Universität Trier

Fachbereich I – Psychobiologie

Impact of selected neural and endocrine Stress Factors on the Startle Eye Blink

Inaugural-Dissertation zur Erlangung des Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

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Eingereicht am:

10.09.2010

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This dissertation thesis and the presented research were performed at the

Division of Clinical Physiology
Institute of Psychobiology – University of Trier
Trier, Germany

supported by the

International Research Training Group "Psychoneuroendocrinology of Stress: From Molecules and Genes to Affect and Cognition"

Funded by the German Research Foundation (Deutsche Forschungsgemeinschaft: DFG)
Project GRK 1389/1

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Acknowledgements

This thesis and the work it is based on would not have been accomplished without the support of many dear friends and colleagues that cannot all be enumerated here. I especially want to thank Prof. Schächinger for all possible kinds of support one can imagine, personal advice and opportunities, and Prof. Anton for being available as a supervisor and reviewer. Without the technical support of Dr. Curio, none of the data would exist, but also, he was a never ceasing source of moral support and wisdom. Also, Dr. Port was indispensable for technical support, access to equipment and software as well as technical guidance. I was especially lucky to find a most reliable source of methodological knowledge, last minute support, good humor and wit in Prof. Blumenthal. Also, I thank Prof. Oitzl and the Leiden group for their advice and the sharing of valuable contacts. I furthermore have to thank Dr. Schulz for his help with startle analysis, Ms. Reichert for last minute E-Prime programming, Ms. Karcher for her help with the data analysis and Mr. Best for his help with conducting the LBNP study and organizing the FOV-group. I furthermore want to thank all my former colleagues (now friends) for new ideas, good teamwork, coffee and cookies.

I especially want to thank my parents for making me who I am, being happy with the result most of the time, for supporting my good decisions and especially for supporting my less good decisions. Furthermore, I thank my brother for setting a benchmark that I am still trying to reach. Last but not least, I thank Carina for being there, for being most admirably patient, even more for not being patient, for giving meaning to everything.

Abstract

The term “stress” was first introduced by Hans Selye, who described a non-specific “general adaptation syndrome” after the confrontation of the organism with a noxious stimulus. This “doctrine of non-specificity” has been challenged and it is now assumed that different stressors evoke different central and peripheral neuroendocrine responses via stressor-specific central nervous system (CNS) pathways. A stressor may be any external or internal stimulus. The organism adapts in the sense of a protective response. Hence, the neuroendocrine stress response represents an adaptive mechanism with the purpose to restore homeostasis (a concept introduced by Cannon 1929). It consists of a variety of neuroendocrine components, mainly separable into autonomic nervous system (ANS) and hypothalamus-pituitary-adrenal (HPA) responses of variable amplitude and duration, largely depending on the stress eliciting stimulus and actual context. Response components may have differential effects on behavior, as may other physiological stress correlates.

The central nervous system is in a state of alarm during acute stress, and defense networks become activated. In such a situation, stress impacts on behaviors, ranging from simple reflexes to more complex social actions (such as mating behavior). This dissertation thesis will focus on the effects of physiological stress correlates on a simple defensive reaction, the protective startle eye blink response. Startle reflexes are elicited by sudden intense stimuli (such as noise bursts). The motor reaction is characterized by protective (mostly flexion) muscle contractions, and is accompanied by ANS responses. The startle reflex may be influenced by pre-existing activation of the defense networks, as evidenced by increased startle during stressful circumstances and aversive context in animals and humans (e.g. threat of shock or social stressor). Although it has been established that the stress response has an impact on protective reflexes, such as the startle eye blink, it is difficult to disentangle the relative contribution of individual neuroendocrine mechanisms and other physiological alterations present during stress. Thus, the current work aimed to separate the impact of several physiological stress correlates, and to investigate their effect on the reflexive startle eye blink response.

Study I and Study II focus on the manipulation of HPA activity. Study I investigates fast effects of (excess) administration of the stress hormone cortisol on startle eye blink magnitude and prepulse inhibition of startle eye blink. Study II focuses on the effects of pharmacological reduction of plasma cortisol levels on startle eye blink magnitude. In Study III, the effects of

arterial and cardiopulmonary baroreceptor unloading (simulating minor blood loss stress) on the startle eye blink reflex are investigated.

It is demonstrated that small amounts of intravenous (IV) cortisol rapidly disrupt prepulse inhibition of startle without affecting startle magnitude, and that pharmacological suppression of cortisol production by metyrapone increases startle eye blink magnitude. Cardiopulmonary and arterial baroreceptor unloading independently increase startle eye blink magnitude.

These results demonstrate that neuroendocrine stress mechanisms and other physiological alterations during stress may have direct impact on the response magnitude of protective and defensive reflexes. These effects may be interpreted with respect to adaption and survival in hostile environments.

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Index of Publications

This doctoral thesis consists in general of three chapters (and, in addition, four chapters that represent a general introduction and discussion), which are published as 'Original Articles' in international peer reviewed journals. All articles are presented here in the originally published form, except for changes in formatting (i.e. figure and table labeling, labeling of headings and reference styles).

Index of Abbreviations

11-β-HSD-1	11-β-hydroxysteroiddehydrogenase type-1 enzyme
μV	microvolt
Ω	ohm
ACTH	adrenocorticotropic hormone
Ag/AgCl	silver/silver chloride
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ANS	autonomic nervous system
AU	arbitrary unit
BMI	body mass index
cm	centimeter
CNS	central nervous system
CPU	central processing unit
CRH	corticotropin releasing hormone
CVLM	caudal ventrolateral medulla
dB	decibel
dB(A)	decibel (A-weighted scale)
dl	deciliter
dZ/dt	first time derivative of transthoracic electrical impedance
ECG	electrocardiogram
EEG	electroencephalogram
EMG	electromyogram
GABA	gamma-aminobutyric acid
GR	glucocorticoid receptor

H	height
HF	high frequency band
HPA	hypothalamus-pituitary-adrenal
HRV	heart rate variability
Hz	hertz
ICG	impedance cardiography
IML	intermedio-lateral cell column
ISI	inter-startle interval
ITI	inter-trial interval
IV	intravenous
kg	kilogram
L	length
l	liter
LBNP	lower body negative pressure
LC	locus coeruleus
LCD	liquid crystal display
LF	low frequency band
m	meter
mg	milligram
min	minute
ml	milliliter
mm	millimeter
mmHg	millimeter mercury
MR	mineralocorticoid receptor
mRNA	messenger ribonucleic acid
ms	millisecond
mV	millivolt
NA	nucleus ambiguus

NAC	nucleus accumbens septi
NaCl	sodium chloride
nmol	nanomol
nRPC	nucleus reticularis pontis caudalis
NTS	nucleus tractus solitarii
NU	normalized unit
PPI	prepulse inhibition of startle
PPTg	pedunculopontine tegmental nucleus
PTSD	posttraumatic stress disorder
PVN	paraventricular nucleus of the hypothalamus
s	second
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SOA	stimulus onset asynchrony
RT	reaction time
RVLM	rostral ventrolateral medulla
W	width

Chapter I - General Rationale

The term “stress” was first introduced by Hans Selye, who described a “general adaptation syndrome” after the confrontation of the organism with a noxious stimulus (Selye, 1936).

According to Selye, stressors evoke a “generalized effort of the organism, to adapt itself to new conditions”. Such adaptive responses of the organism were first seen as a non-specific response (Selye, 1936). This “doctrine of non-specificity” has been challenged and it is now assumed that different stressors evoke different central and peripheral neuroendocrine responses via stressor-specific central nervous system (CNS) pathways (Pacak et al., 1998; Pacak and Palkovits, 2001). A stressor may be any external or internal challenge. The organism adapts in the sense of a protective response. Hence, the neuroendocrine stress response represents an adaptive mechanism with the purpose to restore or maintain homeostasis (a concept introduced by Cannon 1929 (Cannon, 1929)). The concept of “homeostasis” was later extended to describe a state of homeostasis at different functional states of the organism (during rest/fight, cold/heat, different states of nourishment, illness, emotional stress etc.), the so called “allostasis”. Challenges may appear in the external (e.g. physical stimuli, social interactions) or internal (e.g. interoceptive stimuli, metabolic demands) environment. It has been proposed that also the perceived threat to homeostasis may pose a stressor that elicits adaptive stress responses (Pacak et al., 1998; Pacak and Palkovits, 2001).

Adaptation to stressors consists of a variety of neuroendocrine components, mainly separable into autonomic nervous system (ANS) activity and hypothalamus-pituitary-adrenal (HPA) responses of variable amplitude and duration, largely depending on the stress eliciting stimulus and actual context. Adaptation includes restoration of homeostasis to existing environmental challenge as well as preparation of the organism for potential challenge. During acute stress, the central nervous system is in a state of alarm, and defense networks become activated. Neuroendocrine responses to acute stress as well as other physiological stress correlates may have differential effects on behavior, ranging from simple reflexes to more complex social actions (such as mating behavior). The startle reflex is a simple defensive reaction, serving to protect important body parts from potentially harmful stimulation. In general, defensive mechanisms should be enhanced during such states of alarm in order to better protect the organism from potential danger. In line with this, startle eye blink magnitude may be influenced by pre-existing activation of the defense networks: acute stress

(e.g. threat of predictable or unpredictable electric shocks) increases the startle eye blink magnitude (Mol et al., 2007) and psychosocial stress (e.g. public speech and counting task) can potentiate the increase of startle eye blink magnitude generally observed during darkness (Grillon et al., 2007). Acute physiological (Johnson and Adler, 1993; Ermutlu et al., 2005) and psychological (White and Yee, 1997) stress also reduces prepulse inhibition of startle (PPI), for example during threat of shock (Grillon and Davis, 1997).

Although it has been established that stress has an impact on protective reflexes, such as the startle eye blink, it is difficult to disentangle the relative contribution of individual neuroendocrine mechanisms and other physiological alterations present during stress. Thus, the current work aimed to separate the impact of several physiological stress correlates, and to investigate their effect on the reflexive startle eye blink response. Because the stress response consists of several components and different stressors evoke a different response pattern and specific adaptation to the challenge imposed by the stressor, such an investigation cannot comprise a detailed research of all physiological stress correlates that may impact on the startle reflex. Therefore, only individual physiological stress correlates of interest were investigated, serving as an example for the modulation of the startle reflex by stress.

Study I and Study II focus on the manipulation of HPA activity. Study I investigates fast effects of (excess) administration of the stress hormone cortisol on startle eye blink magnitude and prepulse inhibition of startle eye blink. Study II focuses on the effects of pharmacological reduction of plasma cortisol levels on startle eye blink magnitude. In Study III, the effects of arterial and cardiopulmonary baroreceptor unloading (simulating minor blood loss stress) on the startle eye blink reflex are investigated.

The following chapter (Chapter II) will describe the physiological principles of the startle reflex, neuroendocrine response systems and receptors that are of relevance for this dissertation project. In the first section, the startle reflex and prepulse inhibition of startle will be described. The second section will treat the hypothalamus-pituitary-adrenal axis, cortisol, fast (non-genomic) effects of cortisol and the present state of research on the interaction of cortisol with the startle reflex and prepulse inhibition of startle. In the third section the baroreflex, physiology of the baroreceptors and the present state of research on the interaction of baroreceptor activity with the startle reflex will be described.

Chapter III, Chapter IV and Chapter V will present the results of the studies this dissertation is based on. The studies have been published in peer reviewed international journals. They are coauthored by colleagues, as it is usually the case in psychobiological

research. In all publications, the impact of the applicant was substantial, as indexed by the position in the authors list. This procedure complies with the dissertation rules of the Fachbereich I of the University of Trier (kummulative Dissertationsschrift).

Chapter VI will summarize general limitations of the dissertation project and Chapter VII will give a short summary of the obtained results with general conclusions.

Chapter II - General Background

2.1 Startle Reflex and Prepulse Inhibition of Startle

The following sections will treat the startle reflex and prepulse inhibition of startle.

2.1.1 Startle Reflex

The startle reflex is a simple, brainstem relayed protective reflex, first investigated by Landis and Hunt in humans (Landis and Hunt, 1939). It is evoked by abrupt and intense stimulation of any exteroceptive non-gustatory sensory modality, most often investigated by presentation of acoustic noise burst stimuli. The startle response consists of a whole-body motor reaction, serving to protect important body parts such as the eyes or abdomen, but also of a vegetative response component (increasing i.a. heart rate, blood pressure and skin conductance). The reflexive eye-lid closure, mediated by contraction of the musculus orbicularis oculi, may be considered as its most stable and most easily quantified component (Blumenthal et al., 2005). The primary acoustic startle circuit consists of only three synapses. Auditory input is transmitted via the cochlear root neurons to the nucleus reticularis pontis caudalis (nRPC) and further to the facial motor nucleus (Davis et al., 1999). Startle responses can be modulated by a variety of factors, such as attention or emotion, impacting on the startle reflex at the level of the nRPC that receives input from several central structures, such as the limbic system (e.g. central amygdala), bed nucleus of the stria terminalis (Davis, 2006), periaqueductal gray matter or pedunculopontine tegmental nucleus (PPTg) (Koch, 1999).

Thus, the startle reflex is not only part of the organism's protective systems, but also represents an indicator for the activation of central defense networks (Lang et al., 2000).

2.1.2 Prepulse Inhibition of Startle

In the prepulse inhibition of startle paradigm, a weaker, non-startling stimulus precedes the startle eliciting stimulus by a short interval (30 – 500 ms) (Koch, 1999). It is assumed that the processing of the prepulse is protected at the expense of the processing of the startle stimulus, resulting in a reduced startle reflex magnitude (Graham, 1975). Thus, prepulse inhibition can

be considered as a model for sensorimotor gating (Braff and Geyer, 1990). The extent of PPI would then reflect the capability to protect appropriate information processing. In line with this, greater PPI is associated with better strategy formation and execution times, most likely due to more efficient early information processing (Bitsios et al., 2006).

The acoustic prepulse is also first processed by the cochlear root neurons, further relayed via the inferior and superior colliculus and the PPTg to the nRPC (Koch, 1999), where it modulates the processing of the startle stimulus. Similar to the startle reflex itself, PPI is not fixed, but can be modulated by a variety of factors. Central projections modulating prepulse inhibition of startle converge at the nucleus accumbens septi (NAC) and are further relayed via the PPTg to the nRPC (Koch, 1999).

2.2 Hypothalamus-Pituitary-Adrenal Axis

The HPA axis can be activated by a number of stressors, however, psychosocial stressors appear to be most effective (Schwabe et al., 2008).

Several central structures (e.g. noradrenergic pathways, descending cortical and limbic pathways, such as septum, amygdala and hippocampus) project to its starting point, the paraventricular nucleus of the hypothalamus (PVN). Thus, the HPA axis can be seen as one of the most important stress pathways. From the PVN, corticotropin releasing hormone (CRH) is released into the hypophyseal-portal circulation. CRH stimulates the biosynthesis of adrenocorticotropic hormone (ACTH) in corticotrope cells of the anterior pituitary and ACTH release into the peripheral circulation. ACTH stimulates the release of glucocorticoids, in humans cortisol, from the zona fasciculata in the adrenal cortex. Glucocorticoids exert a fast negative feedback at all levels of the HPA axis (Papadimitriou and Priftis, 2009).

2.2.1 Cortisol

Cortisol, released over the course of minutes after a stressful event (Sapolsky et al., 2000) mobilizes stored energy (increasing blood glucose concentration), increases vascular tone (e.g. by sensitization of adrenergic receptors) and induces moderate water retention, thus increasing energy supply to the muscles. It also modulates immune responses and behavior (e.g. reduction of feeding or reproductive behavior).

Cortisol modulates the activity of central structures such as amygdala, hippocampus or ventral prefrontal cortex. Thus, it is not surprising that it can modulate numerous cognitive functions, such as attention, perception, memory, and emotional processing (Erickson et al., 2003). For example, the impact of cortisol on memory processes has received considerable attention. But also, cortisol induces frontal lobe dependent deficits of working memory (Lupien et al., 1999; Young et al., 1999) and increases fear potentiated startle, possibly by modulating amygdala activity (Grillon et al., 2006).

2.2.2 Fast (non-genomic) Effects of Cortisol

According to the classical view, "the bulk" of glucocorticoid effects are mediated via a genomic mechanism (Sapolsky et al., 2000). This process involves several steps until the gene product is expressed and available to take effect in the organism. Even the first step, glucocorticoid dependent transcription, results in detectable mRNA only 7.5 minutes after addition of the steroid 'in vitro' (Groner et al., 1983). Thus, genomic effects cannot be observed before an interval of several minutes has passed. It is generally assumed that effects cannot be observed before an interval of approximately 15 minutes has passed, although commonly effects are expected in the range of hours.

Results that point towards a non-genomic mechanism behind steroid hormone mediated effects have been published many years ago (Spach and Streeten, 1964; Edwardson and Bennett, 1974). However, only recently have the fast, probably non-genomic effects of steroid hormones (i.e. effects that may be observed within seconds) moved into the focus of research (Joels, 1997). Of special interest, such effects have also been demonstrated for neuronal cells. 'In vitro' studies demonstrated glucocorticoid effects in hippocampal tissue, where they increase glutamate content, glutamate release probability and subsequently glutamatergic neurotransmission (Venero and Borrell, 1999; Karst et al., 2005; Olijslagers et al., 2008) or inhibit activity of GABAergic neurons (Stromberg et al., 2005). These effects are, as a consequence of their very rapid onset, assumed to be mediated via a non-genomic mechanism.

2.2.3 Impact of Cortisol on the Startle Reflex and PPI

It has repeatedly been assumed that cortisol impacts on the startle reflex. For example, startle eye blinks are increased at evening, when cortisol levels are low, and decreased at morning (Miller and Gronfier, 2006). It should be noted however, that changes in alertness may influence the results when comparing responses during evening and morning test sessions. The oral application of cortisol affects startle eye blinks in a biphasic pattern, with low doses (5 mg) increasing and high doses (20 mg) decreasing startle eye blinks (Buchanan et al., 2001), matching results of circadian startle magnitude variations. Animal studies indicate that early life stress, sufficient to alter diurnal cortisol secretion, also affects startle eye blinks (Sanchez et al., 2005).

Animal studies also demonstrated an impact of cortisol on PPI. Both chronic exposure to high levels of corticosterone (Ingram et al., 2005) and intracerebroventricular infusion of corticotropin releasing factor (Conti et al., 2002), sufficient to induce endogenous adrenal corticosterone release (Campbell et al., 2004), reduce PPI in mice or rats, respectively. In humans, a reduced prepulse inhibition can be observed in response to acute physiological (Johnson and Adler, 1993; Ermutlu et al., 2005) and psychological (White and Yee, 1997) stress (evidence from studies investigating the P50 component of event related potentials). A reduced prepulse inhibition of startle has been observed during threat conditions (Grillon and Davis, 1997), and in posttraumatic stress disorder (PTSD) patients (Ornitz and Pynoos, 1989; Grillon et al., 1996), a disorder that has been associated with HPA abnormalities. Studies investigating the impact of exogenous cortisol application on PPI in humans are missing so far.

Thus, in Study I, the impact of exogenous cortisol application on startle and PPI was investigated with a focus on fast, probably non-genomic, cortisol effects in a single-blind placebo controlled study. Eye blink responses to startle stimuli (measured by electromyogram (EMG) of the musculus orbicularis oculi) were investigated in 30 healthy male volunteers. Participants were randomly assigned to two groups, each receiving two infusions (1 mg cortisol and 1 mg placebo infused over 7 minutes) in counterbalanced order. The experiment consisted of 2 parts with 4 blocks (‘baseline’, ‘infusion’, ‘post 1’, and ‘post 2’), with infusions of cortisol or placebo being applicated during block 2 of each part. PPI tests were run during the last 5 minutes of each block (minute 3 to 7). During each block, participants received twenty startle probes (50 ms, white noise, 105 dB(A), instantaneous rise time), half of which were preceded by a weak prepulse-tone (50 ms, 440 Hz, 60 dB(A), 10 ms rise/fall time, SOA

120 ms). The stimuli were presented in counterbalanced order with a randomized inter-startle interval (ISI) of 10 to 14 seconds.

For the application of cortisol and placebo, a remote controlled covert infusion paradigm was employed that resulted in elevations of serum cortisol levels comparable to a moderate stress event without participants being aware of start, end or nature (cortisol or placebo) of the infusion. The intravenous (IV) application of cortisol ensures a close relationship between the time of application and the presence of cortisol at the receptors as well as a close relationship between the infused amount of cortisol and available amount of cortisol in the organism. Since cortisol is directly infused into the blood stream and rapidly distributed in the body, this paradigm allows for an analysis of the time course of the observed effects and interpretation of the results in terms of genomic versus non-genomic mechanism of action.

The infusion of 1 mg cortisol resulted in a rapid reduction of PPI, with its maximum at 20 minutes after administration, and PPI returned to baseline after another 20 minutes. Startle magnitude in the absence of a prepulse was not affected. Contrasts to 'baseline' data indicated that significant PPI effects of cortisol administration were already present during the 'infusion' block. This rapid onset implies that the effect of the intravenous cortisol application is probably mediated by a non-genomic mechanism.

The results indicate that the previously observed effects of stress on sensorimotor gating may (also) be mediated by glucocorticoids. The disruption of sensorimotor gating by the stress hormone cortisol may serve the processing of intense and potentially dangerous startling stimuli. Thus, disrupted PPI in the presence of cortisol may allow for more adaptive processing of the stimulus that is eliciting a protective startle reaction, and thus may improve the ability to automatically protect important body parts (such as the eyes, back of the neck, or abdomen) from potential danger (Fendt et al., 2001).

Startle magnitude was not affected by the intravenous application of cortisol. This result appears to be in contrast with the known effects of orally applicated cortisol on startle magnitude. It has been shown that the oral application of larger doses of cortisol, i.e. doses that are sufficient to fully occupy central nervous system corticosteroid receptors (20 mg), results in decreased startle magnitude, while the oral application of smaller doses of cortisol (5 mg) results in an increased startle magnitude (Buchanan et al., 2001). The fact that startle magnitude was not affected by the cortisol infusion in Study I may be explained by the different application route. While only slow, presumably genomic, effects of cortisol can be observed after oral administration, Study I was designed to investigate the fast, presumably

non-genomic, effects. Also, since different doses of cortisol appear to exert opposing effects on startle magnitude, differences of published results to Study I may be explained by dosage related effects.

While cortisol applications that result in complete occupation of CNS corticosteroid receptors appear to reduce startle magnitude, and smaller doses appear to increase it, the effect of cortisol depletion on the startle reflex has not been investigated before. Therefore, in Study II the effect of reduced serum cortisol levels on startle eye blink magnitude was investigated in a single-blind, placebo-controlled design. Eye blink responses to startle stimuli (measured by EMG) were investigated in 50 healthy volunteers. Participants received six acoustic startle stimuli (50 ms, white noise, 105 dB(A), instantaneous rise time, ISI 9 s). Cortisol levels were artificially reduced by inhibiting cortisol synthesis with metyrapone. 25 participants received oral metyrapone (1500 mg), while 24 controls received oral placebo.

Metyrapone is an inhibitor of steroid 11β-hydroxylase. The inhibition of this enzyme blocks endogenous adrenal corticosteroid synthesis by inhibiting the conversion of the inactive precursor 11-deoxycortisol to cortisol (Haynes Jr, 1990), resulting in decreased cortisol levels in humans (Fiad et al., 1994; Rotllant et al., 2002; Hagendorf et al., 2005; Otte et al., 2007). Furthermore, metyrapone inhibits the 11-β-hydroxysteroiddehydrogenase type-1 enzyme (11-β-HSD-1) that converts inactive cortisone into active cortisol in the central nervous system (Raven et al., 1995).

The oral application of metyrapone resulted in a significant increase of startle eye blink responses. This effect may be due to differential occupation of CNS corticosteroid receptors during cortisol depletion, and application of small or large doses of cortisol. However, the effect may also be mediated by CNS mechanisms controlling cortisol feedback, since cortisol depletion results in increased levels of CRH that has been demonstrated to increase startle magnitude (Liang et al., 1992). Further research is needed to clarify the underlying mechanisms.

2.3 Baroreflex

In order to maintain homeostasis, input from peripheral afferents (baroreceptors, chemoreceptors, nociceptors, muscle afferents) and central nervous system afferents is integrated at the nucleus tractus solitarii (NTS), constantly modulating the activity of the ANS outflow (Dampney et al., 2002).

The reflexive adaptation of ANS outflow to altered baroreceptor activity, for example during orthostatic challenge or locomotion, is termed baroreflex. This control mechanism prevents excessive blood pressure fluctuations, maintains a stable cardiac output, secures a pressure head to drive capillary flow at rest (Nosaka, 1996) and permits a controlled increase of cardiovascular activity during exercise.

2.3.1 Physiology of Baroreceptors

Baroreceptors are located in arterial vessel walls of the aortic arch and the carotid sinus (the high pressure system), but also in pulmonary vessel walls and in the atria and ventricles (the low pressure system; baroreceptors in the ventricles respond to alterations in ventricular preload, afterload and contractility). Baroreceptors are stretch sensitive receptors, and therefore blood pressure changes are sensed indirectly via pressure related vessel deformation. At normal blood pressure, baroreceptors are activated by changes in vessel distension. However, they can also be activated by vessel collapse under extreme circumstances. Changes in vessel diameter can be phasic (e.g. due to orthostatic manoeuvres or during a pulse wave) or static (e.g. due to higher or lower blood volume). In general, the firing rate of baroreceptors increases with increasing vessel stretch and decreases vice versa (Dembowsky and Seller, 1995). Baroreceptor subtypes differ in several characteristics, such as minimum threshold and maximum saturation transmural pressure, initial and steady state firing frequency, slope of the stimulus-response curve, or adaptation to different pressure levels (Dembowsky and Seller, 1995). Afferent signals are transmitted via myelinated A-type fibres with fast signal transmission (10-35 m/s) and higher discharge rates that are more sensitive to rapid/pulsatile pressure fluctuations or unmyelinated C-fibres with slow signal transmission (<2.5m/s) and lower discharge rates that are more sensitive to prolonged/static pressure fluctuations (Persson et al., 1989).

Arterial baroreceptor afferents contain more myelinated A-fibres than cardiopulmonary afferents and are more sensitive to rapid/pulsatile fluctuations as they may occur during fast adaptation to changing functional demands and even during a single pressure wave (Persson et al., 1989). Arterial blood pressure may have to adapt quickly in order to suit different demands during different functional states of the organism (“allostasis”), e.g. during combat or at rest. Thus, arterial baroreceptor function may quickly adapt to a new operating point, about which blood pressure is then regulated.

Cardiopulmonary baroreceptor afferents consist mainly of unmyelinated C-fibres (Persson et al., 1989). They are more responsive to prolonged/static pressure changes, respond slower and are less adaptive than the arterial baroreceptors (Dembowsky and Seller, 1995). Cardiopulmonary baroreceptors sense fluctuations in central venous pressure that should (in comparison to arterial blood pressure) remain rather constant and are rather determined by factors affecting long-term blood pressure regulation and volume homeostasis. In line with this, cardiopulmonary baroreceptor activity affects renal resistance vessels rather than skeletal muscle resistance vessels (Thoren, 1979).

Thus, cardiopulmonary baroreceptors may also play a role in maintaining blood pressure at the level about which arterial baroreceptors operate.

Afferents from the baroreceptors travel along with the Nervus vagus (Xth cranial nerve) and the Nervus glossopharyngeus (IXth cranial nerve), to reach the NTS in the medulla oblongata (Jänig 2006).

The NTS constitutes a major integrative center for viscerosensory input (Degtyarenko and Kaufman, 2006). At the NTS, excitatory and inhibitory afferent input to the baroreflex pathway is integrated with input from other peripheral afferents (chemoreceptors, nociceptors, or muscle afferents) and central nervous system afferents (Potts, 2002). Blood pressure and heart rate are then modulated via the parasympathetic and sympathetic nervous system to meet current demands to the cardiovascular system.

The parasympathetic activity is modulated via projections from the NTS to the nucleus ambiguus (NA). From the NA vagal preganglionic neurons (vagal cardiomotor neurons) innervate the heart (Dembowsky and Seller, 1995; Dampney et al., 2002).

The sympathetic nervous system is modulated via projections from the NTS to the caudal ventrolateral medulla (CVLM). Activation of the CVLM results in inhibition of the rostral ventrolateral medulla (RVLM) (that otherwise is tonically activated by the CVLM). The RVLM finally innervates preganglionic sympathetic neurons in the intermedio-lateral cell column (IML) of the spinal cord and thus modulates sympathetic outflow (Aicher et al., 2000; Wang et al., 2001; Dampney et al., 2002).

In short, blood pressure increases will increase baroreceptor activity, resulting in increased parasympathetic activity and reduced sympathetic activity, thereby reducing heart rate (predominantly determined by parasympathetic activity) and blood pressure, thus finally reducing baroreceptor activity and closing the feedback loop.

The NTS also projects to higher CNS structures, such as the limbic system, hypothalamus and thalamus, and the locus coeruleus (LC), which is involved in pain processing, emotion, and regulation of higher cognitive-motor functions (Grindstaff et al., 2000; Henderson et al., 2004; Kimmerly et al., 2005).

In line with this, arterial baroreceptor activity influences electroencephalogram (EEG) activity (Mini et al., 1995; Vaitl et al., 1996), pain perception (Mini et al., 1995; Edwards et al., 2001; Edwards et al., 2003; McIntyre et al., 2006; Edwards et al., 2008), psychomotor reaction times (Edwards et al., 2007; McIntyre et al., 2008b), and memory processes (Moor et al., 2005).

Cardiopulmonary baroreceptor activity may have similar effects, as demonstrated for pain processing (Randich and Maixner, 1984; Sheps et al., 1992; D'Antono et al., 2000) or EEG activity (Vaitl et al., 1996). However, findings are not undisputed since other studies found no impact of cardiopulmonary baroreceptors on pain perception or reaction times (McIntyre et al., 2007; McIntyre et al., 2008a).

2.3.2 Impact of Baroreceptor Activity on the Startle Reflex

As mentioned above, increasing baroreceptor activity inhibits several central processes and among these also processes that are relevant for the protection of the organism, such as the processing of painful stimuli. Importantly, increased arterial baroreceptor activity also decreases startle responsiveness (Nyklicek et al., 2005; Schulz et al., 2009a; Schulz et al., 2009b; Schulz et al., 2009c).

Similar effects have been found for stimulation of cardiopulmonary baroreceptors, although the published literature is far less univocal. Thus, it could be assumed that cardiopulmonary baroreceptors also impact on the startle reflex. However, literature concerning this question is still lacking, one study with protracted head-up/head-down tilt (Vaitl et al., 1996) remained inconclusive.

Therefore, in Study III, the effects of arterial and cardiopulmonary loading/unloading on the startle eye blink reflex and psychomotor reaction times were investigated in a within-participant design. Eye blink responses (measured by EMG), and psychomotor reaction times (measured by button pushes) to startle stimuli were assessed in 12 healthy male volunteers. The experiment consisted of 8 blocks. Participants received 30 acoustic startle stimuli (50 ms, white noise, 105 dB(A), instantaneous rise time, ISI 5 – 7s) during each block, with latencies

of either 230 ms (arterial baroreceptor loading) or 530 ms (arterial baroreceptor unloading) after the cardiac R-wave in randomized order, while they were treated with a series of 5 minutes of lower body negative pressure (LBNP) at levels of 0, -10, -20, and -30 mmHg each, in randomized order. For each participant, every LBNP level was applied two times.

LBNP is a paradigm, during which a negative pressure gradient (conmpared to ambient pressure) is established around the lower limbs, most commonly in an air-tight chamber that has a vacuum pump attached to it. By application of the pressure gradient, the venous flow into the central compartment is diminished and the central venous volume is decreased, resulting in an unloading of cardiopulmonary baroreceptors. The LBNP paradigm is distinguished by little to no somatosensory, proprioceptive, vestibular or affective stimulation. LBNP has only a minor impact on mean arterial blood pressure (Hinghofer-Szalkay et al., 1996; Laszlo et al., 1998; van Hoeyweghen et al., 2001; Franke et al., 2003; Kitano et al., 2005). It can be assumed to predominantly manipulate cardiopulmonary baroreceptor activity. Also, a stepwise increase of LBNP results in an almost linear decrease of central blood volume, allowing for a controlled manipulation in dose-response fashion (van Hoeyweghen et al., 2001; Convertino et al., 2006). To investigate the effects of arterial baroreceptor load on the startle reflex, the presentation of startle stimuli was time-linked to the cardiac cycle. As summarized by Edwards et al. (Edwards et al., 2009), the pulse pressure wave reaches the aortic baroreceptors after R+90ms and the sinus carotis at R+140ms; the baroreceptors begin firing at the onset of the systolic upstroke, peak 100 ms later (R+240ms for sinus baroreceptors) (Angell James, 1971; Coleridge et al., 1987) and end firing after an additional 150 ms. These intervals remain constant, if the ejection dynamics during the systolic phase are not substantially altered. After the pulse wave has passed the baroreceptors at the sinus carotis (R+390), baroreceptor activity quickly decreases and baroreceptor afferents are mostly silent until the onset of the next pulse wave (Chapleau and Abboud, 1987; Koley et al., 1989; Seagard et al., 1990). Due to the quick silencing of baroreceptor activity (Angell James, 1971; Coleridge et al., 1987), a stimulus that is presented after the pulse wave transit should fall into a phase of similar low baroafferent nerve traffic, independent of whether the diastolic phase is going to last longer or shorter. Thus, in Study III, stimuli were presented with specific latencies (230 ms and 530 ms) after hardware-based R-wave detection. Latencies were chosen to synchronize stimulus presentation with baroreceptor loading or unloading, respectively.

The results demonstrate that unloading of cardiopulmonary baroreceptors as well as unloading of arterial baroreceptors increases startle eye blink responsiveness in a continuous

dose-response fashion (the stronger the unloading, the higher the startle magnitude). However, unloading of either group of baroreceptors did not affect psychomotor reaction times.

This is the first study to indicate increased startle eye blink responses to acoustic noise stimuli during unloading of cardiopulmonary baroreceptors. The applied maximal LBNP corresponds with a blood loss of about 500 ml from the central compartment. Stronger startle eye blinks during baroreceptor unloading may provide an adaptive mechanism to a hostile environment, because during states of blood loss stress, which may for example occur as a result of a predator attack, the support of protective mechanisms enhances the organism's chance of survival. Furthermore, the results suggest that 'archaic' automatic brainstem-based protective responses may be favored during haemorrhagic challenge, since voluntary psychomotor reaction times stayed unaffected. Thus, cardiopulmonary baroreceptors may play a role in adapting response strategies during specific stressful circumstances.

Chapter III - Cortisol rapidly disrupts Prepulse Inhibition in healthy Men

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3.0 Abstract

Stress is known to affect sensorimotor gating (measured with prepulse inhibition of startle, or PPI), possibly improving perception of threat signals at the expense of other input during states of arousal. Stress also induces a variety of autonomic nervous system and endocrine responses, such as an activation of the hypothalamic-pituitary-adrenal axis. The latter will result in the release of the stress hormone cortisol which is known to exert rapid and sustained action on several CNS processes. Since previous studies have not clarified whether and which stress response components may mediate effects on sensorimotor gating, this study asked whether a link may exist between cortisol and sensorimotor gating. We tested whether cortisol may affect PPI by assessing PPI before, during, and after non-stressful, covert 1 mg IV cortisol infusions in 27 healthy men in a single-blind and placebo-controlled within-subject design.

Cortisol induced a rapid reduction of PPI, with its maximum at 20 min after administration, and PPI returned to baseline after another 20 min. Startle magnitude in the absence of a prepulse was not affected. This rapid effect of the IV cortisol infusions is probably mediated by a non-genomic mechanism. We conclude that stress effects on sensorimotor gating may be mediated by glucocorticoids. The disruption of sensorimotor gating by the stress hormone cortisol may serve the processing of intense and potentially dangerous startling stimuli.

Keywords: Non-genomic cortisol effects; Prepulse inhibition; Sensorimotor gating; Startle; Stress

3.1 Introduction

Stress profoundly impacts on stimulus processing. It is known to reduce prepulse inhibition of startle (PPI), a measure of sensorimotor gating. The PPI paradigm involves a weaker prepulse stimulus preceding a louder startle eliciting stimulus activating an inhibitory function to protect processing of the prepulse (Graham, 1975). Disrupted PPI has been reported in response to acute stress in healthy individuals (Grillon and Davis, 1997), and in adults (Grillon et al., 1996) and children (Ornitz and Pynoos, 1989) with posttraumatic stress disorder (PTSD). Disrupted PPI can also be observed in animals after stress (Leitner, 1986). A disrupted PPI during states of stress and arousal may indicate improved threat and startle signal perception. Thereby, a disrupted PPI would support stronger reflexive motor responses, such as the eye-lid closure, and contribute to enhanced automatic defensive behavior protecting against the impact of potentially harmful stimuli.

Stress is known to induce a variety of responses, such as activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, the latter resulting in an increased level of circulating glucocorticoids (cortisol in humans, corticosterone in mice and rats). Glucocorticoids have been identified as modulators of PPI in animal studies: chronic exposure to high levels of corticosterone significantly reduces PPI in mice (Ingram et al., 2005). Acute intracerebroventricular infusion of corticotropin-releasing factor, sufficient to induce endogenous adrenal corticosterone release (Campbell et al., 2004), significantly reduces PPI in rats (Conti et al., 2002).

Recently, *in vitro* studies reported rapid non-genomic effects of the brain mineralocorticoid receptor, one of the two receptors mediating corticosteroid responses in the brain (de Kloet et al., 2008). Corticosteroids were found to non-genomically decrease the activity of hippocampal GABAergic neurons (Stromberg et al., 2005) which are crucial for successful sensorimotor gating (Adler et al., 1998) and counteract GABAergic inhibitory control by rapidly increasing glutamate release from the synapses (Venero and Borrell, 1999; Karst et al., 2005; Olijslagers et al., 2008), potentially contributing to reduced PPI.

Based on the recently described non-genomic effects of cortisol on neurotransmission, we hypothesized that cortisol would rapidly disrupt PPI in healthy humans. Cortisol was administered in a concentration that resembles the endogenous cortisol secretion in response to a moderate stress event. We employed a single-blind placebo-controlled within-subject design, and assessed PPI before, during, and after intravenous (IV) infusions of cortisol or placebo.

3.2 Methods and Materials

3.2.1 Participants

Participants were recruited via the campus newsletter and offered a monetary incentive for participation. After routine medical examination on a separate day, 30 male volunteers were randomly assigned to receive either cortisol first, then placebo, or vice versa. Exclusion criteria were any acute or chronic illness, substance abuse (including nicotine at any dose), age below 18 or above 40, a body mass index below 18 or above 30, habitual listening to loud music, exposure to industrial noise (without proper ear protection) during the last 3 weeks, usage of contact lenses, or complete absence of startle eyeblinks (non-responders). Three participants were startle non-responders and thus were not included in the analysis. Of the remaining 27 participants (age: 26.0 years, SEM = 0.75; BMI: 24.3 kg/m², SEM = 0.57), 13 received cortisol first. All participants were asked to refrain from caffeine for 24 hours before the experiment, to sleep sufficiently, and not to perform sports or eat heavy meals on the day of the experiment.

All participants gave their written informed consent. The study was approved by a community-based ethical committee (Landesärztekammer Rheinland-Pfalz).

3.2.2 Procedure

Experiments took place between 2 pm and 5 pm. Participants were seated in a quiet room with dim light. After cannulation of a cubital vein (18 G venflon, vasofix-safety, B.Braun Melsungen Co., Melsungen, Germany), participants were allowed to rest for 45 min. After the resting period, electrodes and headphones were attached and the experiment was started. The experiment consisted of two parts (28 min each, part 2 following part 1 immediately) with four blocks of 7 min duration (‘baseline’, ‘infusion’, ‘post 1’, and ‘post 2’). PPI tests were run during the last 5 min of each block (minute 3-7). After the experiment, participants were decannulated and received their monetary reward.

3.2.3 Intravenous (IV) Drug Administration

We employed a constant background sodium-chloride (NaCl 0.9%, B.Braun Melsungen Co., Melsungen, Germany) infusion (120 ml/h) that was reduced for the cortisol (Hydrocortison 100, Rotexmedica, Trittau, Germany) and placebo (NaCl 0.9%) target infusions to keep flow constant at any time. Infusions were controlled by a CPU-operated modular Fluid-Management System (B.Braun Melsungen Co., Melsungen, Germany) located in an adjacent room. Because of absence of visual or auditory infusion-related cues, participants were not able to detect infusion onset or offset.

During the second block ('infusion') of each part, 1 mg cortisol or placebo was infused over the 7 min duration in a single-blind design. Thus, every participant received cortisol and placebo, while the order of application was counterbalanced across the participants.

3.2.4 Stimulus Presentation

PPI tests were run during the last 5 min of each block (minute 3-7). Twenty startle probes (50 ms, white noise, 105 dB(A), instantaneous rise time), half of which were preceded by a weak prepulse-tone (50 ms, 440 Hz, 60 dB(A), 10 ms rise/fall time, SOA 120 ms) were presented in counterbalanced order with a randomized inter-startle interval of 10-14 s (mean: 12 s) during each block. A constant background noise of 50 dB(A) was employed to reduce the influence of ambient noise. The experimental room was sound attenuated. All stimuli were delivered by E-Prime 1.2 software (Psychology Software Tools Inc., Pittsburgh, PA, USA) via headphones for binaural stimulation (Sennheiser Electronic GmbH & Co KG, Wedemark, Germany). The headphones for binaural stimulation covered the entire ear and further reduced ambient noise (passive noise reduction >10-40 dB(A), according to Sennheiser product information).

3.2.5 Cortisol Concentrations in Blood Plasma

In a pilot study (healthy male participants; same exclusion criteria as described in Section 2.2.1.; n = 8), we measured the amount of circulating cortisol in blood plasma in response to the 7 min infusion of 1 mg cortisol. Data revealed an increase in serum cortisol by 21.23 ± 3.60 ng/ml from 73.51 ± 7.46 ng/ml (before cortisol infusion) to 94.74 ± 9.32 ng/ml (after

cortisol infusion). This is in the range of mild to moderate stress responses in healthy volunteers (Kok et al., 1995).

3.2.6 Data Recording and Analysis

Data were recorded by Physiocorder software (Institute of Biomedical Engineering, University of Stuttgart, Stuttgart, Germany) at 1 kHz sampling rate and 16 bit resolution. Electromyogram (EMG) responses to startle stimuli were recorded with Ag/AgCl electrodes (ARBO H124SG, Tyco Healthcare, Kendall, Neustadt, Germany) at the left musculus orbicularis oculi by a Biopac MP150 system (Biopac Systems Inc., Goleta, CA, USA) (hardware band-pass filter: 10-500 Hz, notch filter: 50 Hz, software filter: 28 Hz high pass cut-off). The raw signal was rectified and integrated offline (time constant: 10 ms) with AcqKnowledge software (Biopac Systems Inc., Goleta, CA, USA). Startle responses were processed offline with a proprietary C++ based semi-automated PC program running on WinXP platform and manually confirmed by a trained psychophysiologist who was not aware of the sequence of placebo or cortisol infusion.

3.2.7 Statistical Analysis

PPI was calculated per block as percent reduction of EMG magnitude in prepulse-paired condition compared to startle-alone condition:

PPI (%) = 100 - (100 * magnitude prepulse-paired / magnitude startle-alone).

For startle and PPI data, a 2 × 4 × 2 mixed-design ANOVA was employed on the combined data set, with the within-subject factors ‘drug’ (‘cortisol’, ‘placebo’) and ‘block’ (‘baseline’, ‘infusion’, ‘post 1’, ‘post 2’), and the between-subject factor ‘order’ (‘cortisol first’, ‘placebo first’). To further investigate the time course of the effects, we calculated contrasts to ‘baseline’ data for ‘infusion’, ‘post 1’, and ‘post 2’ data.

Furthermore, since the results of the second infusion may be affected by the first infusion (differential carryover), the data acquired during the first infusion was also tested in a 4 × 2 mixed-design ANOVA with the within-subject factor ‘block’ (‘baseline’, ‘infusion’, ‘post 1’, ‘post 2’) and the between-subject factor ‘drug’ (‘cortisol’, ‘placebo’). Statistical analysis was calculated with SPSS 15 software (SPSS Inc., Chicago, IL, USA). All p-values of factors with

more than two levels were adjusted with Greenhouse-Geisser correction, but non-adjusted degrees of freedom are reported in text. A result of $p<0.05$ was considered significant.

3.3 Results

3.3.1 Startle Magnitude

Startle magnitude in the startle-alone condition habituated across blocks ($F_{(3,75)} = 16.91$; $p < 0.001$; $\eta^2 = 0.403$; main effect 'block'). Cortisol did not affect startle magnitude (main effect 'drug') nor its habituation (interaction effect 'drug' × 'block') (see Figure 3.1.A). The order of drug administration ('cortisol first' or 'placebo first') did not contribute to any interaction effect.

3.3.2 Prepulse Inhibition of Startle (PPI)

The infusion of 1 mg cortisol significantly reduced PPI ($F_{(3,75)} = 5.69$; $p = 0.001$; $\eta^2 = 0.185$; interaction effect 'drug' × 'block') (see Figure 3.1.B). Contrasts to 'baseline' data interacted with 'drug' ('cortisol', 'placebo'), revealing significant PPI effects of cortisol administration for 'infusion' data ($p = 0.018$), 'post 1' data ($p = 0.017$) and 'post 2' data ($p = 0.002$). The effect of cortisol infusion on PPI was not affected by the order of drug application, i.e. allocation of the subject to 'cortisol first' or 'placebo first' groups ($F_{(3,75)} = 1.70$; $p = 0.184$; $\eta^2 = 0.064$; interaction effect 'drug' × 'block' × 'order').

When placebo followed cortisol administration ('cortisol first' group), we could observe that PPI slowly returned to baseline values from 'infusion' to 'post 2' blocks during part 2 of the experiment, indicating the recovery of PPI.

We also analyzed the first part separately in a between groups design, due to potential effects of PPI recovery during part 2 of the experiment. A 4 × 2 mixed-design ANOVA was employed with the within-subject factor 'block' ('baseline', 'infusion', 'post 1', 'post 2') and the between-subject factor 'drug' ('cortisol', 'placebo'). The interaction of 'block' and 'drug' remained significant ($F_{(3,75)} = 3.10$; $p = 0.041$; $\eta^2 = 0.110$).

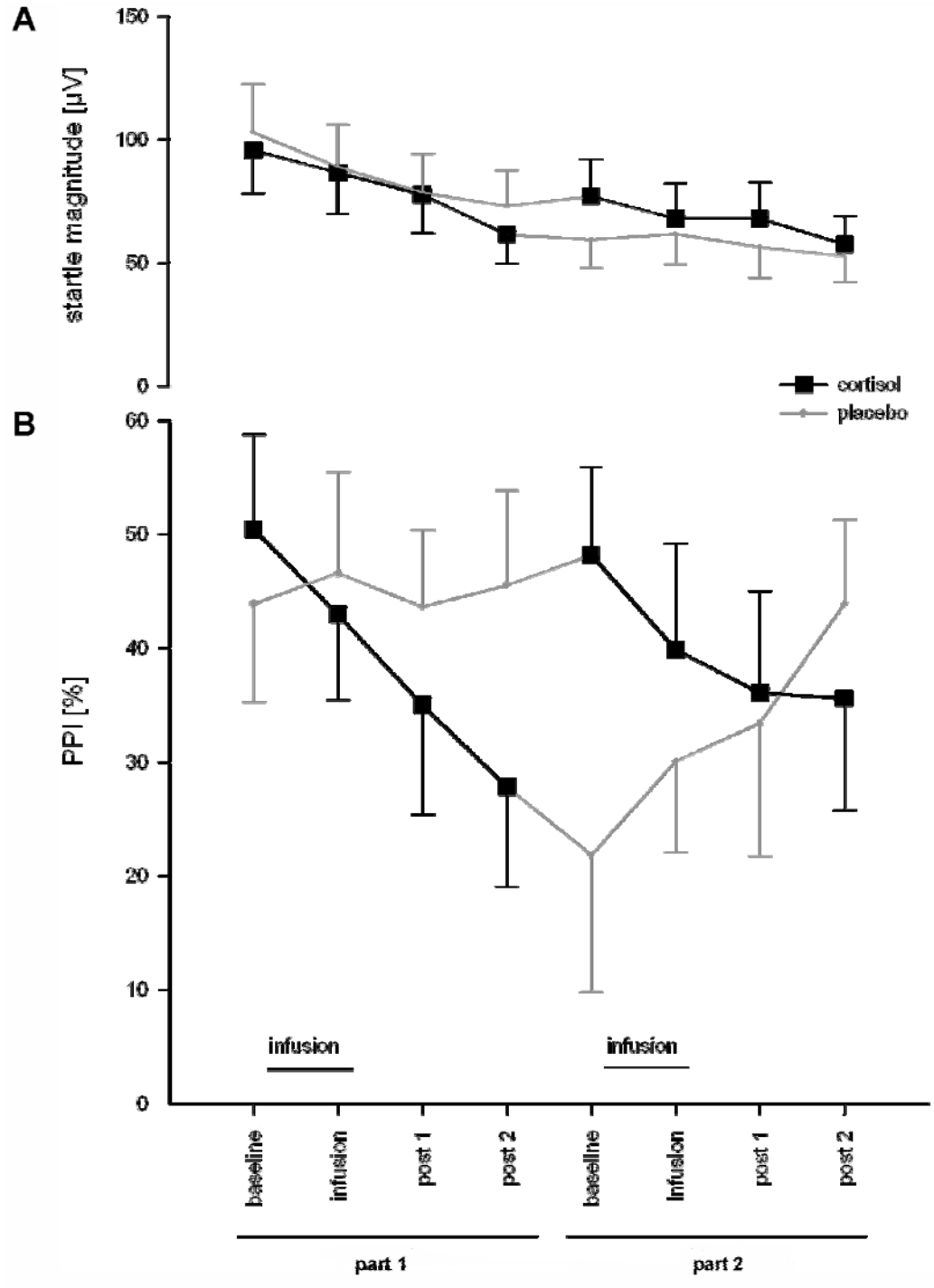

Figure 3.1 Startle magnitude in µV (A) and prepulse inhibition (PPI) of startle in percentage of reduction (B) before, during, and after IV infusion of 1mg cortisol and placebo (0.9% NaCl) in healthy young men. Lines represent two experimental groups with counterbalanced order of drug administration (i.e. one group receiving first cortisol then placebo, the other group receiving first placebo then cortisol). Black: cortisol; grey: placebo. Horizontal bars indicate the duration of infusion, lasting 7 min. Each treatment condition (part 1, part 2) lasted 28 min and was separated into four blocks of 7 min. PPI was tested during the last 5 min of each block. Data represent mean ± SEM.

3.4 Discussion

This is the first human study to report rapid effects of low dose exogenous IV cortisol infusions (mimicking the cortisol response to a mild to moderate stressor) on PPI of startle eyeblink. The effect was unrelated to startle eyeblink magnitude. The disruption of PPI suggests a generally diminished capacity for sensorimotor gating, and a resultant increase in processing of the threatening startle stimulus, in the presence of moderate elevations of the stress hormone cortisol. We found that the disruptive effect of cortisol on PPI occurs within minutes. It reached its maximum about 20 min after infusion and PPI responsiveness returned to baseline values after another 20 min, as could be observed in the group that received cortisol first. In the 'cortisol first' group, the similarity in the level of PPI between the 'post 2' block of part 1 (cortisol application) and the immediately-following 'baseline' block of part 2 (placebo application) may indicate a carryover effect of residual cortisol suppressing PPI during the placebo baseline block. This effect dissipated rapidly over the course of the placebo blocks in this group, as blood cortisol would be expected to decrease. Alternatively, the subsequent increase in PPI across these blocks may have been due to a possible genomic effect of cortisol. In either case, the analysis of the first part of the experiment alone, in which 'drug' was defined as a between-subject factor, suggests that the significant impact of cortisol on PPI was not carried by the drug effects in part 2 of the experiment.

This is not the first study linking sensorimotor gating deficits to stress mechanisms in humans. EEG studies have found impaired P50 auditory gating (another frequently used measure of sensorimotor gating in healthy subjects) after physiological (Johnson and Adler, 1993; Ermutlu et al., 2005) or psychological stress (White and Yee, 1997). Deficits in PPI can be seen in healthy participants in threat-of-shock conditions (Grillon and Davis, 1997) and in adults (Grillon et al., 1996) and children (Ornitz and Pynoos, 1989) with posttraumatic stress disorder (PTSD). However, stress is known to induce a variety of responses, such as activation of the autonomic nervous system and the HPA axis, the latter resulting in an increased level of circulating cortisol in humans. Human studies separating the components which may be responsible for sensorimotor gating deficits during the stress response are missing in the published literature. This study was designed to isolate effects mediated by the stress-hormone cortisol. We used a low dose of cortisol, mimicking the physiological endogenous cortisol response to a mild to moderate stressor (Kok et al., 1995; Goldman et al., 2007). Thus, our data suggest that cortisol has the potential to induce sensorimotor gating deficits during stress.

Traditionally, cortisol effects are expected rather late (in the range of hours) after stress exposure, due to the genomic mechanism of action that was the focus of research until recently. However, acute PPI reduction observed in our study, its rapid onset, and its recovery within <60 min, suggest a non-genomic mechanism of action for the stress hormone cortisol. These rapid, non-genomic effects of cortisol are being investigated with increasing frequency, and ours is not the first study to show such effects on central nervous system functioning (Venero and Borrell, 1999; Karst et al., 2005; Stromberg et al., 2005; de Kloet et al., 2008; Olijslagers et al., 2008). Thus, our results add to existing evidence in demonstrating that cortisol can significantly, and rapidly, modulate PPI.

The reduced capacity to protect the processing of the weaker prepulse stimulus improves the processing of the stronger, potentially more stress-relevant, startle stimulus. Thus, disrupted PPI in the presence of cortisol (e.g. early in the course of a stressful event) may allow for more adaptive processing of the stimulus that is eliciting a protective startle reaction. We suggest that this improves the ability to automatically protect important body parts (such as the eyes, back of the neck, or abdomen) from potential danger (Fendt et al., 2001). Therefore, the disruption of PPI by cortisol during stress may serve to express extensive automatic protection against intense, potentially dangerous, startling stimuli, thereby fostering survival in healthy humans.

Since PPI is influenced by a variety of central nervous system structures, and cortisol may impact virtually every cell in the central nervous system, several mechanisms may account for the observed PPI deficits during stress. For example, in humans, PPI has successfully been modulated by targeting serotonergic (Mann et al., 2008) and dopaminergic activity (Oranje et al., 2004). Cortisol affects dopaminergic neurotransmission, and HPA axis activation increases dopamine concentrations in the brain (Rothschild et al., 1985; Imperato et al., 1989). Administration of exogenous corticosterone in rats enhances dopamine release in the nucleus accumbens, a brain structure with well-known modulatory effects on PPI (Piazza et al., 1996; Yamada et al., 1998). PPI is also affected by GABAergic and glutamatergic neurotransmission (Swerdlow et al., 2001), and cortisol interacts with these transmitter systems as well (Karst et al., 2005; Stromberg et al., 2005). Only recently, *in vitro* studies detected rapid non-genomic effects of the brain mineralocorticoid receptor, one of the two receptors mediating corticosteroid responses in the brain (de Kloet et al., 2008), and corticosteroids were found to rapidly decrease GABAergic activity (Stromberg et al., 2005)

and increase glutamate release from synapses (Venero and Borrell, 1999; Karst et al., 2005; Olijslagers et al., 2008).

Stress, cortisol, and sensorimotor gating deficits may also play a role in the etiology of psychopathology. For example, elevated cortisol levels (Muck-Seler et al., 2004; Keller et al., 2006; Kluge et al., 2007; Mittal et al., 2007), and impaired negative feedback control of glucocorticoids (Kiriike et al., 1988; Catapano et al., 1990; Lammers et al., 1995; Nelson and Davis, 1997), have been reported for affective disorders, schizophrenia, compulsive disorders, and other psychiatric conditions that are associated with deficits in sensorimotor gating (summarized in Braff et al., 2001).

The rapid effects of cortisol on PPI, and its rapid recovery, also have some general, practical implications for future experimental designs, such as suggesting that stress effects on sensorimotor gating should be evaluated soon after stress induction. Conversely, paradigms that employ PPI should consider the rapid disruptive effects of stress-induced cortisol release and ensure stable baseline conditions by sufficient resting intervals.

3.4.1 Limitations

This is a neuropharmacological study investigating the effect of physiological doses of an exogenous stress hormone (cortisol) on sensorimotor gating (PPI), without participants experiencing concomitant stress. This combination was realized by a computer-controlled infusion protocol which assured that subjects were not only unaware (single-blind design) about the substance (placebo or cortisol) that they received, but also about the timing, onset, and offset of the infusions (covert infusion protocol). Thus, PPI may respond differently to cortisol when subjects experience acute or chronic stress with full awareness. The current study was not designed to test this.

Assessing blood concentration of cortisol would have allowed for more detailed analysis of the pharmacodynamic relationship between cortisol and PPI. We did not collect blood or saliva samples since this may have influenced startle, startle habituation, and/or PPI. However, plasma level changes are very reliable during IV drug application, as we illustrated in our separate intervention study.

Due to the time limit of the experiment, possible later responses (occurring after 60 min) resulting from a genomic mechanism may have escaped observation. These would be in accordance with previous literature, but in this study, we focused on rapid effects of cortisol.

3.4.2 Conclusion

Our results demonstrate a rapid disruption of sensorimotor gating, measured with prepulse inhibition of startle, in healthy humans by covert IV infusion of 1 mg cortisol, an amount in the range of the cortisol response to a mild to moderate stressor. Reduced PPI of startle may assist to preserve the processing of potentially dangerous stimuli in healthy participants during stress. However, it may also indicate impaired information processing, since reduced PPI of startle was found in a variety of stress-sensitive clinical disorders.

3.5 Role of funding Source

This study was funded by the German Research Foundation (Deutsche Forschungsgemeinschaft: DFG), grant GRK 1389/1 and the Netherlands Organization for Scientific Research (NWO-DN 95-429) to the International Research Training Group (IRTG). The German Research foundation had no further role in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

3.6 Conflict of Interest

All authors declare that they have no potential conflicts of interest.

3.7 Acknowledgements

T.D. Blumenthal would like to acknowledge the support of the Sara Jo Brownlow Shearer Fund at Wake Forest University.

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Chapter IV - Endogenous Cortisol Suppression with Metyrapone enhances acoustic Startle in healthy Subjects

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4.0 Abstract

Previous human studies have shown that excess cortisol sufficient to fully occupy central nervous system (CNS) corticosteroid receptors may reduce startle eye blink. The present study tested whether cortisol depletion and the resulting reduction in activity of CNS corticosteroid receptors has the opposite effect. In a single-blind, placebo-controlled, randomized study, eye blink EMG responses to 105 dB acoustic startle stimuli were assessed in 25 healthy subjects who received oral metyrapone (1500 mg) to suppress endogenous cortisol production, while 24 controls received oral placebo. As expected, metyrapone significantly reduced saliva cortisol, indicating effective endogenous cortisol suppression. Startle eye blink responses were significantly increased in the metyrapone group. Short-term habituation of the startle reflex was not different between groups. Our results suggest that startle is enhanced during depletion of cortisol. This effect may be mediated by CNS mechanisms controlling cortisol feedback.

Keywords: Cortisol, Metyrapone, Acoustic startle, Short-term habituation

4.1 Introduction

Stress-induced release of hypothalamus-pituitary-adrenal (HPA) hormones plays an important role in human affective disorders such as depression (e.g. Varghese and Brown, 2001) and anxiety disorders (e.g. Yehuda et al., 1991), has an anxiogenic effect (Shepard et al., 2000; Grillon et al., 2007), and facilitates fear conditioning (Jackson et al., 2006). HPA derived stress hormones are known to impact startle (Davis, 2006), and a recent review (Risbrough and Stein, 2006) suggested startle methodology as a translational tool for investigating the association between stress-induced HPA system changes and affective disorders.

The startle response, a protective response shown by many species to an abrupt and intense stimulation, is potentiated during exposure to aversive, threatening stimuli and is diminished by appetitive, pleasant stimuli (Bradley et al., 1999; Grillon and Baas, 2003). Further, evidence exists (mostly animal-based) for an impact of stress hormones on startle responsiveness. Studies in rats revealed a dose-dependent increase in acoustic startle response magnitude after intracerebroventricular infusions of corticotropin-releasing hormone (CRH: Swerdlow et al., 1986; Liang et al., 1992a). This effect was blocked by pretreatment with a CRH antagonist (Swerdlow et al., 1986; Swerdlow et al., 1989; Liang et al., 1992a). In a similar fashion, blockade of negative feedback via glucocorticoid receptor (GR) antagonists increased acoustic startle responding in rats (Korte et al., 1996). After neonatal treatment with high doses of the peptide fragment $ACTH_{4\text{-}10}$ adult rats showed an increase in acoustic startle responding (McGivern et al., 1987), whereas peripheral injections of corticosterone led to a decrease in startle responding (Sandi et al., 1996). The latter effect is compatible with an inverse relationship between peripheral cortisol levels and startle, as suggested by human studies showing larger startle eye blinks during evening than morning, in contrast to diurnal cortisol levels being highest in the morning and lowest in the evening (Miller and Gronfier, 2006).

There is preliminary evidence to suggest that not only startle response magnitude, but also habituation of the startle reflex, is modulated by stress hormones. Startle habituation is the normal phenomenon of startle response attenuation following repetitive stimulation (Pilz and Schnitzler, 1996; Koch, 1999), and represents a very simple form of implicit learning (Kandel, 2000). Post-traumatic stress disorder (PTSD) is a specific type of anxiety disorder that is characterized by altered HPA axis activity with low levels of peripheral cortisol (Kellner and Yehuda, 1999; Oquendo et al., 2003), high levels of CRH (Bremner et al., 1997; de Kloet et al., 2008), and evidence of nonhabituated exaggerated startle reactions (Shalev et al., 2000; Garrick et al., 2001; Ladwig et al., 2002). The common physiological background of these findings may be excessive central release of CRH, acting on the bed nucleus of the stria terminalis, to promote anxiety initiated by non-specific stimuli (Marshall and Garakani, 2002).

There are only a few human studies published that have investigated the effect of stress hormones on startle. A biphasic effect of escalating doses of oral hydrocortisone (cortisol) was found, with significantly diminished startle eye blink magnitudes after 20 mg compared to 5 mg cortisol (Buchanan et al., 2001). An oral 20 mg cortisol dose will be readily absorbed in the gastrointestinal tract, and is higher than the normal human average total daily

endogenous cortisol production (Weitzman et al., 1971). Thus, the diminished startle eye blink reactivity found by Buchanan et al. (2001) occurred when central GR and mineralocorticoid receptors (MR) were likely to be completely occupied with their natural active ligand, cortisol. Based on this assumption, it may be expected that the opposite condition, that is cortisol depletion and the resulting reduction in activity of central GR and MR receptors, will result in the opposite effect, namely an enhancement of startle. This, however, has never been reported.

Thus, the current study was undertaken to investigate the effect of pharmacological suppression of endogenous cortisol by metyrapone on human startle responsiveness. Metyrapone inhibits the conversion of the inactive precursor 11-deoxycortisol to cortisol by the adrenal enzyme 11-beta-hydroxylase (Haynes Jr, 1990), and leads to reduced circulating cortisol, increased CRH and ACTH, and an accumulation of 11-deoxycortisol (Fiad et al., 1994; Rotllant et al., 2002; Hagendorf et al., 2005; Otte et al., 2007). This substance not only exerts effects upon adrenocortical steroid synthesis, but also inhibits the 11-β-hydroxysteroiddehydrogenase type-1 enzyme (11-β-HSD-1) that regenerates active cortisol from inactive cortisone in the central nervous system (Raven et al., 1995). This induces an increase of steroids proximal to the enzyme block, accompanied by cortisol depletion and a reduction of MR and GR receptor activity that is followed by an increase of CRH and adrenocorticotropic hormone (ACTH) due to the impaired negative cortisol feedback (Jahn et al., 2003). Based on previous human (Buchanan et al., 2001) and animal (Sandi et al., 1996) data on exogenous excess cortisol, which showed a decrease in startle magnitude with higher cortisol levels, we expected endogenous cortisol suppression with metyrapone to result in enhanced startle reactivity. Since HPA hormones may have an effect on startle habituation, we chose a between-subject design which is less sensitive to the effects of treatment order (e.g. treatment vs. placebo first) than the more powerful cross-over design, but which allowed us to address whether short-term habituation of startle is affected by metyrapone.

4.2 Methods

4.2.1 Participants

Fifty-four healthy volunteers were recruited at Trier University by announcements posted on a web page, with follow-up emails. Twenty-seven subjects were randomly assigned to either a

metyrapone group (20 females and 7 males, mean age: 24, range: 20-30 years) or a placebo group (17 females and 10 males, mean age: 26, range: 19-40 years). Exclusion criteria were chronic physical or mental disease, intolerance to lacteal products, allergies to any pharmaceutical product, use of any pharmaceuticals, use of nicotine or tobacco on a regular basis, a body-mass-index above 30 or below 18 kg/m^2, pathologic laboratory findings (hemogram, renal values, liver values), illicit substance use within the last two years, acute medical or psychiatric symptoms, or participation in a pharmaceutical study within the last three months. All female participants reported the regular use of oral contraceptives, but were still tested for pregnancy with a commercial urine kit. Ethical permission was obtained from the local ethics committee (in accordance with the Declaration of Helsinki) and volunteers gave informed consent before attending the trial for moderate monetary incentive.

4.2.2 Manipulation of the HPA-Axis

Participants entered the laboratory at 8.00 a.m.. At 8.30 a.m. they received either 750 mg metyrapone or placebo together with a snack which included dairy products, to reduce the likelihood of gastrointestinal metyrapone side effects. A further dose of 750 mg was administered together with a sandwich lunch at 12.00 p.m.. Previous studies have shown significantly reduced plasma cortisol levels at about 6 hours after intake of the initial metyrapone dose (Young et al., 1997; Broadley et al., 2005). Subjects were not allowed to leave the laboratory until the end of the experiment, but were free to engage in low arousing activities like reading. They refrained from smoking and consuming caffeinated beverages during the session. All subjects tolerated the metyrapone treatment well, and reported no side effects.

4.2.3 Collection and Determination of Salivary Cortisol

Saliva samples for a diurnal cortisol profile (6.30 a.m., 6.45 a.m., 7.00 a.m. 7.15 a.m., 7.30 a.m., 8.00 a.m., 11.00 a.m., 13.00 p.m., 15.00 p.m. and 20.00 p.m.) were collected on two consecutive days one week before the test day, and an additional two samples were taken on the test day immediately before and after the eye blink protocol (see below). Saliva was collected by the subjects themselves using standard Eppendorf tubes (1.5 ml, Eppendorf, Hamburg; Germany). Cortisol data of one participant was lost due to incomplete saliva

sampling. Saliva samples were stored at -20°C and analyzed for cortisol with a time-resolved fluorescence immunoassay as repeat determination (Dressendörfer et al., 1992). Intra- and interassay variability were below 5 % and 12 %, respectively.

4.2.4 Procedure

At 13:30 p.m., participants completed the German version of the state-trait-anxiety-inventory (STAI, Laux et al., 1981) to test for differences in anxiety between the groups. At 14:00 p.m., 2 h after administration of the second dose of metyrapone or placebo, the subjects were prepared for electromyographic (EMG) eye blink recording (electrode attachment and headphone placement). They were told that they would hear brief, intense tones, and were instructed to sit quietly during the recording period, and neither speak nor move. They were asked to look in the direction of a fixation cross which was attached to the opposing wall. After collection of a saliva sample, eye blink responses to sudden noise bursts (white noise, 105 dB(A), 50 msec, instantaneous rise time) presented binaurally via headphones (Sennheiser Inc.) were recorded by EMG (see below). The acoustic startle protocol consisted of six startle probes with a fixed inter-trial interval (ITI) of 9 s. The startle session lasted about 1 min, and was followed by collection of a saliva sample. After the startle experiment, participants attended an implicit learning task that was unrelated to the previous experiment. The results of that experiment are reported elsewhere.

4.2.5 EMG-Data Acquisition and Analysis

The eye blink response was measured by recording EMG activity of the orbicularis oculi muscle beneath the left eye, using standard procedures (Blumenthal et al., 2005). The raw EMG-signal was recorded with a BIOPAC MP 150 at a sampling rate of 1000 Hz with a notch filter of 50 Hz, and a band-pass filter of 28 to 500 Hz. Data were rectified and integrated with a time constant of 10 ms.

EMG blink responses were identified by means of proprietary computer-assisted scoring software using the largest peak in the time interval of 50-150 ms after the startle stimulus onset, relative to a stable baseline 50 ms before startle-stimulus onset. All trials were analyzed with respect to zero-response (no visible startle response) and artifacts (i.e. excessive background noise and voluntary or spontaneous eye blinks at or near the startle stimulus

onset). Startle magnitudes were computed, including zero-responses and excluding artifacts. Five of the 54 participants were excluded because they showed no reliable startle response due to absence of eye blink responses or excessive blinking. The final sample size consisted of 49 subjects, with 25 subjects in the metyrapone group (18 females and 7 males, mean age: 24, range: 20-30 years), and 24 subjects in the placebo group (15 females and 9 males, mean age: 26, range: 19-40 years).

4.2.6 Statistical Analysis

For both, metyrapone and placebo group, cortisol data of the diurnal profiles were averaged at each time over the two consecutive pre-test days (pre-administration) and analyzed with a group (metyrapone vs. placebo) × time of collection ANOVA. To evaluate the impact of administration of metyrapone on cortisol, data from the two measures on the startle test day were averaged (post-administration), and then compared to cortisol measured at a comparable time on the pre-test days (one week earlier). These four cortisol measures were subjected to a treatment (metyrapone vs. placebo) × time (pre- vs. post-administration) ANOVA to check the effect of drug manipulation. The EMG eye blink magnitudes were analyzed with a treatment (metyrapone vs. placebo) by trial (1-6) repeated measures ANCOVA with sex as a covariate. Differences in anxiety between the groups were evaluated with t-tests for both trait and state anxiety.

For all statistical analyses (performed with SPSS for Windows, Statistical Package of the Social Sciences, Version 11.5), the level of significance (α) was .05 and in case of violation of the assumption of homogeneity of variances the Greenhouse-Geisser-adjustment was applied and adjusted p-values are reported, with uncorrected degrees of freedom and epsilon-values. Significant main effects or interactions were further analyzed with Bonferroni-adjusted paired t-tests and effect sizes (partial eta squared: η_p^2) are reported.

4.3 Results

4.3.1 Saliva Cortisol Levels

As illustrated in Figure 4.1, the diurnal cortisol profiles collected prior to metyrapone treatment showed the typical pattern, a significant effect of time ($F_{9,468}$ = 108.072, p < .001, η_p^2 = .675, ε = .435), with increased cortisol levels during the morning and gradually decreasing levels during the day. There was no main effect of group ($F_{1,52}$ = 1.349, p = .251) and no interaction between group and time ($F_{9,468}$ = .372, p = .824, ε = .435), indicating that the diurnal cortisol profiles did not significantly differ between the placebo and metyrapone groups.

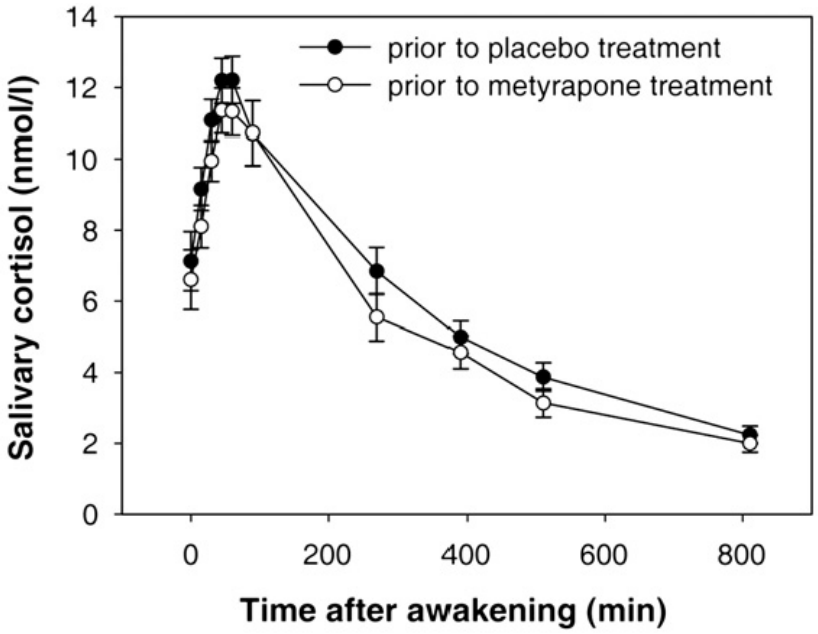

Figure 4.1 Diurnal saliva cortisol profiles (averaged data over two consecutive days) of placebo and metyrapone group, indicating that groups did not differ in cortisol prior to treatment.

The manipulation check revealed a significant interaction of treatment (placebo vs. metyrapone) and time (pre- vs. post-treatment; F1,51 = 11.451, p < .001, ηp2 = .183; placebo-

pre = 4.958 (SE = .473), placebo-post = 4.656 (SE = .399), metyrapone-pre = 4.563 (SE = .464), metyrapone-post = 1.780 (SE = .391)). Post-treatment cortisol levels were significantly decreased in the metyrapone group, ($t26 = 5.752$, $p < .001$), but not in the control group, ($t25 = .545$, $p = .591$).

4.3.2 Startle Reflex Magnitude

Startle reactivity was significantly increased in the metyrapone group compared to the placebo group ($F_{1,46} = 4.284$, $p < .05$, $\eta_p^2 = .085$; see Figure 4.2). The startle reflex habituated for both metyrapone and placebo groups, ($F_{5,230} = 3.458$, $p < .05$, $\eta_p^2 = .07$, $\varepsilon = .716$), but there was no interaction of treatment and time ($F_{5,230} = .212$, $p = .916$, $\varepsilon = .716$), indicating that the decline of startle response magnitude with repeated stimulation was not different between the groups. The ANCOVA revealed neither a sex effect ($F_{1,46} = 2.628$, $p = .112$) nor a treatment × sex interaction ($F_{5,230} = .717$, $p = .566$, $\varepsilon = .716$).

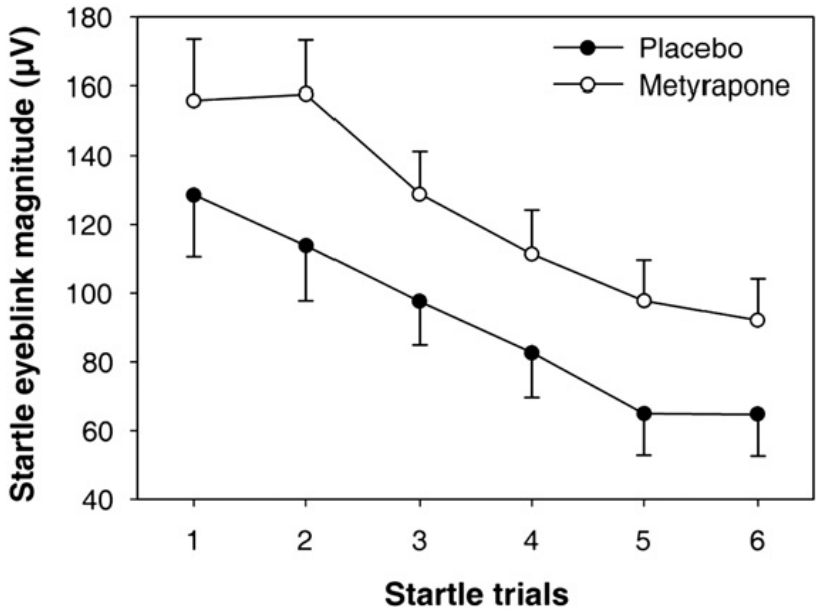

Figure 4.2 Startle reactivity after treatment with metyrapone or placebo and habituation within 6 startle trials. Startle magnitude is significantly increased in the metyrapone group compared to the placebo group ($F_{1,46} = 4.284$, $p < .05$, $\eta_p^2 = .085$), and there is a significant decline in startle response magnitudes over the 6 trials for both metyrapone and placebo groups ($F_{5,230} = 3.458$, $p < .05$, $\eta_p^2 = .07$, $\varepsilon = .716$).

4.3.3 Anxiety Scores

Metyrapone had no significant effect on either trait anxiety (t_{52} = .854, p = .397; placebo = 39.96 (SE = 1.82), metyrapone = 37.67 (SE = 1.98)) or state anxiety (t_{52} = -.622, p = .537; placebo = 35.19 (SE = 1.44), metyrapone = 36.48 (SE = 1.51)).

4.4 Discussion

This placebo-controlled study was performed to investigate the effects of endogenous cortisol suppression by metyrapone on acoustic startle eye blink responses. As expected, administration of metyrapone effectively suppressed endogenous cortisol production. At the same time, metyrapone treatment enhanced startle eye blink response magnitude significantly, but did not affect the short-term habituation of this response. The enhancement of eye blink responses after reduction of central corticosteroid receptor activity is consistent with previous animal (Sandi et al., 1996) and human (Buchanan et al., 2001) research reporting reduced startle responsiveness following corticosteroid administration at a dose which is sufficient to fully activate central GR and MR receptors with their natural ligand. It has already been demonstrated in previous research that metyrapone treatment, and interruption of the negative cortisol feedback loop, increases central CRH activity (Jahn et al., 2003). Since central CRH enhances startle response magnitude (Liang et al., 1992b), it seems reasonable to assume that increased central CRH mediated the observed metyrapone effect on startle eye blink responsiveness in this study, although we were not able to measure central CRH.

Suppression of endogenous cortisol production by metyrapone did not affect startle reflex habituation in the present study. Metyrapone induces an HPA activity pattern of reduced peripheral cortisol and increased central CRH similar to the pattern found in PTSD. Also, PTSD can involve impaired startle reflex habituation (Shalev et al., 2000). However, the pathological state of PTSD usually exists for a prolonged time period before a clinical diagnosis is established, time enough to allow for functional or even structural adaptations of the CNS. Thus, a 6 h treatment with metyrapone may be too short to affect the brain mechanisms that determine startle reflex habituation. Our findings are in line with previous studies in rodents showing that adrenalectomy (Davis and Zolovick, 1972) or treatment with oral corticosterone (Ardayfio and Kim, 2006) does not affect startle habituation and, therefore

suggest that an intact adrenal-pituitary system is not crucial for habituation of the startle reflex.

Another factor that is known to enhance startle reactivity is anxiety (Bradley et al., 1999; Grillon and Baas, 2003). If metyrapone treatment and/or increased CRH had had an anxiogenic effect in our sample, that may have been visible in increased post-treatment state anxiety scores. However, we did not find differences in anxiety scores between treatment groups, suggesting that an effect of metyrapone treatment on self-reported anxiety in this study sample can be excluded.

There are also other substances affected by metyrapone treatment, such as serotonin, growth hormone, dehydroepiandrosterone, and progesterone and its metabolite 3α-hydroxy-5α-pregnane-20-one (Korte-Bouws et al., 1996; Hirani et al., 2002; Jahn et al., 2003) that we did not control for, which might have had an influence on startle.

There are two implications of our study: First, our results complement previous studies of reduced startle due to excess cortisol in showing that cortisol depletion has the opposite effect on startle. As such, our results suggest that startle methodology may be a useful tool to index the activity level of central networks affecting HPA regulation over the whole range of relative hypo- to hypercortisolism. Since startle is sensitive to alterations of the HPA axis, it may prove useful in further studies in patient groups characterized by HPA axis changes. Startle methodology is widely available and does not pose a great burden on subjects. Second, our findings suggest that acute metyrapone-treatment induces a startle eye blink pattern similar to the pattern found in PTSD (Shalev et al., 2000). This interpretation is corroborated by the endocrinological profile of metyrapone, which induces low peripheral cortisol and high central CRH levels. However, this interpretation is in contrast to the anxiolytic effects of metyrapone previously reported (Roozendaal et al., 1996). Future studies will have to replicate these findings.

Several limitations of the current study have to be mentioned. The experimental between-subjects design chosen is less statistically powerful than a within-subject cross-over design, where placebo control data would have been assessed for all participants. However, the expectation of a treatment effect on habituation over sessions may complicate the interpretation of a cross-over study. Thus, we decided to use a cohort study. Another potential limitation of this study was the time of day when the startle eye blink protocol took place. We assessed the effects of metyrapone for all subjects in the afternoon, when basal cortisol levels are low in comparison to morning cortisol. Although we found a significant suppression of afternoon cortisol levels, the treatment effect might have been greater if data acquisition had

been performed in the morning. If there is the capacity to watch the participants overnight, to reschedule the data acquisition to the morning, this could also be considered in a replication study. Also, this study involved healthy young volunteers, and it would be interesting to investigate whether clinical populations, for example adrenalectomized patients, would have shown the same results or different findings. Finally, plasma ACTH should be assessed in future studies since it may influence CNS processes.

In summary, the present results indicate that acute cortisol depletion is associated with enhanced startle reactivity, but does not seem to affect habituation of the acoustic startle response. Thus, the PTSD-like pattern of cortisol and CRH levels after short-term metyrapone administration results in the same phenotype of enhanced startle responsiveness.

4.5 Acknowledgments

This study was supported by the University of Trier and the International Research Training Group "Psychoneuroendocrinology of Stress – From Molecules and Genes to Affect and Cognition", funded by the German Research Foundation (Deutsche Forschungsgemeinschaft: DFG), grant GRK 1389/1. Sonja Roemer, Frauke Nees, Steffen Richter and Hartmut Schachinger are members of the International Research Training Group "Psychoneuroendocrinology of Stress – From Molecules and Genes to Affect and Cognition", funded by the German Research Foundation (Deutsche Forschungsgemeinschaft: DFG), grant GRK 1389/1. Terry Blumenthal would like to acknowledge the support of the Sara Jo Brownlow Shearer Fund at Wake Forest University.

The experiments comply with the current law of the country in which they were performed.

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Chapter V - Cardiopulmonary Baroreceptors affect reflexive Startle Eye Blink

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5.0 Abstract

Baroafferent signals originating from the 'high pressure' arterial vascular system are known to impact reflexive startle eye blink responding. However, it is not known whether baroafferent feedback of the 'low pressure' cardiopulmonary system loading status exerts a similar effect.

Lower Body Negative Pressure (LBNP) at gradients of 0, -10, -20, and -30 mmHg was applied to unload cardiopulmonary baroreceptors. Acoustic startle noise bursts were delivered 230 and 530 ms after spontaneous R-waves, when arterial baroreceptors are either loaded or unloaded. Eye blink responses were measured by EMG, and psychomotor reaction time by button pushes to startle stimuli. The new finding of this study was that unloading of cardiopulmonary baroreceptors increases startle eye blink responsiveness. Furthermore, we replicated the effect of relative loading/unloading of arterial baroreceptors on startle eye blink responsiveness. Effects of either arterial or cardiopulmonary baroreceptor manipulations were not present for psychomotor reaction times. These results demonstrate that the loading status of cardiopulmonary baroreceptors has an impact on brainstem-based CNS processes.

Keywords: Acoustic startle reflex; Arterial baroreceptors; Cardiopulmonary baroreceptors; Lower body negative pressure; Reaction time

5.1 Introduction

There is growing evidence that neural visceral afferent signals have an impact on higher central nervous system (CNS) processes, such as emotion and cognition (Damasio, 2003; Wiens, 2005). Many of the afferent signals, which ascend via vagal, glossopharyngeal, and thoracic afferent nerve fibers, originate from phasically active thoracic or abdominal

structures, such as the lungs, gastrointestinal organs, and the central cardiovascular system (Critchley et al., 2004). Visceral afferents play an important role in adaptation and homeostasis, and neural baroreceptor feedback is required for controlling heart rate, blood pressure, cardiac workload, and vascular resistance, but has also been found to affect central nervous system (CNS) functions which are not directly linked to cardiovascular regulation, such as pain processing (Dworkin, 2000). Baroreceptors are located in arterial vessel walls of the aortic arch and the carotid sinus (the high pressure system), but also in pulmonary vessels and atria (the cardiopulmonary low pressure system). Due to their distribution and due to the blood vessel wall characteristics, arterial baroreceptors respond to changes in arterial pressure, whereas cardiopulmonary baroreceptors respond to changes in central venous pressure which, during healthy conditions, is proportional to changes in central venous volume. Loading of baroreceptors enhances their neural output, unloading induces the opposite effect.

The CNS structures prominently involved in both baroreflex pathways are located in the brainstem. Information from arterial and cardiopulmonary baroreceptors converges in the nucleus tractus solitarii (NTS), and is further relayed to the nucleus ambiguous and the ventrolateral medulla (Masuda et al., 1991; Aicher et al., 1995; Jeske et al., 1995; Jänig, 2006). Both baroreceptor systems project, via the NTS, to similar CNS structures (e.g., Anterior cingulum, Insular cortex, Locus coeruleus) which have been shown to be involved in pain processing, emotion, and regulation of higher cognitive-motor functions (Grindstaff et al., 2000; Henderson et al., 2004; Kimmerly et al., 2005).

Several psychophysiological effects of loading or unloading arterial baroreceptors have been described. Enhanced arterial baroreflex afferent feedback activity impacts on EEG activity (Mini et al., 1995; Vaitl et al., 1996), attenuates pain perception (Mini et al., 1995; Edwards et al., 2001; Edwards et al., 2002; Edwards et al., 2003; McIntyre et al., 2006; Edwards et al., 2008), and induces a prolongation of psychomotor reaction times (Vaitl et al., 1996; Edwards et al., 2007; McIntyre et al., 2008b), and unloading vs. loading of arterial baroreceptors may affect memory processes (Moor et al., 2005). Furthermore, neural arterial baroreceptor afferent feedback transmission has an inhibitory effect on simple brainstem reflexes, such as the startle response (Rau et al., 1993; Nyklicek et al., 2005; Schulz et al., 2009b; Schulz et al., 2009a). The startle eye blink reflex is a protective reflex, which is reliably evoked by presentation of abrupt and intense acoustic noise stimuli. This reflex is affected by spontaneous neural arterial baroreceptor afferent feedback transmission, since lower startle responsiveness was found when stimuli were presented during the early cardiac cycle phase, when arterial baroreceptors are loaded, as compared to the late cardiac cycle

phase, when arterial baroreceptors are relatively unloaded. This effect relies on intact neural afferent signal transmission, and it is absent in diabetic autonomic neuropathy (Schulz et al., 2009b).

However, in contrast to arterial baroreceptors, relatively little is known about the significance of cardiopulmonary baroreceptors for psychophysiological processes. The loading status of cardiopulmonary baroreceptors may change with everyday activities, such as altering body position from laying/supine to standing upright (Pump et al., 1997; Pump et al., 2001b; Pump et al., 2001a). It may be reduced during moderate states of dehydration (i.e. due to reduced drinking or increased water loss), as well as substantial blood loss (Abboud et al., 1979; Wada et al., 1995). Furthermore, emotional stress may increase central venous volume and pressure (Brod et al., 1979), as well as pulmonary artery pressure (Schachinger et al., 2000), and thus affect the loading status of cardiopulmonary baroreceptors. An impact of cardiopulmonary baroreceptor activity on pain processing (Randich and Maixner, 1984; Sheps et al., 1992; Vaitl et al., 1996; D'Antono et al., 2000) and EEG theta-band activity (Vaitl and Gruppe, 1990) has been reported, and the direction of effects is similar to those of arterial baroreceptor manipulations. Since relative arterial baroreceptor unloading is associated with increased startle eye blink responsiveness, it may be assumed that cardiopulmonary baroreceptor unloading induces a similar effect, but this has never been investigated. The current research project was designed to replicate the finding that relative unloading of arterial baroreceptors enhances startle responsiveness, and, at the same time, to investigate whether unloading of cardiopulmonary baroreceptors affects startle responsiveness in the same direction. Statistical interaction effects of arterial and cardiopulmonary baroreceptors on startle eye blink responsiveness will also be tested, as well as baroreceptor loading and unloading effects on a psychomotor reaction time task (button press) to startling stimuli.

This study employed Lower Body Negative Pressure (LBNP), which induces central vascular volume changes with only minor impact on mean arterial blood pressure (Hinghofer-Szalkay et al., 1996; Laszlo et al., 1998; van Hoeyweghen et al., 2001; Franke et al., 2003; Kitano et al., 2005), and thus may be considered as being ‘non-hypotensive’. One of the greatest advantages of LBNP is related to its dose-response characteristics; that is, increasing LBNP stepwise increases its effects (van Hoeyweghen et al., 2001; Convertino et al., 2006). Furthermore, it can be assumed that LBNP has very little body position-related effect on somatic muscle tone and stimulation-associated perceptions, since body and limb position remain largely unchanged during LBNP. Thus, no change in proprioceptive sensations is

expected. Also, LBNP is not painful and it does not trigger aversive sensations. However, as there are no studies focusing on potential mood changes during LBNP, and since mood changes may have an impact on startle, this study will assess affective changes in order to control for this.

To clarify the roles of arterial and cardiopulmonary baroreceptors in modulating startle processing we conducted a within-participant experiment with 12 healthy men. The participants received 240 acoustic startle noises in total, presented via headphones, with latencies of either 230 ms (arterial baroreceptor loading) or 530 ms (arterial baroreceptor unloading) after the cardiac R-wave while each participant was treated with a series of 5 min of LBNP at levels of 0, -10, -20, and -30 mmHg each, in randomized order. For each participant, every LBNP level was applied two times. Surface electromyogram (EMG) of the orbicularis oculi muscle was recorded and the participants were asked to respond to each noise as quickly as possible by pressing a response key. The latter was done because several studies have shown that arterial baroreceptors affect psychomotor reactions (Weisz, 1996; Stewart, 2006; Edwards et al., 2007; McIntyre et al., 2008b), but little is known about the effect of cardiopulmonary baroreceptors on psychomotor function. In general, we expected to find an inhibitory effect of both baroreceptor types on startle responsiveness and psychomotor reactions.

5.2 Methods

5.2.1 Participants

We studied 12 healthy male non-smokers who participated voluntarily and received a small monetary incentive of € 25.00. Mean age was 25 years (range: 20 to 34 years; SD = 4.0), mean resting heart period was 1048 ms (SD = 170), and mean BMI was 24 kg/m² (SD = 2.3). All participants had a regular sinus rhythm. Exclusion criteria were BMI below 18 or above 30 kg/m², and a height of more than 1.95 m (because of the LBNP-chamber size). Participants with any chronic or acute diseases, cardiovascular disorders, or known hereditary vascular diseases (e.g. aneurysms) in their family history were excluded from the study. Women were not included so as to increase homogeneity of our sample. Participants underwent a routine medical history and screening that included a standard electrocardiogram (ECG) recording, resting blood pressure measurement, and blood analysis (standard haematology and

chemistry). None of the participants showed any pathological findings and total serum cholesterol levels were lower than 180 mg/dl for each participant.

All participants gave written informed consent. The study was approved by the community-based ethical committee of the 'Landesärztekammer Rheinland-Pfalz, Mainz, Germany'.

5.2.2 Apparatus

The LBNP-device consists of a steel 120 × 60 × 50 (L × W × H) cm chamber (Curio Medizinelektronik, Bonn, Germany) fixed on a table that accommodates the participant's legs. An adjustable bicycle seat is placed in the chamber to ensure a stable position of the participant. The chamber is sealed with a neoprene cover at the level of the iliac crest. An adjustable acrylic glass cover avoids deformation of the neoprene during negative pressure gradients. Attached above the participant's head is a LCD monitor for written instructions during the experiment. The negative pressure within the chamber is electronically controlled and infinitely variable from -10 mmHg to -60 mmHg. Any selected negative pressure within the chamber was reached in less than 5 s.

The vacuum pump was positioned in a soundproof box at a distance of 1 m from the participant. Ambient noise level in the laboratory was at 44 dB(A), the maximum ambient noise level with the vacuum pump at maximum performance level was 47 dB(A).

5.2.3 Stimulus Control

All stimuli and written instructions were delivered by E-Prime 1.2 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA). Startle stimuli consisted of 50 ms white noise at 105 dB(A) with instantaneous rise time. The headphones for binaural stimulation (Sennheiser electronic GmbH & Co KG, Wedemark, Germany) covered the entire ear and reduced ambient noise markedly (passive noise reduction > 10-40 dB(A), according to Sennheiser product information). No participant was able to correctly report acoustic activity of the vacuum pump when headphones were attached. The experimenter sat in the same room for safety reasons, but out of sight of the participant. Movement sounds or other indicators of the experimenter's presence could not be heard by the participant due to the sound-reducing properties of the headphones.

5.2.4 Ratings

Participants rated affective valence, momentary arousal, and perceived intensity of ambient noise at every LBNP level. The affective rating scale was a paper-pencil based single item 20 cm visual analog rating scale. Questions were presented on separate pages to avoid carry-over effects. Anchors were "very unpleasant" on the left and "very pleasant" on the right for affective valence, "very low" and "very high" for arousal, and "none" and "very high" for ambient noise intensity. Ratings were measured in millimeters and transferred into a range of 0 to 100.

5.2.5 Recording Parameters

Standard Ag/AgCl electrodes (ECG Tyco Healthcare H34SG Ag/AgCl electrodes of 45 mm diameter) were used for ECG and impedance cardiography (ICG, see below). ECG electrodes were attached to obtain a standard lead II configuration. ICG spot-electrodes were attached on both sides of the neck, below each clavicula, on both sides of the arcus costae and on the lower abdomen in the mid-clavicular line. ECG and EMG were recorded with 1000 Hz sampling rate at 16 bit resolution by a Biopac MP150 system, with amplifier modules ECG100C and EMG100C. Hardware band-pass filter settings were 0.5 to 500 Hz for ECG and 10 to 500 Hz for EMG. The R-waves were identified by a customized ECG detection device (Curio Medizinelektronik, Bonn, Germany). Accuracy of R-wave detection in sinus rhythm was higher than 99.8%, with latency below 3 ms (internal lab report).

ICG was recorded with 1000 Hz sampling rate at 14 bit resolution by a newly developed research impedance module manufactured at the Institute of Medical Technology (Prof. J. Nagel, Dr. J. Port), University of Stuttgart, Germany.

Systolic, diastolic, and mean blood pressure were measured with a Dinamap monitor (Dinamap SX 1846, Critikon, US). A handheld reaction time-button was placed into the participant's dominant hand, and responses were recorded by E-Prime software (see above). Data were stored to hard disk with Labview-based software (National Instruments, US).

5.2.6 Psychophysiological Data Analysis

The EMG signal was further filtered in software with a passband of 28 to 500 Hz (van Boxtel et al., 1998), and then integrated offline with a 5 ms Boxcar filter (the equivalent of a 10 ms

time constant) (Blumenthal, 1994; Blumenthal et al., 2005) with AcqKnowledge software (Biopac Systems Inc., Goleta, CA, USA). It was then processed offline with a proprietary C++ based semi-automated PC program running on a WinXP platform. The algorithm identified response peaks in the rectified and integrated signal in the time interval of 20 to 150 ms after the startle probe onset. The baseline period was defined as the 50 ms interval prior to the acoustic stimulation. All response data were manually confirmed. Signals with electrical and physiological artifacts, such as coinciding eye blinks or other facial muscular activity, were rejected from analysis and defined as missing. If responses were not visible in the typical response latency range of a particular participant, response magnitude was set to zero. Zero response data were included in the averaging procedure, with startle response magnitude as the final output measure (Blumenthal et al., 2005). Averaging was done per participant and according to whether startle was elicited in the cardiac systole or diastole, in each LBNP condition.

Cardiovascular data were processed with WinCPRS software (WinCPRS 1.6, Absolute Aliens Oy, Turku, Finland). Interbeat interval, T-wave amplitude, and heart rate variability were calculated from the ECG. The spectral analysis of interbeat intervals was done with WinCPRS software using a FFT routine. The R-R interval time series was linearly interpolated and resampled with a sampling rate of 5 Hz, the resampled data was tapered using a Hanning window and the windowed data zero padded to the next power of 2. The FFT spectrum was smoothed using a sliding triangular weighting function in order to increase the number of freedoms and thus improve the statistical relevance of the spectrum.

The low frequency band (LF) was defined as 0.06 to 0.14 Hz and the high frequency band (HF) was defined as 0.2 to 0.5 Hz. Normally, the low frequency cut-off of the high frequency band is 0.15 Hz. However, we used here a higher cut-off frequency, so as to exclude the frequency at which startle stimuli were presented (0.14 to 0.2 Hz; corresponding to an ISI of 5 to 7 s).

The normalized power of the low frequency band as well as the LF/HF ratio (Malliani et al., 1994; Montano et al., 1994; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997; Zaza and Lombardi, 2001) was calculated, because they reflect sympathetic activity more accurately than the absolute power of the low frequency band of heart rate variability, which is also influenced by parasympathetic tone (Akselrod et al., 1985; Pomeranz et al., 1985). The pre-ejection period and left ventricular ejection time were calculated from the ECG and dZ/dt signal. The pre-ejection period was calculated as the interval from R-wave to B-point

(corresponding to the opening of the aortic valve), and left ventricular ejection time was calculated as the interval from B-point to X-point (corresponding to the closure of the aortic valve) in the dZ/dt signal. Stroke volume was calculated from the impedance signal by the Kubicek formula (Kubicek et al., 1966). Respiratory frequency was calculated with WinCPRS software from changes in the thorax impedance signal (Ernst et al., 1999).

5.2.7. Experimental Procedure

The participants visited the lab on a separate day for the medical screening and to familiarize themselves with the experimental setting. On the experimental day, the participants were helped into the LBNP-device, electrodes for ECG, ICG, and EMG were attached, and the Dinamap cuff was fastened to the non-dominant arm. The participants were instructed to relax, avoid unnecessary movement, and press the response button as quickly as possible whenever they perceived a noise stimulus. They received instructions by written information presented on the overhead monitor.

At the beginning of the experimental session, six startle probes were administered as habituation trials, and were not further analyzed. Each participant was then exposed to eight LBNP blocks of either 0, -10, -20, or -30 mmHg pressure gradients, with each LBNP level applied twice to each participant. Dependent variables were averaged across the two instances of each LBNP level. Exposure to LBNP blocks followed a pseudo-randomized order. Each block lasted 5 min. After each block was a break of 3 min. The first minute of each block was used for technical adjustments and blood pressure measurement. The startle protocol was started after the first minute and lasted between 3 and 3.5 min. The difference in startle protocol duration was due to the cardiac cycle dependent elicitation and randomization of startle stimulus presentation. Blood pressure was measured again during the last minute of exposure to LBNP. Intermittent cuff blood pressure readings were taken before and after the startle protocol to avoid interference.

A startle protocol consisted of 30 startle probes that were administered with a randomized interstimulus interval of 5 to 7 s. Half of these startle probes were presented 230 ms after an R-wave (cardiac systole), and the other half were presented 530 ms after an R-wave (cardiac diastole), in randomized order. The timing for stimulus presentation in cardiac systole and diastole was chosen according to previous publications (Ring and Brener, 1992; Ring et al., 1994; Edwards et al., 2001; Edwards et al., 2007; Schulz et al., 2009b; Schulz et al., 2009a).

5.2.8 Statistical Analysis

Startle EMG responses were t-transformed to control for between participant differences in startle eye blink responsiveness. For startle data, a 4 × 2 ANOVA with repeated measures design was employed with the factors (i) LBNP level and (ii) cardiac cycle phase. For reaction time data, a similar procedure was used, but the covariate "mean individual reaction time" (the mean of all reaction time data available for that individual) was introduced to control for between participant differences in psychomotor reaction times. For cardiovascular data, an ANOVA with repeated measures design with one factor (LBNP level) was employed, with the respective cardiovascular parameters as a dependent variable. Subjective ratings were transferred from the visual analog scale into a scale from 0 to 100 arbitrary units (AU) and tested with an ANOVA with repeated measures design with one factor (LBNP level) and the respective rating as the dependent variable.

All p-values of within-subjects-factors with more than two conditions were adjusted with Greenhouse-Geisser correction, but non-adjusted degrees of freedom are reported in text. A result of $p \leq 0.05$ was considered significant. Statistical analysis was conducted with SAS software (SAS Institute Inc., Cary, NC, USA).

5.3 Results

5.3.1 Cardiovascular and respiratory Data

Impedance cardiography derived stroke volume could not be calculated for one participant, because B-points were not identifiable in the dZ/dt signal. Systolic blood pressure ($F(3, 33) = 4.79$, $p = 0.02$, $\eta^2 = 0.30$) and interbeat interval ($F(3, 33) = 13.14$, $p = 0.002$, $\eta^2 = 0.54$) decreased with increasing LBNP. However, mean blood pressure ($F(3, 33) = 0.16$, $p = 0.82$) remained unchanged, underlining the non-hypotensive character of the intervention. Diastolic blood pressure increased only slightly ($F(3, 33) = 2.64$, $p = 0.081$, $\eta^2 = 0.19$), due to the increase of peripheral resistance induced by LBNP. Thorax impedance was increased by increasing levels of LBNP ($F(3, 33) = 52.60$, $p < 0.001$, $\eta^2 = 0.83$) reflecting a reduction of conductive fluids (i.e. blood) in the central compartment, while stroke volume was reduced ($F(3, 30) = 25.62$, $p < 0.001$, $\eta^2 = 0.72$), reflecting reduced diastolic filling of the heart.

The pre-ejection period increased (10.4 ms difference for -30 mmHg LBNP on average), and the left ventricular ejection time decreased during higher LBNP (25.6 ms difference for -30 mmHg LBNP on average). Thus, while at -30 mmHg LBNP the interbeat interval decreased by 133 ms on average, the electromechanical systole decreased by only 15 ms on average. A summary of systolic time intervals at different levels of LBNP is given in Table 1. The T-wave amplitude decreased with increasing LBNP ($F(3, 33) = 21.63$, $p < 0.001$, $\eta^2 = 0.66$). Absolute power in the low and high frequency band of the heart rate variability decreased with increasing LBNP gradients; however, this decrease was only significant for the high frequency band ($F(3, 33) = 5.48$, $p = 0.03$, $\eta^2 = 0.33$), indicating parasympathetic withdrawal. Total power of the heart rate variability also decreased slightly, but not significantly. Normalized high frequency power of the heart rate variability decreased ($F(3, 33) = 9.95$, $p = 0.002$, $\eta^2 = 0.48$), while the low frequency power ($F(3, 33) = 9.92$, $p = 0.002$, $\eta^2 = 0.47$) and the LF/HF ratio increased ($F(3, 33) = 9.19$, $p = 0.001$, $\eta^2 = 0.46$) with increasing LBNP, suggesting decreasing parasympathetic and increasing sympathetic activity. The respiratory frequency decreased with increasing LBNP from 0.31 Hz (SE = 0.014) at 0 mmHg to 0.28 Hz (SE = 0.015) at -30 mmHg ($F(3, 33) = 10.26$, $p = 0.001$, $\eta^2 = 0.48$).

Mean values and standard errors for cardiovascular data for the different LBNP levels are summarized in Table 1.

Table 5.1 Summary of cardiovascular parameters observed at 0, -10, -20, and -30 mmHg of LBNP.

Parameter	LBNP 0 mm Hg		LBNP −10 mm Hg		LBNP −20 mm Hg		LBNP −30 mm Hg	
	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)
Systolic blood pressure [mm Hg]	112	(2.0)	112	(1.9)	110	(1.4)	109[a]	(1.6)
Mean blood pressure [mm Hg]	84	(1.8)	84	(1.9)	84	(2.3)	83	(1.9)
Diastolic blood pressure [mm Hg]	65	(1.9)	67	(2.3)	67	(2.2)	68[a]	(2.2)
Interbeat interval [ms]	1055	(44.0)	1032[a]	(40.4)	984[a]	(34.1)	922[a]	(29.4)
T-wave amplitude [mV]	0.30	(0.03)	0.29[a]	(0.02)	0.28[a]	(0.02)	0.26[a]	(0.03)
Total power [ms^2]	57.43	(1418)	5388	(1332)	4768	(1263)	4223	(847)
High frequency [ms^2]	956	(290)	816	(262)	531	(118)	254[a]	(45)
Low frequency [ms^2]	1910	(668)	1420	(334)	1158	(332)	1144	(214)
Normalized high frequency [NU]	0.49	(0.04)	0.48	(0.05)	0.45	(0.04)	0.33[a]	(0.03)
Normalized low frequency [NU]	0.50	(0.04)	0.51	(0.05)	0.53	(0.04)	0.67[a]	(0.03)
LF/HF ratio	1.18	(0.19)	1.36	(0.27)	1.33	(0.19)	2.10[a]	(0.26)
Thorax impedance [Ω]	12.6	(0.46)	12.9[a]	(0.44)	13.2[a]	(0.44)	13.5[a]	(0.42)
Stroke volume [ml]	79	(8.3)	72[a]	(9.9)	58[a]	(9.7)	55[a]	(9.6)
Pre-ejection period [ms]	78.3	(4.3)	80.6	(5.0)	84.6[a]	(4.9)	88.7[a]	(5.7)
Left ventricular ejection time [ms]	334.9	(11.4)	330.7	(14.7)	322.2[a]	(14.7)	309.2[a]	(14.8)
Electromechanical systole [ms]	413.2	(12.4)	411.3	(14.6)	406.8	(15.3)	397.9[a]	(15.3)
Respiratory frequency [Hz]	0.31	(0.014)	0.30	(0.013)	0.28[a]	(0.014)	0.28[a]	(0.015)

* Significant differences relative to LBNP of 0 mmHg (based on the within-participant contrasts of the ANOVA with repeated measures design).

5.3.2 Ratings

Statistical test revealed no significant effect of LBNP on affective valence or perceived intensity of ambient noise. Valence ratings were 63.5 (SE= 3.7) for 0 mmHg, 62.6 (SE= 3.2) for -10 mmHg, 63.0 (SE= 3.6) for -20 mmHg, and 62.0 (SE= 3.2) for -30 mmHg. Given the scale range of 0 to 100, this represents a neutral-to-pleasant state. Ambient noise ratings were 7.6 (SE= 2.4) for 0 mmHg, 7.5 (SE= 2.0) for -10 mmHg, 7.8 (SE= 2.1) for -20 mmHg, and 8.1 (SE= 2.1) for -30 mmHg. Given the scale range of 0 to 100, this represents a very low subjective ambient noise level. However, a significant effect of LBNP on momentary arousal was revealed ($F(3, 33) = 5.02$, $p = 0.01$, $\eta^2 = 0.32$), indicating higher subjective arousal with increasing LBNP gradients. Momentary arousal ratings were 38.0 (SE= 5.2) for 0 mmHg, 34.6 (SE= 5.7) for -10 mmHg, 41.1 (SE= 4.8) for -20 mmHg, and 43.0 (SE= 5.5) for -30 mmHg. Given the scale range of 0 to 100, this represents a low to medium arousal for lower LBNP levels and a medium subjective arousal state for higher LBNP.

Mean ratings and standard errors are summarized in Table 2.

Table 5.2 Ratings of momentary arousal, affective valence, and ambient noise at 0, -10, -20, and -30 mmHg of LBNP.

	LBNP 0 mm Hg		LBNP −10 mm Hg		LBNP −20 mm Hg		LBNP −30 mm Hg	
	Mean	(SE)	Mean	(SE)	Mean	(SE)	Mean	(SE)
Momentary arousal [AU]	38.0	(5.2)	34.6	(5.7)	41.1[a]	(4.8)	43.0[a]	(5.5)
Effective valence [AU]	63.5	(3.7)	62.6	(3.2)	63.0	(3.6)	62.0	(3.2)
Ambient noise [AU]	7.6	(2.4)	7.5	(2.0)	7.8	(2.1)	8.1	(2.1)

* Significant differences relative to LBNP of 0 mmHg (based on the within-participant contrasts of the ANOVA with repeated measures design).

5.3.3 Startle Data

Startle magnitude increased with increasing levels of LBNP ($F(3, 33) = 4.53$, $p = 0.01$, $\eta^2 = 0.29$). Subsequent analysis revealed a significant linear trend of pressure gradients on startle magnitude ($F(1, 11) = 15.4$, $p = 0.002$); quadratic and cubic trends were not significant.

Also, a significant main effect of the cardiac cycle phase on startle magnitude was found ($F(1, 11) = 4.59$, $p = 0.05$, $\eta^2 = 0.29$). No significant interaction of LBNP level × cardiac cycle phase could be detected ($F(3, 33) = 0.05$, $p = 0.98$).

A graphical representation of startle amplitude with standard errors for the respective LBNP conditions and cardiac cycle phase is given in Figure 6.1.

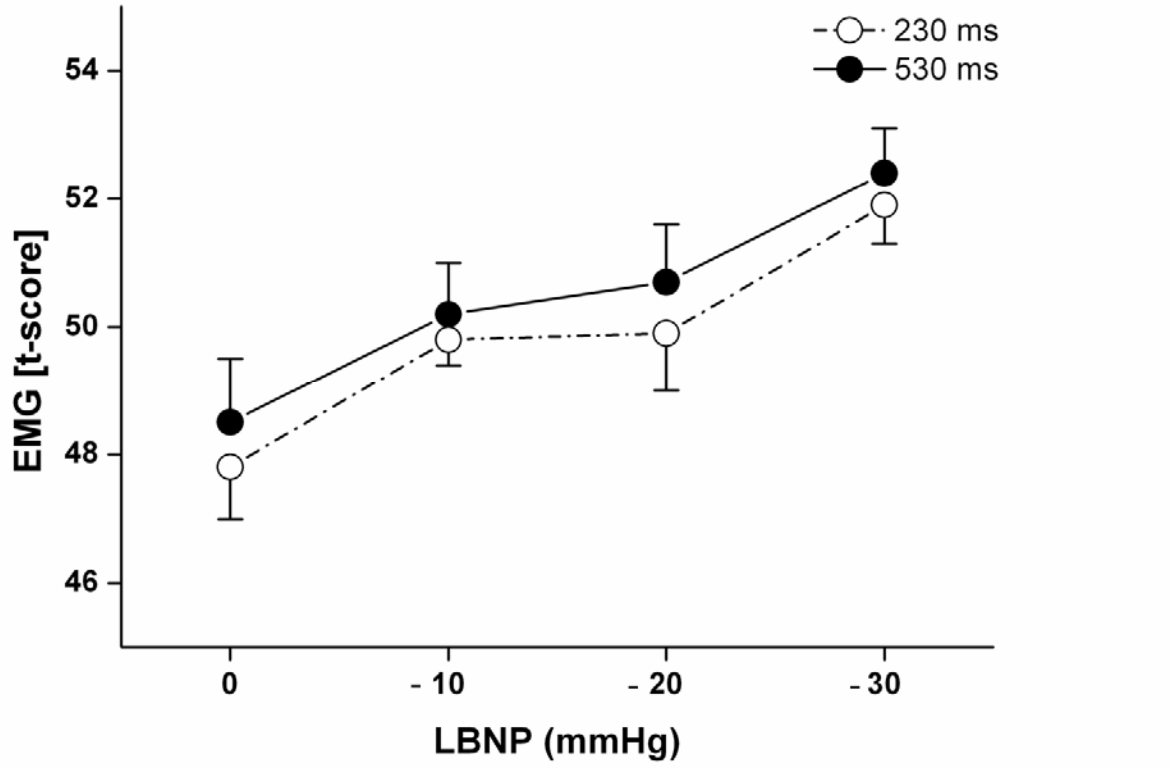

Figure 5.1 Mean EMG responses to startle stimuli (t-scored) at 0, -10, -20, and -30 mmHg of LBNP. Lines represent early (systolic) cardiac cycle phase (empty circles) and late (diastolic) cardiac cycle phase (filled circles). Error bars represent ±1 standard error of mean.

5.3.4 Reaction Time Data

For the reaction times to startle stimuli, no significant effects of LBNP ($F(3, 33) = 0.15$, $p = 0.93$) or cardiac cycle phase ($F(1, 11) = 0.16$, $p = 0.70$) were observed. An interaction of cardiac cycle phase and LBNP on reaction time ($F(3, 33) = 2.82$, $p = 0.05$, $\eta^2 = 0.20$) was present, but disappeared when mean individual reaction time was included as a covariate.

A graphical representation of reaction times with standard errors for the respective LBNP conditions and cardiac cycle phase is given in Figure 6.2.

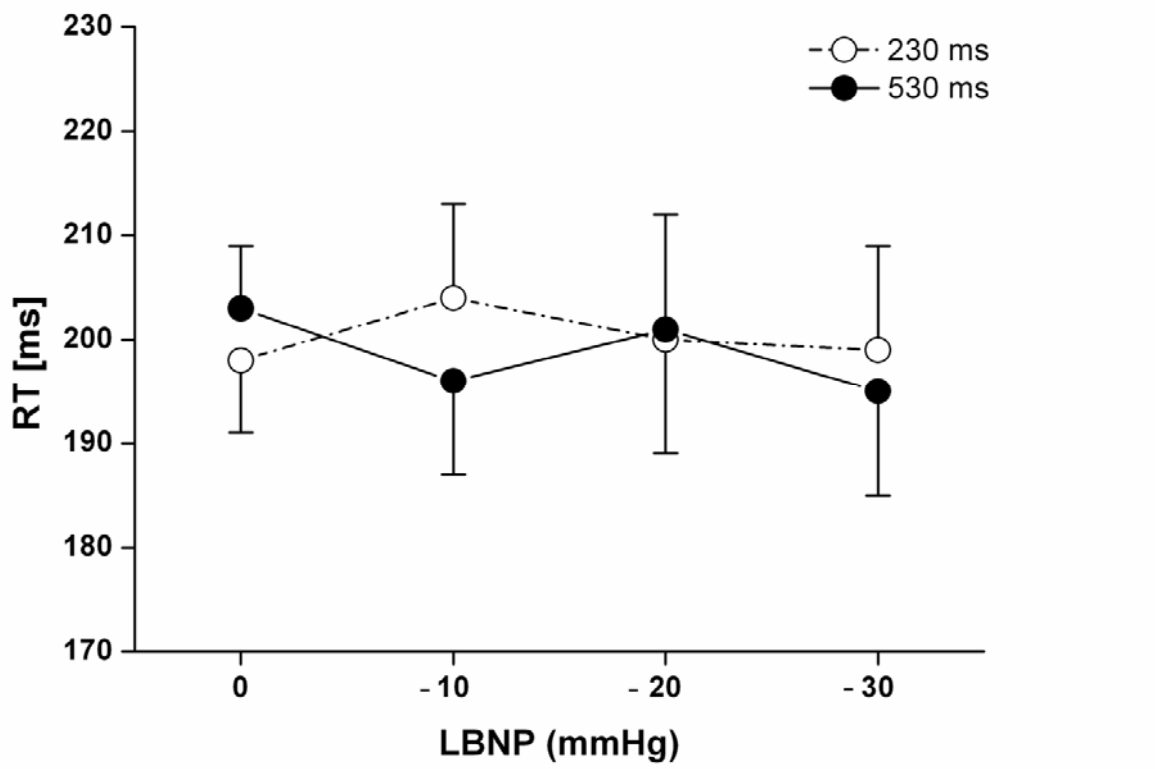

Figure 5.2 Mean reaction times to startle stimuli at 0, -10, -20, and -30 mmHg of LBNP. Lines represent early (systolic) cardiac cycle phase (empty circles) and late (diastolic) cardiac cycle phase (filled circles). Error bars represent ±1 standard error of mean.

5.4 Discussion

The main aim of the current study was to investigate whether manipulation of cardiopulmonary 'low pressure' baroreceptor activity influences startle responsiveness. The principle new finding of this study is that cardiopulmonary baroreceptor unloading enhances startle eye blink responsiveness in a continuous dose-response fashion (the stronger the unloading, the higher the startle magnitude).

Furthermore, we assessed the effects of relative arterial baroreceptor loading/unloading on startle eye blink responsiveness to indicate feasibility of the research methodology. We could replicate the previously described effect of increased startle eye blink responsiveness by relative arterial baroreceptor unloading. This replication serves as validation of the research approach.

Some previous studies have suggested that loading of arterial baroreceptors can inhibit psychomotor reactions (Vaitl et al., 1996; Stewart, 2006; Edwards et al., 2007; McIntyre et al., 2008b), but this study, as well as other studies (Thompson and Botwinick, 1970; Weisz, 1996) did not find such an effect. Furthermore, this study did not find an effect of cardiopulmonary baroreceptor unloading on psychomotor reaction time.

Because of the assumed orthogonal variation of cardiopulmonary and arterial baroreceptor activity, we tested whether manipulations of both receptor types would statistically interact with respect to reflexive startle eye blink data, but such effects were not present. No effects of arterial or cardiopulmonary baroreceptor manipulation were found for voluntary psychomotor reaction times in this study, which is well in line with previous research. Three previous studies have addressed potential effects of baroreceptors on higher CNS functions. Two studies (McIntyre et al., 2007; McIntyre et al., 2008a) used fixed coupling of reflex-evoking stimuli to electrocardiogram R-wave occurrence in participants who lay supine with their legs raised (which results in cardiopulmonary baroreceptor loading) or lowered, and showed an inhibitory effect of arterial, but not of cardiopulmonary, baroreceptors on reaction times. However, a further study (Vaitl and Gruppe, 1990) used simulated micro-gravity head-up head-down tilt technology, and found a summative contribution of arterial and cardiopulmonary baroreceptor afferent feedback on EEG theta-band activity, so that the question of baroreceptor influences on higher CNS structures remains unresolved and needs to be investigated by future research, which also may address interactions of arterial and cardiopulmonary baroreceptors. Such interactions have been described in previous research on the level of cardiovascular homeostatic neural processes

(Victor and Mark, 1985; Cooper and Hainsworth, 2001; Brown et al., 2003), suggesting that the two baroreceptor systems have the potential to interact in a complex way. This study is not able to address these interactions because of the following limitations: LBNP represents a static unloading of cardiopulmonary baroreceptors, in contrast to a phasic loading and unloading of arterial baroreceptors. Interactions (or absence of interactions) found in this study may, thus, not be generalizable to other static/phasic manipulation patterns. Furthermore, LBNP may affect the temporal dynamics of the cardiac ejection, so that the chosen latencies of stimulus presentation may not be optimal during every LBNP gradient. However, the pre-ejection period and left ventricular ejection time data described in this study argue that the temporal cardiac dynamics of the cardiac ejection are subject to minor changes only. As summarized by Edwards et al. (Edwards et al., 2009), neural baroreceptor activity peaks 100 ms after the pulse pressure wave reaches the sinus carotis, i.e. at R+240ms (Angell James, 1971a; Coleridge et al., 1987). After the pulse pressure wave has passed the baroreceptors at the sinus carotis, baroreceptor activity quickly decreases (Angell James, 1971b; Coleridge et al., 1984) until baroreceptor afferents are mostly silent after an additional 150 ms (R+390ms) until the onset of the next pulse pressure wave (Chapleau and Abboud, 1987; Koley et al., 1989; Seagard et al., 1990). Accordingly, even at altered interbeat intervals during LBNP, all stimuli should have been appropriately placed.

We observed cardiovascular responses to LBNP which were comparable to previous reports (Hinghofer-Szalkay et al., 1996; Laszlo et al., 1998; van Hoeyweghen et al., 2001; Franke et al., 2003; Cooke et al., 2004; Kitano et al., 2005). Thorax impedance increased while stroke volume decreased, reflecting blood pooling in the legs and subsequent reduction of blood volume in the central venous compartment. Systolic blood pressure and interbeat intervals decreased while diastolic blood pressure increased slightly. These changes reflect the cardiovascular adjustments to LBNP, such as changed cardiac mechanics due to reduced end-diastolic volume and an increased peripheral resistance induced by increasing LBNP. Mean arterial blood pressure remained unaffected by the LBNP intervention, showing that the LBNP procedure can be regarded as a non-hypotensive manipulation of central venous blood volume. The application of LBNP resulted in decreased normalized heart rate variability in the high frequency band, suggesting vagal withdrawal, increased normalized heart rate variability in the low frequency band (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Berntson et al., 1997), increased LF/HF ratio (Malliani et al., 1994; Montano et al., 1994; Zaza and Lombardi, 2001), and reduced T-wave amplitude (Contrada et al., 1989; Furedy et al., 1992; Furedy et al.,

1996), suggesting increased sympathetic activity. It should be noted that respiratory frequency significantly decreased with increasing LBNP gradients, so that an effect of respiration on heart rate variability cannot be excluded. However, the decrease was rather small (0.03 Hz), and respiratory frequency remained well within the high frequency band. Since a decrease of respiratory frequency is associated with an increase in high frequency heart rate variability (Angelone and Coulter, 1964; Schachinger et al., 1991) (given that the respiratory frequency is greater than 0.1 Hz), an increase in high frequency heart rate variability would be expected due to the respiratory frequency change, but the opposite effect (a decrease) was found. Thus, the induced changes in heart rate variability reflect true parasympathetic withdrawal, serving as a manipulation check in our study, to document the previously described impact of LBNP on autonomic nervous system function (Laszlo et al., 1998; Franke et al., 2003; Kitano et al., 2005). The cardiovascular and autonomic nervous system changes are well in line with increased arousal ratings observed during increased LBNP levels in this study.

This is the first study to indicate increased startle eye blink responses to acoustic noise stimuli during unloading of cardiopulmonary baroreceptors. Stronger reactions to startle stimuli, either voluntarily, as in the case of psychomotor responses, or automatically, as in the case of reflexive startle eye blink, are protective and potentially adaptive when confronted with hostile environmental demands. LBNP has been introduced as a model for haemorrhagic stress (Rea et al., 1991; Cooke et al., 2004; Cooke and Convertino, 2005; Convertino et al., 2006; Cooke et al., 2008), and the cardiovascular changes, especially the direction of heart rate variability changes observed in this study, correspond to autonomic function changes seen after moderate blood loss of about 500 ml (Haberthur et al., 2003). Indeed, it is likely that during states of blood loss stress, which may occur as a result of a predator attack, the support of protective mechanisms enhances the organism's chance of survival. Protective startle responses are favored during negative affect and stress (Grillon et al., 2007; Lissek et al., 2007; Mol et al., 2007), and were found to correlate positively with cardiovascular stress responses (Gautier and Cook, 1997). On the other hand, stressful and attention-demanding tasks may attenuate startle (Neumann, 2002). In the current study, participants did not perceive the application of LBNP as unpleasant. It therefore seems unlikely that the observed increased startle magnitude is mediated by a negative affective state. Rather, our results suggest that baroafferent processes may play a role in adjusting protective response strategies during stressful circumstances, since unloading of baroreceptors, as would occur during states of blood loss, was associated with an enhancement of protective automatic reactions.

Previous studies have found reaction time, especially the pre-motor 'cognitive' component of psychomotor reactions, to be shorter during unloading of arterial baroreceptors (Stewart, 2006; Edwards et al., 2007; McIntyre et al., 2008b), but others have not (Thompson and Botwinick, 1970; Weisz, 1996). In line with the latter finding, our results did not show an effect of either arterial or cardiopulmonary baroreceptors on reaction times. Hence, this supports the assumption that automatic protective reactions (such as reflexive eye blinks) are more likely to be disinhibited during blood loss stress than are voluntary psychomotor reactions, suggesting that 'archaic' automatic brainstem-based protective responses may be favored during haemorrhagic challenge.

In conclusion, these results demonstrate effects of cardiopulmonary as well as arterial baroreceptors on protective reflexive startle eye blink responses. Our results support the speculation that cardiopulmonary baroreceptors may play a role in adapting response strategies during stressful circumstances, such as favouring automatic brainstem relayed responses, while having no such effect on voluntary psychomotor reactions. Our results, furthermore, suggest that effects on startle need to be taken into consideration in investigations employing procedures which affect cardiopulmonary baroreceptor loading status, i.e. changes in body position or changes in fluid intake or output, or after evoking other forms of central venous blood loss.

5.5 Acknowledgements

Steffen Richter, André Schulz and Hartmut Schächinger are members of the International Research Training Group (IRTG) "Psychoneuroendocrinology of Stress: From Molecules and Genes to Affect and Cognition", funded by the German Research Foundation (Deutsche Forschungsgemeinschaft: DFG), grant GRK 1389/1.

Terry Blumenthal would like to acknowledge the support of the Sara Jo Brownlow Shearer Fund at Wake Forest University.

We thank our students Daniel Best, Katharina Beck, Anna Börner and Madlen Kotermann for their help in recruiting participants, data acquisition, and analysis.

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Chapter VI - General Limitations

The presented studies have in common that they focus on the effect of physiological stress correlates on reflexive startle eye blink. It is important to emphasize that these studies do not investigate the impact of natural stress, neither on the startle reflex, nor on prepulse inhibition of startle. Rather, it was the goal to investigate the individual contributions of separate components of the stress response. Thus, it may be quiet likely that participants would react differently when they experience 'real life' stressful situations.

Also, any experimental manipulation involves a certain amount of sensory feedback (as for example during the application of LBNP) or may be associated with potentially stressful circumstances before the actual experiment (as, for example, the placement into the LBNP box, or the application of a venous catheter, or the ingestion of a pill). The applied methods were all chosen to reduce the immediate sensory feedback due to the experimental procedure and sufficient resting periods were included into the study design to reduce any confounding influence of such manipulations. Valence and arousal ratings were obtained to control for any aversive impact of the applied methods.

The present studies were not designed to assess or control central processes other than those reflected by startle and PPI measurements. Thus, we can only speculate about the CNS processes occurring during the manipulation. The manipulation of (serum) cortisol levels likely has had an impact on HPA axis activity due to negative feedback mechanisms, resulting in altered levels of CRH and ACTH, which may have had an impact on the startle reflex and PPI.

Thus, the exact nature of centrally induced changes can not be elucidated with the present methods and remains to be investigated in future studies.

Chapter VII - General Conclusions

The current work aimed to separate the impact of several physiological stress correlates on the protective eye blink reflex. In Study I, it was demonstrated that exogenous stress hormone cortisol (excess) administration, resulting in increased cortisol levels as they may occur during moderate stress events, rapidly impairs prepulse inihibition of startle and thus may contribute to the previously described impairment of PPI during stress. This may improve the capability to detect potentially harmful stimuli in a stressful environment and thus may improve the ability to automatically protect important body parts (such as the eyes, back of the neck, or abdomen) in an environment where the probability that such stimuli indicate real danger is increased. Startle eye blink magnitude remained unaffected by the cortisol administration.

In Study II, it was demonstrated that the pharmacological suppression of endogenous cortisol production, resulting in decreased cortisol levels, enhances startle reflex magnitude. Although a similar effect was observed for the application of small doses of cortisol, a significant reduction of startle magnitude has been demonstrated for high doses of cortisol. This effect may either be due to a differential activation of glucocorticoid and mineralocorticoid receptors at different cortisol levels (due to differences in receptor affinity) or due to an impact of cortisol levels on CRH (via altered negative feedback).

In Study III, it was demonstrated that the unloading of cardiopulmonary baroreceptors, as well as arterial baroreceptors, as it may occur during blood loss stress, increases startle eye blink magnitude. Increasing the magnitude of protective startle reflexes may indeed be of advantage when central venous pressure, or arterial blood pressure, is reduced, for example during blood loss after a predator attack, or during disease and infection, when cytokines and fever induce peripheral blood pooling. It was furthermore demonstrated that simple psychomotor reactions are not affected by cardiopulmonary baroreceptors, suggesting that blood loss stress induces enhancement of automatic rather than voluntary protective reactions.

In summary, these results demonstrate that neuroendocrine stress mechanisms and other physiological alterations during stress may have direct impact on the response magnitude of protective and defensive reflexes. The effects may all be described as supporting adaption and survival in hostile environments.

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbst verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe. Zudem wurde die Arbeit an keiner anderen Universität zum Erlangen eines wissenschaftlichen Grades eingereicht.

Trier, den 10.09.2010

Dr. med. Steffen Richter