Stress and the brain: The brain as a coordinator and target of the human stress response

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The experimental data presented in chapters two and three of this work were published as "Original Research Articles" in international peer-reviewed journals.

Chapter 2.1 Böhringer, A., Schwabe, L., Richter, S., & Schachinger, H. (2008). Intranasal insulin attenuates the hypothalamic-pituitary-adrenal axis response to psychosocial stress. *Psychoneuroendocrinology, 33*, 1394-1400.

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INDEX OF ABBREVIATIONS

ACTH	adrenocorticotropic hormone
AgRP	agouti gene-related protein
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ANS	autonomic nervous system
AUC	area under the curve
AVP	arginine vasopressin
BMI	body mass index
CART	cocaine amphetamine–related transcript
CBG	corticosteroid-binding globulin
CNS	central nervous system
CPT	cold pressor test
CRH	corticotrophin releasing hormone
CSF	cerebrospinal fluid
DMH	dorsomedial hypothalamus
ECG	electrocardiogram
GABA	γ-aminobutyric acid
GC	glucocorticoid
GLM	general linear model
GR	glucocorticoid receptor
HPA	hypothalamic-pituitary-adrenal
mPFC	medial prefrontal cortex
MR	mineralocorticoid receptor
mRNA	messenger ribonuleic acid
NPY	neuropeptide Y
NTS	nucleus tractus solitarii

POMC	pro-opiomelanocortin
PVN	paraventricular nucleus
SNS	sympathetic nervous system
TSST	Trier Social Stress Test

1. INTRODUCTION

1.1. Introduction and outline

The study of stress has gained broad interest during the past decades. Going back to the pioneering works of Walter B. Cannon and Hans Selye, hundreds of studies aimed to investigate how the organism manages to keep homeostasis in the context of constantly changing, stressful environments (Goldstein & Kopin, 2007). This work told us much about the functional mechanisms of the body's major stress systems, i.e. the HPA axis and the ANS. Now, however, more than fifty years after H. Selyes initial studies, the focus of interest in stress research has shifted to the brain as the central organ of the stress response. The brain recognizes and evaluates potential threats, i.e. stressors, to the body. It initiates behavioural and physiological responses that allow the individual to adapt successfully to the stressor. It is furthermore also a target of stress and changes structurally and functionally in response to stressors (Joels & Baram, 2009; McEwen, 2008). Stress along with its protective and adverse effects on the individual can thus only be understood if we learn more about the brain's role in the organism's response to stressors. Today, however, we are only beginning to understand how the brain orchestrates the various stress mediators into a well organised stress reaction and how these mediators in turn shape brain function.

The present work deals with the brain as a coordinator and target of the human stress reaction. Specifically, it addresses the question of (i) how the brain integrates feedback from bodily systems involved in the control of metabolic homeostasis into a well coordinated stress reaction and (ii) how different stress components affect learning and memory, two important aspects of human brain function.

This work is organized into three consecutive chapters. Chapter one offers a short overview upon the essential components of the stress reaction and discusses recent work on effects of stress on learning and memory processes. It is shown that the brain not only acts as a coordinator of the stress response but also serves as a

target of stress mediator action. Experimental results illustrating this dual role of the brain in stress are presented in chapters two and three. Chapter two shows that the stress-related activity of the HPA axis is affected by centrally acting insulin, suggesting that the brain coordinates the stress response according to ongoing metabolic needs. In contrast, chapter three reports results from two experimental studies investigating the effects of stress induced arousal on memory retrieval and stress induced cortisol on learning processes. These data show that the stress response affects learning and memory, two important aspects of human brain function.

1.2. Defining stress, stressor and stress response

"Stress" is a popular term nowadays. It is part of our every-day speech and a frequent topic in newspapers and television. However, the term stress is often used in ambiguous ways, sometimes referring to a specific type of event, sometimes to particular bodily processes. The present work therefore starts with a short definition of "stress" and the closely related terms "stressor" and "stress response".

Stress. Numerous definitions of stress have been made in the past that vary in the extend to which they emphasize stressful events, responses, or individual appraisals of situations as the central characteristics of stress (Goldstein & Kopin, 2007). A commonality among these diverging definitions is that they view stress as a state in which environmental demands are sensed by an individual and require adaptive responses to maintain bodily homeostasis. Here, I will follow a recent suggestion that builds up on this general homeostatic conceptualization of stress and define stress as "a condition where expectations, whether genetically programmed, established by prior learning, or deduced from circumstances, do not match the current or anticipated perceptions of the internal or external environment, and this discrepancy between what is observed or sensed and what is expected or programmed elicits patterned, compensatory responses" (Goldstein & Kopin, 2007).

Stressor. A "stressor" may be viewed as a stimulus that disrupts homeostasis and leads to patterned compensatory responses. Stressors can be divided into four main categories (Pacak & Palkovits, 2001): (1) physical stressors that have either a negative or, in some situations, a positive psychological component; (2) psychological stressors that reflect a learned response to previously experienced adverse conditions; (3) social stressors reflecting disturbed interactions among individuals; and (4) stressors that challenge cardiovascular and metabolic homeostasis. Two of the experimental studies presented in the present work deal with psychosocial stressors. The third study employs a mixed stressor which combines characteristics of a physical (low temperature) and a psychosocial stressor.

Stress response. The entirety of compensatory responses that allow for maintaining homeostasis in threatening situations is usually referred to as the "stress response". The stress response is characterized by the activation of specific bodily systems (discussed below). However, various neuroendocrine and hormonal mediators were discovered that mediate responses to stressors (Joels & Baram, 2009). The complex interactions among these mediators allow the organism to respond adequately to different types of stressors and stressful situations.

1.3. The stress response: Biological fundamentals

A stressor was defined above as an internal or external demand that disrupts bodily homeostasis and leads to patterned compensatory responses. The latter are characterized by the activation of the body's two primary compensatory systems, i.e. the ANS and the HPA axis. (Kandel, Schwartz, & Jessell, 2000; Klinke, Pape, & Silbernagl, 2005; Ulrich-Lai & Herman, 2009). The anatomy of these "primary" stress systems is introduced below.

The ANS allows for generating immediate responses to stressor exposure. It is classically subdivided into a sympathetic and a parasympathetic division (Klinke et al., 2005). The sympathetic trunk consists of preganglionic neurons located in the intermediolateral cell column of the thoracolumbal spinal cord which project to pre-

and paravertebral ganglia. These postganglionic pre- and paravertebral neurons in turn project to end organs and chromaffin cells of the adrenal medulla which secret adrenaline and noradrenaline into the systemic circulation. Sympathetic innervation of end organs and systemically acting adrenalin and noradrenalin provoke rapid alteration in physiological states. Within seconds, the heart rate speeds up, the blood pressure rises, and resources are mobilized from energy stores. In combination with effects of centrally acting monoamines and peptides the organism reaches a state of heightened alertness, vigilance and arousal which prepares for immediate attempts to cope with the stressor. This early phase of the stress response was first characterized by Walter B. Cannon and colleagues and represents the classical "fight or flight" reaction (Goldstein & Kopin, 2007). The parasympathetic trunk of the ANS consists of craniosacral preganglionic nuclei which project to postganglionic neurons in or near the end organs. Parasampathetic actions are generally opposite to those of the sympathetic system and may help to modulate sympathetic activity during stressor exposure (Ulrich-Lai & Herman, 2009).

The second major stress system is the HPA axis. The HPA axis is a conglomerate of neuroendocrine structures with the most important components being specific hypothalamic nuclei, the pituitary, and the adrenal cortex (Klinke et al., 2005). The HPA axis's final common output pathway from the brain is the PVN of the hypothalamus (Pecoraro et al., 2006). This structure contains CRH synthesizing neurons that also synthesize AVP. Axons from CRH cells project to the median eminence where they release their contents into the primary portal vasculature. After vascular transport to and diffusion from the secondary capillary network in the anterior pituitary, CRH and AVP stimulate glandular corticotrope cells to release ACTH into the systemic circulation. Elevated plasma ACTH then stimulates cells in the zona fasciculata of the adrenal cortex to synthesize and secrete GCs (Pecoraro et al., 2006). The actions of GCs characterize the second wave of stress mediator effects which lasts from minutes to hours after stressor exposure. Although GCs have immediate effects (Olijslagers et al., 2008), they are mostly known for their delayed

actions on gene transcription. Importantly, GCs promote the mobilization of energy stores and potentiate numerous sympathetically mediated effects, such as peripheral vasoconstriction. Moreover, they have important effects on higher-level cognitive processes, such as learning and memory, and thus shape the individual's learning history towards the stressor (Joels, Pu, Wiegert, Oitzl, & Krugers, 2006).

1.4. The central regulation of the stress response

1.4.1. Bottom-up and top-down control

Although the entire brain is involved in the maintenance of energy homeostasis and responds to stressor exposure some neural circuitries are of particular importance for the initiation and coordination of an adequate stress response. These involve ascending neural systems in the brainstem, specific hypothalamic nuclei, and descending inputs from the limbic forebrain (Ulrich-Lai & Herman, 2009). The present section will give an overview upon these neural systems.

Brainstem systems. Major homeostatic perturbations, such as blood loss, respiratory distress, traumatic pain, or inflammation are conveyed to the brainstem, where they modulate activity of the sympathetic and parasympathetic branch of the ANS and the HPA axis (Herman et al., 2003; Ulrich-Lai & Herman, 2009). Sympathetic responses involve reflex arcs that communicate with areas in the medulla oblongata and preganglionic sympathetic neurons in the intermediolateral cell column of the spinal cord (Klinke et al., 2005). Inputs to parasympathetic preganglionic neurons in the medulla oblongata and the sacral spinal cord modulate vagal tone to the heart and lungs and help to control the duration of autonomic responses (Kandel et al., 2000; Klinke et al., 2005; Ulrich-Lai & Herman, 2009). Both, parasympathetic and sympathetic systems show ascending projections to higher-order autonomic sites in the hindbrain, midbrain, and forebrain. Theses higher-order centres receive additional information from the hypothalamus and the limbic forebrain and project back to preganglionic sympathetic and parasympathetic

neurons to modulate autonomic responses in accordance with higher-level neural information (Kandel et al., 2000).

Neuronal populations in the brainstem also activate the HPA axis in response to stressor exposure. Catecholaminergic projections from the NTS to the parvocellular division of the PVN of the hypothalamus increase stress-related HPA axis activity (Plotsky, Cunningham, & Widmaier, 1989), whereas destructions of ascending noradrenergic and adrenergic neurons reduce HPA axis responses to stimuli that signal homeostatic perturbations (Buller, Xu, Dayas, & Day, 2001). Moreover, serotonergic fibres from the median raphe nuclei in the midbrain project to the parvocellular PVN and serotonin activates the HPA axis via serotonin receptors on PVN neurons (Lowry, 2002).

Paraventricular and dorsomedial hypothalamus. The PVN is a principle integrator of stress related signals (Herman & Cullinan, 1997). Various inputs from other brain regions converge upon neurons of its parvocellular subdivision. These neurons synthesize CRH and AVP and thus represent the final-common output pathway for neuronal regulation of the HPA axis (Herman & Cullinan, 1997). The PVN furthermore projects to autonomic targets in the brainstem and the spinal cord, such as the intermediolateral cell column, the parabrachial nucleus, the dorsal motor nucleus of the vagus nerve, and the NTS, and thus modulates ANS activity in response to stressors (Ulrich-Lai & Herman, 2009).

The DMH projects to the pre-autonomic and neuroendocrine cell groups in the PVN and receives ascending catecholaminergic and descending cortico-striatal inputs, thus positioning this structure as another central coordinator of the stress response (Pecoraro et al., 2006). Local stimulation of the DMH increases heart rate, blood pressure, and HPA axis responses to a psychological challenge (Bailey & Dimicco, 2001), whereas inhibition attenuates stress-induced cardiovascular activity (Stotz-Potter, Willis, & DiMicco, 1996). The DMH is thus crucially involved in the regulation of autonomic and HPA axis responses to psychogenic stressors.

Limbic stress circuits. The limbic system is a set of functionally interacting brain structures located along the corpus callosum. Important limbic structures are the amygdala, the hippocampus, and the mPFC. These regions receive ascending associational information from subcortical and cortical areas involved in higher-order sensory processing, attention, and arousal and modulate stress-related activity of the HPA axis and the ANS via descending projections to subcortical action sites (Herman, Ostrander, Mueller, & Figueiredo, 2005; Ulrich-Lai & Herman, 2009).

The amygdala consists of several subnuclei which are essentially involved in the initiation of autonomic and endocrine stress responses (Herman et al., 2005). Whereas its central nucleus is crucial for the regulation of autonomic and endocrine responses to systemic stressors (Sawchenko, Li, & Ericsson, 2000; Xu, Day, & Buller, 1999), the medial and basolateral nuclei initiate HPA axis responses to psychological stressors (Dayas, Buller, & Day, 1999).

The hippocampus exerts an inhibitory influence on the HPA axis (Herman et al., 2005). Hippocampal stimulation decreases GC secretion in rats (Dunn & Orr, 1984) and hippocampal damage increases stress-induced GC secretion (Jacobson & Sapolsky, 1991). This inhibitory influence on the HPA axis is stressor specific with only responses to psychogenic stressors being affected by hippocampal lesions (Herman & Mueller, 2006).

The mPFC can be subdivided in different subregions which are crucially involved in coordinating stress responses. Its prelimbic part exerts an inhibitory influence on HPA axis responses to psychological stressors (Figueiredo, Bruestle, Bodie, Dolgas, & Herman, 2003; Radley, Arias, & Sawchenko, 2006), specifically influencing the duration but not the peak levels of GC secretion (Ulrich-Lai & Herman, 2009). Moreover, inhibition of the prelimbic mPFC enhances heart rate responses to psychological stimuli, which is consistent with a role of this region in inhibiting autonomic stress responses (Akana, Chu, Soriano, & Dallman, 2001). In contrast, the infralimbic mPFC is involved in initiating autonomic and HPA responses to psychogenic stimuli, suggesting a stimulatory role of this region in

stress responses (Radley *et al.*, 2006; Ulrich-Lai & Herman, 2009). The different parts of the mPFC thus fulfil different roles in coordinating the stress response, with the prelimbic mPFC having inhibitory and the infralimbic mPFC having stimulatory functions.

The hippocampus and the mPFC furthermore mediate negative feedback inhibition of stress-related and basal HPA axis activity by GCs secreted from the adrenals. This effect is conveyed by central GRs and MRs and of particular importance for the regulation of HPA axis responses to psychogenic stressors (Herman et al., 2005; Ulrich-Lai & Herman, 2009).

1.4.2. Intra-hypothalamic coordination

Between the above described bottom-up and top-down inputs are intrahypothalamic nuclei and networks that coordinate behavioural, neuroendocrine, and autonomic output systems (Ulrich-Lai & Herman, 2009). These include the five preoptic hypothalamic nuclei, neurons in the lateral hypothalamus, and the suprachiasmatic nucleus of the hypothalamus (Ulrich-Lai & Herman, 2009). Of particular importance is furthermore the mediobasal hypothalamus, which includes the nucleus arcuatus. The nucleus arcuatus receives limbic efferents and has neuropeptidergic and GABAergic projections to the PVN through which it regulates feeding and energy expenditure and also HPA axis activity (Pecoraro et al., 2006). This system thus provides a means to gate responses to stressors with respect to ongoing physiological processes.

The arcuate nuclei are situated between the third ventrical and the median eminence. They contain NPY/AgRP and POMC/CART producing neurons that are highly sensitive to many energy signals and form an essential part of the central melanocortin system (Cone, 2005). In general, stimulation of NPY/AgRP neurons promotes feeding (orexigenic) and reduces metabolic rate, whereas stimulation of POMC/CART neurons inhibits feeding (anorexigenic) and increases metabolic rate (Pecoraro et al., 2006). Important adipostatic hormones that affect arcuate NYP/agRP

and POMC/CART neurons are the pancreatic hormone insulin and the adipocytic hormone leptin. Both are secreted in proportion to body fat mass and thus provide information about long-term availability of energy resources (Cone, 2005).

An increasing body of evidence suggests that centrally acting mediators of energy homeostasis, such as NYP and leptin, indeed affect stress response systems (Rohleder & Kirschbaum, 2007). For example, intracerebroventricular injections of the orexigenic peptide NPY increase hypothalamic CRH mRNA or immunoreactivity (Haas & George, 1987; Suda *et al.*, 1993), and increase peripheral concentrations of ACTH and corticosterone in rats (Brunton, Bales, & Russell, 2006; Wahlestedt *et al.*, 1987). In contrast, the anorexigenic hormone leptin down-regulates CRH gene expression in the PVN of adrenalectomized mice (Arvaniti, Huang, & Richard, 2001). Orexigenic regulators of energy homeostasis thus stimulate, whereas anorexigenic regulators exert an inhibitory tone on the HPA axis activity. These effects are presumably conveyed by action sites within the nucleus arcuatus.

1.5. Stress and brain function: Stress effects on learning and memory

Learning and memory are essential aspects of human brain function. This is particularly true under conditions of stress, where the ability to learn and interpret the predictive significance of environmental stimuli is crucial for the selection of appropriate coping strategies towards the threatening agent. Many studies have shown that stress affects learning and memory and ample evidence suggests that GCs and catecholamines are primary effectors of these stress effects on cognitive processing (Joels *et al.*, 2006). For example, GCs modulate spatial learning in rats (Oitzl & de Kloet, 1992) as well as memory consolidation (Kuhlmann & Wolf, 2006) and memory retrieval (de Quervain, Roozendaal, Nitsch, McGaugh, & Hock, 2000) in humans. However, the effects of stress mediators on learning and memory are complex, being sometimes facilitative and sometimes detrimental. Factors such as the time point of stress mediator action, the cooccurrence of autonomic or emotional

arousal, and the type of stressor affect the direction of the effect (Lupien, Maheu, Tu, Fiocco, & Schramek, 2007; Roozendaal, McEwen, & Chattarji, 2009). The long standing question of how stress affects learning and memory thus still remains to be answered.

Recently, a neurobiological model was suggested that attempts to account for the controversial effects of stress mediators on learning and memory (Joels et al., 2006). The authors show that studies in which stressor exposure occurred during or shortly after learning mostly find enhancing effects of stress on later memory performance. In contrast, studies in which stress was induced before or a reasonable amount of time after learning report impairing effects of stress on memory retrieval. Based on these findings Joels and colleagues (Joels et al., 2006) conclude that the time point of stress onset crucially influences the direction of stress effects on learning and memory processes. Moreover, they propose a unifying theory in which the time-dependent effects of stress are explained by diverging actions of stress related transmitters and hormones on brain cell and network function. In this theory, a first wave of stress mediator actions that is characterized by heightened activity of the ANS, central effects of noradrenaline, CRH, and early non-genomic actions of GCs, facilitates the formation of new memories and shapes perception and attention towards the stressor. In contrast, the delayed effect of GCs on gene-transcription that takes place tens of minutes after stressor exposure suppresses information unrelated to the stressor and enhances memory consolidation of stressor related information. If stress is thus experienced during learning, the early effects of stress mediator actions and the late effect of GCs on memory consolidation will enhance later memory performance. If, however, stressor exposure occurred at a longer time before learning or shortly before memory retrieval the GC mediated suppression of activity will impair new learning and the retrieval of old information.

1.6. The present studies

The foregoing sections introduced the term stress and gave an overview upon the essential components of the stress reaction. It was shown that limbic and brain stem areas are crucially involved in the initiation of the stress response and that intra-hypothalamic sites shape the stress response according to ongoing metabolic needs. Moreover, it was demonstrated that peripheral and central stress mediators modulate learning and memory, acting back on the brain and shaping brain function. The brain is thus at the centre of stress, being on the one hand the central coordinator of the stress response but on the other hand also a target of stress mediator action (Figure 1.6.1).

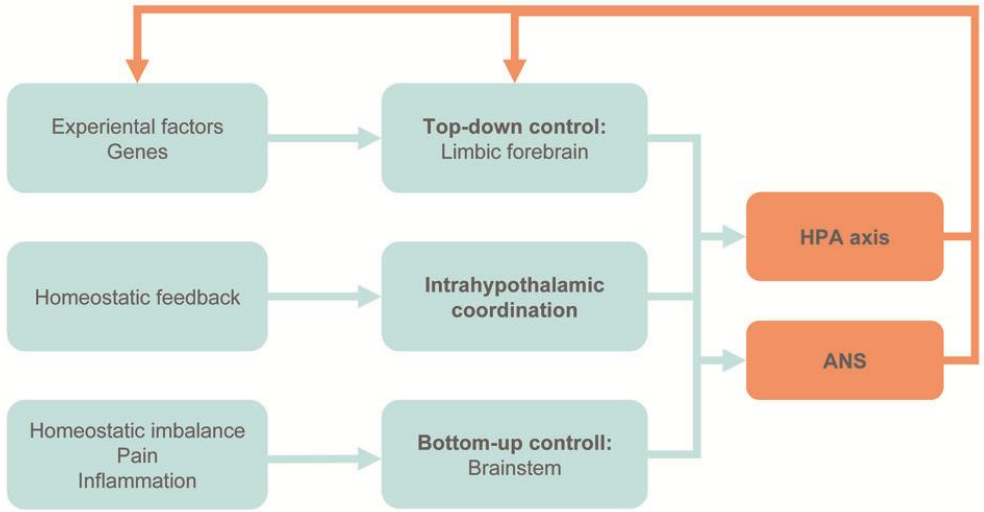

Figure 1.6.1. The brain as a coordinator and target of the stress response

Building up on this dual role of the brain in stress, the following chapters present experimental results referring to the brain's role as a coordinator (chapter two) and target (chapter three) of the human stress response. Study I (chapter 2.1) focuses on the question of how homeostatic feedback signals involved in the regulation of energy homeostasis modulate the stress response according to ongoing metabolic needs. Specifically, it was asked if the pancreatic peptide hormone insulin influences the response to a psychosocial stressor at the level of the central nervous system. Insulin is the most important anabolic hormone of the organism and

crucially involved in the regulation of the peripheral glucose metabolism. Pointing to a role of central insulin in the regulation of energy homeostasis, intracranial application of insulin reduces food intake and body fat (Air, Benoit, Blake Smith, Clegg, & Woods, 2002; Hallschmid *et al.*, 2004; Niswender, Baskin, & Schwartz, 2004). Insulin was shown to modulate the basal activity of the HPA axis and this effect is presumably mediated by central action sites (Fruehwald-Schultes, Kern, Born, Fehm, & Peters, 2001; Hallschmid, Benedict, Schultes, Born, & Kern, 2008). However, it remains to be elucidated if central insulin furthermore modulates HPA axis responses to stressor exposure. Here, it was hypothesized that centrally acting insulin, as other anorexigenic hormones and peptides involved in the regulation of energy homeostasis (Rohleder & Kirschbaum, 2007), attenuates the HPA axis response to stressor exposure.

In contrast to chapter two, the following chapter three focuses on the brain's role as a target of stress mediator action. Two studies are presented in which the effect of a psychosocial and a mixed psychosocial/physical stressor on learning and memory was investigated. Study II (chapter 3.1) aimed to further disentangle the effects of different stress components on post-stress memory performance. Specifically, it was asked if stress-induced subjective arousal, a marker of early stress mediator action, predicts post-stress memory retrieval. Based on previous work suggesting enhancing effects of subjective arousal on cognitive performance (Matthews & Davies, 2001; Matthews & Westerman, 1994; Revelle & Loftus, 1992) it was hypothesized that stress-induced subjective arousal would facilitate post-stress memory performance. In contrast to study II, study III (chapter 3.2) investigated effects of pre-learning stress on subsequent memory retrieval. Based on the model by Joels and colleagues (Joels et al., 2006) it was hypothesized that pre-learning stress facilitates later memory retrieval. Moreover it was tested if effects of pre-learning stress on memory retrieval are modulated by the affective valence of the to-be-remembered stimulus material. This was expected since previous work showed that stress-effects on memory are influenced by the affective content of the tested

stimulus material (Cahill, Gorski, & Le, 2003; Roozendaal, Okuda, de Quervain, & McGaugh, 2006).

2. THE BRAIN AS A COORDINATOR OF THE STRESS RESPONSE

2.1. Study I: Intranasal insulin attenuates the hypothalamic-pituitary-adrenal axis response to psychosocial stress

Andreas Böhringer, Lars Schwabe, Steffen Richter, Hartmut Schächinger

2.1.1. Abstract

Previous studies have shown that intranasally administered insulin exerts an inhibitory influence on the basal HPA axis activity. To date, however, it remains unclear as to whether intranasal insulin does furthermore affect HPA axis responsiveness in situations of stress. Here, we tested whether intranasally administered insulin attenuates the HPA axis response to psychosocial stress.

Fifty minutes before being exposed to the TSST, 26 healthy young male participants received a single intranasal dose of human insulin (40 I.U.) or placebo in a placebo controlled, double-blind between-subject design. Plasma cortisol, saliva cortisol, heart rate, and blood pressure were measured at resting baseline and in response to the TSST.

Plasma cortisol ($p<.001$) and saliva cortisol ($p=.002$) increased in response to stress, as did heart rate ($p<.001$) and blood pressure ($p<.001$). Intranasal insulin did not influence plasma or saliva cortisol, heart rate, blood pressure, blood glucose, and plasma insulin levels at baseline. However, intranasal insulin diminished the saliva cortisol (two-way ANOVA; treatment by time interaction: $p=.05$) and plasma cortisol (two-way ANOVA; treatment by time interaction: $p=.05$) response to the TSST without affecting heart rate, and blood pressure stress reactivity.

Our data show that a single intranasal insulin administration effectively lowers stress-induced HPA axis responsiveness. Intranasal insulin may offer a therapeutic potential to prevent hyperactivity of the HPA system.

2.1.2. Introduction

Activation of the HPA axis is crucial for successful regulation of energy homeostasis during situations of stress (Sapolsky, Romero, & Munck, 2000). However, hyperactivity of the HPA system is associated with several wide spread diseases like depression, arterial hypertension, visceral obesity, and the metabolic syndrome (Bjorntorp, 2001; Chrousos, 2000; Parker, Schatzberg, & Lyons, 2003; Wirtz *et al.*, 2006), where it contributes to the manifestation of these pathological states. To date our knowledge about the inhibitory control over the HPA axis activity is sparse and identification of factors that inhibit HPA axis activity may help to develop new therapeutic approaches against diseases characterized by HPA axis hyperactivity.

The pancreatic peptide hormone insulin plays a significant role in HPA axis regulation (Chan *et al.*, 2005; Fruehwald-Schultes *et al.*, 1999; Fruehwald-Schultes, Kern, Born, Fehm, & Peters, 2001). Circulating insulin reaches the CNS via a saturable active transport mechanism across the blood-brain-barrier and binds to brain specific insulin receptors that are found with high density in hypothalamic nuclei and limbic structures (Plum, Schubert, & Bruning, 2005; Unger, Livingston, & Moss, 1991). These brain structures are known to be involved in the regulation of HPA axis activity (Herman, Ostrander, Mueller, & Figueiredo, 2005) and animal data indicate that insulin effects on the HPA axis are indeed mediated by actions on central nervous sites (Davis et al., 1995). In humans, intranasal insulin administration is an easy applicable tool for analyzing central nervous insulin effects (Fehm, Perras, Smolnik, Kern, & Born, 2000; Hallschmid *et al.*, 2004). Intranasally administered insulin reaches the CSF without being absorbed into the blood stream (Born et al., 2002). Thus, this application method allows investigating central nervous insulin effects without confounding influences of peripheral insulin actions that are seen with systemic insulin infusions. Recently, it was shown that long-term treatment (eight weeks) with intranasally administered insulin reduces the morning HPA axis activity in lean (Benedict et al., 2004) and obese (Hallschmid, Benedict, Schultes, Born, & Kern, 2008) individuals and could thus offer a therapeutic way to treat

hyperactivity of the HPA axis. Nevertheless, it remains unclear as to whether intranasally administered insulin may affect the HPA axis response to mental stress. This, however, would be of particular interest since human research revealed that HPA axis activation is closely linked to psychosocial challenge (Dickerson & Kemeny, 2004; Schwabe, Haddad, & Schachinger, 2008).

The present study examined the role of intranasally administered insulin on the HPA axis response to psychosocial stress. The Trier Social Stress Test (TSST) (Kirschbaum, Pirke, & Hellhammer, 1993) was used as a psychosocially relevant stressor. This procedure is very effective in activating the HPA axis and has a straight forward relation to every day stress experiences (Dickerson & Kemeny, 2004). Changes in total plasma cortisol, saliva cortisol, heart rate, and blood pressure were measured as indices of HPA axis and cardiovascular responses to the stress challenge, respectively. Based on previous reports about inhibitory influences of intranasal insulin administration on the basal HPA axis activity (Benedict et al., 2004; Hallschmid et al., 2008) we hypothesized that intranasal insulin administration before TSST onset would attenuate the cortisol secretion in response to the stress challenge, as compared to placebo administration.

2.1.3. Methods

Participants

Twenty-six young, healthy male university students between 20 and 31 years of age participated in this study. Exclusion criteria were as follows: any acute or chronic disease, smoking of cigarettes, familiarity with the TSST, a presence or history of mental illness, use of systemic medication, current participation in another clinical study, fasting glucose above 5.5 mmol/l, BMI below 18 or above 25, the presence of a depressive disorder (screened with the German version of the Patient Health Questionnaire (PHQ-D, (Loewe, Spitzer, Zipfel, & Herzog, 2002)). Participants were required to fast for 6h before arrival in our laboratory. All participants gave

voluntary written informed consent and were compensated for their participation. The study was conducted in accordance with the declaration of Helsinki and was approved by the Ethical Committee of the State's Medical Association (Landesärztekammer Rheinland-Pfalz).

One participant of the insulin group was excluded from further analyses because he showed baseline cortisol values that were 2 standard deviations above the average baseline cortisol values. Furthermore, one participant of the placebo group was excluded because he did not meet exclusion criteria as turned out during the experiment.

Procedure

All participants arrived between 1330 h and 1530 h in our laboratory and were screened for exclusion criteria by the responsible physician. Participants were then randomly assigned to the insulin group (n = 12) or the placebo group (n = 12). Afterwards, a catheter (Vasofix, B.Braun, Melsungen, Germany) was inserted in an antecubital vein 105 minutes before start of the Trier Social Stress Test (TSST) to allow blood sampling at several time points across the experiment. ECG electrodes were attached according to a standard lead II configuration. The ECG was used for automated detection of heart rate. Fifty-five minutes before the stress challenge the first blood and the first saliva sample were obtained. Directly thereafter (fifty minutes before TSST onset), either 0.4 ml (containing 40 I.U) insulin (Actrapid® 100, Novo Nordisk) or a corresponding volume of placebo (dilution buffer without insulin; kindly provided by Dr. Manfred Hallschmid, University of Lübeck, Germany) were administered intranasally to the participants. The timing of intranasal insulin administration and the amount of 40 I.U applied were chosen according to foregoing studies investigating acute effects of intranasal insulin on endocrine and cognitive parameters (Born et al., 2002; Hallschmid et al., 2008). Next, all participants drank 0.3 l of water in order to standardize the intake of liquid. Ten minutes prior to the TSST heart rate monitoring was started. In order to avoid

influences of orthostatic reactions on heart rate changes during the TSST all participants were asked to change to a standing position before. Three minutes prior to the TSST the second blood-sample and the second saliva sample were collected and blood pressure was measured. The TSST was started for all participants between 1600 h and 1800 h. Immediately after it's termination the third blood and saliva samples were obtained and blood pressure was measured again. Furthermore, participants evaluated on two 10-point rating-scales how stressful and insecure they felt during the TSST. Additional blood and saliva samples were collected 10, 20, 30, 45, 60, and 90 minutes after termination of the stress test. Heart rate sampling was stopped 10 minutes after the end of the TSST. During the stay in our laboratory participants were not allowed to eat or drink anything. During the waiting periods between blood sampling they were obliged to restrict themselves to calm and non-arousing activities, such as reading newspapers.

The Trier Social Stress Test (TSST)

A detailed protocol of the TSST was described elsewhere (Kirschbaum et al., 1993). Briefly, the TSST is a standardized laboratory stressor consisting of a free speech and a mental arithmetic task in front of an audience and a video camera. Participants were introduced to the task and instructed to prepare a presentation in which they had to promote their candidacy for a job. After a 3-minute preparation period, they were asked to give a 5-minute free speech. Thereafter, participants were introduced to the mental arithmetic task, also standing in front of the audience. Subjects were required to count backwards from 2023 in steps of 17 as fast and accurate as possible for 5 minutes; upon a mistake they had to stop and start again at 2023.

Biochemical Analyses

Blood and saliva sampling. In order to determine the plasma cortisol, plasma insulin, and plasma glucose concentrations venous blood samples were collected in

EDTA coated tubes (Monovette, Sarstedt, Germany). Samples were stored on ice for a maximum of 10 minutes and than centrifuged for 10 minutes at 6 ˚C 1200g. Plasma was stored at -20 ˚C until analyses of cortisol and insulin concentrations. Plasma glucose concentrations were determined prior to freezing. Saliva samples were collected in Eppendorf tubes (Eppendorf, Hamburg, Germany), stored at room temperature until completion of the session and than kept at -20 ˚C until analyses. After thawing for biochemical analyses, saliva samples were centrifuged at 2000g for 10 minutes.

Cortisol. Total plasma cortisol was determined at all time-points of measurement with a commercial competitive enzyme amplified sensitivity immunoassay (ELISA, Immuno Biological Laboratories, IBL, Hamburg, Germany). Lower detection threshold of this assay was 6.9 ng/ml. The intraassay variation ranged between 4.0% and 4.7%, the interassay variation between 5.0% and 9.6%. Saliva cortisol levels were determined employing a competitive solid phase time-resolved fluorescence immunoassay with fluoromeric end point detection (DELFIA). This method was described in detail elsewhere (Dressendorfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992). The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% -9.0%.

Glucose and Insulin. Blood samples drawn 55 and 3 minutes prior to the stress challenge were used to analyze plasma insulin and glucose concentrations. Plasma glucose was determined by the hexokinase method (Olympus Analyzer, Olympus Life and Material Science Europe GmbH, Hamburg, Germany). Plasma insulin concentrations were determined by an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). The lower detection threshold of this assay was 1.39 pmol/l. Interassay reproducibility ranged between 2.68% and 3.08%. The intraassay precision was 0.93%.

Heart rates

Heart rate was derived from a single standard lead II ECG configuration employing telemetric HP 78100A transmitter and HP 78101A receiver system (Hewlett Packard Corp.). ECG was sampled by 1 kHz with 12bit resolution. Beat detection was performed offline by WinCPRS (Absolute Aliens Oy, Turku, Finland) as was artifact control.

Heart rate measurements were taken continuously 10 minutes before, during, and 10 minutes after the TSST. The mean pre- and post-TSST heart rates as well as the mean task (preparation for speech, speech, arithmetic task) specific heart rates during the TSST were calculated for each participant.

Blood pressure

Blood pressure was measured 3 minutes before and 1 minute after the TSST with a Criticon Dinamap device (SX1846 Dinamap Criticon, Tampa, FL).

Psychological assessment

All participants rated on two rating scales ranging from 0 (not at all) to 10 (very much) how stressed and insecure they felt during the TSST.

Statistical analyses

Data are presented as mean ± SEM. Kolmogorov-Smirnov tests revealed normal distribution for all variables. Effects of the pharmacological intervention as well as the stress challenge on endocrine, metabolic, and cardiovascular parameters were analyzed by two-way mixed design analyses of variance (ANOVAs) with the within-subject factor ‘time’ (timepoint of measurement) and the between-subject factor ‘treatment’ (insulin vs. placebo). Additionally, we calculated the area under the time response curve with respect to increase (AUC; (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003)) according to the trapezoid rule for each

participant and used one-way ANOVA to reveal influences of the pharmacological manipulation on the stress provoked cortisol secretion. The AUC was referenced to the cortisol concentration measured 55 minutes before TSST onset. Psychological variables were analyzed by one-way ANOVAs with the between-subject factor 'treatment' (insulin vs. placebo). Huynh-Feldt corrections were used for all analyses including repeated measures factors and only corrected results are shown. A p-value ≤ 0.05 two-sided was considered significant.

Missing-data. Due to technical error post-TSST blood pressure data of one participant in the placebo group was lost.

2.1.4. Results

Demographic variables

Both groups were comparable in age (insulin: 24.2 ± 0.9 yr; placebo: 25.3 ± 1.1 yr; $F_{1,22} = .60$; $p = .45$), weight (insulin-group: 72.8 ± 2.3 kg: placebo-group: 76.2 ± 2.6 kg; $F_{1,22} = .94$; $p = .34$), and body mass index (insulin-group: 21.7 ± 0.6 kg/m^2; placebo-group: 22.5 ± 0.5 kg/m^2; $F_{1,22} = 1.3$; $p = .28$).

Pre-stress endocrine and metabolic measurements

The insulin and placebo groups did not differ in their plasma cortisol (TSST – 55 min: $F_{1,22} = .36$; $p = .56$; TSST – 3 min: $F_{1,22} = .38$; $p = .55$) and saliva cortisol (TSST – 55 min: $F_{1,22} = 1.53$; $p = .23$; TSST – 3 min: $F_{1,22} = .84$; $p = .37$) concentrations before onset of the TSST (Figures 2.1.1 and 2.1.2, Table 2.1.1). Furthermore, groups showed comparable baseline glucose and insulin values (all ps > .57; see Table 2.1.1). Whereas insulin ($F_{1,22} = .55$; $p = .46$) and glucose ($F_{1,22} = .29$; $p = .60$) values remained unchanged over time in both groups, plasma cortisol ($F_{1,22} = 16.98$; $p < .001$; $\eta^2 = .43$) and saliva cortisol ($F_{1,22} = 11.70$; $p = .002$; $\eta^2 = .34$) concentrations declined within the pre-stress interval. This change in cortisol was comparable under both treatment conditions (*treatment* by *time* interaction; plasma cortisol: $F_{1,22} < .01$; $p = .97$; saliva cortisol $F_{1,22} =$

.14; p = .72) indicating that the observed decline, most likely due to diurnal cortisol rythmicity, was not affected by the insulin application. Thus, intranasal insulin administration did neither alter the circulating amount of insulin nor did it influence pre-stress cortisol, or blood glucose values.

	Time	Insulin-group	Placebo-group
Plasma Cortisol (ng/ml)	TSST – 55 min	63.03 ± 4.34	58.95 ± 5.29
	TSST – 3 min	52.51 ± 3.93	48.65 ± 4.92
Saliva Cortisol (nmol/l)	TSST – 55 min	3.57 ± .50	2.82 ± .35
	TSST – 3 min	2.61 ± .53	2.05 ± .31
Glucose (mmol/l)	TSST – 55 min	4.92 ± .10	4.99 ± .08
	TSST – 3 min	4.90 ± .08	4.89 ± .10
Insulin (pmol/l)	TSST – 55 min	26.50 ± 3.67	29.65 ± 3.81
	TSST – 3 min	27.85 ± 4.61	31.85 ± 5.39

Table 2.1.1. Pre-stress saliva cortisol, plasma cortisol, plasma glucose and plasma insulin concentrations. Values are presented as mean ± SEM.

Endocrine, cardiovascular, and subjective responses to stress

Cortisol. Both groups showed a strong increase in plasma cortisol ($F_{2.1,47.6}$ = 75.18; p < .001; η^2 = .77) and in saliva cortisol ($F_{2.3,49.8}$ = 24,16; p >.001; η^2 = .52) in response to the stress challenge, indicating that the TSST reliably activated the HPA axis (Figure 2.1.1 and Figure 2.1.2). Most relevant to the specific goals of the present study, significant *treatment* by *time* interactions revealed that the insulin and the placebo groups differed regarding their cortisol response to the stress test (plasma cortisol: $F_{2.2,47.6}$ = 3.12; p = .05; η^2 = .13; saliva cortisol: $F_{2.2,49.8}$ = 2.90; p = .05; η^2 = .12). One-way ANOVAs with the dependent variables plasma cortisol AUC and saliva cortisol AUC showed that both the plasma cortisol ($F_{1,22}$ = 4.67; p = .04; η^2 = .18) and saliva cortisol ($F_{1,22}$ = 4.17; p = .05; η^2 = .16) response to the TSST were significantly lower in the insulin group as compared to the placebo group. Insulin administration prior to the TSST reduced the mean plasma cortisol AUC by 49 percent (Figure 2.1.1) and the mean saliva cortisol AUC by 68 percent (Figure 2.1.2).

Heart rate and blood pressure. Changes in cardiovascular parameters in response to the TSST are summarized in table 2.1.2. Both groups had comparable cardiovascular values prior to the stress test (heart rate: $F_{1,22} = 2.36$; $p = .14$; systolic blood pressure: $F_{1,22} = .41$; $p = .53$;. diastolic blood pressure: $F_{1,22} = .47$; $p = .46$). Moreover, the TSST elicited a significant increase in heart rate and systolic as well as diastolic blood pressure in both treatment groups (all ps < .001; all $\eta^2 > .59$) indicating a cardiovascular stress reaction. However, in contrast to the observed effects on the HPA axis activity two way ANOVAs revealed that the cardiovascular stress reaction was not influenced by intranasal insulin administration (*treatment* by *time* interactions and main effects *treatment* for heart rate and blood pressure data: all ps > .24).

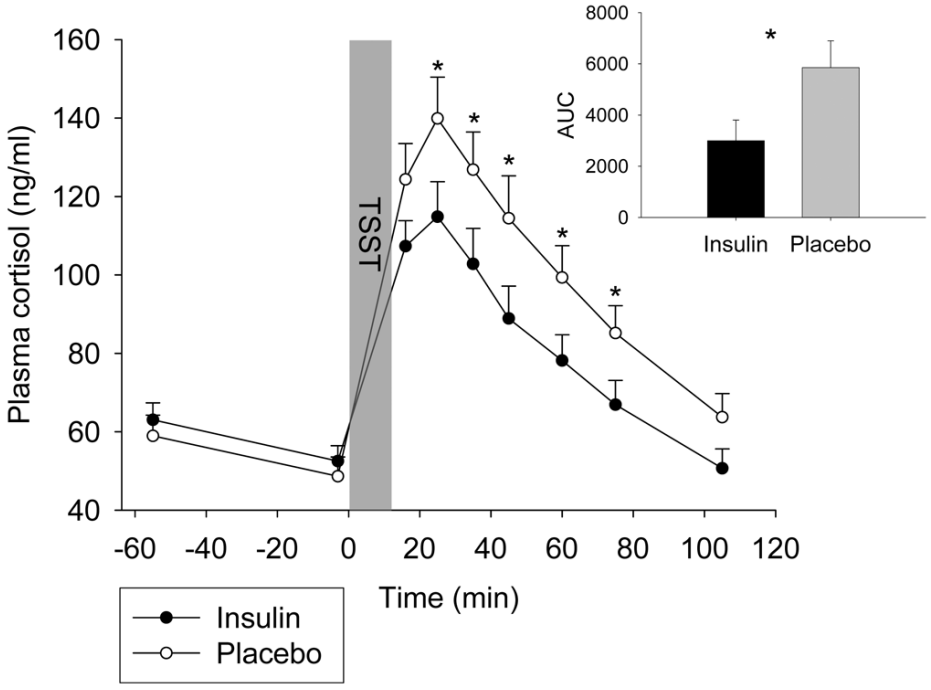

Figure 2.1.1. Mean (±SEM) plasma cortisol concentrations before and after the TSST. *Insert:* Mean (±SEM) plasma cortisol AUC calculated in reference to the plasma cortisol value measured 55 minutes before TSST onset. The plasma cortisol AUC in the insulin-group (*black bar*) reached only 51% of the plasma cortisol AUC in the placebo-group (*grey bar*); comparisons at single time-points were done by means of one-way ANOVA; * - p≤.05

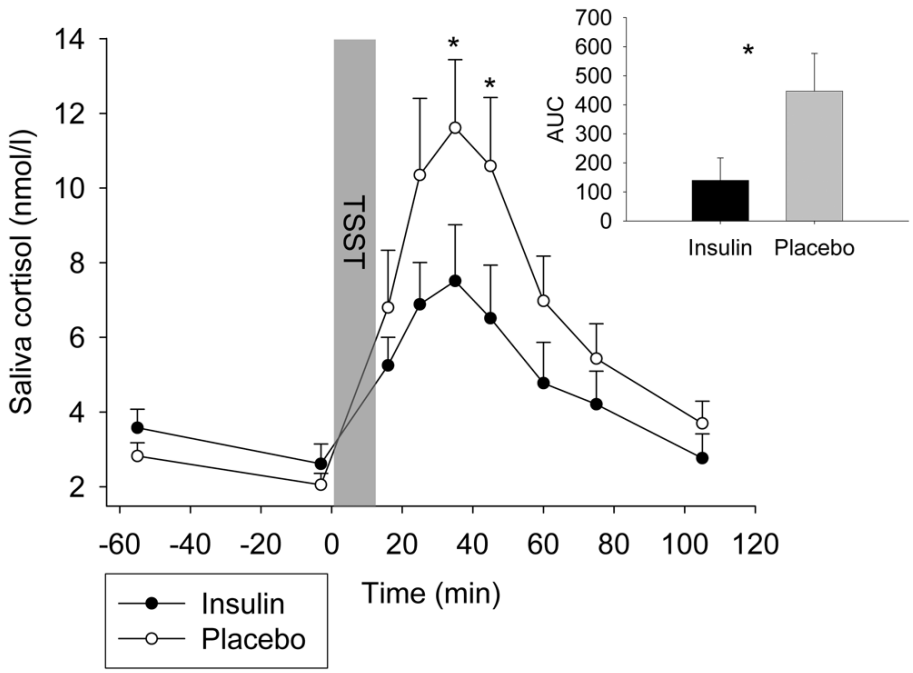

Figure 2.1.2. Mean (±SEM) saliva cortisol concentrations before and after the TSST. *Insert:* Mean (±SEM) saliva cortisol AUC calculated in reference to the saliva cortisol value measured 55 minutes before TSST onset. The saliva cortisol AUC in the insulin-group (*black bar*) reached only 32% of the saliva cortisol AUC in the placebo-group (*grey bar*); comparisons at single time-points were done by means of one-way ANOVA; * - p≤.05

Psychological measures. All participants were asked to report on two 10-point rating scales ranging from 0 (not at all) to 10 (very much) how stressed and insecure they felt during the TSST. The two groups did not differ regarding their ratings of stressfulness (insulin-group: 7.33 ± .60; placebo-group: 6.92 ± .54; $F_{1,22} = 0.26$; $p < .61$) and insecurity (insulin-group: 6.83 ± .60; placebo-group: 5.50 ± .53; $F_{1,22}=2.77$; $p = .11$). Since ratings of insecurity tended to be higher in the insulin-group as compared to the placebo-group we analyzed if differences in perceived insecurity mediated group differences in the stress provoked cortisol secretion. Controlling for insecurity by means of ANCOVAs with cortisol concentration and AUC as dependent variable and insecurity as covariate did not change the results.

	Time	Insulin-group	Placebo-group
Heart rate (min^{-1})	Pre-TSST	71.8 ± 2.0	66,6 ± 2.8
	Preparation	83.1 ± 3.8	83.9 ± 5.5
	Speech	88.6 ± 5.4	92.7 ± 5.2
	Arithmetic	89.4 ± 3.8	89.2 ± 4.2
	Post-TSST	78.5 ± 3.4	74.1 ± 2.9
BP sys (mmHg)	TSST – 1 min	121.3 ± 3.1	124.3 ± 3.4
	TSST + 1 min	140.0 ± 3.9	146.2 ± 4.3
BP dia (mmHg)	TSST – 1 min	72.3 ± 2.5	75.1 ± 2.5
	TSST + 1 min	83.9 ± 2.5	86.2 ± 2.3

Table 2.1.2. Cardiovascular responses to the TSST. Values are presented as mean ± SEM. BP sys = systolic blood pressure; BP dia = diastolic blood pressure.

2.1.5. Discussion

The present study provides the first evidence that intranasally administered insulin attenuates the HPA axis response to psychosocial stress in healthy young men. It is known that intranasal insulin reaches the CSF without entering the blood stream (Born et al., 2002) and circulating insulin levels were comparable among the insulin and the placebo group before onset of the stress challenge in the current study. Thus, although peripheral insulin actions cannot be ruled out because we measured plasma insulin at two time points only, we suggest that the blunted HPA axis response to the TSST found in the insulin-group is most likely due to insulin effects on central nervous sites.

In line with our hypothesis we found an attenuating effect of intranasally administered insulin on the HPA axis response to psychosocial stress. This result extends previous reports about inhibitory influences of intranasally administered insulin on the basal HPA axis activity (Benedict et al., 2004; Hallschmid et al., 2008) to the domain of stress related HPA axis activity. Previous studies that investigated effects of insulin on the stress-induced HPA axis activity focused on physiological stressors solely. Most of them examined the effects of hypoglycemia stress at

different levels of systemic hyperinsulinemia. These studies provided rather inconsistent results. Some authors reported enhancing (Davis, Mellman, & Shamoon, 1993; Lingenfelser *et al.*, 1996) others attenuating (Kerr, Reza, Smith, & Leatherdale, 1991) or no effects (Diamond *et al.*, 1991; Fisher, Bruning, Lannon, & Kahn, 2005) of insulin on the HPA axis response to hypoglycemia. This discrepancy might be due to differences in sample characteristics (e.g. testing healthy subjects or participants with insulin dependent diabetes). One study investigated effects of brain insulin signaling on the HPA axis response to hypoglycemia stress (Davis et al., 1995). The authors found that a selective increase in the level of insulin in the blood perfusing the brain enhances the cortisol response to hypoglycemic stress in dogs, as compared to peripheral insulin infusion. This suggests a stimulatory effect of insulin on the HPA axis response to hypoglycemia at the CNS level. In the present study, however, we obtained an attenuated HPA axis response to psychosocial stress following administration of intranasal (i.e. centrally acting) insulin. This discrepancy in CNS insulin effects on the HPA axis might be owing to the different species studied or differences in the way of insulin delivery. Furthermore, the obviously diverging insulin effects may be explained by the different stressors used, i.e. physiological/metabolic vs. mental stressors. Importantly, it was suggested that HPA axis reactions to simple systemic stressors like hypoglycemia rely crucially on brainstem and direct systemic projections to the hypothalamus (Pacak & Palkovits, 2001). In contrast, stressors requiring interpretative processing like psychological stress tests involve an activation of limbic and higher-order brain structures (Herman & Cullinan, 1997; Herman *et al.*, 2005). Thus, specific insulin actions on brainstem, limbic, and higher-order brain structures may account for diverging insulin effects on the HPA axis response to physiological stressors like hypoglycemia and psychological stressors like the TSST.

Limbic structures, particularly the hippocampus and the amygdala, have been shown to play an important modulatory role in HPA axis regulation with the hippocampus having inhibitory and the amygdala having excitatory influences

(Herman et al., 2005). Interestingly, both structures express insulin receptors (IRs) at a high density (Unger et al., 1991). It is tempting to speculate that insulin exerts its modulating effect on the HPA axis via its influence on hippocampal or amygdaloid neurons. Furthermore, it is well known that insulin has profound effects on hypothalamic nuclei involved in the regulation of energy homeostasis (Benoit, Clegg, Seeley, & Woods, 2004; Niswender, Baskin, & Schwartz, 2004; Plum *et al.*, 2005). In particular, neurons within the arcuate nucleus of the hypothalamus are affected by insulin (Benoit *et al.*, 2002; Schwartz *et al.*, 1992). Since it was shown that the ARC is crucially involved in normal regulation of HPA axis activity (Bell, Bhatnagar, Akana, Choi, & Dallman, 2000) modulation of arcuate neurons could be another route of insulin action on the HPA axis.

Intranasal insulin administration did not alter the basal HPA axis activity in the present study, and the plasma and saliva cortisol concentrations declined comparably in the insulin and the placebo group over the pre-stress interval. While this finding is in line with a previous study that revealed attenuating effects of chronic but not of acute intranasal insulin treatment on the HPA axis activity (Benedict et al., 2004) another study suggests that intranasal insulin administration may acutely decrease the circulating amount of cortisol (Hallschmid et al., 2008). In contrast to this attenuating effect of intranasally administered insulin on the basal HPA axis activity, other studies showed that high levels of systemic hyperinsulinemia during euglycemic glucose clamps could directly activate the HPA axis secretory activity in rats (Chan et al., 2005) and humans (Fruehwald-Schultes et al., 1999; Fruehwald-Schultes et al., 2001). The discrepancy between studies involving intranasal insulin administration and hyperinsulinemic glucose clamp techniques may be explained by diverging insulin effects at central and peripheral levels of the HPA axis. Importantly, results from an in-vitro study suggest that systemic hyperinsulinemia may affect the steroid hormone synthesis in adrenal cells that are not reached by intranasal insulin (Penhoat, Chatelain, Jaillard, & Saez, 1988).

Here, we did not find effects of intranasal insulin on the cardiovascular stress reaction. Such dissociations between the HPA axis stress reactivity and markers of autonomic arousal have previously been reported (Fries, Hellhammer, & Hellhammer, 2006; Kirschbaum *et al.*, 1997; Kirschbaum *et al.*, 1993; Schommer, Hellhammer, & Kirschbaum, 2003). Intranasally administered insulin appears to be another modulator of stress reactivity that influences specifically the endocrine stress reaction without having effects on corresponding autonomic markers and cardiovascular parameters.

Some limitations of the present study have to be discussed. First, we focused on male participants only since it is known that estradiol and progestins modify the endocrine stress reaction to psychosocial stress (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Further studies will have to corroborate our findings in women. Second, our study was based upon a rather small sample size. Future studies involving a bigger sample size may offer the opportunity to investigate influences of intranasal insulin on the endocrine stress reaction and subjective responses to psychosocial stress in more detail.

In conclusion, the present study demonstrates that intranasal insulin attenuates the HPA axis response to psychosocial stress in healthy male subjects. This finding points to a modulatory role of brain insulin signaling in the regulation of HPA axis activity. Furthermore, our data suggest that the intranasal route of insulin delivery may offer a new therapeutic approach to prevent increased excitability of the HPA system.

2.1.6. References

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3. THE BRAIN AS A TARGET OF STRESS

3.1. Study II: A combination of high stress-induced tense and energetic arousal compensates for impairing effects of stress on memory retrieval in men

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3.1.1. Abstract

Stress can both impair and enhance memory retrieval. GCs mediate impairing effects of stress on memory retrieval. Little is known, however, about factors that facilitate post-stress memory performance. Here, we asked whether stress-induced arousal mediates facilitative stress effects on memory retrieval. Two arousal dimensions were separated: Tense arousal, which is characterized by feelings ranging from tension and anxiety to calmness and quietness, and energetic arousal, which is associated with feelings ranging from energy and vigour to states of fatigue and tiredness. Fifty-one men (mean ± SEM: 24.57 ± .61 years) learned emotional and neutral words. Memory for these words was tested 165 min later, after participants were exposed to a psychosocial stress or a non-arousing control condition. Changes in heart rate, self-reported (energetic and tense) arousal, and saliva cortisol in response to the stress/control condition were measured. Overall, stress impaired memory retrieval. However, stressed participants with high increases in both tense and energetic arousal performed comparable to controls. Neither cortisol nor autonomic arousal predicted memory performance after controlling for changes in energetic and tense arousal. The present data indicate that stress-induced concurrent changes in tense and energetic arousal can compensate for impairing effects of stress on memory retrieval. This finding could help to explain some of the discrepancy in the literature on stress and memory.

3.1.2. Introduction

Stress has multiple effects on physiology and cognition. Typically, acute stress leads to the secretion of GCs from the adrenal cortex, an increase in cardiovascular activity, and an increase in subjective feelings of arousal. These responses facilitate adaptation and prepare the individual to cope successfully with stressful situations. With regard to cognition, it is well established that stress affects memory retrieval (Joels, Pu, Wiegert, Oitzl, & Krugers, 2006; Roozendaal, McEwen, & Chattarji, 2009). However, the direction of this effect is still a matter of debate. Stress can both facilitate (Buchanan & Tranel, 2008; Domes, Heinrichs, Reichwald, & Hautzinger, 2002; Nater *et al.*, 2007; Schwabe *et al.*, 2009) or impair (Buchanan, Tranel, & Adolphs, 2006; de Quervain, Roozendaal, & McGaugh, 1998; Kuhlmann, Piel, & Wolf, 2005; Lupien *et al.*, 1997) retrieval performance. The neurobiological mechanisms mediating these effects are only partly understood. A recent neurobiological model suggests that GCs (with cortisol being the most important GC in humans) and concurrent autonomic arousal interactively mediate *impairing* effects of stress on memory retrieval (Roozendaal, 2002; Roozendaal, Okuda, de Quervain, & McGaugh, 2006). To date, however, it remains unclear which factors account for *enhancing* effects of stress on retrieval performance. A better knowledge of such factors might improve our understanding of stress effects on memory and could thus proof beneficial for the development of strategies counteracting possible detrimental effects of stress on memory performance.

Stress is typically associated with an increase in emotional arousal (Schlotz *et al.*, 2008). Arousal is a state of heightened alertness and responsiveness to sensory inputs which is accompanied by changes in subjective mood and an increase in physiological activity (Adamantidis & de Lecea, 2008; Thayer, 1989). Besides physiological measures such as heart rate and blood pressure, self-report has been established as a measure of arousal (Thayer, 1989). Studies using subjective measures identified two separate dimensions describing a current arousal state: energetic

arousal and tense arousal (Thayer, 1989). Tense arousal is identifiable through feelings that range from tension and anxiety to states of calmness and quietness. Energetic arousal, on the other hand, is characterized by feelings ranging from energy and vigour to states of fatigue and tiredness. Interestingly, energetic and tense arousal are not only distinct with respect to subjective experience but are also associated with different patterns of brain activity (Thayer, 1989).

It has long been known that moderate levels of arousal can enhance performance in various cognitive tasks (Revelle & Loftus, 1992; Yerkes & Dodson, 1908). More recent research suggests that this is particularly true for an increase in energetic arousal. One study showed that energetic arousal facilitates whereas tense arousal is not associated with or even reduces performance in controlled visual and memory search tasks (Matthews & Westerman, 1994). This finding suggests that stress-induced energetic arousal may mediate facilitative effects of stress on memory retrieval. To date, however, it is unknown whether memory retrieval can be facilitated by high energetic arousal. In fact, most studies on stress and memory (retrieval) did not pay attention to possible effects of subjective arousal.

Here, we hypothesized that a stress-induced increase in arousal facilitates post-stress memory retrieval. Healthy participants learned a list of emotional and neutral words. Emotionality of the words was varied since previous work revealed that stress effects on memory retrieval are crucially influenced by the affective characteristics of the to-be-remembered word material (Kuhlmann *et al.*, 2005). Prior to retention testing for these words, participants were exposed to a psychosocial laboratory stress test or a non-arousing control condition. Changes in subjective and autonomic arousal as well as saliva cortisol were measured before and after the stress/control condition. We expected an enhancing influence of energetic arousal and no or an inhibitory influence of tense arousal on memory retrieval. In addition, we investigated how arousal effects relate to effects of the stress-induced cortisol secretion on memory retrieval and how these effects are influenced by the emotionality of the to-be-remembered stimulus material.

3.1.3. Methods

Participants

Fifty-one young, healthy male university students between 18 and 31 years of age participated in the present study (mean ± SEM: 24.57 ± .61 years). Since estradiol and progestins are known to change the endocrine response to psychosocial stress tests like the TSST (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999), only male participants were included. Participants were excluded from the study if they met any of the following criteria: any acute or chronic disease, smoking of more than five cigarettes per day, familiarity with the TSST, a presence or history of mental illness, use of systemic medication, current participation in another clinical study, BMI below 18 or above 28, the presence of a depressive disorder. These criteria were assessed by a physical examination (including amongst others a screening for cardiovascular or chronic respiratory diseases) and a standardized screening for psychiatric diseases. Presence of a depressive disorder was screened with the German version of the Patient Health Questionnaire (PHQ-D; (Loewe, Spitzer, Zipfel, & Herzog, 2002). Participants were asked to refrain from eating meals, drinking coffee or alcohol and severe physical exercise in the 2 h before the experiment. All participants gave voluntary written informed consent and were compensated for their participation. The study protocol was approved by the Ethical Committee of the State's Medical Association (Landesärztekammer Rheinland-Pfalz).

Procedure

Participants arrived between 1330 h and 1530 h in our laboratory. If all requirements were met, a word list containing 10 negative, 10 positive, and 10 neutral words was presented on a piece of paper. Participants were instructed to read the list aloud twice and to rate every word regarding its imageability (difficult to imagine vs. easy to imagine) on a bipolar seven-point rating scale (data not shown). Subjects were not told that memory for these words would be tested later on. Afterwards, the first saliva sample was taken and the participants were randomly

assigned to the stress- (n = 33) or the control-condition (n = 18). We used a between-subject design to avoid effects of test-repetition and to prevent overt rehearsal strategies that might have influenced delayed memory testing.

More participants were assigned to the stress group because it was planned to split this group into subjects with high vs. low stress-induced increases in emotional arousal later on (see statistical analyses section). ECG electrodes were attached according to a standard lead II configuration. The ECG was used for automated detection of heart rate within the course of the experimental session. In order to standardize activity patterns following word learning all participants answered questionnaires assessing health and subjective well-being for a duration of 45 minutes after presentation of the word list. Thereafter, they were obliged to restrict themselves to calm and non-arousing activities (e.g. reading newspapers), apart from the researchers. The second saliva sample was collected 60 minutes before the stress or control procedure respectively. Ten minutes prior to the stress/control procedure heart rate monitoring was started and participants answered a questionnaire assessing momentary mood (MDBF Version A; (Steyer, Schwenkmezger, Notz, & Eid, 1994)). In order to avoid influences of orthostatic reactions all participants were asked to change to a standing position before. Three minutes before stressor (control procedure) onset a third saliva sample was collected. The stress or control procedure was started for all participants between 1600 h and 1800 h (135 minutes after word learning). This time-interval between word learning and the stress/control task was chosen since previous work showed that GCs affect memory consolidation when administered immediately after stimulus encoding but not when administered several hours later (McGaugh, 1989). The time-interval of 135 minutes used here thus allows for studying isolated effects of stress on memory retrieval. Immediately after the stress/control procedure a fourth saliva sample was collected. Furthermore, participants evaluated on three 10-point rating-scales how stressful, anxious, and insecure they felt during the task. Heart rate sampling was stopped 10 minutes after the stress/control procedure. At this time point all participants answered again a

questionnaire assessing momentary mood (MDBF Version B; (Steyer *et al.*, 1994)). Immediately thereafter (165 minutes after word learning), participants completed a paper-pencil based free recall test for the words presented at the beginning of the experimental session. Additional saliva samples were collected 10, 20, 30, 45, and 60 minutes after cessation of the stress/control procedure. During the stay in our laboratory participants were not allowed to smoke, to eat or drink anything except water. At the end of the experimental session all participants were asked to indicate if they expected a memory test for the words presented at the beginning of the experiment. No participant expected a test of memory retrieval for these words.

Stress and control condition

Participants in the stress condition completed the TSST. A detailed protocol of the TSST was described elsewhere (Kirschbaum, Pirke, & Hellhammer, 1993). Briefly, the TSST is a standardized laboratory stressor consisting of a free speech and a mental arithmetic task in front of an audience (a man and a woman) and a video camera. Participants were introduced to the task and instructed to prepare a presentation in which they had to promote their candidacy for a job. After a 3-minute preparation period, they were asked to give a 5-minute free speech. Thereafter, participants performed an arithmetic task for 5 minutes, also standing in front of the audience. Subjects were required to count backwards from 2023 in steps of 17 as fast and accurate as possible; upon a mistake they had to stop and start again at 2023.

Participants in the control condition firstly read aloud a non-arousing popular science newspaper article standing in an empty room. Afterwards, they were asked to do simple paper-pencil based arithmetic. Both tasks lasted 5 minutes. Participants in the control condition were informed that they would not be tape-recorded or videotaped.

Word list

Construction of the word list was based on a study by Schwibbe et al. (Schwibbe, Räder, Schwibbe, Borchard, & Geiken-Pophanken, 1994). The authors let university students evaluate 1698 German words regarding their emotional valence on a bipolar 7-point rating-scale ranging from -3 (negative) to +3 (positive). Ten positive (valence; M ± S.E.M.: 1.24 ± .06), 10 negative (-1.50 ± .06), and 10 neutral (.00±.01) two-syllable nouns from this data pool were selected for presentation on the word list. There were no differences in word frequency between the three valence categories (univariate ANOVA: $F_{2,27} = .003$; $p = .99$; word frequency norms were taken from a German internet data base).

Assessment of physiological and psychological stress responses

Saliva cortisol sampling and biochemical analyses

Saliva was passed from the mouth to Eppendorf tubes (Eppendorf, Hamburg, Germany) by commercial plastic tubes and was collected 135, 55, and 3 minutes before as well as 1, 10, 20, 45, and 60 minutes after the stress/control task. Samples were stored at -20 °C until analyses. Saliva cortisol was measured with a time-resolved fluorescence immunoassay. The intra-assay coefficient of variation was between 4.0% and 6.7%, and the corresponding inter-assay coefficients of variation were between 7.1% -9.0%. The lower detection limit of this method is .43 nM for a 50µl saliva sample (Dressendorfer, Kirschbaum, Rohde, Stahl, & Strasburger, 1992).

Assessment of autonomic arousal

Heart rate was derived from a single standard lead II ECG configuration employing telemetric HP 78100A transmitter and HP 78101A receiver system (Hewlett Packard Corp.). ECG was sampled by 1 kHz with 12bit resolution. Beat detection was performed offline by WinCPRS (Absolute Aliens Oy, Turku, Finland) as was artifact control.

Heart rate measurements were taken continuously 10 minutes before, during and 10 minutes after the stress or control task. The mean pre- and post task heart rates as well as the mean heart rates during the task were calculated for each participant.

Assessment of subjective arousal and further psychological variables

Mood was assessed ten minutes before and ten minutes after the stress or control procedure with two parallel versions of a German mood questionnaire (Mehrdimensionaler Befindlichkeitsfragebogen; MDBF; (Steyer *et al.*, 1994)). The MDBF measures momentary mood on three bipolar dimensions: (1) wakefulness – sleepiness; (2) calmness – restlessness; (3) pleasant – unpleasant mood. This three-dimensional conceptualization of mood has frequently been confirmed (Schimmack & Reisenzein, 2002; Thayer, 1989). Here, we follow the terminology suggested by Thayer who used the terms energetic arousal and tense arousal to describe the two mood dimensions associated with activity (Thayer, 1989). We employed the MDBF-Wakefulness vs. Sleepiness-Scale to measure energetic arousal and the MDBF-Calmness vs. Restlessness-Scale to measure tense arousal.

In addition, all participants rated on three scales ranging from 0 (not at all) to 10 (very much) how stressed, anxious and insecure they felt during task participation.

Data analyses

Kolmogorov-Smirnov tests revealed that all variables tested were normally distributed. We used methods based on the GLM in order to analyze effects of stress induced arousal on memory retrieval. The GLM approach allows for investigating interacting influences of categorical and continuous variables on a dependent variable in one single analysis. Here, we calculated a GLM with the within-subject factor *word valence* (positive, negative, neutral) and the between-subject factors *change in energetic arousal, change in tense arousal, change in heart rate*, and the *maximum increase in cortisol* to test for significant influences of these variables on memory

retrieval. The GLM included main effects for all mentioned variables. Moreover, the interaction term *change in energetic arousal* × *change in tense arousal* was entered into the model. The latter was done because past research suggests that energetic arousal and tense arousal may interactively predict cognitive performance (Matthews & Westerman, 1994). All variables were centered before entering into the GLM. Next, further illustrative analyses were conducted in order to disentangle significant interaction effects revealed by the GLM analysis. Specifically, a median split on *change in energetic arousal* and *change in tense arousal* within the stress group was used to create four new subgroups representing different combinations of changes in tense and energetic arousal. An ANOVA was used to compare memory performances between these four groups and the control-group. The change in energetic arousal, tense arousal, and feelings of pleasantness in response to the stress or control task was calculated by subtracting post-task measurements (MDBF-Version B) from pre-task measurements (MDBF-Version A). The change in heart rate was expressed (i) as the increase in heart rate from pre-TSST to the highest heart rate during the TSST and (ii) as the increase in heart rate from pre-TSST to post-TSST. The maximum increase in saliva cortisol was expressed as the individual increase in cortisol from the last measurement before the TSST or control task to the highest individual cortisol value after the respective task. Since both measures of heart rate were highly correlated they were included in separate analyses. Cortisol, heart rate data, and further subjective reactions to the stress or control task were analyzed by means of one-way and two-way mixed design analyses of variance (ANOVAs). Follow-up tests of ANOVA effects were done using the Tukey-HSD correction and only corrected p-values are shown. In case of ANOVAs with repeated measurements the Greenhouse-Geisser correction was employed where appropriate. Only corrected p-values and df are shown. Pearson product-moment correlations were calculated to assess associations among variables. A p-value ≤ .05 two-tailed was considered significant. Data are presented as mean ± S.E.M.

3.1.4. Results

Subjective and physiological stress responses

Psychological measures. Subjective responses to the stress and the control task are summarized in table 3.1.1. Ratings of wakefulness ($F_{1,49}$ = 1.62; p = .21), calmness ($F_{1,49}$ = 2.00; p = .16), and pleasant mood ($F_{1,49}$ = .15; p = .70) were comparable between the stress and the control group at baseline, i.e. 10 min before the stress/control procedure. Compared to the control group the stress group showed a stronger increase in restlessness, i.e. tense arousal, ($F_{1,49}$ = 52.36; p < .0001; η^2 = .52) and a stronger decrease in pleasant mood ($F_{1,49}$ = 10.22; p = .002; η^2 = .17). Wakefulness, i.e. energetic arousal, however, increased comparable in both groups ($F_{1,49}$ = .01; p = .94), probably due to the fact that both groups were exposed to a cognitive task. Moreover, participants in the stress group felt more stressed, insecure, and anxious during the TSST than participants in the control group during the control task (all p < .001). Next, we analyzed associations among measures of arousal and further subjective responses to the TSST. Results of these analyses are reported in table 3.1.2. While the change in tense arousal was positively correlated with perceived stress, anxiety, insecurity, and the decline in mood no such associations were found for the change in energetic arousal.

	Stress (n=33)	***Control (n=18)***
Wakefulness/Sleepiness (pre-task)	*13.9 ± .37*	*12.8 ± .61*
Wakefulness/Sleepiness (post-task)	*15.5 ± .44*	*14.4 ± .76*
Calmness/Restlessness (pre-task)	*16.4 ± .44*	*15.1 ± .70*
Calmness/Restlessness (post-task)	*10.3 ± .54*	*16.4 ± .37*
Pleasant mood (pre-stress)	*15.4 ± .50*	*15.7 ± .55*
Pleasant mood (post-stress)	*12.0 ± .56*	*15.4 ± .63*
Stress	*6.64 ± .36*	*2.00 ± .20*
Insecurity	*5.97 ± .42*	*1.67 ± .21*
Anxiety	*3.64 ± .40*	*1.67 ± .30*
HR (pre-task)	*70.4 ± 1.62*	*73.4 ± 2.45*
HR (interview/reading)	*85.3 ± 2.70*	*75.5 ± 2.32*
HR (arithmetic)	*88.1 ± 2.03*	*75.3 ± 2.03*
HR (post-task)	*76.5 ± 2.01*	*73.3 ± 2.34*

Table 3.1.1. Subjective and heart-rate responses to the stress and control task. Wakefulness/Sleepiness = Score on MDBF Scale „wakefulness vs. sleepiness“, low values indicate low wakefulness, i.e. high sleepiness; Calmness/Restlessness = Score on MDBF Scale “calmness vs. restlessness”, low values indicate low calmness, i.e. high restlessness; Pleasant mood = Score on MDBF Scale “pleasantness vs. unpleasantness”; Stress, Insecurity, Anxiety = perceived subjective feelings of stress, insecurity, and anxiety during the stress or control task as rated on 10-point rating scales; HR = heart rate. The Trier Social Stress Test (TSST) was used as a psychosocial stress challenge; Data presented as mean ± SEM.

	Subjective arousal		Autonomic arousal	
	CE	**CT**	**PostHR**	**MaxHR**
CE			-.03	-.01
CT	-.12		.05	.02
CP	-.04	.61**	-.23	-.17
Stress	-.14	.48**	-.02	.15
Insecurity	-.05	.54**	.08	.13
Anxiety	-.15	.42*	.06	-.04

Table 3.1.2. Pearson product-moment correlations among measures of arousal and further subjective reactions to the TSST (n=33). CE = change in energetic arousal; CT = change in tense arousal; CP = change in pleasantness; PostHR = change in heart rate from pre to post TSST; MaxHR = maximum change in heart rate; Stress, Insecurity, Anxiety = Subjective feelings during the TSST as measured on 10-point rating scales; the positive correlation between CP and CT is due to the bipolar conceptualization of the MDBF rating-scales. It indicates that an increase in unpleasantness (i.e. a decline in pleasantness) was associated with an increase in restlessness. ** = p≤.01; * = p≤.05

Heart rate. Heart rate data are summarized in table 3.1.1. A 2 *group* (stress group, control group) by 4 *time* (pre-task, reading, arithmetic, post-task) mixed design ANOVA indicated that participants in the stress group showed a stronger increase in heart rate than participants in the control group (*group* by *time* interaction: $F_{1.8,89.4} = 17.98$; $p < .0001$; $\eta^2 = .27$). Heart rates were comparable between both groups before ($F_{1,49} = 1.06$; $p = .31$) and after ($F_{1,49} = .97$; $p = .33$) the task. Moreover, no associations were found among subjective arousal measures and autonomic arousal as measured by the change in heart-rate (maximum change and change pre-TSST to post-TSST). We furthermore asked if changes in energetic and tense arousal would be associated with different heart rate response patterns to the TSST. To this end, we run a GLM with repeated measurements on heart rate data within the stress group and included the independent variables change in energetic arousal, change in tense arousal, and the interaction term between these variables. The critical *time* by *change in energetic arousal* by *change in tense arousal* interaction was not significant ($F_{2,52} = .53$; $p = .56$), indicating that tense and energetic arousal were not associated with different heart rate response patterns to the TSST.

Saliva cortisol. Stress and control participants showed comparable cortisol values before stressor/control procedure onset (all ps > .20; Figure 3.1.1). However, groups differed regarding their cortisol responses to the stress or control procedure respectively. A 2 *group* (stress group, control group) by 9 *time* (timepoint of measurement 1 to 9) mixed design ANOVA revealed that cortisol responses were higher in the stress group as compared to the control group (*group* by *time* interaction: $F_{2.2,107.2} = 15.24$; $p <. 0001$; $\eta^2 = .24$). In analogy to heart rate data, a GLM analysis within the stress group revealed that stress-induced changes in energetic and tense arousal were not associated with different cortisol response patterns to the TSST (*time* by *change in energetic arousal* by *change in tense arousal* interaction: $F_{2,61} = 2.23$; $p = .12$).

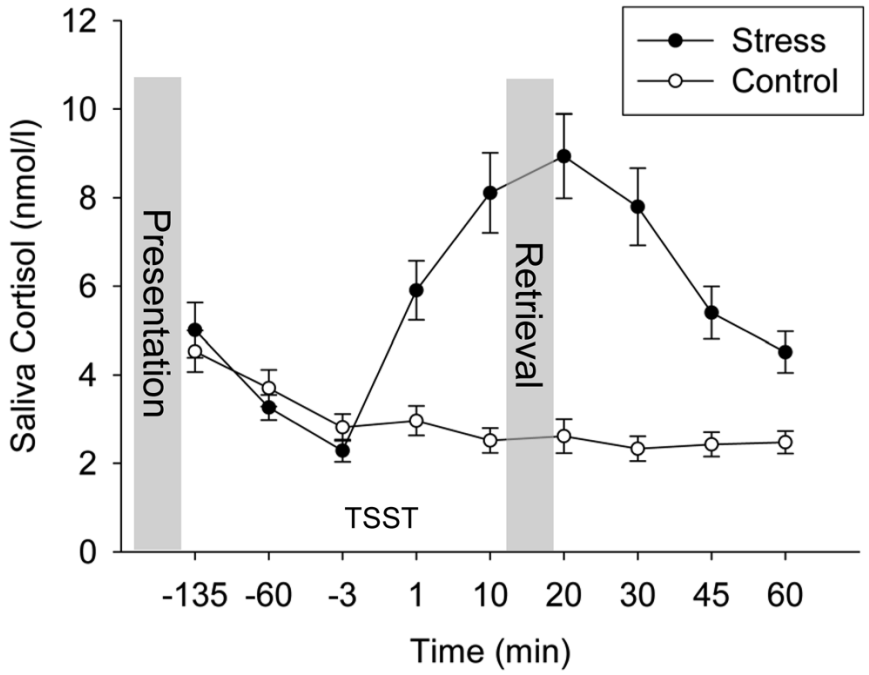

Figure 3.1.1. Saliva cortisol response to the stress and the control task. Saliva cortisol increased in the stress group (n=33) but not in the control group (n=18) (ANOVA, *time* by *group* interaction, p<.0001); Presentation = presentation of the word list; Retrieval = free recall of words learned 165 min earlier; Data presented as mean ± S.E.M.

Memory performance

Effects of stress on memory retrieval. Stress prior to retention testing reduced retrieval performance ($F_{1,49} = 8.07$; $p = .007$; $\eta^2 = .14$; Table 3.1.3). Although this effect

appeared to be especially pronounced for negative words, the referring *group* by word *valence* interaction did not reach statistical significance ($F_{2,98} = 1.78$; $p = .17$).

	Stress	Control
positive	38.2 ± 2,1	44.4 ± 3.6
negative	25.5 ± 2.6	37.8 ± 2.8*
neutral	23.0 ± 2.0	26.7 ± 2.7
total	28.9 ± 1.6	36.3 ± 2.1**

Table 3.1.3. Memory performance in percent for positive, negative, neutral words, and in total in the stress group (n=33) and the control group (n=18). Total memory retrieval (i.e. percentage of words retrieved independent of emotional word category) was significantly lower in the stress group compared to the control group; Data presented as mean ± SEM; * = p≤.05; ** = p≤.01

Effect		P
CE	$F_{1,27} = 4.64$	0.04
CT	$F_{1,27} = 2.05$	0.16
CE x CT	$F_{1,27} = 4.50$	0.04
CT x Valence	$F_{2,54} = 3.26$	0.05
Cortisol	$F_{1,27} = 0.94$	0.34
PostHR	$F_{1,27} = 0.00$	0.98
MaxHR	$F_{1,27} = 0.01$	0.94

Table 3.1.4. Influences of tested independent variables on memory retrieval of positive, negative, and neutral words within the stress group as revealed by a GLM analysis. CE = change in energetic arousal; CT = change in tense arousal; Cortisol = maximum increase in saliva cortisol; PostHR = change in heart rate from pre to post TSST; MaxHR = maximum change in heart rate; Valence = word valence.

Influences of arousal, cortisol, and word valence on memory retrieval. A GLM including the independent variables *word valence* (positive, negative, neutral) *change in energetic arousal*, *change in tense arousal*, *change in heart rate*, and *the maximum increase in cortisol* was calculated within the stress group. This analysis revealed a significant main-effect of the factor *change in energetic arousal* ($F_{1,27} = 4.64$; $p = .04$; $\eta^2 = .15$), a significant *change in energetic arousals* by *change in tense arousal* interaction ($F_{2,27} = 4.50$; $p = .04$; $\eta^2 = .14$), and a significant *change in tense arousal* by *word valence* ($F_{2,54} =$

3.26; $p < .05$; $\eta^2 = .11$) interaction. These effects are analyzed in detail in the following paragraphs. None of the other effects reached significance (see Table 3.1.4). For reasons of comparability with previous studies, a separate GLM was calculated within the stress group that included the factors *word valence* (positive, negative, neutral) and *maximum increase in saliva cortisol* only. This analysis revealed that the stress related cortisol secretion tended to predict post-stress memory retrieval ($F_{1,31}$ = 3.32; $p = .07$; $\eta^2 = .10$). There was no interaction between the increase in saliva cortisol and the factor word valence ($F_{2,62} = .43$; $p = .66$). Correlational analyses indicated a positive association among the maximum increase in cortisol and overall memory performance ($r = .31$, $p = .07$). However, after controlling for changes in energetic and tense arousal the association among increase in saliva cortisol and memory retrieval no longer tended to be significant ($F_{1,28} = 1.05$; $p = .32$). We furthermore analyzed if absolute levels of energetic or tense arousal measured before and after the TSST would predict post-stress memory retrieval. No such associations were found (all $p > .18$).

Change in energetic arousal by change in tense arousal interaction. The significant *change in energetic arousal* by *change in tense arousal* interaction indicated that both variables predicted memory performance interactively. We therefore analyzed the interaction among these variables and not the significant effect of change in energetic arousal alone. As reported above, energetic arousal changed comparably in both the stress and the control group. It was thus analyzed if effects of changes in self-reported arousal on memory retrieval differed among stressed and non-stressed participants. A GLM including the variables *word valence* (positive, negative, neutral), *group* (stress, control) *change in energetic arousal*, *change in tense arousal* as well as the interaction term between these variables and the factor *group* were included as independent variables into the analysis. This analysis revealed a significant main effect of the factor *group* ($F_{1,43} = 5.16$; $p < .03$; $\eta^2 = .11$) and a significant *group* by *change in energetic arousals* by *change in tense arousal* interaction ($F_{2,43} = 3.20$; $p = .05$; $\eta^2 = .13$). The significant interaction indicated that (i) only the combination of change in

energetic arousal and change in tense arousal predicted memory performance and (ii) that this influence differed between the stress and the control group. As already reported the *change in energetic arousal* by *change in tense arousal* interaction was significant in the stress group. However, an additional analysis showed that neither the main effects *change in energetic arousal* ($F_{1,14}$ = .35; p = .57) or *change in tense arousal* ($F_{1,14}$ = .05; p = .82) nor the interaction term between these variables reached significance in the control group ($F_{1,14}$ = 1.79; p = .20), indicating that self-reported arousal did not affect memory performance in the control group.

Next, further illustrative analyses were conducted in order to analyze the significant two-way interaction within the stress group in more detail. Specifically, a median split on *change in energetic arousal* and *change in tense arousal* within the stress group was used to create four new groups representing different combinations of changes in energetic arousal and tense arousal (data shown as mean ± S.E.M.): [1] low energetic arousal (.00 ± .57)/low tense arousal (2.00 ± 1.00), [2] low energetic arousal (-1.00 ± .27)/high tense arousal (9.88 ± .67), [3] high energetic arousal (4.50 ± .60)/low tense arousal (4.30 ± 4.5), [4] high energetic arousal (3.00 ± .44)/high tense arousal (7.89 ± .89). Next, a 5 *group* (high energetic arousal/low tense arousal, low energetic arousal/high tense arousal, high-energetic arousal/high tense arousal, low energetic arousals/low tense arousal, control group) ANOVA on the number of words retrieved was calculated (Figure 3.1.2). This analysis indicated significant group differences (main effect *group*: $F_{4,46}$ = 5.53; p = .001; η^2 = .33). Tukey-HSD corrected follow-up tests revealed that participants in the stress group with low change in energetic arousal but high change in tense arousal performed worse than participants with high change in energetic arousal and high change in tense arousal (p = .009) and participants in the control group (p = .007). No other pair wise comparisons were significant after alpha error correction. A contrast analysis, however, indicated that the memory performance of participants in the stress group with high changes in energetic arousal and tense arousal was comparable to participants in the control group and that both groups differed from the other three

groups in the stress condition ($F_{1,46}$ = 16.46; p < .001; η^2 = .26). Furthermore, participants in the stress group with low change in energetic arousal but high change in tense arousal performed worse than the other four groups ($F_{1,46}$ = 11.37; p = .002; η^2 = .20).

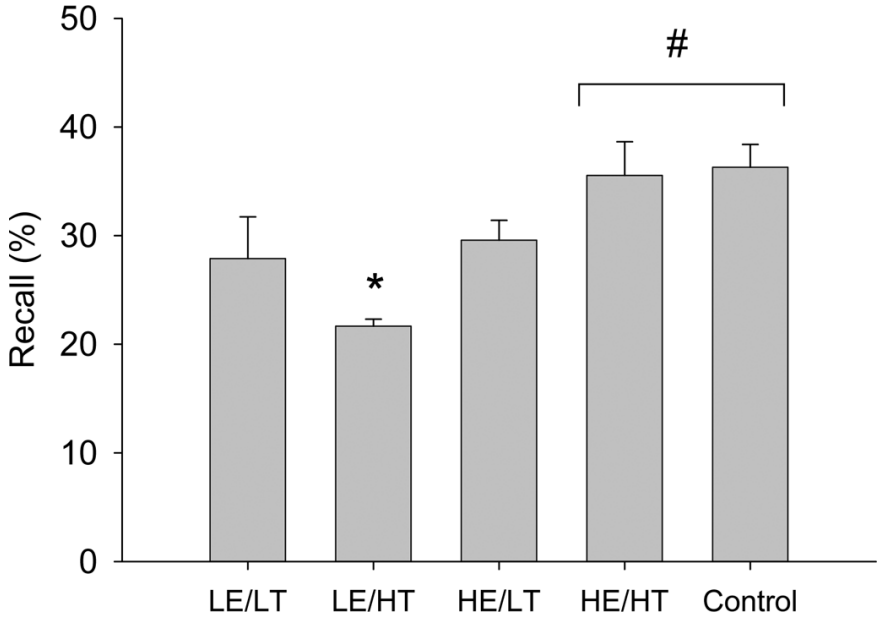

Figure 3.1.2. The x-axis represents subgroups of participants with different combinations of high and low changes in energetic and tense arousal in response to the TSST as well as the control group. LE/LT = low change in energetic arousal and low change in tense arousal (n=8); LE/HT = low change in energetic arousal and high change in tense arousal (n=8); HE/LT = high change in energetic arousal and low change in tense arousal (n=8); HE/HT = high change in energetic arousal and high change in tense arousal (n=9); Control = no-stress group (n=18). Contrast analyses based on ANOVAs revealed that HE/HT and control participants performed both better than the other three groups (# = p≤.05) and that LE/HT showed lowest memory performance of all groups (* = p≤.05). Recall% = percentage of words retrieved from a list of words learned 165 min before; Data presented as mean ± S.E.M.

Change in tense arousal by word valence interaction. The significant *change in tense arousal* by *word valence interaction* indicated that the impact of stress-induced change in tense arousal on memory retrieval was influenced by word valence. Additional analyses showed that *change in tense arousal* tended to be correlated with negative words (r = .31; p = .08) but was not significantly associated with positive (r = -.11; p = .54) or neutral words (r = -.14; p = .45).

3.1.5. Discussion

Here, we asked whether stress-induced changes in arousal facilitate post-stress memory retrieval. Although we did not find a memory enhancement by

arousal our data show that stress-induced arousal can compensate for impairing effects of stress on memory performance. We will discuss this result in more detail in the following paragraphs.

As hypothesized, stress-induced arousal was associated with post-stress memory retrieval. A multifaceted picture of arousal effects on memory performance emerged. Overall, stress impaired post-stress memory retrieval. However, within the stress group, participants with high stress-related changes in both energetic arousal and tense arousal showed best memory retrieval; they performed similar to control participants. This suggests that concurrent increases in energetic and tense arousal may compensate for impairing effects of stress on memory retrieval. Overall, this finding is in agreement with previous studies that found facilitative influences of heightened arousal and alertness on cognitive performance in attentional and vigilance tasks and tests of declarative memory (Aston-Jones, 2005; Revelle & Loftus, 1992). Particularly, the facilitative influence of energetic arousal on memory performance was expected. Studies on sleep deprivation showed that increased subjective feelings of sleepiness are associated with impaired cognitive performance (Matsumoto, Mishima, Satoh, Shimizu, & Hishikawa, 2002; Thomas *et al.*, 2000) and research on individual differences revealed that heightened energetic arousal predicts high performance in sustained attention, visual or memory search, and letter transformation tasks (Matthews & Davies, 2001; Matthews, Davies, & Lees, 1990). Surprisingly, however, an increase in energetic arousal alone did not affect memory performance in the control group. Moreover, only a combination of high change in energetic arousal and tense arousal was associated with unimpaired memory performance in the stress group. This latter finding is of particular interest. Based on previous work (Matthews *et al.*, 1990; Matthews & Westerman, 1994), we expected that a high change in tense arousal would impair memory retrieval. In support of this assumption and in contrast to unimpaired memory performance in participants with concurrent high increase in energetic and tense arousal, memory performance was worst in participants with high stress-induced change in tense arousal but low

change in energetic arousal. This complex pattern of arousal effects suggests that the neural and peripheral systems underlying both arousal states interact and that this interaction determinates effects of arousal on post-stress memory retrieval.

Stress-induced tense arousal was accompanied by states of heightened anxiety and fearfulness which are known to induce activation in limbic brain areas, such as the amygdala (Davidson, 2003; Wang *et al.*, 2005). This finding is in line with evidence that tense arousal is associated with activation in limbic brain structures (Thayer, 1989). Ample evidence suggests that impairing effects of GCs on memory retrieval require noradrenergic activation within the amygdala (de Quervain, Aerni, & Roozendaal, 2007; Kuhlmann & Wolf, 2006; Okuda, Roozendaal, & McGaugh, 2004; Roozendaal *et al.*, 2006). It is thus tempting to speculate that activation of limbic brain areas by isolated tense arousal mediated impairing effects of stress on memory retrieval. In contrast, energetic arousal was suggested to be associated with general mobilization of physiological and cognitive capabilities that may have a neurophysiologic correlate in heightened activation of the brainstem reticular formation (Thayer, 1989). More recent research on neurobiological mechanisms of wakefulness and arousal identified several brain regions, such as the locus coeruleus and different hypothalamic nuclei, as well as noradrenergic, cholinergic, dopaminergic, and serotonergic transmitter systems that are involved in the regulation of wakefulness and arousal (Aston-Jones, 2005; Jones, 2003). Brain activation induced by energetic arousal might modulate activation in the amygdala (or in brain areas connected to the amygdala) induced by tense arousal, compensating for impairing effects of isolated tense arousal on memory retrieval. However, further studies are needed to test this hypothesis directly.

Numerous studies suggest that effects of arousal on cognitive performance are nonlinear, following an inverted-U relationship (Diamond, Campbell, Park, Halonen, & Zoladz, 2007). Our finding of impairing as well as protective effects of stress-induced arousal on memory retrieval is in line with this literature. Recently, it was shown that norepinephrine and dopamine, which are interactively involved in the

control of stress-induced arousal, have inverted-U-shaped influences on prefrontal cortex (PFC) physiology and cognition (Arnsten, 2009; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007). This brain region is a key player in cognitive control and is involved in many cognitive domains, including episodic memory (Gilboa, 2004). The PFC could therefore be a crucial moderator of nonlinear effects of subjective arousal on cognitive performance and memory retrieval. Future studies will focus on the validity of this theory.

The complex interactive arousal effect found in the present study might explain some of the discrepancy in the literature on effects of cortisol and arousal on post-stress memory retrieval. It is well established that emotional arousal induced by affective stimuli (de Quervain *et al.*, 2007) or psychosocial stress (Kuhlmann *et al.*, 2005) is a prerequisite for impairing effects of cortisol on memory retrieval. However, some authors found better memory retrieval in high-cortisol responders than low responders to a laboratory stressor (Domes *et al.*, 2002; Nater *et al.*, 2007; Schwabe *et al.*, 2009). Our results suggest that the strength and combination of stress-induced change in energetic and tense arousal critically affect the direction of stress effects on memory retrieval. Under conditions of isolated increase in tense arousal or relatively specific activation of affective systems (de Quervain *et al.*, 2007) GCs might interact with arousal induced activity in the limbic system and impair post-stress memory retrieval. In contrast, a combination of high increase in energetic and tense arousal might override these impairing effects and lead to unimpaired or even facilitated memory performance. This could offer a parsimonious explanation for unexpected positive effects of GCs on post-stress memory performance reported previously (Domes *et al.*, 2002; Nater *et al.*, 2007; Schwabe *et al.*, 2009).

Our data indicate that the pattern of stress-induced change in subjective arousal is a better predictor of individual differences in post-stress memory retrieval than the absolute level of arousal at a specific point in time. A predisposition to react to stress with high increase in tense arousal but low increase in energetic arousal might thus represent a vulnerability factor that leads to impaired memory retrieval

in stressful situations. In contrast, individuals with high change in energetic as well as tense arousal might be protected against such detrimental effects of stress. This finding might have relevance for the development of therapeutic approaches against detrimental effects of stress on memory and cognition.

We found no association among post-stress memory performance and a measure of autonomic arousal, i.e. heart-rate. This could be due to the fact that we measured heart-rate only until 10 minutes after the TSST and assessed subjective arousal only thereafter. However, the dissociation between subjective and autonomic arousal measures might also be due to the multifaceted structure of the arousal construct. Past research showed that a generalized arousal component can be found that accounts for a substantial amount of behavioral variance in single forms of arousal such as sexual behavior or fear (Garey *et al.*, 2003; Pfaff, Ribeiro, Matthews, & Kow, 2008). Moreover, data suggest that self-report may be a better indicator of generalized arousal than single physiological measures (Thayer, 1989). In an early study, Thayer investigated associations between physiological (heart rate, finger blood volume, skin conductance) and psychological arousal reactions to a laboratory stress task (Thayer, 1970). He found that intercorrelations between the physiological functions were very low. However, after combining physiological measures to form a single general arousal index, self-report measures correlated substantially with this general arousal index. Our data suggest that self-report measures of arousal might proof particularly beneficial as predictors of memory performance because they represent a generalized arousal component.

Previous work showed that the affective characteristics of the to-be-remembered stimuli mediate effects of stress on memory performance (Buchanan, 2007; Schwabe, Bohringer, Chatterjee, & Schachinger, 2008). In the present study, the general effect of stress on memory retrieval was not influenced by the valence of the learned words. Earlier studies indicated that stimulus arousal has a stronger impact on stress-related memory phenomena than stimulus valence (Buchanan & Lovallo, 2001; Buchanan & Tranel, 2008; Cahill, Gorski, & Le, 2003; Kuhlmann *et al.*, 2005).

Thus, the absence of a valence effect in the present study might be due to the fact that we tested memory for stimuli that differed along the valence but not the arousal dimension. However, we found some evidence that arousal effects on memory performance are modulated by the affective characteristics of the presented stimulus material. Within the stress group, the change in restlessness tended to correlate positively with retrieval of negative words whereas no association was found with retrieval of positive or neutral words. It is well established that the match between affective characteristics of the to-be-remembered stimuli and the mood state at retrieval affects memory performance (Buchanan, 2007; Lewis, Critchley, Smith, & Dolan, 2005). Here the change in tense arousal was correlated with a decline in pleasantness. Mood congruency effects may thus have mediated the facilitative effects of tense arousal on memory retrieval.

In sum, the present findings demonstrate that stress-induced arousal as measured by self-report predicts post-stress memory retrieval. Importantly, our data suggest that a certain pattern of combined high change in energetic arousal and high change in tense arousal compensates for impairing effects of stress on memory performance. This finding may help to explain some of the discrepancies in the literature on stress effects on memory retrieval. Moreover, it may proof beneficial for the development of new strategies against detrimental effects of stress on memory performance, e.g. in stressful working environments or stressful testing situations.

3.1.6. Acknowledgement

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3.2. Study III: Effects of pre-learning stress on memory for neutral, positive and negative words: different roles of cortisol and autonomic arousal

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3.2.1. Abstract

Stress can have enhancing or impairing effects on memory. Here, we addressed the effect of pre-learning stress on subsequent memory and asked whether neutral and emotionally valent information are differentially affected by specific stress components, autonomic arousal and stress-induced cortisol. Ninety-six healthy men and women underwent either a stressor (modified cold pressor test) or a control warm water exposure. During stress, participants showed comparable autonomic arousal (heart rate, blood pressure), while 60 percent showed an increase of cortisol (responders vs. 40 percent non-responders). Ten minutes after the cold pressor test neutral, positive and negative words were presented. Free recall was tested 1 and 24 hours later. Overall, positive and negative words were better recalled than neutral words. Stress enhanced the recall of neutral words independently of cortisol response. In contrast, the free recall of negative words was enhanced in cortisol responders in the 1-hour but not 24-hour test which might suggest different effects of cortisol on consolidation and reconsolidation processes. Recall for positive words was unaffected by stress-induced cortisol. To summarize, (i) pre-learning stress can enhance memory for neutral words independently of cortisol and (ii) stress effects on memory for negative words appear to rely on stress-induced cortisol elevations, the absence of this effect for positive words might be at least partly due to differences in arousal evoked by positive versus negative words.

3.2.2. Introduction

Stress affects memory in many ways. Stress within a short period after learning facilitates memory (Roozendaal, 2000), but stress shortly before testing impairs memory (de Quervain, Roozendaal, & McGaugh, 1998; Kuhlmann, Piel, & Wolf, 2005). The influence of stress prior to learning is less clear. Several studies indicated that declarative memory can be impaired when people are exposed to stress before learning (Elzinga, Bakker, & Bremner, 2005; Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996b; Lupien, Gaudreau, Tchiteya, Maheu, Sharma, Nair, Hauger, McEwen, & Meaney, 1997; Payne, Jackson, Ryan, Hoscheidt, Jacobs, & Nadel, 2006); but other studies found enhanced memory performance in individuals stressed before learning (Domes, Heinrichs, Reichwald, & Hautzinger, 2002; Nater, Moor, Okere, Stallkamp, Martin, Ehlert, & Kliegel, 2007; Smeets, Giesbrecht, Jelicic, & Merckelbach, 2007). This discrepancy might be explained by such diverse factors as the different memory functions tested (long-term vs. working memory), the sample size of the study (Kirschbaum et al. (1996) tested only 13 subjects) and the time of testing (morning vs. afternoon), which is a factor crucial for the direction of the stress (hormone) effect on memory (see the review by Het, Ramlow, & Wolf, 2005).

There is a body of literature suggesting that cortisol, the adrenocortical hormone that is released during stress in humans, is a primary effector in the effects of stress on memory functions (de Kloet, Oitzl, & Joels, 1999; Het et al., 2005; Lupien & McEwen, 1997). A recent model proposes that cortisol released around the time of learning facilitates ongoing learning processes and thus would predict memory enhancing effects of stress experienced shortly before learning (Joels, Pu, Wiegert, Oitzl, & Krugers, 2006). Furthermore, it has been suggested that the effects of stress (hormones) are mediated via the basolateral amygdala (Roozendaal, 2000; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006). According to Roozendaal (2000), stress affects memory only if the actions of cortisol and autonomic arousal

converge in the basolateral amygdala, which then modulates memory processes in other brain structures. Importantly, several studies show that relative to neutral items, positively and negatively valenced stimuli elicit significantly greater activity in the amygdala, which suggests that emotional but not neutral words are processed by the amygdala (Garavan, Pendergrass, Ross, Stein, & Risinger, 2001; Hamann & Mao, 2002). This raises the question whether the assumptions of Roozendaal (2000) hold for both emotional and non-emotional information. Is a co-occurence of autonomic arousal and cortisol required for stress effects on memory for both emotional and non-emotional stimuli? Indeed, there is some evidence that the effects of pre-learning stress on memory depend on the emotionality of the material to be learned. Both Elzinga et al. (2005) and Payne et al. (2006) showed that stress prior to learning affected the recall of non-emotional information, but did not affect memory for emotional information. However, none of these studies separated the contributions of stress-induced cortisol and autonomic arousal.

Although stress is typically defined as an elevation in cortisol levels, individuals differ considerably in their cortisol responses. While some individuals show persistently high cortisol responses to stress, others show little or no such responses (Kirschbaum, Pruessner, Stone, Federenko, Gaab, Lintz, Schommer, & Hellhammer, 1995). Comparing individuals who show autonomic and cortisol responses to a task (cortisol responders) with others who respond with autonomic changes but without increases in cortisol (cortisol non-responders), provides the opportunity to assess the influences of stress-induced cortisol elevations and to separate these from effects of autonomic arousal. For instance, Buchanan, Tranel and Adolphs (2006) exposed participants to a cold pressor stress or control condition before testing them for previously learned words. The authors split the stressed subjects into cortisol responders and cortisol non-responders to dissect the effects of cortisol and autonomic activity on memory retrieval and found cortisol responders impaired relative to non-responders. Thus, Buchanan et al. (2006) concluded that stress-induced cortisol affects memory retrieval independently of autonomic activity.

A very recent study used the same strategy to disentangle the contribution of autonomic arousal and stress-induced cortisol on the effect of pre-learning stress on subsequent memory (Nater et al., 2007). In line with the model of Joels et al. (2006), Nater and colleagues (2007) found that participants with high cortisol responses had better recall performance than participants that showed low cortisol responses to the stressor. These authors, however, did not differentiate between emotional and non-emotional stimuli.

The present study aimed to test the influence of pre-learning stress on the memory for neutral, positive and negative terms. Therefore, we exposed participants to a modified cold pressor test (videotaped hand immersion into ice water) shortly before they saw a list of neutral, positive and negative words. Earlier studies indicated that the cold pressor test reliably causes stress expressed for example as increases in skin conductance (Buchanan et al., 2006) and high levels of discomfort (Cahill, Gorski, & Le, 2003). Based on the theoretical framework of Joels and colleagues (2006), we hypothesized a memory enhancing effect of stress shortly before learning. In order to dissect the possible contributions of stress-induced cortisol and autonomic arousal on memory for neutral, positive and negative words, we subdivided the stressed participants into cortisol responders and cortisol non-responders. If cortisol is required for stress effects on amygdala-mediated emotional memory only, then cortisol responders should show better memory performance than cortisol non-responders for positive and negative words but not for neutral words.

3.2.3. Methods

Participants

Ninety-six healthy volunteers (age: M=23.3 yrs, SD=3.2 yrs; 48 women: age range 19-36 yrs, BMI: 21.8 ± 2.6 kg/m^2; 48 men: age range 20-37 yrs, BMI: 23.3 ± 2.7 kg/m^2) recruited at the University of Trier participated in this study. Individuals who

met any of the following criteria, which were assessed in a standardized interview by a physician, were excluded from participation: medical illness within the prior 3 weeks; current or lifetime psychopathology; cardiovascular disorders; skin diseases; left-handedness; current treatment with psychotropic medications, narcotics, beta-blockers or steroids; current alcohol or tobacco use; or BMI (BMI=weight (in kg) / height (in m)^2) lower than 19 or higher than 26. To avoid menstrual cycle effects in women only oral contraceptive using women were included. Moreover, subjects were asked to refrain from fatty meals, caffeine and excessive exercise within the 4 hours prior to the experimental session on day 1 and the 4 hours prior to retention testing on the following day.

Participants were paid 20 € for participation. All participants provided written informed consent. The study protocol was approved by the local ethics committee.

Procedure

The time line of the experiment is shown in figure 3.2.1. To control for the diurnal cycle of cortisol, all testing was carried out between 2pm and 5.30pm. Participants were randomly assigned to the control and stress group. Sexes were counterbalanced with n=24 women and n=24 men per group.

After subjects were informed about the study procedure, they sat in a chair and baseline measurements of cortisol, heart rate (ECG) and blood pressure (Finapres) were taken.

Stress protocol: Participants were then informed that they will be exposed to a cold pressor test (CPT), videotaped and were requested to look into the camera during CPT. They were told that the video recordings would be analyzed for facial expression and asked to provide consent that the recordings can be used for scientific purposes later on. Participants were videotaped during the CPT in order to include characteristics of the TSST; (Kirschbaum, Pirke, & Hellhammer, 1993), i.e. to strengthen the social-evaluative character of the task which is known to boost cortisol responses (Dickerson & Kemeny, 2004). After signing the declaration of consent

participants immersed their right hand up to and including the wrist into ice cold water (0-2°C). Subjects were told that they should try to keep their hand as long as possible in the water, at maximum 3 minutes, but could remove their hand at their discretion. The experimenter asked them repeatedly to concentrate

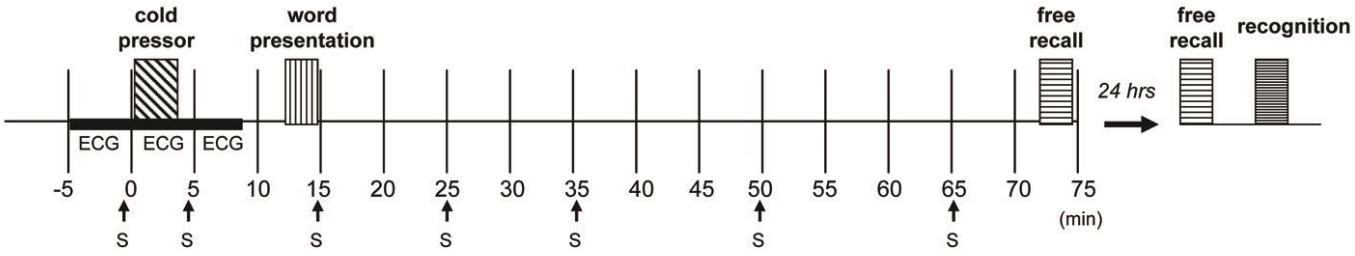

Figure 3.2.1. Sequence of events during the experimental session. t = 0 denotes the beginning of the cold pressor test. While ECG was recorded also blood pressure was measured. S - saliva sample.

on their right hand. All participants kept their hand in the water for the 3 minutes and were instructed at this point to take their hand out of the water.

Participants in the control group submerged their right hand for 3 minutes in warm water (35-37°C); there was no camera.

To verify the efficacy of the stress protocol, heart rate, blood pressure and saliva cortisol measurements were taken at several time points across the experiment.

Subjective stress: Immediately after the hand immersion participants in both groups rated on an 11-point scale ranging from 0 ("not at all") to 100 ("extremely") how stressful, painful and unpleasant the cold pressor and control condition, respectively, had been.

Word presentation: Ten minutes after cessation of the stress manipulation the learning phase started. This interval between stress and learning was suggested previously by other authors (Domes et al., 2002; Kirschbaum et al., 1996). Moreover, after a 10minute interval specific sensations associated with the cold pressor (in particular hyperaemia) are most likely gone. Participants were presented a list of 18 words (see Word material). To make sure that words were really encoded, subjects were instructed to read each of the words aloud and rate its emotional valence on a scale from -3 ("very negative") to 3 ("very positive"). They were not informed that

memory for these words would be tested subsequently. During the 60 minute break between word presentation and the free recall test, participants remained in a separate room. Subjects were allowed to bring an own book and read during the waiting period, except when saliva samples were taken.

1h-free recall: One hour after rating the words participants completed a free recall test in which they wrote as many words as they could remember on a sheet of paper. There was no time limit for the completion of the free recall test. All participants finished within 5 minutes. They were not told about the retention tests which followed 24hours later.

24h-free recall: The following day, subjects returned to the laboratory and completed a free recall test again. They were told that they have as much time as needed to recall and write down the words presented the day before. The recall test took no longer than 5 minutes.

Recognition test: Immediately after the 24h-free recall task participants completed a recognition memory test. Participants heard 36 words (18 words they had rated the day before and 18 new ones) and were asked to say "old" or "new" as to indicate whether or not they remembered rating the word on the previous day. New words were valence-matched to the learned words. The order of new and old words was random but constant for all subjects.

To assess the participants' ability to discriminate between previously presented and new words we used signal detection theory parameters hit (i.e. identification of previously presented words as "old"), false alarm (i.e. missclassification of new words as "old") and the sensitivity index d' (computed as z [p(hit)] - z [p(false alarm)]; see Wickens, 2002). A perfect hit rate of 100 percent was corrected and set to 97.5 percent (18 "old" words; $\frac{17}{18} + \frac{1}{18} \times 0.5 = 0.975$) as suggested by Wickens (2002). Accordingly, if a participant made no error of commission, the false alarm rate was set to 2.5 percent.

Word material

A separate group of 67 subjects (38 women, 29 men; age: M = 25.9 yrs, SD = 5.7 yrs) was presented a list of 85 German two-syllable nouns and asked to rate the emotional valence of these words on a 7-point scale ranging from -3 ("very negative") to 3 ("very positive"). Words were accepted as negative if their mean was smaller than -2.0 (SD < 0.5), as positive if the mean was higher than 2.0 (SD < 0.5), and as neutral if the mean valence score was between 0.5 and -0.5. Thirty-six words (16 neutral, 10 positive, 10 negative) were selected and divided into two valence-matched lists, each containing 8 neutral words (e.g. street, cup), 5 positive words (e.g. love, sun) and 5 negative words (e.g. torture, murderer).

Cardiovascular data and analysis

Heart rate and blood pressure measurements were taken 5 minutes before (baseline), during (test) and 5 minutes after hand immersion (post).

Heart rate was derived from a single standard lead II ECG configuration employing telemetric HP 78100A transmitter and HP 78101A receiver system (Hewlett Packard Corp.). ECG was sampled by 1 kHz with 12bit resolution. Beat detection was performed offline by WinCPRS (Absolute Aliens Oy, Turku, Finland) as was artifact control.

Continuous blood pressure was recorded using the Finapres system (Ohmeda, Englewood, CO, USA); a cuff was placed on the middle finger of the left hand which was put on a box to keep the hand at heart-level. Beat-to-beat systolic and diastolic blood pressure were determined offline with the help of WinCPRS software. Owing to technical failure we lost the blood pressure data of 6 subjects of the control group and 7 subjects of the stress group.

Collection of saliva and biochemical analyses

Saliva was collected by the subjects using customary straw 1 minute before (-1), immediately after (+5), 10 minutes after (+15), 20 minutes after (+25), 30 minutes

after (+35), 45 minutes after (+50) and 60 minutes after (+65) the modified cold pressor or control condition.

The saliva was put directly into standard Eppendorf tubes (1,5ml, Eppendorf, Hamburg; Germany), stored at room temperature until completion of the session, and then kept at -20°C until analysis. After thawing for biochemical analysis, the fraction of free cortisol in saliva (salivary cortisol) was determined using a time-resolved immunoassay with fluorometric detection, as described in detail elsewhere (Dressendorfer & Kirschbaum, 1992).

Cortisol responders and non-responders

To dissect the possible contributions of autonomic arousal and the adrenocortical stress hormone cortisol on memory performance we split the participants who had completed the modified cold pressor test into cortisol "non-responders" and "responders". Cortisol non-responders are subjects who show a stress-induced increase in autonomic parameters such as heart rate and blood pressure but not in cortisol. Cortisol responders, on the other hand, show both an increase in autonomic activity and cortisol in response to a stressor (Buchanan & Tranel, 2007; Buchanan et al., 2006; Fehm-Wolfsdorf, Soherr, Arndt, Kern, Fehm, & Nagel, 1993). Comparing unstressed control subjects and cortisol non-responders provides the opportunity to assess the contribution of autonomic arousal on memory whereas the comparison of cortisol non-responders and cortisol responders indicates the effect of stress-induced cortisol on memory performance.

Post-hoc, we characterized subtypes of cortisol profiles; a cortisol increase of at least 1.5 nmol/l relative to the individual baseline (i.e. the cortisol concentration 1 minute before the beginning of the CPT) was used to subdivide participants into cortisol responders and cortisol non-responders, respectively. Other authors used a median-split to assess the effect of stress-induced cortisol (Nater et al., 2007). While this is appropriate to distinguish cortisol high and low responders, an absolute cut-off is required when trying to separate cortisol responders and non-responders. The

chosen cut-off criterion (cortisol increase of at least 1.5 nmol/l) has been suggested earlier by Fehm-Wolfsdorf and colleagues (1993; see also Lupien et al., 1997).

Statistical analyses

In order to examine the possible interactions between stress, sex and word valence, memory data were subjected to 3 (group: controls, cortisol non-responders and cortisol-responders) × 2 (sex) × 3 (valence: neutral, positive and negative) ANOVAs. Significant main effects were further analyzed using Bonferroni adjusted post-hoc tests. In case of significant interactions, we first analyzed simple main effects by means of ANOVA. To pursue this analysis interaction contrasts were performed. All calculations were done with SPSS-statistical package (version 14.0; SPSS Inc.). Reported p-values are two-tailed. $P < .05$ was accepted as statistical significance. Analyses include the partial η^2 as measure of effect size where appropriate. Following the conventions by Cohen (1988) partial $\eta^2 = 0.01$ is considered a small effect, partial $\eta^2 = 0.06$ a medium-sized and partial $\eta^2 = 0.14$ a large effect.

3.2.4. Results

Effectiveness of the stress induction

Autonomic and cortisol measurements as well as participants' subjective stress ratings verified the stress-induction by the modified cold pressor test (CPT).

Autonomic stress responses

Stressed participants showed an increase in autonomic stress indices while controls did not. As shown in table 3.2.1 systolic and diastolic blood pressure were significantly increased in response to the modified cold pressor test (group × time interaction: both Fs > 25, both ps < .001, both $\eta^2 > .24$; group: both Fs > 32, both ps < .001, both $\eta^2 > .25$; time: both Fs > 15, both ps < .001, both $\eta^2 > .17$). Similarly, we

obtained a significant group × time interaction for heart rate ($F_{(2,176)} = 7.36$, $p < .01$, $\eta^2 = .08$; group: $F_{(1,89)} = 1.06$, $p = .31$, $\eta^2 < .01$; time: $F_{(2,176)} = 12.41$, $p < .001$, $\eta^2 = .12$) indicating that heart rate changed in subjects in the stress group but not in controls.

Interestingly, heart rate was increased in stressed participants already before the stress manipulation. This is most likely due to the announcement of the cold pressor test and video recording and questions the value of the pre-stress measurement as a baseline. A measurement prior to the announcement of the stress procedure would have been useful.

Moreover, we found significantly higher systolic and diastolic blood pressure in men compared to women, whereas women had higher heart rates than men (all Fs > 5, all ps < .03, all $\eta^2 > .05$). However, there were no significant interactions between sex and the other factors (all Fs < 3, all ps > .10, all $\eta^2 < .02$) which suggests that the effects of the stress manipulation were equivalent for both sexes.

	Cold pressor			Control manipulation		
	Heart rate	Systolic bp	Diastolic bp	Heart rate	Systolic bp	Diastolic bp
Before	**73.2 ± 1.5**	126.0 ± 2.2*	71.6 ± 1.9	70.1 ± 1.5	118.3 ± 2.4	68.0 ± 1.9
During	**73.5 ± 1.5**	**151.6 ± 3.1**#	**86.8 ± 2.3**#	70.0 ± 1.6	117.8 ± 2.3	65.4 ± 1.9
After	69.2 ± 1.4	127.7 ± 2.2#	72.8 ± 1.9#	69.5 ± 1.5	118.5 ± 2.0	66.6 ± 1.5

Table 3.2.1. Heart rate, systolic and diastolic blood pressure before, during and after the experimental manipulation. Increased heart rate, systolic and diastolic blood pressure (bp) indicate the success of the stress-induction. No change in these measures in the control group. Note the increased heart rate in subjects of the stress group before the experimental manipulation: These participants were informed that they have to immerse their hand in ice-cold water and will be videotaped. Data represent means ± SEM. Bold - p<.01 within group; * p<.05 between groups, # p<.01 between groups.

Salivary cortisol responses

Cortisol was increased in participants of the stress group (group $F_{(1,91)} = 4.17$, $p < .05$; $\eta^2 = 0.05$; Figure 3.2.2a) with a different time course from controls (time $F_{(6,546)} = 8.12$, $p < .0001$, $\eta^2 = .08$; time × group $F_{(6,546)} = 5.96$, $p < .0001$, $\eta^2 = .29$). There were no differences between men and women in cortisol response ($F_{(1,91)} = 1.54$, $p = .22$, $\eta^2 < .01$), nor was there an interaction between sex and one of the other factors (Fs

< 1, *p*s > .40, η^2 < .01) meaning that cortisol was elevated comparably in both men and women.

Inspection of individual data revealed a subgroup of 19 "cortisol non-responders" in the stressed subjects (Figure 3.2.2b). Participants were classified as

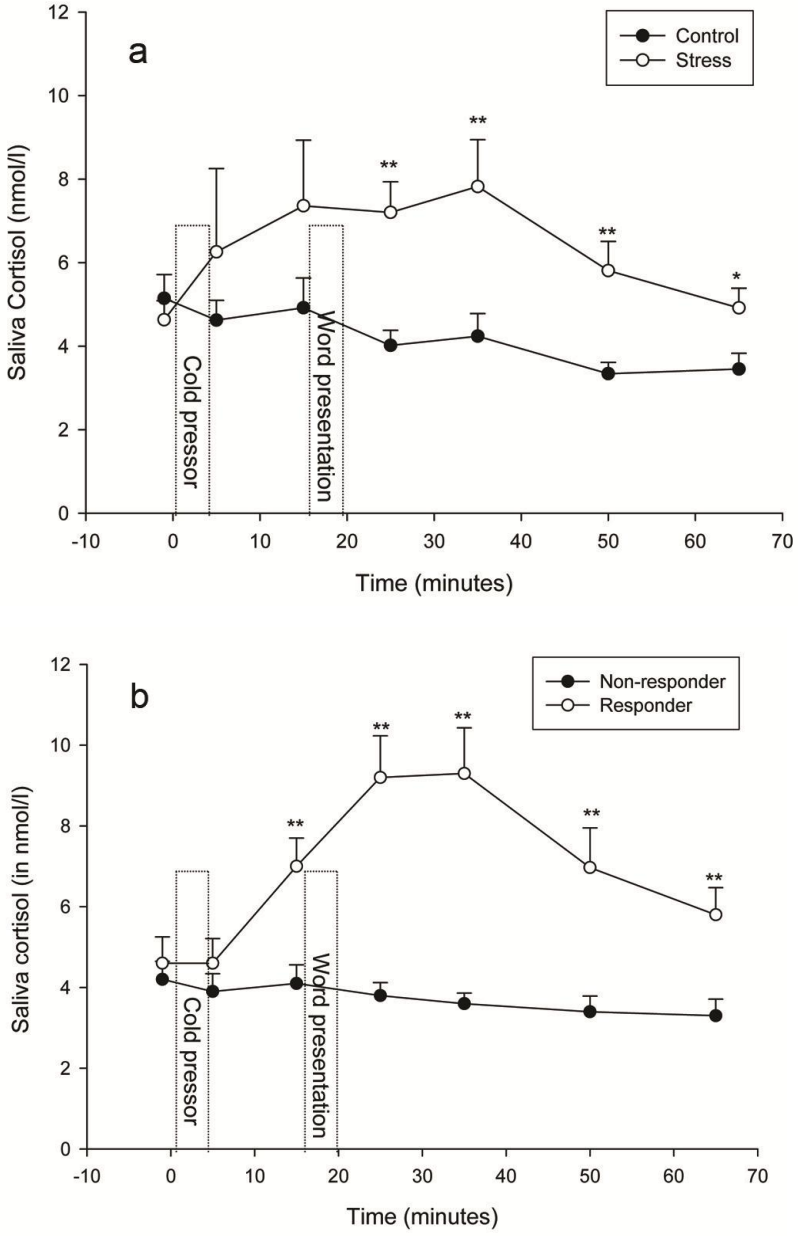

Figure 3.2.2. Salivary cortisol in nanomoles per liter (mean ± SEM) was measured at several time points throughout the experiment. The boxes in the graph denote the time point and duration of the cold pressor stress or control manipulation as well as the time point of the word presentation. (a) Comparison of stress group and control group (n=48 per group). Subjects in the stress group exhibited significantly higher cortisol concentrations than controls. (b) Comparison of stressed participants with an increase in cortisol of at least 1.5 nmol/l relative to baseline (responders; n=29) and those who did not show such an increase (non-responders; n=19). Note that words were presented during the cortisol rise. * p<.05; ** p<.01.

"cortisol non-responder" if they showed an increase in salivary cortisol concentrations of less than 1.5 nmol/l relative to baseline, otherwise they were classified as "cortisol responder". While 60 percent (29 out of 48) of the stress group were classified as cortisol responders, only 4 percent (2 out of 48) of the control subjects were cortisol responders ($\chi^2(1) = 34.73$, $p < .0001$). The two cortisol responders to the control condition (both were female) were excluded from further analyses. Men and women were comparable with respect to the number of cortisol responders and cortisol non-responders ($\chi^2(2) = 3.15$, $p = .21$).

Importantly, cortisol responders and cortisol non-responders did not differ with respect to their increase in heart rate, systolic and diastolic blood pressure (all Fs < 1.57, all ps > .22, all $\eta^2 < .01$), i.e. they were similar in their autonomic arousal.

Please note that we report saliva cortisol concentrations here. The rise in saliva cortisol is about 10 minutes delayed compared to plasma and serum cortisol concentrations (see Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999; Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). Thus, although this is not reflected in saliva cortisol, groups did most likely differ in their (plasma) cortisol concentrations during learning already.

Subjective stress ratings

As expected, participants in the stress group rated the experimental manipulation as significantly more stressful, painful and unpleasant than did controls (all ts > 7, all ps < .0001).

Effects of stress on memory

Free recall one hour after learning

This study investigated the effect of stress prior to learning on memory for neutral and emotional information. As shown in figure 3.2.3a, stress and stress-induced cortisol elevations had differential effects on memory for neutral, positive and

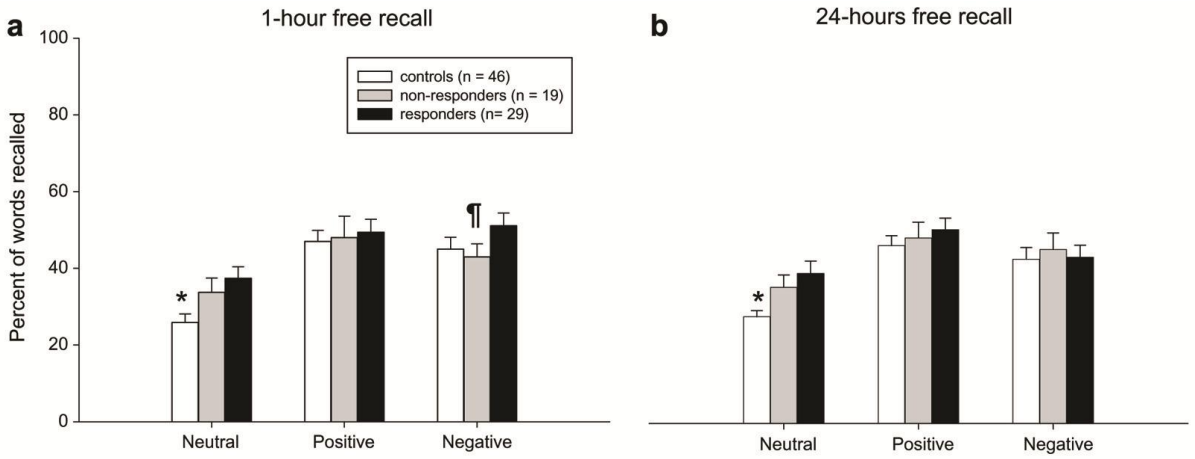

Figure 3.2.3. Recall of neutral, positive and negative words in controls, cortisol non-responders and cortisol responders (a) 1 hour after encoding and (b) 24 hours after encoding. Results are expressed as percentage of 1 hour delayed and 24 hours delayed recall, respectively; bars represent M ± SEM. * Significant difference from the other two groups; ¶ Significant difference from cortisol responders.

negative words (group × valence $F(4,174) = 2.53$, $p = .04$, $\eta^2 = .06$). Analyses of simple main effects indicated that controls, cortisol non-responders and cortisol responders differed in their recall performance for neutral ($F(2,91) = 5.30$, $p < .01$, $\eta^2 = 0.11$) and negative ($F(2,91) = 2.83$, $p = .06$, $\eta^2 = .06$) but not for positive words ($F(2,91) = 0.07$, $p = .93$, $\eta^2 < .01$). These differences were pursued by interaction contrasts comparing controls and cortisol non-responders as well as cortisol non-responders and cortisol responders. For neutral words, we obtained significantly better recall in cortisol non-responders than in controls ($p < .04$; cortisol responders vs. controls: $p < .01$) while there was no difference between cortisol non-responders and cortisol responders ($p = .33$). For negative words, however, controls and cortisol non-responders were similar in their memory performance ($p = .22$) whereas cortisol responders recalled more words than cortisol non-responders ($p = .02$; cortisol responders vs. controls: $p = .09$). Furthermore, we found a main effect of group ($F(2,87) = 3.07$, $p = .05$, $\eta^2 = .06$) indicating that cortisol responders tended to recall more words than controls (Bonferroni adjusted post-hoc test; $p = .06$). Additionally, there was a significant main effect of word valence ($F(2,174) = 25.97$, $p < .001$, $\eta^2 = .23$). Bonferroni adjusted post-hoc tests revealed that there was a better memory performance for both positive and negative words compared to neutral words (both ps < .01; positive vs. negative: $p >$

.50). We found no significant effect of sex on 1-hour recall ($F_{(1,87)}$ = 2.11, p = .17, η^2 = .01), nor was there an interaction between sex and one of the other factors (all Fs < 1, all ps > .60, all η^2-values < .01).

Free recall 24 hours after learning

Stress 10 minutes prior to learning affected recall performance on the following day (figure 3.2.3b). Again, we found different effects of stress and stress-induced cortisol on memory for neutral, positive and negative words (group × valence $F_{(4,174)}$ = 2.42, p < .05, η^2 = .05). Significant group differences were obtained for neutral ($F_{(2,91)}$ = 6.62, p < .01, η^2 = .13) but neither for positive ($F_{(2,91)}$ = 0.97, p = .38, η^2 = .02) nor for negative words ($F_{(2,91)}$ = 0.14, p = .87, η^2 < .01). Contrasts indicated that cortisol non-responders recalled more neutral words than controls (p = .04; cortisol responders vs. controls: p < .01) while cortisol non-responders and cortisol responders showed a comparable memory performance for neutral words (p = .21). There was a significant main effect of group on memory performance ($F_{(2,87)}$ = 4.15, p = .02, η^2 = .09) with cortisol responders recalling more words than controls (Bonferroni adjusted post-hoc tests; p < .05). Moreover, participants showed significantly better recall performance for both positive and negative words compared to neutral words (valence $F_{(2,174)}$ = 19.36, p < .001, η^2 = .18; Bonferroni adjusted post-hoc tests: negative/positive vs. neutral: both ps < .01; negative vs. positive: p = .21). There was no main effect of sex on 24h-recall ($F_{(1,87)}$ = 1.08, p = .30, η^2 < .01), nor was there an interaction between sex and the other factors (all Fs < 1.5, all ps > .22, all η^2-values < .01).

Correlations between percent of neutral (r = .81), positive (r = .71) and negative words (r = .70) recalled in the 1h- and 24h-free recall tests were high. Words recalled on day 2 were essentially the same as those recalled the day before.

Recognition memory

Recognition memory as assessed by signal detection indices of performance (discriminability d') was remarkably good in all participants (table 3.2.2). It was not affected by group ($F_{(2,89)} = 0.46$, $p = .64$, $\eta^2 < .01$), nor was there an interaction of group and one of the other factors (all Fs < 1, all ps > .35, all η^2-values < .02). Men and women were similar in their recognition memory ($F_{(1,89)} = 0.05$, $p = .82$, $\eta^2 < .01$; sex × valence $F_{(2,178)} = 0.14$, $p = .87$, $\eta^2 < .01$). However, recognition performance was significantly influenced by word valence ($F_{(2,178)} = 19.13$, $p < .001$, $\eta^2 = .18$) [1]: Negative words were best recognized, neutral words worst (Bonferroni adjusted post-hoc tests: all ps < .01).

	Controls		Cortisol non-responders		Cortisol responders	
d'	men	women	men	women	men	women
neutral words	2.20 ± 0.19	2.25 ± 0.31	2.13 ± 0.29	2.35 ± 0.21	2.51 ± 0.19	1.96 ± 0.15
positive words	2.76 ± 0.15	2.80 ± 0.33	2.39 ± 0.31	2.39 ± 0.20	2.60 ± 0.24	2.41 ± 0.22
negative words	2.80 ± 0.14	3.07 ± 0.28	2.74 ± 0.18	2.60 ± 0.26	2.83 ± 0.22	2.71 ± 0.20

Table 3.2.2. Recognition performance for neutral, positive and negative words expressed as sensitivity index d' in men and women of the 3 groups. Recognition performance was very high in all participants. Perfect performance: d' = 3.57; Hit rate of 90 percent and false alarm rate of 10 percent: d' = 2.56. Data represent M ± SEM.

Ratings of word material

Participants' ratings of the presented words confirmed the classification of words as positive, negative and neutral. Neutral words were rated significantly lower in valence than positive words ($t_{(94)} = 41.86$, $p < .0001$) and significantly higher in valence than negative words ($t_{(94)} = 47.82$, $p < .0001$). Valence ratings were independent of experimental group (group $F_{(2,91)} = 0.01$, $p = .91$, $\eta^2 < .01$; group × valence $F_{(4,184)} = 0.26$, $p = .88$, $\eta^2 < .01$).

[1] We used the total error rate to correct.

3.2.5. Discussion

The main aim of this study was to assess the involvement of specific stress components, autonomic arousal and stress-induced cortisol, in the effect of pre-learning stress on the memory for neutral and emotional stimuli. Overall, our data indicate that autonomic arousal (measured by heart rate and blood pressure) and stress-induced cortisol are differentially involved in the effects of pre-learning stress on memory for neutral, negative and positive words.

For neutral words, we obtained enhanced recall in stressed compared to control subjects both in the 1-hour and 24-hours delayed recall tests while there was no difference between cortisol responders and cortisol non-responders suggesting that autonomic arousal but not cortisol facilitated memory recall for neutral words. Participants were stressed prior to learning, thus stress could have affected memory encoding as well as memory consolidation and (at least on day 1) retrieval. In our view, it is relatively unlikely that stress affected memory retrieval of neutral words because the interval between stress and retention testing was relatively long (about 70 minutes), i.e. the stress induced autonomic arousal was most likely over at the time of the 1-hour free recall. Rather, the observed influence of stress on recall of neutral words might be a consolidation effect. This would be in line with earlier findings showing consolidation enhancing effects of autonomic activity (Nielson, Radtke, & Jensen, 1996; for a review: McGaugh, 2006). It is noteworthy, that there was a very high correlation between memory for neutral words in the 1-hour and 24-hours delayed recall tests. This may be because the act of retrieval strengthens the memory for the information recalled (Sara, 2000).

A different picture emerged for emotional words. Let us consider the effect of pre-learning stress on memory for negative words first. At 1-hour after learning, recall of negative words was enhanced in cortisol responders compared to cortisol non-responders while cortisol non-responders and controls performed similarly.

Thus, different from neutral words 1-hour delayed recall of negative words was affected by stress-induced cortisol elevations. We argue that this difference is due to a differential involvement of the amygdala in the processing of neutral and negative material. The amygdala complex has been identified as part of the neural circuitry critical for emotional reactivity and emotional memory (Gallagher & Chiba, 1996; LeDoux, 2000; McGaugh, Cahill, & Roozendaal, 1996). It is supposed to process emotionally valent but not neutral stimuli (Garavan et al., 2001; Hamann & Mao, 2002). Recent ideas regarding the amygdala's role in mediating stress effects on memory emphasize the interaction of sympathetic and adrenocortical systems. In other words, modulation of memory processes by the amygdala requires a co-occurrence of autonomic activity *and* glucocorticoids (Roozendaal, 2000).

At the 24-hour recall test, however, the effect of cortisol on memory for negative words disappeared. Both cortisol responders and cortisol non-responders performed similarly to participants in the control group. Interestingly, except a slight overall reduction in performance from the 1h- to the 24h-test, the only significant change appeared in cortisol responders for negative words. In contrast to previous studies showing retrieval impairing effects of stress (hormones) (Buchanan & Tranel, 2007; Buchanan et al., 2006; de Quervain et al., 1998; Kuhlmann et al., 2005), this pattern of results suggest that cortisol, which was still elevated at the time of the 1-hour recall, may have had an enhancing effect on retrieval. Alternatively, our findings for negative words could be due to differential effects of stress-induced cortisol on consolidation and reconsolidation processes. Increased glucocorticoid concentrations after learning facilitate memory consolidation (Buchanan & Lovallo, 2001; Cahill et al., 2003; Sandi, Loscertales, & Guaza, 1997). In particular, it has been reported that brief stress can enhance early, i.e. synaptic, consolidation processes via an activation of endogenous plasticity mechanisms (such as long-term potentiation) in the hippocampus and the amygdala (see Diamond, Campbell, Park, Halonen, & Zoladz, 2007). This might explain the enhanced memory for negative words 1-hour after encoding. The retrieval of the words, however, activates a reconsolidation

process. Reconsolidation refers to the process in which a memory item is rendered transiently malleable after its reactivation (Dudai, 2006; Nader, Schafe, & LeDoux, 2000). We argue that the still elevated cortisol concentrations during the 1-hour delayed recall, i.e. during memory reactivation, impaired the fragile memory trace and thus nullified the memory benefit of cortisol responders for negative words 24 hours later. Indeed, several studies show memory impairing effects of glucocorticoids administered around the time of the reactivation of emotional memories (Aerni, Traber, Hock, Roozendaal, Schelling, Papassotiropoulos, Nitsch, Schnyder, & De Quervain, 2004; Cai, Blundell, Han, Greene, & Powell, 2006; Maroun & Akirav, 2007; Soravia, Heinrichs, Aerni, Maroni, Schelling, Ehlert, Roozendaal, & De Quervain, 2006). Cai and colleagues (2006), for example, demonstrated in rats that the administration of glucocorticoids immediately after reactivation of previously acquired contextual fear diminishes subsequent recall of the fear. Interestingly, recent clinical trials suggest that postreactivation treatment with mild doses of cortisol has beneficial (i.e. impairing) effects on established fear or trauma memories in patients suffering from specific phobia (Soravia et al., 2006) or post-traumatic stress disorder (Aerni et al., 2004).

If stress-induced cortisol facilitates the early consolidation of negative stimuli and impairs their memory trace during reactivation and if this is found in negative but not neutral words, presumably because these effects are mediated via the amygdala which processes emotional information: then why did we not find the same effects for positive items? Why was the recall performance for positive words unaffected by stress and cortisol both on day 1 and day 2? A possible answer lies in the arousal associated with the presented material. Stress effects on memory for emotional stimuli depend also on the emotional arousal produced by the material to be learned (e.g. De Quervain, Aerni, & Roozendaal, 2007; Roozendaal et al., 2006). Roozendaal and colleagues (2006) reported that corticosterone injections after training in an object recognition task enhanced memory in rats that were naïve to the training context, i.e. for which the training situation was arousing. In rats that were

previously habituated to the training context, i.e. in which novelty-induced arousal was reduced, there was no effect of post-training corticosterone administration. In the same line, De Quervain et al. (2007) found that cortisol administration impairs memory retrieval for emotionally high-arousing words but not for medium- or low-arousing words.

Looking at the positive (e.g. sun, love, pleasure, vacation) and negative words (e.g. torture, murderer, violence, bomb) that were used in the present study, it appears reasonable to assume differences between both stimulus classes regarding the arousal level. Positive words were most likely less arousing than negative words, which could explain the absence of a stress (hormone) effect on memory for positive words. As many other studies in the field (Elzinga et al., 2005; Kuhlmann et al., 2005; Tops, van der Pompe, Baas, Mulder, den Boer, F., & Korf, 2003), we did not measure the emotional arousal associated with the words. This is to be considered as a limitation of the present study and future research will have to corroborate our interpretation by systematically varying the valence *and* arousal associated with the test material.

Importantly, neutral words are usually less well recalled than emotional words (see also Abercrombie, Kalin, Thurow, Rosenkranz, & Davidson, 2003; Buchanan & Lovallo, 2001; Payne et al., 2006). Here, the induction of stress made the recall performance for neutral words more similar to that for emotional words. This could be interpreted in light of the frequently reported inverse u-shaped relationship between arousal and memory performance (for a review: Baldi & Bucherelli, 2005). Accordingly, the enhanced memory for emotional words would be attributable to the higher arousal level associated with these stimuli. The stress prior to word presentation might have substituted the lack of arousal associated with neutral words at least partly and thus increased their memorability.

Enhanced memory for emotional relative to neutral words was observed in the recognition test 24-hours after encoding, too. Interestingly, recognition performance was also better for negative than for positive words. This might be due

to the higher arousal associated with negative compared to positive words, as argued above. We did not find an effect of the modified cold pressor test on recognition memory. However, recognition performance was exceedingly good in all participants. Especially, recognition memory for emotional words was close to perfect. These "ceiling effects" limit the value of the stress-recognition analyses and are probably due to the rather small number of words presented.

In line with recent studies of Smeets et al. (2007) and Domes et al. (2002) we obtained memory enhancing effects of pre-learning stress. We also corroborate the findings of Nater and colleagues (2007) who reported better memory performance in participants with a high cortisol response to stress administered before learning than in those that showed a low cortisol response. However, our results extend these previous findings in a very important point. None of the aforementioned studies controlled the valence of the presented material. Here, we provide evidence that the effects of pre-learning stress and stress-induced cortisol depend on the valence of the presented material.

Recently, a model was presented to account for the effects of acute stress on memory (Joels et al., 2006). The core of that model is that stress enhances memory if it is experienced around the time of learning. We stressed subjects within 10 minutes prior to learning and obtained results in line with the model of Joels and colleagues (2006). It is noteworthy that according to the framework of Joels et al. (2006) one would expect different effects of pre-learning stress on memory performance, if the stress-learning interval is extended (e.g. 30 minutes). GCs initiate a gene-mediated pathway which will bring the brain in a "consolidation mode" and suppress the processing of unrelated information. If encoding occurs some time after stressor exposure, this gene-mediated process will have developed and learning will be most likely impaired (Joels et al., 2006).

Although, we did not find an effect of sex on memory performance, it cannot be excluded that the mechanism underlying the effect of stress on memory is at least partly different in men and women. Women's cortisol responses to stress depend

critically on menstrual cycle phase (Kirschbaum et al. 1999). To keep this factor constant only oral contraceptives taking women were included in the present study. Women using oral contraceptives show blunted saliva cortisol responses to stress compared to men (Kirschbaum et al., 1999; Kirschbaum, Platte, Pirke, & Hellhammer, 1996). This is most likely owing to an ethinyl-estradiol induced increase in CBG, which in turn lowers the (salivary) free cortisol, i.e. the biologically active cortisol fraction (Kirschbaum et al., 1999). Thus, our finding that men and women (on contraceptives) showed similar cortisol responses is rather surprising and suggests that in women more cortisol was released from the adrenal cortex. This might have been due either to an increased sensitivity of the adrenal cortex to ACTH or to a higher HPA axis activation leading to higher ACTH secretion. ACTH effects on memory have been reported repeatedly (Izquerdo & Dias, 1985; Izquerdo, Barros, Medina, & Izquerdo, 2002). Thus, if the comparable saliva cortisol concentrations in men and women were owing to increased ACTH levels in women, this might have affected our results in women at least partly.

Finally, two further study limitations have to be addressed. First, the stress manipulation was announced to the cold pressor group at the beginning of the study. Thus, different expectations in the control and stress group might be potentially confounded with the results. Second, a more consistent control task during the waiting period might be valuable in future studies because this could help to avoid possible differences in rumination about the presented material.

For decades, the effects of stress on memory function have been viewed as mainly disruptive (e.g. Sapolsky, 1996). Results from this experiment extend previous reports indicating that stress may also have enhancing effects on memory formation and suggest a differential involvement of specific stress components, autonomic activity and stress-induced cortisol, in these effects, depending on the emotional valence of the learned material.

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3.2.7. References

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ERKLÄRUNG

nach § 9, Abs. 1 der Promotionsordnung des Fachbereichs I der Universität Trier vom 13.11.2008.

Hiermit versichere ich, dass ich die vorliegende Arbeit selber verfasst und keine außer den angegebenen Hilfsmitteln und Referenzen benutzt habe. Die Arbeit wurde bisher weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde vorgelegt.

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