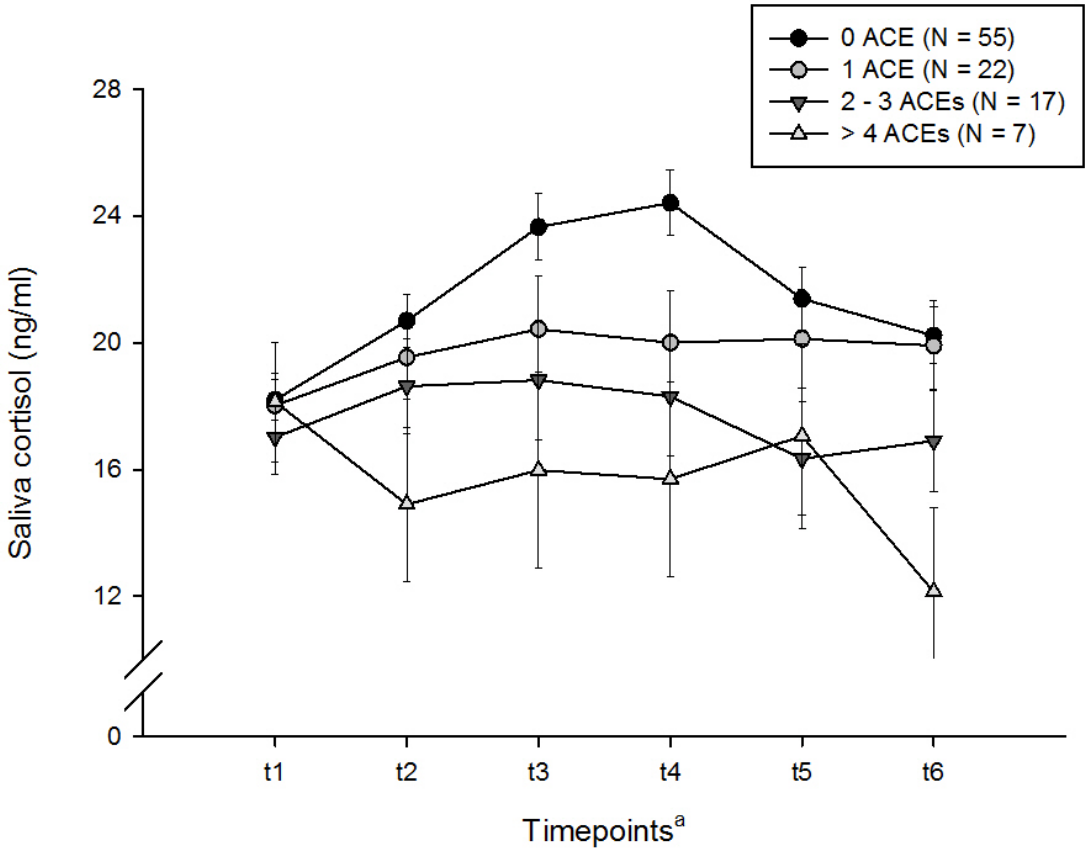


**Fig. 2.** Cortisol responses to the MIST are depicted for women who experienced 0, 1, 2-3 or up to 4 or more ACEs that lasted more than a year. Values represent cortisol ± standard error of the mean for the *ACEs>one year group* controlled for oral contraceptive use and BMI. Higher ACE sum scores are associated with blunted cortisol responses. [a]Timepoints relative to the MIST: t1 = -1min, t2 = +1min, t3 = +10min, t4 = +25min, t5 = +40min, t6 = +55min.

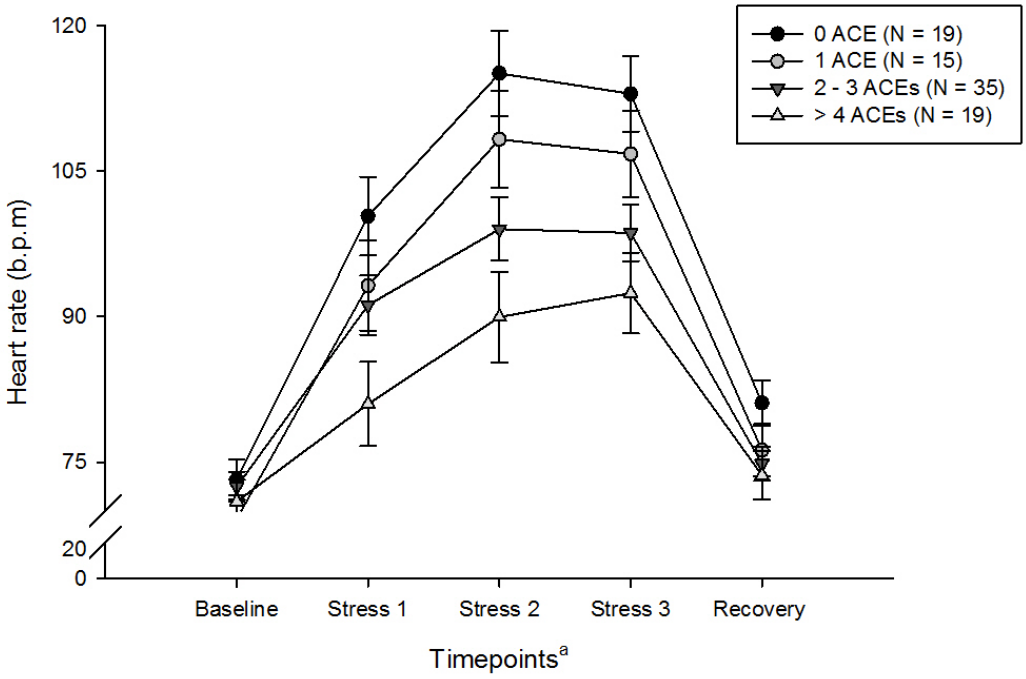


**Fig. 3.** Heart rate responses to the MIST are depicted for women who experienced 0, 1, 2-3, or 4 or more ACEs. Values represent average heart rate (beats per minute) ± standard error of the mean for the *ACE total groups* controlled for oral contraceptive use and BMI. Higher ACE sum scores are associated with attenuated heart rate reactivity.

[a]Timepoints: Heart rate averaged for Baseline, Stress 1, Stress 2, Stress 3, and Recovery periods in reference to the various sections of the experiment.

Appendix B

Voellmin A., Entringer, S., Moog, N., Wadhwa, P.D.W., & Buss, C. (2013).

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# Maternal positive affect over the course of pregnancy is associated with the length of gestation and reduced risk of preterm delivery

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ABSTRACT

*Objective:* The association between maternal psychological state during pregnancy and birth outcomes is well established. The focus of previous studies has been on the potentially detrimental consequences of maternal stress on pregnancy and birth outcomes, particularly shortened gestation and increased risk of preterm birth. Despite a growing literature linking positive affect with favorable health outcomes this construct has received little attention in the context of pregnancy. Therefore, in the current study, we tested the hypothesis that maternal positive affect during pregnancy is associated with beneficial consequences in terms of increased length of gestation and reduced risk of preterm birth above that of the absence of stress.
*Methods:* In 169 pregnant women maternal positive affect and perceived stress were serially assessed at 15.2± 0.9 weeks (T1; mean ± SD), 19.7 ± 0.9 weeks (T2) and 30.7 ± 0.7 weeks (T3) gestation. Pregnancy and birth outcomes were abstracted from the medical record.
*Results:* Higher maternal positive affect and a steeper increase in maternal positive affect over pregnancy were positively associated with length of gestation ($p < .05$) and reduced risk of preterm delivery ($p < .01$), whereas maternal perceived stress was not significantly associated with shorter length of gestation ($p > .10$).
*Conclusions:* These findings suggest that maternal positive affect may be beneficial for outcomes related to the length gestation, and that this effect cannot be accounted for by the lower stress levels associated with higher positive affect. Interventions to increase maternal positive affect may be beneficial for fetal development.


## Introduction

The belief that a mother's emotional state during pregnancy may influence the development of her fetus has persisted across time and culture. This has stimulated research on maternal psychological state during pregnancy and various pregnancy and birth outcomes. One of the most consistent findings in this literature is the observed association between higher levels of maternal psychological stress during pregnancy and shortened length of gestation and increased risk of preterm birth [1–14]. Although a growing body of literature has examined and demonstrated that positive affect is independently associated with more favorable health outcomes [15] this question has received relatively little attention in the context of pregnancy and birth outcomes.

Several studies, for example, have shown associations between positive affect and improved cardiovascular function, with positive affect being related to accelerated recovery from cardiovascular reactivity [15–17], decreased blood pressure in ambulatory assessments [18,19], and elevated parasympathetic activation [20]. Positive affect also has been linked to lower cortisol concentrations over the course of the day [21,22] and to higher antibody responses to hepatitis B vaccination [23].

One of the few studies on positive maternal affect during pregnancy found that women with stronger personal resources (mastery, self-esteem, optimism) had higher birth weight babies, even after controlling for the effects of gestational age at birth, psychosocial stress, and other variables [4]. Another study reported that maternal dispositional optimism was related to higher infant birth weight [24]. A more recent study described associations of positive state of mind and emotional stability in the immediate post-partum period with having experienced a normal delivery, however, positive affect was assessed in the immediate post-partum period and the positive delivery experience may have caused the higher positive affect in

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these women and not vice versa [25]. Thus, there is some preliminary evidence suggesting that maternal positive affect may be beneficial in the context of pregnancy and birth outcomes.

The objective of the present study was to assess the relationship between positive affect and length of gestation. We hypothesized that high maternal positive affect would be associated with longer length of gestation, and that this association would be significant even after controlling for the effects of maternal stress levels.

## Method

### Participants

Data for the present analysis were collected in the context of a longitudinal pregnancy and birth outcomes study conducted by the University of California, Irvine Development, Health and Disease Research Program. All study procedures were approved by the institutional review board and all participants provided written, informed consent.

The study population comprised a population-based cohort of 169 pregnant women assessed serially over the course of gestation (at 15.2 ± 0.9 weeks (T1; mean ± SD), 19.7 ± 0.9 weeks (T2) and 30.7 ± 0.7 weeks (T3)) and followed through birth. Women who participated in at least two study visits during pregnancy were included in the current analyses, which allowed assessing rate of change in positive affect over the course of gestation. Subjects were English-speaking adult women with singleton, intrauterine pregnancies. Exclusion criteria included tobacco, alcohol, or other drug use in pregnancy, use of in vitro fertilization/reproductive technology, and uterine or cervical abnormalities.

Furthermore, women who had an elective cesarean section (n = 66) and women who had missing information about mode of delivery (n = 25) were excluded from the present analyses. The final sample included 169 women. Socio-demographic characteristics of the sample are displayed in Table 1.

**Table 1**
Participant characteristics of the study sample (n = 169)

| Variable | Total sample (n = 169) | Low positive affect (n = 85) | High positive affect (n = 84) |
|---|---|---|---|
| *Sociodemographic characteristics* | | | |
| Maternal age[a] | 28.6 ± 5.6 yrs | 27.2 ± 5.3 yrs | 30.0 ± 5.5 yrs[b] |
| Race/ethnicity[c] | | | |
| Non-Hispanic White | 70 (41.4%) | 35 (41.2%) | 35 (41.7%) |
| Hispanic White | 58 (34.3%) | 33 (38.8%) | 25 (29.8%) |
| Other | 41 (24.3%) | 17 (20.0%) | 24 (28.5%) |
| Annual family income[c] | | | |
| Under $20,000 | 28 (16.6%) | 17 (22.1%) | 11 (13.8%)[b] |
| Between $20,000 and $50,000 | 43 (25.5%) | 29 (37.6%) | 14 (17.5%)[b] |
| Between $50,000 and $80,000 | 35 (20.7%) | 16 (20.8%) | 19 (23.7%)[b] |
| Over $80,000 | 51 (30.2%) | 15 (19.5%) | 36 (45.0%)[b] |
| Marital status[c] | | | |
| Separated/divorced from or not living with baby's father | 15 (8.9%) | 13 (17.1%) | 2 (2.5%)[b] |
| *Pregnancy-related characteristics* | | | |
| Obstetric risk[c] | 48 (28.4%) | 27 (31.8%) | 21 (25.0%) |
| Parity[c] (≥1) | 93 (55.0%) | 46 (54.1%) | 47 (56.0%) |

*Note*. A median split was performed to create high and low positive affect groups.
[a] Values represent mean ± SD.
[b] Difference between high and low positive affect group significant at $p < .05$.
[c] Values represent frequency n (% of total sample or group).

### Study protocol

Study participants were assessed serially at least two and up to three times over the course of gestation. Gestational age was determined by best obstetric estimate with a combination of last menstrual period and early uterine size, and was confirmed by obstetric ultrasonographic biometry using standard clinical criteria [26]. Pregnancy and birth outcomes were abstracted from medical charts. Sociodemographic information (i.e., marital status, family income and maternal age at delivery) was assessed by interview.

### Measures

#### Maternal positive affect

Positive affect was assessed using a questionnaire on attitudes towards pregnancy, adapted from prior research in pregnancy [27,28]. This self-report questionnaire consists of 7 positive and 6 negative feelings towards pregnancy. Participants read statements such as "In the last week, I often felt happy about being pregnant" and responded with answers ranging from 1 (never), 2 (rarely), 3 (sometimes), 4 (often) to 5 (always). At each assessment a sum score for positive attitudes toward pregnancy, termed positive affect, was computed from the 13 items, for which purpose the negative items were reverse coded. Thus, scores could range from 13 to 65 with higher scores reflecting higher positive affect. Average scores at each of the three assessments are depicted in Table 2.

#### Maternal perceived stress

At each study visit, current levels of perceived stress were measured with the Perceived Stress Scale [29]. The PSS consists of 12 items that are designed to measure how uncontrollable, unpredictable and overloaded participants find their lives. Responses are given on a 5-point Likert scale from 0 to 4. For each participant, an average score was computed over all time points of assessment and used as a covariate in the analyses.

#### Obstetric conditions and birth outcomes

Length of gestation was abstracted from medical charts after delivery and assessed as a continuous variable by completed weeks gestation. Obstetric risk was defined as the presence of major medical complications in the index pregnancy, i.e., gestational diabetes, vaginal bleeding, placenta abruptio, pregnancy-induced hypertension, preeclampsia, or infection. Information on presence of any of these conditions was retrieved by medical interviews with the pregnant women at each of the three pregnancy visits and by medical chart abstraction. Obstetric risk was then coded as a dichotomous variable as previously described [14].

### Data analysis

Previous studies have reported that both average levels of psychological state as well as its change over the course of pregnancy can have an impact on pregnancy and birth outcomes [30,31]. Therefore, we assessed whether mean positive affect scores changed over gestation and determined the associations between level as well as rate of change of positive affect over gestation with birth outcomes.

**Table 2**
Overview of positive affect and perceived stress scores over the course of gestation

| Variable | T1 (15.2 ± 0.9[a]) | T2 (19.7 ± 0.9[a]) | T3 (30.7 ± 0.7[a]) |
|---|---|---|---|
| Positive affect (mean ± SD) | 52.4 ± 8.0 | 53.9 ± 7.6 | 54.2 ± 8.2 |
| Perceived stress (mean ± SD) | 2.2 ± 0.6 | 2.2 ± 0.7 | 2.2 ± 0.7 |

[a] Average weeks gestation at assessment ± SD.

Gestational age at the time of study visit was centered at the gestational week of the first study visit (T1), i.e. time zero in the centered variable is equivalent to the mean gestational age at T1 (15.18 weeks). A linear regression model was fitted using the positive affect scores as outcomes and the centered gestational age as predictor. For each participant, the intercept of the regression line was used as level of positive affect at the first study visit and the slope of the regression line as a measure of change in maternal positive affect. Both variables were used as predictors for gestational length in a linear regression model.

The demonstration of associations between positive affective states and biological parameters may simply reflect the absence of negative affect, leading to ambiguous results. Therefore, Steptoe (2005) suggests controlling for negative affect in order to investigate the impact of positive affect on psychobiological outcomes [32]. We therefore controlled for perceived stress as an indicator of negative affect in all analyses.

Furthermore, to account for the potential confounding influence of other factors that could be associated with gestational length and/or positive affect, the following variables were included as covariates: parity, maternal age, race/ethnicity, family income, marital status, and presence of obstetric risk.

Additionally, a logistic regression was conducted with the same predictors and covariates and preterm birth (<37 completed weeks of gestation) as the outcome of interest. The assumptions for logistic regression were met. Non-multicollinearity of the predictors was indicated by tolerance values of the predictors >.04 and variance inflation factor (VIF) <3 [33,34].

All analyses were performed using IBM SPSS Statistics version 20.

### Results

In our sample, the mean length of gestation at birth was 38.9 ± 2.1 weeks (±SD), and ranged from 26.3 to 42.0 weeks. 20 of these deliveries (11.8%) were preterm (≤37 completed weeks gestation). The average levels of positive affect and perceived stress at each pregnancy assessment are depicted in Table 2. Mean positive affect significantly increased over gestation ($F_{(1.7;249.8)} = 5.63$; $p = .006$), specifically values at the first assessment were significantly lower than at the second and third assessment. Perceived stress did not change over gestation ($p > .10$).

As expected, maternal mean positive affect and mean perceived stress were inversely correlated ($r = -0.644$, $p < .001$).

*Association between positive affect and length of gestation*

Table 3 shows the results for the regression model predicting gestational length by the intercept and slope of positive affect, with parity, maternal age, race/ethnicity, family income, marital status, obstetric risk and mean perceived stress score as covariates. Both the positive affect score at mean gestational age at T1 (intercept) and the rate of change over the course of gestation (slope) significantly and positively predicted length of gestation, indicating that more positive affect toward pregnancy in the early second trimester as well as a steeper increase in positive affect over gestation are associated with longer duration of pregnancy. Specifically, a 1SD (7.97 point) increase in the intercept (representing positive affect levels in the early second trimester) is associated with a 4.57 day increase in length of gestation. Furthermore, a 1SD (0.76 point) increase in the rate of change in positive affect over gestation is associated with 3.50 days increase in pregnancy duration. These effects are independent of negative affect, as measured by the perceived stress scale, and are significant even after controlling for the effects of several potential confounders. The only other significant predictor of gestational length was presence of any obstetric risk condition, which – if present – decreased gestational length by approximately 1 week.

**Table 3**
Results of the linear regression model showing the association between positive affect and the covariates included in the model and length of gestation

| Variable | Coefficient estimate | SE[a] | t-Value | *p*-Value |
|---|---|---|---|---|
| Positive affect intercept | 0.082 | 0.029 | 2.81 | .006* |
| Positive affect slope | 0.658 | 0.267 | 2.46 | .015* |
| Perceived stress | −0.076 | 0.393 | −0.19 | .848 |
| Parity | 0.015 | 0.182 | 0.09 | .933 |
| Maternal age | −0.027 | 0.038 | −0.70 | .482 |
| Non-Hispanic White | −0.209 | 0.428 | −0.49 | .626 |
| Hispanic White | 0.075 | 0.467 | 0.16 | .873 |
| Obstetric risk | −1.020 | 0.376 | −2.71 | .008* |
| Family income | 0.083 | 0.061 | 1.36 | .175 |
| Marital status | −1.059 | 0.589 | −1.80 | .074 |

[a] SE = standard error of the mean.
* $p < .05$.

*Association between positive affect and preterm birth*

Table 4 shows the results for the logistic regression model predicting preterm delivery by the intercept and slope of positive affect, with parity, maternal age, race/ethnicity, family income, obstetric risk, marital status, and perceived stress included as covariates. Positive affect at T1 (intercept) was significantly and inversely related to preterm delivery (OR = 0.843; [95% CI = 0.757–0.939]; $p = .002$). This means that 1 point increase in positive affect intercept is associated with an 18.6% reduced risk of delivering preterm (for OR < 1: % = [(OR/1) − 1] * 100). However, positive affect slope was not a significant predictor of preterm delivery (OR = 0.551; [95% CI = 0.210–1.445]; $p = .226$), indicating that the change in positive affect over pregnancy did not differ in mothers who delivered preterm as compared to mothers who delivered at term.

Presence of an obstetric condition was associated with increased risk for preterm birth, family income was negatively associated with preterm birth and being married to or living with the baby's father was positively associated with preterm birth risk, which was probably due to the fact that the group of women living with their babies' father was disproportionately larger than the group of women not cohabiting with the father. Perceived stress and the other covariates were not significantly related to preterm delivery (see Table 4 for details). In Fig. 1, positive affect at each time point in gestation is depicted for women who delivered at term versus preterm.

## Discussion

Results from the present study suggest that higher levels of positive affect in pregnancy are associated with longer length of gestation and with a reduced risk of delivering preterm. The level of positive affect in the early second trimester of pregnancy as well as the rate of increase in positive affect over the course of pregnancy were positively associated with a longer length of gestation. The magnitude of this effect was such that every point increase in positive affect in the early second trimester was associated with an 18.6% reduced risk of delivering preterm. Moreover, the association between maternal positive affect and length of gestation was independent of maternal perceived stress, suggesting that it is not solely the absence of maternal stress that accounts for the beneficial effect of maternal positive affect on length of gestation.

Several empirical studies have identified high maternal psychosocial stress as a risk factor for adverse pregnancy and birth outcomes [1,2,7,35]. We and others have reported that after accounting for the influence of established obstetric and socio-demographic risk factors, maternal psychosocial stress is significantly and independently associated with an increased risk of preterm birth and restricted fetal growth [3–6]. Alterations in stress-related maternal–placental–fetal endocrine and immune physiology have been suggested as pathways that may underlie this association [1]. In support of this suggested biological transmission pathway, elevated levels of maternal cortisol,

**Table 4**
Results of the logistic regression model showing the association between positive affect and the covariates included in the model and preterm delivery (<37 weeks gestation)

| Variable | Wald | *p*-Value | OR[a] | 95% CI[a] for OR[b] |
|---|---|---|---|---|
| Positive affect intercept | 9.66 | .002* | 0.843 | 0.76–0.94 |
| Positive affect slope | 1.47 | .226 | 0.551 | 0.21–1.45 |
| Perceived stress | 0.33 | .568 | 0.647 | 0.15–2.88 |
| Parity | 0.00 | 1.000 | 1.000 | 0.53–1.90 |
| Maternal age | 2.88 | .090 | 1.133 | 0.98–1.31 |
| Non-Hispanic White | 1.06 | .304 | 2.258 | 0.48–10.68 |
| Hispanic White | 0.51 | .477 | 0.508 | 0.08–3.29 |
| Obstetric risk | 9.02 | .003* | 10.588 | 2.27–49.39 |
| Marital status | 4.81 | .028* | 18.574 | 1.37–252.77 |
| Family income | 5.92 | .015* | 0.711 | 0.54–0.94 |

*Note*. Nagelkerke $R^2_N = .365$; Model $\chi^2_{(10)} = 31.92$, $p < .01$.
[a] CI = confidence interval.
[b] OR = odd's ratio.
* $p < .05$.

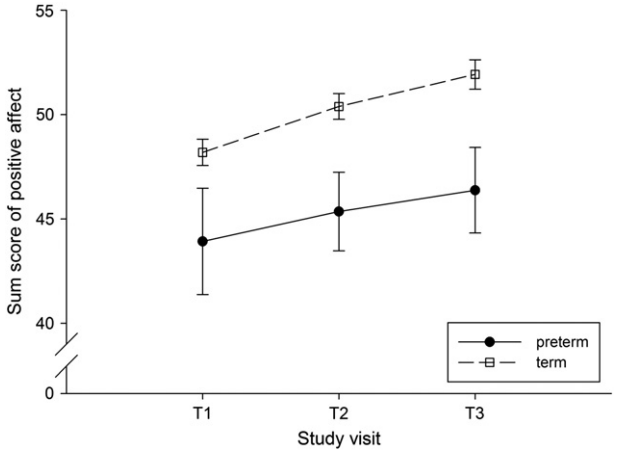


**Fig. 1.** Positive affect at 15.2 ± 0.9 weeks (T1), 19.7 ± 0.9 weeks (T2) and 30.7 ± 0.7 weeks (T3) gestation is depicted for women who delivered at term versus preterm. Values represent sum scores (±standard error of the mean) for positive affect residualized by race/ethnicity, parity, maternal age, obstetric risk, family income, marital status, and perceived stress. Higher maternal positive affect was associated with decreased risks of preterm delivery.

pro-inflammatory cytokines and placental corticotrophin-releasing hormone (CRH) have been shown to be associated with shorter length of gestation [9,12,14,36–39] and reduced fetal growth [6,14,40].

It is possible that maternal positive affect may exert its beneficial effects on length of gestation by also impacting maternal–placental–fetal endocrine and immune physiology. In non-pregnant individuals associations have been reported between positive affect or optimism and lower cortisol concentrations, attenuated cortisol awakening responses, lower stress reactivity, and a lower inflammatory milieu [16,22,41–43]. Also, in the very small number of studies that addressed the association between maternal positive affect or positive life events and stress in pregnancy, preliminary evidence suggests that positive affect may buffer the effect of stress. A study by Nierop and colleagues reported that a higher number of psychosocial resources was associated with dampened stress reactivity in pregnant women [44]. After a mental stress test, higher resources and daily uplifts were associated with a blunted alpha-amylase reactivity and lower psychological stress. Higher resources predicted lower cortisol stress reactivity of borderline significance [44]. Furthermore, a recent study on 60 pregnant women demonstrated that positive life events predicted significantly lower third-trimester maternal morning cortisol levels across the cortisol awakening response [45]. These findings suggest that positive affect dampens endocrine and psychological responses to stress in pregnancy, which may be protective for the developing fetus.

Theoretical perspectives on resilience and positive affect suggest that the experience of positive emotions may be important in helping resilient individuals recover quickly from stress. According to the broaden-and-build theory of positive emotions [46,47], negative emotions narrow momentary thoughts and actions to produce autonomic nervous system activation that prepares the body for specific action. Positive emotions are believed to play a homeostatic role by inhibiting autonomic arousal and by returning to a cardiovascular equilibrium.

Our report has some limitations. Because our assessment of positive affect was based on a self-report questionnaire, and was not assessed on a daily basis, it is possible that participants were inaccurate in their recall of positive affect over the past week. We suggest that future studies should use ecological momentary assessment of positive affect. Another limitation is the absence of additional possible confounding or mediating variables that were not assessed such as social support and health behaviors related to diet and physical activity. A meta-analysis of studies of positive affect and mortality has found the resilient effects of positive affect persist after accounting for the effects of behavioral factors [48].

Despite these limitations, to the best of our knowledge, this is the first study to show in a prospective, longitudinal design that positive affect is associated with length of gestation and decreased risk of preterm delivery. In this study population maternal positive affect in pregnancy was a better predictor of gestational length than perceived stress. Therefore, our data support the premise that interventions aimed to increase maternal positive affect may be beneficial for fetal development. More empirical research is needed to examine the role of maternal positive affect as a resilience factor and its effect on pregnancy physiology, and maternal health promoting behavior and fetal development.

## Conflict of interest

The authors have no competing interests to report.

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## References

[1] Wadhwa PD, Entringer S, Buss C, Lu MC. The contribution of maternal stress to preterm birth: issues and considerations. Clin Perinatol 2011;38:351–84.
[2] Alder J, Fink N, Bitzer J, Hosli I, Holzgreve W. Depression and anxiety during pregnancy: a risk factor for obstetric, fetal and neonatal outcome? A critical review of the literature. J Matern Fetal Neonatal Med 2007;20:189–209.
[3] Hobel CJ, Goldstein A, Barrett ES. Psychosocial stress and pregnancy outcome. Clin Obstet Gynecol 2008;51:333–48.
[4] Rini CK, Dunkel-Schetter C, Wadhwa PD, Sandman CA. Psychological adaptation and birth outcomes: the role of personal resources, stress, and sociocultural context in pregnancy. Health Psychol 1999;18:333–45.
[5] Glynn LM, Wadhwa PD, Dunkel-Schetter C, Chicz-Demet A, Sandman CA. When stress happens matters: effects of earthquake timing on stress responsivity in pregnancy. Am J Obstet Gynecol 2001;184:637–42.
[6] Wadhwa PD, Sandman CA, Porto M, Dunkel-Schetter C, Garite TJ. The association between prenatal stress and infant birth weight and gestational age at birth: a prospective investigation. Am J Obstet Gynecol 1993;169:858–65.
[7] Beydoun H, Saftlas AF. Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. Paediatr Perinat Epidemiol 2008;22:438–66.
[8] Dunkel Schetter C. Psychological science on pregnancy: stress processes, biopsychosocial models, and emerging research issues. Annu Rev Psychol 2011;62:531–58.
[9] Entringer S, Buss C, Andersen J, Chicz-DeMet A, Wadhwa PD. Ecological momentary assessment of maternal cortisol profiles over a multiple-day period predicts the length of human gestation. Psychosom Med 2011;73:469–74.
[10] Wadhwa PD, Dunkel-Schetter C, Chicz-DeMet A, Porto M, Sandman CA. Prenatal psychosocial factors and the neuroendocrine axis in human pregnancy. Psychosom Med 1996;58:432–46.
[11] Sandman CA, Glynn L, Schetter CD, Wadhwa P, Garite T, Chicz-DeMet A, et al. Elevated maternal cortisol early in pregnancy predicts third trimester levels of placental corticotropin releasing hormone (CRH): priming the placental clock. Peptides 2006;27:1457–63.
[12] Hobel CJ, Dunkel-Schetter C, Roesch SC, Castro LC, Arora CP. Maternal plasma corticotropin-releasing hormone associated with stress at 20 weeks' gestation in pregnancies ending in preterm delivery. Am J Obstet Gynecol 1999;180:S257–63.
[13] Erickson K, Thorsen P, Chrousos G, Grigoriadis DE, Khongsaly O, McGregor J, et al. Preterm birth: associated neuroendocrine, medical, and behavioral risk factors. J Clin Endocrinol Metab 2001;86:2544–52.
[14] Wadhwa PD, Garite TJ, Porto M, Glynn L, Chicz-DeMet A, Dunkel-Schetter C, et al. Placental corticotropin-releasing hormone (CRH), spontaneous preterm birth, and fetal growth restriction: a prospective investigation. Am J Obstet Gynecol 2004;191:1063–9.
[15] Dockray S, Steptoe A. Positive affect and psychobiological processes. Neurosci Biobehav Rev 2010;35:69–75.
[16] Bostock S, Hamer M, Wawrzyniak AJ, Mitchell ES, Steptoe A. Positive emotional style and subjective, cardiovascular and cortisol responses to acute laboratory stress. Psychoneuroendocrinology 2011;36:1175–83.
[17] Tugade MM, Fredrickson BL, Barrett LF. Psychological resilience and positive emotional granularity: examining the benefits of positive emotions on coping and health. J Pers 2004;72:1161–90.

[18] Ong AD, Allaire JC. Cardiovascular intraindividual variability in later life: the influence of social connectedness and positive emotions. Psychol Aging 2005;20:476–85.
[19] Steptoe A, Wardle J, Marmot M. Positive affect and health-related neuroendocrine, cardiovascular, and inflammatory processes. Proc Natl Acad Sci U S A 2005;102:6508–12.
[20] Bhattacharyya MR, Whitehead DL, Rakhit R, Steptoe A. Depressed mood, positive affect, and heart rate variability in patients with suspected coronary artery disease. Psychosom Med 2008;70:1020–7.
[21] Brummett BH, Boyle SH, Kuhn CM, Siegler IC, Williams RB. Positive affect is associated with cardiovascular reactivity, norepinephrine level, and morning rise in salivary cortisol. Psychophysiology 2009;46:862–9.
[22] Steptoe A, Gibson EL, Hamer M, Wardle J. Neuroendocrine and cardiovascular correlates of positive affect measured by ecological momentary assessment and by questionnaire. Psychoneuroendocrinology 2007;32:56–64.
[23] Marsland AL, Cohen S, Rabin BS, Manuck SB. Trait positive affect and antibody response to hepatitis B vaccination. Brain Behav Immun 2006;20:261–9.
[24] Lobel M, DeVincent CJ, Kaminer A, Meyer BA. The impact of prenatal maternal stress and optimistic disposition on birth outcomes in medically high-risk women. Health Psychol 2000;19:544–53.
[25] Hernandez-Martinez C, Val VA, Murphy M, Busquets PC, Sans JC. Relation between positive and negative maternal emotional states and obstetrical outcomes. Women Health 2011;51:124–35.
[26] O'Brien GD, Queenan JT, Campbell S. Assessment of gestational age in the second trimester by real-time ultrasound measurement of the femur length. Am J Obstet Gynecol 1981;139:540–5.
[27] Mancuso RA, Schetter CD, Rini CM, Roesch SC, Hobel CJ. Maternal prenatal anxiety and corticotropin-releasing hormone associated with timing of delivery. Psychosom Med 2004;66:762–9.
[28] Gurung RAR, Dunkel-Schetter C, Collins N, Rini CM, Hobel CJ. Psychosocial predictors of prenatal anxiety. J Soc Clin Psychol 2005;24:497–519.
[29] Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983;24:385–96.
[30] Glynn LM, Schetter CD, Wadhwa PD, Sandman CA. Pregnancy affects appraisal of negative life events. J Psychosom Res 2004;56:47–52.
[31] Glynn LM, Schetter CD, Hobel CJ, Sandman CA. Pattern of perceived stress and anxiety in pregnancy predicts preterm birth. Health Psychol 2008;27:43–51.
[32] Steptoe A, Wardle J. Positive affect and biological function in everyday life. Neurobiol Aging 2005;26:108–12.
[33] Meyers R. Classical and modern regression with applications. 2nd ed.Boston: Duxbury; 1990.
[34] Menard S. Applied logistic regression analysis. Thousand Oaks: Sage; 1995.
[35] Muglia LJ, Katz M. The enigma of spontaneous preterm birth. N Engl J Med 2010;362:529–35.
[36] Buss C, Entringer S, Reyes JF, Chicz-DeMet A, Sandman CA, Waffarn F, et al. The maternal cortisol awakening response in human pregnancy is associated with the length of gestation. Am J Obstet Gynecol 2009;201:e1–8.
[37] Santhanam U, Avila C, Romero R, Viguet H, Ida N, Sakurai S, et al. Cytokines in normal and abnormal parturition: elevated amniotic fluid interleukin-6 levels in women with premature rupture of membranes associated with intrauterine infection. Cytokine 1991;3:155–63.
[38] Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection. J Clin Invest 1990;85:1392–400.
[39] Murtha AP, Sinclair T, Hauser ER, Swamy GK, Herbert WN, Heine RP. Maternal serum cytokines in preterm premature rupture of membranes. Obstet Gynecol 2007;109:121–7.
[40] Bolten MI, Wurmser H, Buske-Kirschbaum A, Papousek M, Pirke KM, Hellhammer D. Cortisol levels in pregnancy as a psychobiological predictor for birth weight. Arch Womens Ment Health 2011;14:33–41.
[41] Steptoe A, O'Donnell K, Badrick E, Kumari M, Marmot M. Neuroendocrine and inflammatory factors associated with positive affect in healthy men and women: the Whitehall II study. Am J Epidemiol 2008;167:96–102.
[42] Brydon L, Walker C, Wawrzyniak AJ, Chart H, Steptoe A. Dispositional optimism and stress-induced changes in immunity and negative mood. Brain Behav Immun 2009;23:810–6.
[43] Polk DE, Cohen S, Doyle WJ, Skoner DP, Kirschbaum C. State and trait affect as predictors of salivary cortisol in healthy adults. Psychoneuroendocrinology 2005;30:261–72.
[44] Nierop A, Wirtz PH, Bratsikas A, Zimmermann R, Ehlert U. Stress-buffering effects of psychosocial resources on physiological and psychological stress response in pregnant women. Biol Psychol 2008;78:261–8.
[45] Pluess M, Wurmser H, Buske-Kirschbaum A, Papousek M, Pirke KM, Hellhammer D, et al. Positive life events predict salivary cortisol in pregnant women. Psychoneuroendocrinology 2012;37:1336–40.
[46] Fredrickson BL. The role of positive emotions in positive psychology. The broaden-and-build theory of positive emotions. Am Psychol 2001;56:218–26.
[47] Fredrickson BL, Levenson RW. Positive emotions speed recovery from the cardiovascular sequelae of negative emotions. Cogn Emot 1998;12:191–220.
[48] Chida Y, Steptoe A. Positive psychological well-being and mortality: a quantitative review of prospective observational studies. Psychosom Med 2008;70:741–56.

Appendix C

Völlmin, A., Winzeler, K., Schläfli, F., Schäfer, V. & Bader, K. (submitted).

Die Effektivität des Montreal Imaging Stress Task bei jungen, gesunden Frauen. *Zeitschrift für Klinische Psychologie und Psychotherapie – Forschung und Praxis*.

# Die Effektivität des Montreal Imaging Stress Task bei jungen, gesunden Frauen

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**Zusammenfassung**

**Theoretischer Hintergrund:** Der *Montreal Imaging Stress Task* (MIST) scheint eine effektive Methode zur Provokation physiologischer und emotionaler Stressreaktionen zu sein (Dedovic et al., 2005). Bis jetzt wurde der MIST vorwiegend in Studien unter Verwendung von bildgebenden Verfahren eingesetzt und validiert. **Fragestellung:** Führt der MIST auch bei gesunden Frauen ausserhalb des MRT zu einer effektiven Stressreaktion? **Methode:** Während einer Ruhephase, gefolgt von der Stressinduktionsphase und einer Erholungsphase wurden physiologische und subjektive Stressparameter von 97 jungen gesunden Frauen unter kontrollierten Laborbedingungen gemessen. **Ergebnisse:** Die Probandinnen wiesen nach dem MIST signifikant erhöhte Cortisolwerte sowie erhöhte kardiovaskuläre und elektrodermale Reaktionen auf. **Schlussfolgerungen:** Der MIST löste eine signifikante Stressreaktion aus, und stellt folglich auch ausserhalb des MRT ein valides, effektives Mittel zur Stressinduktion bei gesunden, jungen Frauen dar.

# The Efficacy of the Montreal Imaging Stress Task in Young Healthy Women

**Abstract**

**Background:** The *Montreal Imaging Stress Task* (MIST) seems to be an effective tool to provoke physiological and emotional stress (Dedovic et al., 2005). Until now, the MIST has been used and evaluated predominantly in MRT studies. **Objective:** Does the MIST also lead to an effective stress reaction in healthy women outside the MRT scan? **Methods:** In a sample of 97 young healthy females, physiological and subjective measures of stress were measured under controlled laboratory conditions in a baseline, experimental and relaxation phase. **Results:** The MIST led to significantly heightened cortisol, cardiovascular, and electrodermal responses. **Discussion:** Our results show that the MIST leads to a valid stress response outside the MRT scan and therefore can be used as an adequate tool to induce stress in healthy, young women.

# Einleitung

Die Provokation von Stressreaktionen unter kontrollierten Laborbedingungen ist eine etablierte Strategie der Stressforschung. Sie ermöglicht die Messung psychophysiologischer Reaktionen unter Ausschluss möglichst vieler Störfaktoren. Eine bewährte Provokationsmethode stellt im Humanbereich der mentale Stresstest dar. Dieser beinhaltet standardisierte psychische und soziale Aufgabenstellungen für den Probanden, die beim Individuum mit grosser Wahrscheinlichkeit eine Stressreaktion auslösen.

Eine bedeutsame endokrine Stressreaktion wird experimentell am zuverlässigsten ausgelöst, wenn ein Stresstest Unkontrollierbarkeit suggeriert und bewirkt, dass ein Aspekt des Selbst durch die soziale Bewertung durch andere Personen bedroht wird (Dickerson & Kemeny, 2004). Diese Anforderungen erfüllt der Trier Social Stress Test (TSST; Kirschbaum, Pirke & Hellhammer, 1993), der seit längerem als Stresstest erster Wahl gilt. Beim TSST werden Probanden gebeten, nach einer kurzen Vorbereitungsphase vor einem Publikum eine kurze freie Rede zu halten und danach Kopfrechenaufgaben zu lösen. Die konföderierten Zuhörer dürfen dabei kein verbales oder nonverbales Feedback geben. In zahlreichen Untersuchungen konnte eine reliable Stimulation der Hypothalamus-Hypophysen-Nebennierenrinden-Achse (HHNA) sowie des Sympathischen Nervensystems (SNS) durch den TSST aufgezeigt werden (Foley & Kirschbaum, 2010). Er führt bei den Probanden durchschnittlich zu einer zwei- bis dreifach erhöhten Ausschüttung von Speichelcortisol und weist mit 70 – 85% hohe Responderraten auf (Kudielka, Hellhammer & Kirschbaum, 2007).

Da der Einsatz des TSST bei Untersuchungen mit bildgebenden Verfahren wegen der Bewegungsartefakte problematisch ist, entwickelte eine Arbeitsgruppe um Pruessner (Dedovic et al., 2005) in Anlehnung an den TSST den *Montreal Imaging Stress Task* (MIST). Beim MIST lösen die Versuchspersonen im Sitzen Kopfrechenaufgaben am Computer und geben ihre Lösungen per Knopfdruck ein. Das Computerprogramm generiert gezielt Aufgaben, die leicht über dem Leistungsniveau der Person liegen. Für eine zuverlässige Aktivierung der HHNA wurde die Komponente der Bedrohung durch soziale Bewertung eingebaut, indem der Misserfolg der Versuchspersonen von der Studienleitung beobachtet und kritisiert wird.

Die Effektivität des MIST konnte in mehreren Magnetresonanztomografie (MRT) und Positronen-Emissions-Tomographie (PET) Studien nachgewiesen werden (Pruessner, Champagne, Meaney & Dagher, 2004; Pruessner et al., 2008; Soliman et al., 2008; Soliman et al., 2011; La Marca, et al., 2010; Jones, Steeden, Pruessner, Deanfield, Taylor & Muthurangu, 2011; Lord, Steiner, Soares, Carew & Hall, 2011). Pruessner et al. (2010) berichteten allerdings in ihrer Übersichtsarbeit, dass der MIST im Vergleich zum TSST als moderater psychologischer Stressor einzuschätzen sei, da er schwächere Cortisolreaktionen auslöse und geringere Responderraten aufweise.

La Marca et al. (2011) untersuchte die Wirkung des MIST erstmals ausserhalb eines Computertomographen an einer Stichprobe männlicher Probanden und fand signifikant erhöhte Cortisolspiegel sowie eine erhöhte Herzfrequenz während des Stresstests im Vergleich zu einer Kontrollbedingung.

In der vorliegenden Studie soll erstmals die Effektivität des MIST bei gesunden, jungen weiblichen Probandinnen in sitzender Position umfassend untersucht werden.

Dazu wurden endokrine, kardiovaskuläre, elektrodermale und subjektive Parameter zur Messung der Stressreaktion verwendet. Es wurde angenommen, dass die Anwendung des MIST bei weiblichen Probandinnen zu einer signifikanten Erhöhung des Blutdrucks, der Herzfrequenz, der elektrodermalen Aktivität und einer vermehrten Speichelcortisolausschüttung führt.

## Methode

### *Stichprobe und Ablauf*

Die Probandinnen wurden an zwei Berufsschulen für Gesundheit in Basel per E-Mail, die eine Kurzbeschreibung der Studie enthielt, rekrutiert. Interessierte meldeten sich per E-Mail oder Telefon für die Studienteilnahme an, mit der Annahme, dass der Zusammenhang zwischen kognitiver Leistung und körperlichen Reaktionen untersucht werden sollte. Weibliches Geschlecht und ein Alter zwischen 18 und 25 Jahren stellten die Einschlusskriterien dar. Ausschlusskriterien waren akute körperliche oder psychische Erkrankungen, Schwangerschaft, starker oder regelmässiger Nikotinkonsum (> 5 Zigaretten/Tag) und die Einnahme von psychotropen Substanzen oder von Medikamenten, welche die Aktivität des Nervensystems oder die adrenocortikale Reaktion

beeinflussen (z.B. Steroide oder Benzodiazepine). Im Rahmen eines Vorscreenings, das schriftlich erfolgte, wurden die Ein- und Ausschlusskriterien grob abgeklärt. Potentiell für die Studie in Frage kommende Personen wurden anschliessend zu einem diagnostischen Untersuchungstermin eingeladen. Dieser beinhaltete neben der Erhebung soziodemographischer Angaben eine detaillierte Abklärung der Teilnahmebedingungen. Das Vorliegen einer psychischen Störung wurde mithilfe des Strukturierten Klinischen Interviews für DSM-IV (SKID-I; Wittchen, Wunderlich, Gruschwitz & Zaudig, 1997) überprüft. Am Ende dieses Termins unterzeichneten die Probandinnen eine schriftliche Einverständniserklärung zur Teilnahme an der Studie. Das Studienprotokoll entsprach der Deklaration von Helsinki und war von der örtlichen Ethikkommission geprüft und genehmigt worden. Die Probandinnen wurden für ihre Teilnahme finanziell entschädigt. Insgesamt wurden 97 Frauen im Alter von 18 bis 25 Jahren ($M$ = 21.79, $SD$ = 1.57) in die Studie aufgenommen.

Die Durchführung des Stresstests fand jeweils zwischen 15:00 und 18:00 Uhr statt, um zirkadiane Schwankungen des Cortisolspiegels zu kontrollieren (Stone et al., 2001). Die Probandinnen waren zuvor angewiesen worden, vor dem Experiment auf körperliche Anstrengungen, Koffein, Zigarettenkonsum, üppige Mahlzeiten oder Getränke mit niedrigem pH-Wert zu verzichten (Kirschbaum & Hellhammer, 1994). Die Einnahme oraler Kontrazeptiva wurde als Kontrollvariable erhoben. Bei Personen, die keine oralen Kontrazeptiva einnahmen, fand der Stresstest während der Lutealphase ihres Menstruationszyklus statt (Kirschbaum, Kudielka, Gaab, Schommer & Hellhammer, 1999).

Nachdem die Probandin auf einem Sessel vor einem Monitor Platz genommen hatte, befestigte die Versuchsleiterin eine Blutdruckmanschette um deren linken Arm. Für die Messung der elektrodermalen Aktivität wurden zwei Elektroden auf dem Handballen der linken Hand fixiert. Das Elektrokardiogramm wurde anhand eines Einthoven-Dreiecks mittels drei Elektroden auf den Thorax verklebt. Es folgte eine zehnminütige Habituationsphase, um das Einpendeln der elektrodermalen Aktivität zu gewährleisten. Anschliessend wurde die erste Speichelprobe erhoben (Messzeitpunkt -10 Min.; siehe Abbildung 1). Gleichzeitig gab die Versuchsperson auf einer visuellen Analogskala (VAS) an, wie gestresst sie sich gerade fühlte. Danach wurde die erste Blutdruckmessung durchgeführt. Angaben zu weiteren Messungen sind Abbildung 1 zu entnehmen.

In Anschluss an eine fünf-minütigen Ruhephase, während der ein neutraler Dokumentarfilm gezeigt wurde, erhielt die Probandin die Instruktionen zum MIST. Dem durchschnittlich 20 Minuten dauernden Stresstest folgte eine 55 Minuten dauernde Erholungsphase, während der in regelmässigen Abständen Speichelproben erhoben sowie Blutdruckmessungen vorgenommen wurden (siehe Abbildung 1). Während der ersten zehn Minuten der Erholungsphase wurden ausserdem weiterhin Herzfrequenz und elektrodermale Aktivität abgeleitet. Am Ende der Erholungsphase, nach Erhebung der letzten Speichelprobe, wurde die Probandin vollständig über den Stresstest aufgeklärt (Debriefing) und unterschrieb eine zweite Einverständniserklärung, in der sie der Weiterverwendung ihrer im Rahmen des Stresstests gewonnenen Daten zustimmte.

**ABBILDUNG 1 BITTE HIER EINFÜGEN**

*Messinstrumente und Interventionen*

*Montreal Imaging Stress Task.* Zur Provokation einer psychophysiologischen Stressreaktion wurde eine adaptierte Version des Montreal Imaging Stress Tasks (MIST; Dedovic et al., 2005) verwendet. Der MIST stellt einen standardisierten, computerisierten psychosozialen Stresstest dar, der aus drei Rechenblöcken besteht. Dabei werden die Versuchspersonen gebeten, die auf dem Bildschirm präsentierten Kopfrechenaufgaben so schnell und korrekt wie möglich zu lösen. Das Computerprogrammpasst sich dem individuellen Leistungsniveau der jeweiligen Versuchsperson an, so dass nur 45-50% der Aufgaben korrekt gelöst werden können. Während dem Rechnen werden der Versuchsperson über einen Zeitbalken die verbleibende Zeit zum Lösen der Aufgabe und ein Feedback zur Richtigkeit der Antwort angezeigt. Zudem wird der Versuchsperson bei jeder Aufgabe die individuelle Leistung im Vergleich zu einer (fiktiven) Normstichprobe als deutlich unterdurchschnittlich rückgemeldet.

Neben diesen vom Computer generierten Leistungsfeedbacks erhält die Versuchsperson zudem sozial-evaluative Feedbacks durch eine Versuchsleiterin und eine Studienleiterin. Die Versuchsleiterin befindet sich während des gesamten Tests im selben Raum wie die Versuchsperson.

Die Studienleiterin befindet sich in einem separaten Raum, die Versuchsperson wird jedoch darauf aufmerksam gemacht, dass die Studienleitung die Leistung über einen externen Bildschirm live mitverfolgt. Nach dem ersten Rechenblock wendet sich die Versuchsleiterin per Telefon an die Studienleiterin, um abzuklären, wie bei der ungenügenden Leistung weiter zu verfahren ist. Nach wiederholtem Misserfolg der Probandin im zweiten Rechenblock wird die Studienleitung erneut telefonisch kontaktiert, worauf diese im Labor erscheint. Um den Druck auf die Probandin zu erhöhen, weist die Studienleiterin auf die Kosten des Experiments hin und teilt mit, dass mit solch schlechter Leistung die Daten unbrauchbar seien. Die Versuchsperson wird explizit auf die mangelhafte Leistung hingewiesen und aufgefordert, ihre Leistung in einem letzten Versuch zu verbessern. Gegen Ende des dritten Rechenblocks teilt die Versuchsleiterin der Probandin mit, dass sie das Experiment nun planmäßig weiterführen würden und die Daten vielleicht trotzdem in gewisser Weise brauchbar seien. Daraufhin verlässt sie den Raum.

*Psychophysiologische Daten.* Das Elektrokardiogramm (EKG) und die elektrodermale Aktivität wurden anhand eines Vitaport-3-Datenerfassungssystems (TEMEC Instruments B.V., Niederlanden) aufgezeichnet. Das EKG wurde über *Lead-II* mit drei Wegwerf-Elektroden (RedDot™, 2248-50, 3M Health Care, Deutschland) auf dem Thorax abgeleitet und diente der Messung der Herzfrequenz. Die elektrodermale Aktivität wurde mit Hilfe von zwei Silber/Silberchlorid Elektroden gemessen. Ein automatisches Blutdruckmessgerät mit Armmanschette (CRITIKON DINAMAP™ 1846 SX, USA) wurde für die oszillatorische Blutdruckmessung verwendet. Zur Erhebung des Speichelcortisols wurden Salivetten (Sarstedt, Sevelen, Schweiz) eingesetzt.

*Subjektive Stressbeurteilung.* Die subjektive Stressbeurteilung wurde anhand einer visuellen Analogskala (VAS) von 0 „gar nicht gestresst“ bis 100 „sehr gestresst“ erfasst.

### *Datenbereinigung und Analysen*

*Herzfrequenz und elektrodermale Aktivität.* Die Samplingrate für die Aufzeichnung der elektrodermalen Aktivität betrug 16 Hz. Die Samplingrate für die Aufzeichnung des EKG betrug 1024 Hz, und es wurden ein physikalischer Tiefpassfilter von 512 Hz sowie ein physikalischer Hochpassfilter von 0.5 Hz verwendet. Die elektrodermale Aktivität und die EKG-Daten wurden mit

dem Computerprogramm *Anslab* analysiert (Matlab-basierte Toolbox zur Auswertung peripherphysiologischer Daten, Autonomic Nervous System Laboratory, Wilhelm & Peyk, 2005). Für das Auslesen der EKG-Daten wurden offline ein Tiefpassfilter von 40 Hz und wiederum ein Hochpassfilter von 0.5 Hz eingesetzt. Um weiteres Rauschen zu eliminieren, wurden die Daten der elektrodermalen Aktivität zusätzlich anhand eines Tiefpassfilters von 1 Hz korrigiert. Artefakte wurden von Hand bereinigt. Die Herzfrequenz (Anzahl der Herzschläge pro Minute) wurde jeweils anhand des EKG berechnet. Für die Herzfrequenz und die elektrodermale Aktivität wurden Mittelwerte über die gesamte Ruhephase, die gesamte MIST-Durchführung und die gesamte Erholungsphase gebildet. Die während der MIST-Durchführung erhobenen Daten wurden zudem weiter aufgegliedert, und es wurden Mittelwerte für jeden der drei Rechenblöcke sowie für die beiden Zwischenphasen mit sozialem Feedback berechnet.

*Speichelcortisol*. Die Speichelproben wurden nach dem Experiment zunächst im Kühlschrank aufbewahrt und anschliessend bei -22 °C eingefroren. Die Cortisolkonzentrationen im Speichel wurden mittels Enzym-Immunoassay bestimmt (Cooper, Trunkfield, Zanella & Booth, 1989). Die Intra-Assay Variabilität beträgt durchschnittlich 8.27%, die Inter-Assay Variabilität 8.33% (Alpco Diagnostics, 2011).

Um bezüglich der Cortisolreaktion auf den MIST Responder von Non-Respondern unterscheiden zu können, wurde als Cut-off-Wert die durchschnittliche Intra-Assay Variabilität von 8.27% verdoppelt (Van Cauter & Refetoff, 1985). Demnach wurde ein Cortisolanstieg von mindestens 16.53% im Vergleich zu der Ruhebedingung als Response betrachtet. Dabei wurden Cortisolpeaks zu den Zeitpunkten +1, +10, +25 oder +40 Min. nach dem MIST berücksichtigt (Dickerson & Kemeny, 2004; Hellhammer & Hellhammer, 2008).

*Statistische Auswertungen*. Die weiteren Datenanalysen wurden mit Hilfe von IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL) durchgeführt. Anhand von deskriptiven Datenanalysen wurden Ausreisser in den Cortisoldaten sowie in den psychophysiologischen Daten identifiziert (mehr als 3 *SD* über dem Mittelwert der Gesamtstichprobe bzw. hohe Fehlerwahrscheinlichkeit) und nach Field (2009) korrigiert. Eine Probandin musste wegen hoher Fehlerwahrscheinlichkeit ausgeschlossen werden, so

dass insgesamt Cortisoldaten von 96 Personen in die Analysen eingingen. Technische Probleme mit dem Vitaportsystem führten zu Ausfällen von elektrodermalen Daten in der Ruhephase (n = 5).

Für sämtliche statistische Analysen wurde ein Signifikanzniveau von .05 verwendet. Um zu überprüfen, ob sich die erhobenen psychophysiologischen und subjektiven Stressparameter über die experimentellen Bedingungen hinweg veränderten, wurden in einem ersten Schritt einfaktorielle Varianzanalysen mit Messwiederholung mit dem Faktor Zeit bzw. Messzeitpunkt und den Stressparametern als jeweils abhängige Variable durchgeführt. Bei verletzter Normalverteilung wurden die Daten logarithmiert. Bei verletzter Sphärizität wurden die statistischen Kennwerte für entsprechende Varianzanalysen nach Greenhouse-Geisser korrigiert.

T-Tests für abhängige Stichproben wurden angewendet, um zu überprüfen, ob sich die psychophysiologischen und subjektiven Stressparameter während bzw. nach dem Stresstest signifikant von denjenigen der Ruhe- bzw. der Erholungsphase unterschieden. Dabei wurden die Mittelwerte der Ruhephase mit den höchsten während bzw. nach dem MIST erhaltenen Mittelwerten verglichen (Ruhephase zu Peak) sowie die höchsten Mittelwerte während bzw. nach dem MIST mit den Mittelwerten am Ende der Erholungsphase (Peak zu Erholungsphase). Nicht normalverteilte Daten wurden logarithmiert. Falls die Normalverteilung nicht durch eine Datentransformierung hergestellt werden konnte, wurde anstelle eines t-Tests ein Wilcoxon-Test für abhängige Stichproben bzw. anstelle einfaktorieller Varianzanalysen Friedman-Tests verwendet. Aufgrund der besseren Interpretierbarkeit werden die nicht-transformierten Resultate berichtet.

Da die Einnahme oraler Kontrazeptiva die Reaktivität der HHNA beeinflussen kann (Kirschbaum et al., 1999), wurde die Einnahme von oraler Kontrazeptiva als Kovariable mitberücksichtigt. Der Bodymass Index galt als Kovariable für den Blutdruck.

Für die Berechnung der Effektstärken der Varianzanalysen wurde das partielle Eta Quadrat ($\eta_p^2$) gewählt, wobei dieses nach kleinen (.01), mittleren (.06) oder starken Effekten (.14) unterteilt wird (Green, Salkind, & Akey, 2000). Für die t-Tests wurde die Effektstärke mittels Pearsons Korrelationskoeffizienten r berechnet, wobei ein kleiner (.10), mittlerer (.30) und starker (.50) Effekt unterschieden wird (Field, 2009).

## Ergebnisse

*Überprüfung von möglichen Kontrollvariablen*

Der Einfluss oraler Kontrazeptiva auf die Cortisolreaktion wurde zu Beginn der Analysen überprüft. Es zeigte sich, dass sich die Einnahme von oraler Kontrazeptiva signifikant auf die Cortisolwerte auswirkte. So wiesen Probandinnen, welche orale Kontrazeptiva einnahmen (N = 51), vor dem Stresstest signifikant höhere Cortisolwerte auf ($M$ = 20.64, $SD$ = 8.52) als Frauen, die keine orale Kontrazeptiva (N = 45) einnahmen ($M$ = 16.68, $SD$ = 7.18), $t(94) = -2.28$, $p < .05$. In den nachfolgenden Analysen wurde die Baseline als Kovariable in das Modell miteinbezogen. Mit allen anderen erhobenen psychophysiologischen Daten ergaben sich keine signifikanten Zusammenhänge.

*Cortisol*

Wie eine einfaktorielle Varianzanalyse mit Messwiederholung zeigte, veränderte sich der Speichelcortisolspiegel signifikant über die sieben Messzeitpunkte hinweg, $F(5, 464) = 3.86$, $p < .05$, $\eta_p^2 = .04$ (siehe Abbildung 2) unter Berücksichtigung der Einnahme oraler Kontrazeptiva sowie der Baseline Messung. Durchschnittlich zeigten die Probandinnen fünfundzwanzig Minuten nach Ende des MIST die höchsten Cortisolwerte ($M$ = 21.58, $SD$ = 9.33). Im Vergleich zur Ruhemessung zum Zeitpunkt -1 Min. ($M$ = 17.73, $SD$ = 6.74) unterschieden sich die Werte fünfundzwanzig Minuten nach Ende des MIST signifikant, $t(95) = -4.05$, $p < .001$, $r = .38$. Es zeigte sich ein signifikanter Abfall des Speichelcortisols vom Höhepunkt zur letzten Messung fünfundfünfzig Minuten nach Beginn des MIST ($M$ = 18.63, $SD$ = 7.63), $t(95) = -4.14$, $p < .001$, $r = .39$.

Um den Einfluss oraler Kontrazeptiva auf die Cortisolreaktion zu überprüfen, wurden individuelle Reaktivitätswerte berechnet, indem vom höchsten Wert während bzw. nach dem Stresstest der niedrigste Wert der Ruhemessung subtrahiert wurde. Es zeigte sich, dass sich die Reaktivität der Probandinnen ohne orale Kontrazeptiva ($M$ = 10.91, $SD$ = 8.44) nicht signifikant von der Reaktivität der Probandinnen mit oralen Kontrazeptiva ($M$ = 9.54, $SD$ = 7.69) unterschieden; $t(94) = .83$, $p = .408$.

*Elektrodermale Aktivität*

Abbildung 2 verdeutlicht eine signifikante Zunahme der elektrodermalen Aktivität über die Zeit hinweg, $F(1.58, 129.69) = 65.93$, $p < .001$, $\eta_p^2 = .45$. Im Vergleich zur Ruhebedingung ($M = 6.89$, $SD = 4.19$) stieg die elektrodermale Aktivität gemittelt über den gesamten Stresstest signifikant an ($M = 9.63$, $SD = 3.86$), $t(89) = -8.85$, $p < .001$, $r = .68$. Nach Ende des Stresstests kam es wieder zu einer signifikanten Abnahme der elektrodermalen Aktivität im Vergleich zu den während der Stressinduktion gemessenen Werte ($M = 7.75$, $SD = 3.56$), $t(84) = 15.34$, $p < .001$, $r = .82$.

*Herzfrequenz*

Auch die Herzfrequenz veränderte sich signifikant über die experimentellen Bedingungen hinweg, $F(2.42, 218.02) = 190.21$, $p < .001$, $\eta_p^2 = .68$ (siehe Abbildung 2). Sie erreichte während des zweiten Rechenblocks ihren Höhepunkt ($M = 101.03$, $SD = 20.81$). Die dabei gemessenen Werte unterschieden sich signifikant von denjenigen der Ruhebedingung ($M = 71.53$, $SD = 9.15$; $t(94) = -16.18$, $p < .001$, $r = .86$) und der Erholungsphase ($M = 75.72$, $SD = 10.11$; $t(90) = 16.53$, $p < .001$, $r = .87$).

**ABBILDUNG 2 BITTE UNGEFÄHR HIER EINFÜGEN**

*Blutdruck*

Varianzanalysen ergaben zudem signifikante Veränderungen der Blutdruck über die zehn Messzeitpunkte hinweg, unter Berücksichtigung des BMI: systolischer Blutdruck: $F(9, 819) = 10.57$, $p < .001$, $\eta_p^2 = .12$ und diastolischer Blutdruck: $F(3.57, 325.27) = 5.00$, $p < .001$, $\eta_p^2 = .05$. Die Mittelwertvergleiche sind in Tabelle 1 aufgeführt.

**TABELLE 1 BITTE UNGEFÄHR HIER EINFÜGEN**

*Subjektive Stressbeurteilung*

Die subjektiven Stressbeurteilungen der Probandinnen veränderten sich signifikant über die sieben Messzeitpunkte hinweg, $\chi^2(6) = 236.13$, $p < .001$. Ein Wilcoxon Test ergab, dass sich die Versuchspersonen unmittelbar nach dem MIST gestresster fühlen ($M = 55.23$, $SD = 27.52$) als direkt

davor ($M$ = 26.65, $SD$ = 20.52), $z$ = -7.69, $p$ < .001. Der subjektiv erlebte Stress sank nach dem Stresstest wieder signifikant ab ($M$ = 21.60, $SD$ = 22.28), z = -8.19, p < .001.

## Diskussion

Die vorliegende Studie untersuchte die endokrine und kardiovaskuläre Stressinduktion des psychosozialen Stresstest MIST erstmals an einer gesunden, weiblichen Stichprobe. Die Resultate bestätigen die vielfach berichtete Effektivität des MIST: Der MIST führte verglichen mit einer Ruhephase zu signifikant höheren Blutdruckwerten, einer signifikant höheren Herzfrequenz und elektrodermalen Aktivität, einem signifikant höheren Speichelcortisolniveau und zu einem stärkeren Gefühl von subjektiv erlebtem Stress.

Unsere Befunde replizieren die Resultate früherer Studien (Dedovic et al., 2005; La Marca et al., 2011; Pruessner et al., 2004; Pruessner et al., 2008; Soliman et al., 2008) und konnten eine effektive, stressinduzierte Stimulierung der HHNA durch den MIST aufzeigen. Der MIST scheint mit einer Effektstärke von $\eta_p^2$ = .04, welche als leichter bis mittlerer Effekt zu interpretieren ist, eine wie bereits in anderen Untersuchungen berichtete moderate Aktivierung der HHNA auszulösen. Bedeutsam erscheint, dass der MIST bei 76.3% der untersuchten Probandinnen eine signifikante Cortisolreaktion auslöste, definiert als Mindestanstieg von 16.53% im Vergleich zur Ruhebedingung (Van Cauter & Refetoff, 1985). Dieser Befund weicht von den in anderen Untersuchungen des MIST berichteten durchschnittlichen Responderraten von 50% ab (Pruessner et al., 2010).

Im Gegensatz zu den meisten Befunden wurde in unserer Studie der Stresstest ausserhalb eines MRT Scans durchgeführt. Es kann vermutet werden, dass der nähere Kontakt der Versuchs- und Studienleiterin zu den Probandinnen die sozial evaluative Bedrohung erhöhte und es bei mehr Probandinnen zu einem signifikanten Cortisolanstieg kam als in den MRT-Studien.

Die Resultate replizieren auch die effektive, stressinduzierte Stimulierung des Sympathischen Nervensystems. Die Effektstärken der kardiovaskulären und elektrodermalen Parameter weisen auf eine starke Aktivierung des Sympathischen Nervensystems hin. Der MIST führte zu einem signifikanten Anstieg der Herzfrequenz und des Blutdrucks wie bereits in anderen Studien berichtet (La Marca et al., 2011; Jones et al., 2011). Entgegen dem Befund einer an mehrheitlich weiblichen

Probandinnen durchgeführten Studie (Soliman et al., 2008), wurde in dieser Untersuchung zudem eine erhöhte elektrodermale Aktivität während des MIST, verglichen mit der Ruhebedingung, gemessen.

Da das gesamte Experiment von beiden Versuchsleiterinnen nach einem detaillierten, standardisierten Leitfaden, unter kontrollierten Bedingungen und stets zur selben Tageszeit durchgeführt wurde, konnte der Einfluss von Störvariablen stark eingegrenzt werden. Besonders Variablen, welche den Cortisolspiegel beeinflussen können (z.B. starker Nikotinkonsum), wurden durch strenge Ausschlusskriterien oder durch genaue Anweisungen (z.B. innerhalb einer Stunde vor dem Experiment auf Koffein verzichten) kontrolliert. Die interne Validität ist insgesamt deshalb als gut einzuschätzen.

Trotz bestätigter Hypothesen hat die vorliegende Studie auch Einschränkungen. So wurden Frauen, welche orale Kontrazeptiva einnahmen, in die Studie aufgenommen. Wie bereits von anderen Autoren berichtet, scheinen orale Kontrazeptiva einen Einfluss auf das biologisch verfügbare Cortisol zu haben und wurden mit einer gedämpften Speichelcortisolausschüttung nach einem psychosozialen Stresstest in assoziiert (Bouma et. al, 2009; Kirschbaum et al., 1999). In unserer Studie konnte bezüglich der Reaktivität von Cortisol zwar kein Unterschied zwischen Frauen mit und ohne orale Kontrazeptiva festgestellt werden, jedoch unterschieden sich die zwei Gruppen durch ein höheres Speichelcortisolniveau zu Beginn der Stressprovokation. Es kann nicht ausgeschlossen werden, dass sich die Einnahme oraler Kontrazeptiva trotzdem auf Speichelcortisolreaktion ausgewirkt haben könnte. Da in dieser Studie nur freies Cortisol bestimmt wurde sowie auf die Messung von ACTH verzichtet wurde, kann diese Frage nicht abschliessend beantwortet werden.

Die Validität des MIST als Stresstest wurde erstmals an einer gesunden, ausschliesslich weiblichen Stichprobe repliziert. Wie bereits von La Marca (2011) erläutert, liegt der Vorteil des MIST im Vergleich zu anderen effektiven Stresstests (z.B. Trier Social-Stresstest, TSST; Kirschbaum, Pirke & Hellhammer, 1993) in der Reduktion diverser potentieller Störfaktoren wie Bewegungsartefakte, Gehen oder Stehen (Chan, Lin, Chao & Lin, 2007; Nater, La Marca, Florin, Moses, Langhans, Koller & Ehlert, 2006) und Sprechen (Bernardi, Wdowczyk-Szulc, Valenti, Castoldi, Passino et al., 2000; Sloan, Korten, & Myers, 1991). Dies ist besonders für die Messung der

sympathischen Stressparameter von Vorteil. Die Stressforschung verfügt somit mit dem MIST über ein wertvolles Mittel, um auch bei weiblichen gesunden Stichproben im Rahmen von bildgebenden Verfahren und anderen komplexen physiologischen Settings kontrolliert und standardisiert Stress zu induzieren, um wichtigen Fragestellungen zu den Mechanismen von Stress und den damit assoziierten Störungen nachzugehen.

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Literatur

Alpco Diagnostics (2011, February 14). *Cortisol (Saliva) ELISA - For the quantitative determination of cortisol in human saliva.* Retrieved August 2, 2011, from http://www.alpco.com/pdfs/11/11-CORHU-E01-SLV.pdf

Bernardi, L., Wdowczyk-Szulc, J., Valenti, C., Castoldi, S., Passino, C., Spadacini, G., & Sleight, P. (2000). Effects of controlled breathing, mental activity and mental stress with or without verbalization on heart rate variability. *Journal of the American College of Cardiology, 35*, 1462 – 1469.

Bouma, E.M.C., Riese, H., Ormel, J., Verhulst, F.C. & Oldehinkel, A.J. (2009). Adolescents‘ cortisol responses to awakening and social stress; Effects of gender, menstrual phase and oral contraceptives. The TRAILS study. *Psychoneuroendocrinology*, 34 (6), 884 – 893.

Chan, H.L., Lin, M.A., Chao, P.K. & Lin, C.H. (2007). Correlates of the shift in heart rate variability with postures and walking by time-frequency analysis. *Computer Methods and Programs in Biomedicine, 85*, 124 – 130.

Cooper, T. R., Trunkfield, H. R., Zanella, A. J., & Booth, W. D. (1989). An enzyme-linked immunosorbent assay for cortisol in the saliva of man and domestic farm animals. *Journal of Endocrinology, 123,* R13-R16.

Dedovic, K., Renwick, R., Kahlili-Mahani, N., Engert, V., Lupien, S. J., & Pruessner, J. C. (2005). The Montreal Imaging Stress Task: Using functional imaging to inverstigate the effects of perceiving and processing psychosocial stress in human brain. *Journal of Psychiatry & Neuroscience, 30,* 319-325.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*, 355-391.

Foley, P., Kirschbaum, C. (2010). Human hypothalamus-pituitary-adrenal axis responses to acute psychosocial stress in laboratory settings. *Neuroscience and Biobehavior Reviews, 35*, 91 – 96.

Field, A. (2009). *Discovering Statistics Using SPSS.* Los Angeles: SAGE.

Green, S.B., Salkind, N.J. & Akey, T.M. (2000). Using SPSS for Windows (2nd ed.). Englewood Cliffs, NJ: Prentice Hall.

Hellhammer, D. H., & Hellhammer, J. (Eds.). (2008). *Stress – The Brain-Body Connection.* Basel: Karger.

Jones, A., Steeden, J.A., Pruessner, J.C., Deanfield, J.E., Taylor, A.M. & Muthurangu, V. (2011). Detailed assessment of the hemodynamic response to psychological stress using real-time MRI. *Journal of Magnetic Resonance Imaging, 33*, 448 – 454.

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology, 19*, 313-333.

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine, 61,* 154-162.

Kirschbaum, C., Pirke, M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test' - a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28,* 76-81.

Kudielka, B.M., Hellhammer, D.H., Kirschbaum, C. (2007). Ten years of research with the Trier Social Stress Test-revisited. In: Harmon-Jones, E., Winkielman, P. (Hrsg.), Social Neuroscience: Integrating Biological and Psychological Explanations of Social Behavior. The Guilford Press, New York, 56 – 83.

La Marca, R., Waldvogel, P., Thörn, H., Tripod, M., Wirtz, H., Pruessner, J. C. et al. (2011). Association between Cold Face Test-induced vagal inhibition and cortisol response to acute stress. *Psychophysiology, 48,* 420-429.

Lord, C., Steiner, M., Soares, C.N., Carew, C.L. & Hall, G.B. (2011). Stress response in postpartum women with and without obsessive-compulsive symptoms: an fMRT study. *Journal of Psychiatry and Neuroscience, 37*, 78 – 86.

Nater, U.M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M.M. & Ehlert, U. (2006). Stress-induced changes in human salivary alpha-amylase activity – associations with adrenergic activity. *Psychonueroendocrinology, 31*, 49 – 58.

Pruessner, J. C., Champagne, F., Meaney, M. J., & Dagher, A. (2004). Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: A positron emission tomography study using [$^{11}$C]Raclopride. *The Journal of Neuroscience*, *24*, 2825-2831.

Pruessner, J. C., Dedovic, K., Khalili-Mahani, N., Engert, V., Pruessner, M., Buss, C. et al. (2008). Deactivation of the Limbic System during acute psychosocial stress: Evidence from Positron Emission Tomography and Functional Magnetic Resonance Imaging Studies. *Biological Psychiatry, 63,* 234-240.

Pruessner, J. C., Dedovic, K., Pruessner, M., Lord, C., Buss, C., Collins, L. et al. (2010). Stress regulation in the central nervous system: Evidence from structural and functional neuroimaging studies in human populations – 2008 Curt Richter Award Winner. *Psychoneuroendocrinology, 35,* 179-191.

Sloan, R.P., Korten, J.B. & Myers, M.M. (1991). Components of heart rate reactivity during mental arithmetic with and without speaking. *Physiology and Behaviour, 50*, 1039 -1045.

Soliman, A., O'Driscoll, G. A., Pruessner, J., Holahan, A. V., Boileau, I., Gagnon, D. et al (2008). Stress-induced dopamine release in humans at risk of psychosis: a [$^{11}$C]Raclopride PET study. *Neuropsychopharmacology, 33,* 2033-2041.

Soliman, A., O'Driscoll, G.A., Pruessner, J., Joober, R., Ditto, B., Streicker, E. et al. (2011). Limbic response to psychological stress in schizotypy: a functional magnetic resonance imaging study. *Schizophrenia Research, 131*, 184 – 191.

Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer D. et al. (2001). Individual differences in the diurnal cycle of salivary free cortisol: A replication of flattened cycles for some individuals. *Psychoneuroendocrinology, 26,* 295-306.

Van Cauter, E., & Refetoff, S. (1985). Evidence for two subtypes of Cushing's disease based on the analysis of episodic cortisol secretion. *The New England Journal of Medicine, 312,* 1343-1349.

Wilhelm, F. H., & Peyk, P. (2005). *ANSLAB: Autonomic Nervous System Laboratory (Version 4.0)*. Erhältlich bei der SPR Software Repository, http://www.sprweb.org.

Wittchen, H. U., Wunderlich, U., Gruschwitz, S., & Zaudig, M. (1997). *SKID-I: Strukturiertes Klinisches Interview für DSM-IV – Achse I: Psychische Störungen*. Göttingen: Hogrefe.

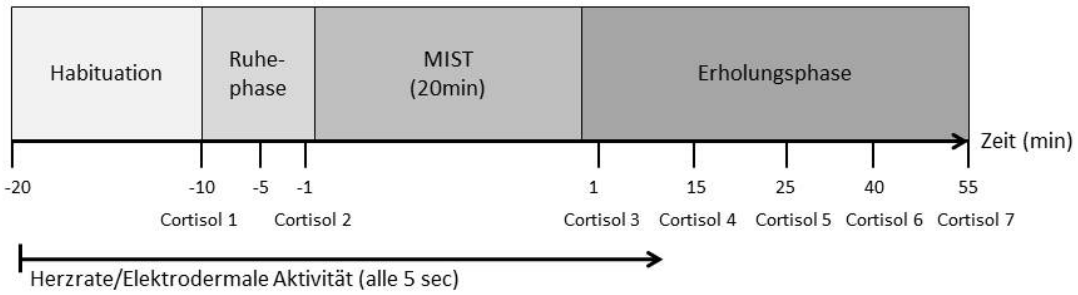


*Abbildung 1.* Ablauf der Untersuchung.

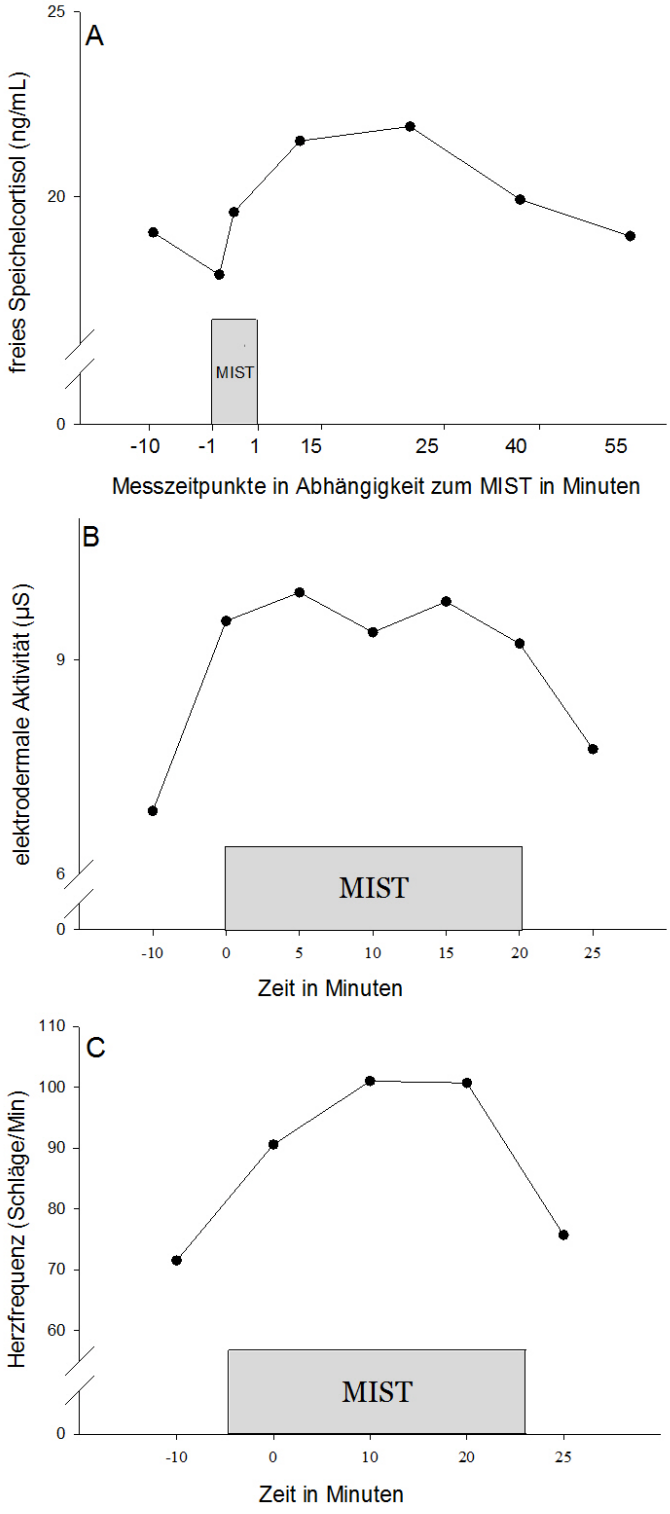


*Abbildung 2*. (A) Speichelcortisol Konzentration (ng/ml), (B) elektrodermale Aktivität (µS) sowie (C) Herzfrequenzveränderungen (Anzahl Schläge/Minute) in Abhängigkeit zum MIST.

*Tabelle 1.* Blutdruck und Puls während der Ruhephase, zu Beginn des dritten MIST Rechnungsblockes und während des letzten Messzeitpunkts der Erholungsphase

| | Ruhephase M(SD) | MIST M(SD) | Erholungsphase M(SD) | Vergleich | Statistik |
|---|---|---|---|---|---|
| BPsys | 111.58 (9.34) | 138.61 (14.79) | 116.70 (10.41) | Ruhe < MIST<br>MIST > Erholung | T (95) = -22.38, p< 0.001; r = .92<br>T (95) = 17.18; p < 0.001; r = .87 |
| BPdia | 68.49 (6.48) | 87.44 (9.77) | 71.60 (11.38) | Ruhe < MIST<br>MIST > Erholung | T (95) = -25.65, p< 0.001; r = .93<br>T (95) = 17.18, p< 0.001; r = .75 |
| Puls | 68.28 (11.11) | 106.13 (19.14) | 71.27 (10.64) | Ruhe < MIST<br>MIST > Erholung | T (95) = -19.05, p< 0.001; r = .89<br>T (95) = 20.07, p< 0.001; r = .90 |