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The Cold Pressor Stress Test: From basic psychophysiology to application

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General Abstract

The last decades of stress research have yielded substantial advancements highlighting the importance of the phenomenon for basic psychological functions as well as physical health and well-being. Progress in stress research heavily relies on the availability of suitable and well validated laboratory stressors. Appropriate laboratory stressors need to be able to reliably provoke a response in the relevant parameters and be applicable in different research settings or experimental designs. This thesis focuses on the Cold Pressor Test (CPT) as a stress induction technique. Three published experiments are presented that show how the advantages of the CPT can be used to test stress effects on memory processes and how some of its disadvantages can be met by a simple modification that retains its feasibility and validity.

The first experiment applies the CPT in a substantial sample to investigate the consolidation effects of post-learning sympathetic arousal. Stressed participants with high increases in heart rate during the CPT showed enhanced memory performance one day after learning compared to both the warm water control group and low heart rate responders. This finding suggests that beta-adrenergic activation elicited shortly after learning enhances memory consolidation and that the CPT induced heart rate response is a predictor for this effect. Moreover, the CPT proved to be an appropriate stressor to test hypothesis about endogenous adrenergic effects on memory processes.

The second experiment addresses known practical limitations of the standard dominant hand CPT protocol. A bilateral feet CPT modification is presented, the elicited neuroendocrine stress response assessed and validated against the standard CPT in a within-subjects design. The bilateral feet CPT elicited a substantial neuroendocrine stress response. Moreover, with the exception of blood pressure responses, all stress parameters were enhanced compared to the standard CPT. This shows that the bilateral feet CPT is a valid alternative to the standard CPT.

The third experiment further validates the bilateral feet CPT and its corresponding control procedure by employing it in a typical application scenario. Specifically, the bilateral feet CPT was used to modulate retrieval of event files in a distractor-response binding paradigm that required lateralized bimanual responses. Again, the bilateral feet CPT induced significant increases in heart rate, blood pressure and cortisol, no such increases could be observed in the

warm water control condition. Moreover, stressed participants showed diminished retrieval compared to controls. These results provide further evidence for the feasibility and validity of the bilateral feet CPT and its warm water control procedure.

Together the experiments presented here highlight the usefulness of the CPT as a tool in psychophysiological stress research. It is especially well suited to test hypothesis concerning stress effects on memory processes and its applicability can be further increased by the bilateral feet modification.

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Index of Publications

This doctoral thesis consists of three chapters (and, in addition, one chapter that represents a general introduction) 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).

Content	***has been published as***
Chapter II	Larra, M.F., Schulz, A., Schilling, T.M., Ferreira de Sa, D.S., Best, D., Kozik, B., and Schachinger, H., 2014. Heart rate response to post-learning stress predicts memory consolidation. *Neurobiology of learning and memory 109,* 74-81.
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Chapter IV	Frings, C., Larra, M.F., Grabener, A., Moeller, B., and Schachinger, H., 2013. Stress disrupts distractor-based retrieval of SR episodes. *Biological Psychology 93,* 58-64.

Index of Abbreviations

°C ..... Degrees Celsius
ACTH ..... Adrenocorticotropic hormone
ADR ..... Adrenaline
Ag/AgCl ..... Silver/silver chloride
ANOVA ..... Analysis of variance
AUCi ..... Area under the curve with respect to increase
AVP ..... Arginine vasopressin
BLA ..... Basolateral nucleus of the amygdala
BMI ..... Body Mass Index
CBG ..... Cortisol binding globulin
cm ..... Centimeter
CPT ..... Cold Pressor Test
CRH ..... Corticotropin-releasing hormone
d' ..... d prime
DA ..... Dopamine
DBP ..... Diastolic blood pressure
e.g. ..... Exempli gratia
ECG ..... Electrocardiogram
GR ..... Glucocorticoid receptor
HPA axis ..... Hypothalamus-pituitary-adrenal axis
HR ..... Heart rate
IV ..... Independent variable
kg ..... Kilogram
kHz ..... Kilo Hertz
LC ..... Locus coeruleus
ln ..... Natural logarithm
MANOVA ..... Multivariate analysis of variance
MAP ..... Mean arterial pressure
min ..... Minute
ml ..... Milliliter

mmHg ..... Millimeter of mercury
MR ..... Mineralocorticoid receptor
ms ..... Milliseconds
NTS ..... Nucleus of the solitary tract
p.m. ..... Post meridiem
PFC ..... Prefrontal cortex
PVN ..... Nucleus paraventricularis
RT ..... Reaction time
SA ..... Sympathoadrenal
sAA ..... Salivary alpha-amylase
SBP ..... Systolic blood pressure
SD ..... Standard deviation
SECPT ..... Socially evaluated Cold Pressor Test
SEM ..... Standard error of the mean
SN ..... Sympathoneural
SNS ..... Sympathetic Nervous System
S-R ..... Stimulus-Response
TSST ..... Trier Social Stress Test

Chapter I: General Background

1.1 Introduction and Outline

The study of stress has essentially contributed to our understanding of the ways in which adverse events are causally linked to physical health and well-being. The last decades have seen fundamental progress in research on the topic of stress. Stress has been shown to be involved in the genesis of a variety of pathological conditions (Chrousos and Kino, 2007; Marin et al., 2011) and to affect diverse psychological processes (Campeau et al., 2011) while recent advancements allowed to trace some of these stress effects to specific actions that stress hormones exert on the brain (Erickson et al., 2003; Lupien et al., 2007; Roozendaal and McGaugh, 2011).

Progress in stress research heavily relies on the availability of suitable and well validated laboratory stressors. Appropriate laboratory stressors need to be able to reliably provoke a response in the relevant parameters and be applicable in different research settings or experimental designs. However, stress responses have been shown to differ according to the type of stressors employed (Dickerson and Kemeny, 2004; Pacak and Palkovits, 2001) and experimental designs often pose restrictions that render an otherwise appropriate stressor unfeasible. The present work focuses on the Cold Pressor Test (CPT) as a stress induction technique. Three published experiments are presented that show how the advantages of the CPT can be used to test stress effects on memory processes and how restrictions of certain experimental designs can be met by a simple modification that retains its feasibility and validity.

This thesis consists of four chapters. In the following chapter I will describe the scientific background to the experimental investigations presented in chapters II to IV. First, I will give a general introduction into the topic of stress in which the basic physiological mechanisms of the stress response, stress effects on the brain and forms of its operationalization in psychobiological experiments are addressed. The second section focuses on the CPT as such a laboratory model of stress. I will briefly describe its physiological mechanisms and effects in different fields of study and discuss problems as well as advantages in its application. Finally, the three experimental investigations are outlined, briefly summarizing their main aims, design, results and final conclusions. The following chapters II to IV contain the published reports on

the three experiments.

1.2 Stress

Stress is a phenomenon referring to the elicitation of a specific response pattern, the "stress response", by a certain class of stimuli termed "stressors". Stressors have been very generally defined as being any perceived or sensed threat to homeostasis or well-being (Ulrich-Lai and Herman, 2009), a mismatch between expectation and perception that elicits a patterned compensatory response (Goldstein and Kopin, 2007) or as any demand on the body that causes a stress response (Selye, 1976). They may be differentiated on the basis of their origin and the kind of threat they pose. Accordingly, four classes of stressors have been suggested (Pacak and Palkovits, 2001): Physical stressors that are directly sensed as pain, cold, noise or chemical agents: Psychological stressors that require evaluation by higher brain areas to be perceived as threat. Social stressors that arise from interactions with other individuals and bodily stressors that pose a demand on cardiovascular or metabolic homeostasis.

While psychological theories on stress focus on the interpretation and evaluation of stressors with respect to available resources (Lazarus, 1999; Lazarus and Folkman, 1984), the physiological response pattern, its mediators and their effects lie at the core of psychobiological stress research. Those will be addressed in the following sections.

1.2.1 The stress response

The stress response is a complex phenomenon comprised of reactions and interactions in behavioral, autonomic, endocrine, and immune systems. Today's recognition of the stress response as a fundamental physiological mechanism was mainly primed by the influential works of Walter Cannon and Hans Selye. In the first half of the 20th century they popularized the topic and lay the foundation for our understanding of the basic principles of the stress response.

Cannon (1939) introduced the concept of homeostasis meaning the maintenance of physiological parameters within an acceptable range. He discovered that a wide variety of

threats to homeostasis including psychosocial factors would lead to an activation of the sympathetic nervous system (SNS) and release of adrenaline (ADR) from the adrenal medulla. Cannon thought these two effectors to act as a unit, the "sympathoadrenal system", that upon activation would produce compensatory and anticipatory adjustments (the "fight or flight response") to restore homeostasis and promote survival.

Selye, who popularized the scientific term stress, defined stress as a nonspecific response pattern to diverse noxious stimuli mainly characterized by an activation of the hypothalamus-pituitary-adrenal (HPA) axis and its effects (Selye, 1950; Selye, 1976). Although Selye(1950) acknowledged that there are also stressor specific responses he did not consider them to be part of the stress response. This doctrine of non-specifity has been challenged and it is now widely acknowledged that the stress response is to some extent specific depending on the type of stressor. Signaling pathways that lead to HPA axis and SNS activation differ according to the type of stressor triggering responses that are commensurate with the nature of the stimulus (Goldstein, 2010; Pacak and Palkovits, 2001).

Modern accounts of the stress response see the SNS and HPA axis as main components of a physiological stress system which is largely controlled by the hypothalamus (Chrousos, 1998; Johnson et al., 1992). The hypothalamus is the principal integrator of stress signals. Brainstem centers that sense systemic stressors as blood loss as well as limbic regions that process psychological stressors project to the nucleus paraventricularis (PVN) of the hypothalamus (McEwen, 2007). The PVN mainly orchestrates the SNS and HPA axis response to stress (Ulrich-Lai and Herman, 2009), these two main components of the stress response will we explained in detail below.

1.2.1.1 Sympathetic Nervous System

The SNS provides a fast physiological response to stressors through neural innervation of its target organs taking effect within seconds. It may be divided into two branches, the sympathoneural (SN) and the sympathoadrenal (SA) arm (Kvetnansky et al., 2009) and there is some evidence that these two branches act partially independent giving rise to specific reactions depending on the type of stressor (Goldstein and Kopin, 2007; Pacak and Palkovits, 2001). Sympathetic preganglionic neurons in both branches are controlled by catecholaminergic and noncatecholaminergic sympathetic premotor neurons located mainly in the hypothalamus and

brainstem. Some systemic stressors that signal major threats to the organism, as blood loss, pain or inflammation, may activate preganglionic neurons without hypothalamic involvement through reflex arcs at the intermediolateral cell column (Pacak and Palkovits, 2001; Ulrich-Lai and Herman, 2009). The SN arm is organized in a two neuron chain consisting of pregranglionic and postganglionic sympathetic neurons. Preganglionic neurons activate postganglionic neurons by release of acetylcholine. Upon activation varicosities of the postganglionic fibers release noradrenaline (NA) at their target organs. They do not form a synaptic junction with cells in their target organs but NA is released via exocytosis over a broad area of the target tissue. In the SA arm preganglionic neurons of the SNS innervate chromaffin cells in the adrenal medulla. Chromaffin cells store mainly ADR but also NA. After excitatory signals arrive from preganglionic neurons, the chromaffin cells secrete ADR and NA into the general circulation via exocytosis causing widespread effects at multiple target sites (for a detailed overview of the SNS see Goldstein, 2009; Palkovits, 2009).

Sympathetic activation thus results in a rise in levels of circulating ADR and NA and leads to an increase in heart rate and force of contraction, peripheral vasoconstriction, increased blood flow to skeletal muscles and energy mobilization (Chrousos and Gold, 1992) giving rise to a general state of arousal that Cannon referred to as fight-or-flight response. However, this response is shortlived and rapidly counteracted by reflex parasympathetic activation (Ulrich-Lai and Herman, 2009).

1.2.1.2 Hypothalamic-Pituitary-Adrenal Axis

The HPA axis acts as interface between the central nervous system (CNS) and the endocrine system mediating the endocrine response to centrally processed stressors. It consists of three core structures, the PVN, the pituitary and the adrenal glands that communicate with each other through specific neurohormones and hormones. The PVN regulates the HPA axis response to stress (Ziegler and Herman, 2002). It receives signals from brainstem centers as well as the limbic system and prefrontal cortex (PFC) allowing for an activation through systemic and directly sensed as well as psychological or anticipated stressors (Herman et al., 2005; Ulrich-Lai and Herman, 2009). During stress the parvocellular neurons of the PVN release regulatory neurohormones, mainly corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), into the hypophysial portal vasculature. Through the portal vessels these neurohormones rapidly reach the anterior pituitary gland where they act synergistically to

stimulate the secretion of adrenocorticotropic hormone (ACTH) into the bloodstream. After its release from the anterior pituitary gland circulating ACTH moves to the cortex of the adrenal glands. Here, it stimulates synthesis and secretion of glucocorticoids mainly in the zona fasciculata. Glucocorticoids, cortisol in humans, are the ultimate endproduct of the HPA axis response to stress and can be expected to increase about ten minutes after stressor onset (for a detailed overview of the HPA axis see Fulford and Harbuz, 2005; Herman, 2009).

To prevent glucocorticoid levels from overshooting HPA axis activity is downregulated by complex negative feedback mechanisms exerted at multiple sites and timescales. Negative feedback is exerted on both the hypothalamus and the pituitary as well as on brain sites projecting to the PVN as limbic structures and the PFC (Dallman, 2007; Herman et al., 2005). An initial rapid feedback develops within seconds by a nongenomic mechanism mediated through membrane receptors (Di et al., 2003). It is short in duration (approximately ten minutes) and sensitive to changes in glucocorticoid concentrations rather than absolute levels (Herman, 2009). Delayed feedback sets in about half an hour after an acute elevation of glucocorticoid levels and may last for hours (Dallman, 2007). It is mediated through genomic mechanisms initialized by nuclear mineralocorticoid and glucocorticoid receptors (Dallman et al., 1992). Together fast and slow negative feedback on HPA axis activity enable the termination of the stress response and ensure that glucocorticoid levels stay within tolerable limits.

1.2.2 Stress effects on the brain

Investigations on stress would probably not play such a prominent role in psychobiological research if not for its profound effects on multiple psychological functions as well as physical and mental health. Everybody has experienced how stress can affect us in our normal functioning and in the last decades substantial advances have been made in our understanding how stress influences brain functions and thereby our experience and behavior.

Stress effects on the brain are mainly mediated by the central actions of the two main endproducts of the stress response, ADR/NA and cortisol (Erickson et al., 2003; Lupien et al., 2007; Roozendaal and McGaugh, 2011). Cortisol traverses the cell membrane and binds to nuclear mineralocorticoid (MR) and glucocorticoid receptors (GR) that then translocate to the cell nucleus to alter gene transcription (de Kloet et al., 1993). Besides these genomic effects

cortisol may also exert faster non-genomic effects through receptors residing in the cell membrane (Falkenstein et al., 2000; Orchinik et al., 1991). Most of the circulating cortisol in the blood is bound to a carrier protein (cortisol binding globulin, CBG) and albumin which renders it physiologically inactive. Unbound cortisol by contrast can cross the blood-brain barrier and readily enters the brain (Mason et al., 2010; Murphy et al., 1967) to act on membrane and nuclear MRs and GRs. Brain structures as the hippocampus, the amygdala and the prefrontal cortex (PFC) express a high density of MRs and GRs allowing for modulations by cortisol during stress (Patel et al., 2000; Sanchez et al., 2000). Indeed, cognitive functions that are associated with these structures as memory (Roozendaal and McGaugh, 2011), attentional (Sanger et al., 2014) and affective processes (Campeau et al., 2011) have been shown to be modulated by cortisol and stress.

Peripheral catecholamines released during stress cannot directly enter the brain (Weil-Malherbe et al., 1959). However, they might affect central processes via vagal afferents projecting to the nucleus of the solitary tract) (NTS; Williams et al., 2000). The NTS heavily projects to the amygdala which in turn sends widespread connections throughout the brain (McGaugh, 2004). In addition, central catecholaminergic pathways mainly originating from the locus coeruleus (LC) and projecting to the limbic system and PFC may contribute to modulatory stress effects on the brain (Berridge and Waterhouse, 2003).

Among the cognitive functions influenced by stress memory processes are probably the most extensively studied within stress research. A prominent model of how ADR/NA and cortisol released during stress may interact in mediating stress effects on memory has been proposed by Roozendaal and McGaugh (2011). Based on a series of rodent experiments they argue that interactions of NA and cortisol at the basolateral nucleus of the amygdala (BLA) cause the retention enhancement of stressful and emotional memories. Specifically, circulating ADR acts on peripheral beta-adrenoreceptors from vagal afferents projecting to the NTS which in turn directly and indirectly (via the LC) changes noradrenergic activation within the BLA (Williams et al., 2000). The amygdala then modulates memory processes through its widespread connections to brain structures mediating memory functions, particularly the hippocampus and caudate nucleus (Ferry et al., 1999; McIntyre et al., 2012). An activation of GRs in the BLA and memory processing areas is required for this effect (Roozendaal et al., 1996). Similarly, cortisol effects on memory require concurrent noradrenergic activation within the BLA (Roozendaal et al., 2006; Setlow et al., 2000).

Animal experiments involving infusions of peripherally and centrally acting adrenoreceptor agonists and antagonists as well as manipulation of GRs and cortisol levels provide compelling evidence for this model (for review see McIntyre et al., 2012; Roozendaal and McGaugh, 2011). The role of endogenously elicited (via Cold Pressor stress) sympathetic arousal in modulating memory consolidation in humans is assessed in one of the experimental investigations presented in this thesis (Chapter II).

1.2.3 Eliciting stress in the laboratory

Establishing ethically acceptable paradigms to elicit stress in humans under laboratory conditions is a continuing challenge. The specifity of stress reactions as a result of the type of stressor applied (Goldstein, 2010) as well as interindividual differences (Gerra et al., 2001; Kajantie and Phillips, 2006) pose the main problem in research with laboratory stressors. Not all of them are qualified to produce a full neuroendocrine stress response in every individual, especially substantial cortisol increases are often lacking (Dickerson and Kemeny, 2004). As has been pointed out above stress effects on the brain rely on rather specific actions of cortisol and catecholamines released during stress, therefore, the choice of an appropriate stressor is crucial. Additionally, depending on the research question further limitations need to be taken into account as certain stressors may be unfeasible e.g. due to their application duration, interference with other experimental paradigms or the lack of a valid control procedure.

Typically, stress experiments follow a sequence beginning with a baseline, or initial rest, period that allows to examine within-subject changes between baseline and task. The stressor is then administered usually followed by a recovery period. A vast variety of stimulations have been used as stressors. Commonly used stress elicitation paradigms are mental arithmetic (e.g. paced subtraction or addition tasks; McCann et al., 1993), exercise (e.g. hand-grip task; Nielsen and Mather, 2015), orthostatic tasks (e.g. head-up tilt; Shoemaker et al., 2001) and psychosocial stressors (e.g. public speaking; Gerra et al., 2001).

Mental arithmetic tasks require effortful control of attention and exercise causes an energy demand both going along with sympathetic activation (Nielsen and Mather, 2015; Peters et al., 1998). Orthostatic changes mainly disrupt cardiovascular homeostasis triggering a fast

autonomic response (Fu et al., 2005). Depending on intensity and duration of exposure increases in cortisol may be observed (Al'Absi et al., 1997; McCann et al., 1993), however, these tasks are primarily employed to study the SNS component of the stress response. Stressors that incorporate a social evaluative component, on the other hand, are particularly well suited to elicit robust HPA axis responses (Dickerson and Kemeny, 2004; Schwabe et al., 2008b). Typical examples of such social stressors are public speaking tasks and the Trier Social Stress Test (TSST), a 20-minute paradigm specifically designed to trigger the HPA axis by a combination of a public speech and mental arithmetic task conducted in front of a panel of judges (Kirschbaum et al., 1993). Although these stressors are best suited to produce a full neuroendocrine stress response including an activation of the HPA axis, they may not always be feasible as they are time consuming, require a cognitive engagement and do not offer a simple control procedure. Also, they rely on the performance and properties of the experimenter, which need to be controlled. Another widely used stress protocol is the CPT which is central to this thesis and will be portrayed in the following sections.

1.3 The Cold Pressor Test

In its core the CPT consists of a procedure in which a limb (usually the dominant hand) is immersed into ice-water for a short period of time (usually 2 to 3 minutes). It was first introduced in the 1930s by Hines and Brown (1932). They employed a routine that started with a resting period during which multiple blood pressure readings were taken. After that the CPT was carried out and a recovery resting period followed both accompanied by blood pressure readings. This format of the reactivity study during rest, stress, and recovery lay the foundation and still is the common adopted procedure in stress studies. Although originally intended as means to experimentally increase blood pressure in studies on hypertension, the CPT has now become a widely used tool in experimental research of different areas and is frequently employed as a laboratory stress protocol.

In the following sections I will briefly summarize what is currently known about the physiological mechanisms and responses triggered by CPT stimulation, its use in different fields of study and finally discuss its main advantages and disadvantages as a laboratory stress protocol.

1.3.1 Physiological mechanism and responses

Exposure to the CPT leads to a stimulation of peripheral thermo- and nociceptors located throughout the skin. Pain and temperature fibers enter the spinal cord in the dorsal roots and cross contralateral to form the spinolateral tract which travels to the thalamus and sends collaterals to the reticular formation. At the medulla level these collaterals may stimulate the rostral ventrolateral medullary pressor area resulting in a reflexive sympathetic discharge towards the heart and the vessels (Nakamura et al., 2008; Velasco et al., 1997). Via brainstem projections CPT stimulation may also affect cortical and subcortical structures as the hypothalamus that further modulate neuroendocrine reactions creating a multifaceted physiological and subjective stress response (Lovallo, 1975; McEwen, 2007; Ulrich-Lai and Herman, 2009).

CPT exposure leads to profound changes in cardiovascular parameters most notably a rise in blood pressure through peripheral vasoconstriction and to a lesser extent cardiac output resulting from an increase in both vascular alpha-adrenergic and cardiac beta-adrenergic drive (Greene et al., 1965; Lovallo, 1975; Yamamoto et al., 1992). However, CPT effects are not restricted to the cardiovascular system. Increases in multiple markers of sympathetic nervous system activity as skin conductance level (Buchanan et al., 2006), plasma chatecholamines (Goldstein et al., 1994; Pascualy et al., 1999; Ward et al., 1983), muscle sympathetic nerve activity (Victor et al., 1987; Yamamoto et al., 1992) and more recently salivary alpha-amylase (sAA) have been reported (Smeets et al., 2008). In addition to the effects on the sympathetic nervous system the CPT has been shown to be capable of activating the HPA axis. McRae et al. (2006) found elevated plasma ACTH concentration after CPT exposition. Also salivary cortisol concentrations have shown to be elevated about 15 minutes after the CPT (al'Absi et al., 2002; Felmingham et al., 2012; Hupbach and Fieman, 2012). On the subjective level participants experience the CPT as painful and report heightened levels of perceived stress and arousal during and immediately after the waterbath (al'Absi et al., 2002; Zoladz et al., 2014).

1.3.2 The Cold Pressor Test in psychophysiological research

The CPT has been employed in a wide range of psychophysiological studies. Originally, it was designed as a standard stimulus to increase blood pressure under laboratory settings and

primarily used in studies on the etiology of hypertension. Blood pressure responses of about 10 to 20 mmHg have been shown to be reliably elicited (Velasco et al., 1997). Furthermore, hyperreactivity in blood pressure responses to CPT stimulation has been reported to be predictive for the development and the severity of essential hypertension (Flaa et al., 2008; Treiber et al., 2003), although conflicting results exist (Lambert and Schlaich, 2004). The CPT has also been used to study sympathetic integrity and basic cardiovascular functioning in healthy and clinical populations as to assess the severity of autonomic dysfunction in diabetes mellitus (Sayinalp et al., 1994) and spinal cord injury (Previnaire et al., 2012). Other studies apply the CPT as pain evoking stimulus to evaluate the analgesic effect of pharmacological and psychological treatments (Abbott et al., 1992; Edwards and Fillingim, 2005).

As the CPT is capable of inducing increases in cortisol and catecholamines, both of which are of major interest in psychophysiological stress research, it is also frequently employed as laboratory stressor. In many studies on stress on behavioral and cognitive processes the CPT has been shown to modulate a range of psychophysiological phenomena. For instance, autonomic startle responses (Deuter et al., 2012) as well as the cardiac modulation of the startle response (Schulz et al., 2011) are affected immediately after CPT exposure. Also, CPT stress impairs the top-down control of attention as reflected in behavioral and electrophysiological indices (Sanger et al., 2014). Finally, a plethora of experiments use the CPT to study stress effects on memory processes. Here, CPT stress has been found to impair retrieval processes while enhancing consolidation of diverse classes of stimuli (Cahill et al., 2003; Duncko et al.,2009; Felmingham et al., 2012; Schwabe et al., 2008a; Schwabe and Wolf, 2010; Smeets et al., 2008).

1.3.3 Advantages and Disadvantages of the Cold Pressor Test

The CPTs frequent use across diverse fields of study and experimental designs is both indicative for its many strengths and also by itself one of its major advantages. Many studies have assessed a multitude of different outcome variables ranging from plasma and salivary concentrations of (neuro-)hormones (Pascualy et al., 2000; Smeets et al., 2008) over electrophysiological parameters (Buchanan et al., 2006; Yamamoto et al., 1992) to subjective reports (al'Absi et al., 2002; Zoladz et al., 2014). A plethora of research has contributed to knowledge about interindividual differences that influence CPT reactions (Flaa et al., 2007; Wu et al., 2010).

This ample level of validation and standardization allows the researcher to quite precisely estimate what outcomes can be expected and what factors need to be taken into account when employing the CPT. Furthermore, unlike other stressors as mental arithmetic or public speaking tasks, the CPT is a passive task in that it does not impose any form of cognitive load on the participant. This helps to reduce conflicts with other experimental measures. For instance, retroactive and proactive interference due to the stressor itself can be avoided when stress effects on memory are to be investigated. Also, the CPT requires only little time in preparation and application which makes it an economic laboratory stressor and also allows for an accurate timing of the intervention. Finally, with the corresponding warm water test a well validated non-stressful control procedure to the CPT is available.

On the other hand, the CPT has been criticized for not being capable of inducing a substantial HPA axis activation (McRae et al., 2006). Indeed, many studies fail to confirm significant increases in cortisol after CPT exposure (Duncko et al., 2009; McRae et al., 2006; Schwabe et al., 2008b). However, this weakness has been addressed by adding a social evaluative component to the CPT (socially evaluated CPT, SECPT) which was found to significantly enhance cortisol responses (Schwabe et al., 2008b). Another objection concerns practical limitations due to the dominant hand immersion that hinders the collection of other measurements also requiring hands during and shortly after the CPT. Moreover, depending on the research question laterality effects due to unilateral hand immersion (Harper et al., 2000; McGinley and Friedman, 2014) may need to be avoided. Taken together, these shortcomings may render the CPT unfeasible with many experimental paradigms.

Thus, whereas the CPT is an advantageous laboratory stressor in many respects, some disadvantages reduce its value within psychophysiological stress research. In Chapters III and IV of this thesis experiments are presented that assess the validity of a modification to the classic CPT addressing these issues.

1.4 Experimental Investigations

In the following section I will summarize the main aims, methods, results and final conclusions of the three experiments presented in Chapters II to IV. The first experiment uses the CPT to evaluate adrenergic influences on memory consolidation. The second experiment introduces a

bilateral feet modification of the standard CPT aimed at solving some of its limitations. The third experiment employs this new CPT version and its corresponding warm water control procedure in a typical application scenario.

1.4.1 Heart rate response to post-learning stress predicts memory consolidation

Stress has been shown to enhance memory consolidation in both humans and animals. This effect is assumed to be based on an interaction of stress induced noradrenergic activation and cortisol within the BLA and hippocampus (see Chapter 1.2.2). Studies employing pharmacological manipulations provide human evidence for this model. However, evidence from human experiments assessing the impact of endogenous sympathetic arousal induced by laboratory stressors is mixed.

This study employs the CPT to investigate the consolidation effects of post-learning sympathetic arousal as indexed by the stress induced heart rate (HR) response. Specifically, we hypothesized that the magnitude of the stress induced HR response would predict memory performance one day after learning. 206 male and female participants saw a set of 52 happy and angry faces immediately before being exposed to the CPT (N = 135) or a control procedure (warm water, N = 71). Memory for the faces and their respective expression was tested twice, after 30 minutes and on the next day. To prevent loss of statistical power when assessing the influence of the HR response within the stress group, we doubled its size with respect to controls thereby enabling us to compare equally sized groups of high HR responders, low HR responders and controls. High HR responders (in comparison to low HR responders as well as to the non-stressful control group) showed enhanced recognition memory one day after learning, whereas there were no group differences in the 30 minute test.

These results show that beta-adrenergic activation elicited shortly after learning enhances memory consolidation and that the stress induced HR response is a predictor for this effect. Moreover, this experiment demonstrates how to make use of the advantages of the CPT procedure and meet its potential limitations. As such, knowledge about its predominantly adrenergic effects allowed for an informed decision on the suitability of the CPT as stressor to test our hypothesis. Furthermore, its short application duration enabled a precise timing after the learning epoch while minimizing carry-over effects on the first memory test. The absence

of cognitive load during the CPT made it possible to avoid effects of retro- and proactive interference when testing memory performance and thus to isolate the pure influence of stress. Finally, with the availability of a control procedure we could follow an experimental between-subjects design while we accounted for the known interindividual variability in heart rate responses to the CPT by doubling the size of the experimental group.

1.4.2 Enhanced neuroendocrine stress response by a bilateral feet compared to a unilateral hand Cold Pressor Test

There are some major practical problems inherent to the classical one hand CPT procedure. As such, the typical unilateral hand immersion produces laterality specific effects (Harper et al., 2000; McGinley and Friedman, 2014) that may create unwanted interference in all studies that require unilateral stimulus presentation or responses in some form. Furthermore, the amount of parameters that can be derived during and shortly after the CPT is limited due to the blocking of one hand. For example measurement of electrodermal activity and beat-to-beat blood pressure both require the placement of sensors on the hand or fingers. Also, local cold of the hands may affect the speed of manual button pushes critical to studies where reaction time is of interest.

Addressing these issues, in this study a simple modification of the classic CPT in which both feet are immersed into ice-water is presented. We assessed feasibility and validity of the bilateral feet CPT version by comparing the elicited neuroendocrine stress response to that of the classical dominant hand CPT in a within-subjects design. 24 participants were exposed to each of both CPT versions on two subsequent days and the sequential order was varied between subjects. Heart rate, blood pressure, sAA and saliva cortisol were measured at baseline and during or after CPT exposition, respectively, along with subjective ratings of pain and stress assessed during the CPT. The change in all of these parameters was evaluated within each stressor version and subsequently compared between both stressors. The feet CPT induced marked increases in heart rate, blood pressure, sAA and cortisol. With the exception of blood pressure, all of these measures were significantly enhanced compared to the hand CPT, which did not lead to significant increases in heart rate or cortisol. Also, subjective stress ratings were higher in the feet than in the hand CPT, however, only during the first two minutes.

This study demonstrates that some of the limitations of the CPT procedure can be met by a simple modification. The bilateral feet CPT induces a substantial neuroendocrine stress response and is thus a valid and feasible alternative to the classic dominant hand CPT. Furthermore, the finding that both cortisol and heart rate responses are enhanced compared to the classic CPT makes the bilateral feet CPT a highly valuable tool in psychophysiological research as these indicators are of crucial interest in most stress studies.

1.4.3 Stress disrupts distractor-based retrieval of SR episodes

In this study the bilateral feet CPT was put to action in a typical application scenario further validating the bilateral feet CPT and its corresponding warm water control procedure. Specifically, we explored the effects of Cold Pressor stress on the phenomenon of distractor-based retrieval of stimulus-response episodes with a sequential priming paradigm, in which the distractor stimuli of the prime trial are sometimes repeated as distractors in the probe trial and the according difference in reaction times is assessed. This paradigm represents a typical application scenario for the bilateral feet CPT as lateral bimanual responses are required and manual response time is the dependent variable, rendering the unilateral hand CPT unfeasible.

22 participants worked through two blocks of the sequential priming paradigm. Immediately before the second block, the bilateral feet CPT or the warm water control procedure was applied and cardiovascular as well as cortisol responses and subjective ratings were assessed. The bilateral feet CPT led to significant increases in blood pressure, heart rate and salivary cortisol. No such increases could be observed in the warm water group which also reported low levels of stress and arousal compared to the cold water group. Furthermore, distractor-response binding was diminished in the second (post-stress) block in the feet CPT but not in the control group which showed enhanced binding.

This study demonstrates that the bilateral feet CPT may be employed to successfully impair retrieval processes paralleling earlier findings obtained with the classic CPT. Moreover, this study replicates our previous results in that both substantial heart rate and cortisol increases can be achieved with the bilateral feet CPT. Finally, the corresponding warm water test is shown to be an appropriate control procedure also for the bilateral feet CPT. In conclusion, these results further confirm the validity of the bilateral feet CPT as a laboratory stress protocol.

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Chapter II: Heart rate response to post-learning stress predicts memory consolidation

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2.0 Abstract

Stressful experiences are often well remembered, an effect that has been explained by beta-adrenergic influences on memory consolidation. Here, we studied the impact of stress induced heart rate (HR) responses on memory consolidation in a post-learning stress paradigm. 206 male and female participants saw 52 happy and angry faces immediately before being exposed to the Cold Pressor Test or a non-stressful control procedure. Memory for the faces and their respective expression was tested twice, after 30 minutes and on the next day. High HR responders (in comparison to low HR responders as well as to the non-stressful control group) showed enhanced recognition memory one day after learning. Our results show that beta-adrenergic activation elicited shortly after learning enhances memory consolidation and that the stress induced HR response is a predictor for this effect.

Keywords: cold pressor stress test, heart rate, memory consolidation, identity memory, recognition memory

2.1 Introduction

Stressful situations often create long lasting memories. Abundant evidence indicates that the high memorability of stressful and arousing events results from an enhancement of consolidation processes (Roozendaal, 2002; Roozendaal and McGaugh, 2011). During stress, activation of the sympathetic nervous system will lead to a state of arousal through beta-adrenergic stimulation of peripheral (i.e. the heart) and central (i.e. the amygdala) target tissues (Chrousos, 1998; Chrousos and Gold, 1992; Johnson et al., 1992). Depending on the type and severity of the stressor (Dickerson and Kemeny, 2004; McRae et al., 2006), activation of the HPA axis will result in a release of cortisol, a steroid hormone that readily passes the blood-brain-barrier (Mason et al., 2010; Murphy et al., 1967; Pardridge and Mietus, 1979). Animal experiments could demonstrate that stress effects on consolidation are driven by beta-adrenergic mechanisms and corticosteroid hormones (McGaugh, 2000; Roozendaal et al., 2009). Specifically, stress leads to beta-adrenoreceptor activation within the basolateral amygdala, and it has been shown that such amygdala activation strengthens memory consolidation via its widespread network of efferent projections to other brain regions (McGaugh, 2004; Roozendaal and McGaugh, 2011).

In line with the animal model, considerable evidence suggests an involvement of the adrenergic/noradrenergic system in human memory regulation (Chamberlain et al., 2006; Lonergan et al., 2012; van Stegeren, 2008). A well replicated finding is that administration of the nonspecific beta-blocker propanolol before learning leads to impaired emotional memory (Cahill et al., 1994; Maheu et al., 2004; O'Carroll et al., 1999a; Strange and Dolan, 2004; van Stegeren et al., 1998). Conversely, enhancing noradrenergic turnover potentiates emotional memories (O'Carroll et al., 1999b). However, these results remain somehow equivocal with respect to the postulated actions on consolidation since the observed effects could theoretically also be explained by influences on encoding. To overcome this problem, a paradigm has been introduced in which adrenergic manipulations are administered post-learning as this allows for a clear attribution to consolidation. Applied after learning, exogenous triggering of beta-adrenergic transmission via administration of adrenaline or yohimbine also led to enhanced memory performance (Cahill and Alkire, 2003; Southwick et al., 2002).

However, albeit the evidence for beta-adrenergic modulation of memory consolidation from studies administering exogenous adrenergic agents, studies seeking to establish a relationship

between *endogenous* markers of post-learning beta-adrenergic activation and memory consolidation have been less conclusive. These studies have typically measured concentrations of salivary alpha-amylase (sAA), an enzyme thought to reflect sympathetic activation via an adrenergic mechanism (Dantzer and Kalin, 2009; Nater et al., 2005; Strahler et al., 2010). An association between memory consolidation and sAA was first reported by Smeets and colleagues (2008). The authors applied the Cold Pressor Test (CPT) immediately after learning of emotional and neutral words. sAA and cortisol concentrations rose significantly after the stress intervention and were both positively correlated to cued recall performance assessed 24 hours later. In contrast, other studies (Bryant et al., 2013; Felmingham et al., 2012) measuring sAA after post-learning administration of CPT could not find an effect of sAA levels on delayed free recall of neutral and emotional pictures. Similarly, two studies applying the Trier Social Stress Test after learning of emotional words (Smeets et al., 2009) or pictures (Preuss and Wolf, 2009) failed to detect any influence of stress induced sAA rise and delayed free recall performance. Nevertheless, endogenously elicited post-learning arousal per se does enhance memory consolidation as has been frequently demonstrated in the above mentioned as well as other studies that unfortunately did not provide any physiological indicator of beta-adrenergic activation (Anderson et al., 2006; Beckner et al., 2006; Cahill et al., 2003; Liu et al., 2007; Nielson and Powless, 2007).

Collecting sAA is a comparatively young approach to the assessment of beta-adrenergic activation and until now there is no consensus on the appropriateness of its use (Bosch et al., 2011). Conversely, there is a long standing tradition in using cardiovascular parameters to quantify beta-adrenergic activation and its impact on multiple aspects of cognition. Most surprisingly, the predictive value of cardiovascular indicators went widely unnoticed in research of stress effects on consolidation. Within this context, the stress induced heart rate (HR) response seems to be an especially promising indicator. Pharmacological agents that have been successfully employed to modify memory show commensurate alterations in HR (Cahill and Alkire, 2003; O'Carroll et al., 1999a) and also change the HR response to stress (Houben et al., 1982; Victor et al., 1987). Furthermore, both tonic and phasic HR responses during encoding have repeatedly been shown to be involved in emotional memory enhancement (Abercrombie et al., 2008; Buchanan et al., 2006; Jennings and Hall, 1980).

Thus, in the current study we attempted to assess the impact of the stress induced heart rate response on memory consolidation in a paradigm of post-learning stress. Using a substantial

sample and the CPT as predominantly adrenergic stressor (Pascualy et al., 2000; Ward et al., 1983) we hypothesized that the magnitude of the stress induced heart rate response would predict memory performance on the next day. 206 male and female participants saw a set of 52 happy and angry faces immediately before being exposed to the CPT or a control procedure (warm water). Memory for the faces and their respective expression was tested twice, after 30 minutes and on the next day. To prevent loss of statistical power when assessing the influence of the heart rate response within the stress group, we doubled its size with respect to controls thereby enabling us to compare equally sized groups of high HR responders, low HR responders and controls.

2.2 Materials and Methods

2.2.1 Sample

206 healthy right-handed men (N = 100) and women (N = 106) (mean age: 23 years, SD: 2.9 years) participated in the experiment. They were randomly assigned to either the stress group (CPT, N = 135, 70 female) or a control condition (warm water bath, N = 71, 36 female). Sex was balanced in the whole sample and across experimental conditions. Subjects were mostly students from the University of Trier, recruited via Email Digest and placard. Participation was limited to right handed, healthy Caucasians with normal weight (Body Mass Index between 19 and 25) and age between 18 and 35 years. Applicants were not included if they showed any evidence of acute or chronic diseases of the circulatory system (deviations from sine rhythm, glaucoma, Raynaud's disease, history of fainting, resting blood pressure above 140/90 mmHg), history of psychiatric disease or family history of arterial hypertension, and cerebral or aortic aneurisms. Blood pressure was measured and normal sine rhythm confirmed during a 10 minutes resting period. Furthermore, the following exclusion criteria were applied: smoking of more than five cigarettes per day, drug intake or current use of medication, increased objective or subjective sensitivity to cold.

A personal screening interview determined if all criteria for inclusion in the study were met. All participants were informed about their right to stop the experiment at any time and gave written informed consent. They were compensated with 30.00 € after completion of the whole experiment.

2.2.2 Procedure

2.2.2.1 General Procedure

The study was conducted over two subsequent days. On the first day, the study protocol started with a ten minute resting period during which baseline measurements for heart rate and blood pressure were taken. Hereafter, the acquisition phase began in which participants were presented with the to-be-remembered stimuli. Immediately following acquisition, the CPT or a control procedure with warm water was carried out. A five minute resting period followed during which heart rate and blood pressure were measured. To prevent any stress effects on memory retrieval, a simple reaction time task was performed before the first memory test took place. The task lasted about 15 min. Thus, about 20 minutes following the stress procedure and 30 minutes after acquisition the first recognition memory test was conducted. The memory test concluded the experimental session for that day.

On the next day, participants returned to the lab for a second memory testing. After completion of the task they were compensated with 30.00 € and dismissed. All experimental sessions were carried out between 13:30 and 18:00 to control for diurnal variations in individual cortisol levels. All procedures were approved by the ethical committee of the state's medical association (Landesärztekammer Rheinland-Pfalz).

2.2.2.2 Acquisition

During acquisition participants saw a set of 52 male faces, half of them with an angry and the other half with a happy expression. Additionally, three faces were shown before and after the actual stimulus set to control for primacy and recency effects. These were not included in any memory tests. Each face was presented on screen for 3 seconds during which participants were instructed to watch it attentively. After presentation of each face they were asked to indicate the expression of the face, to ensure that this element had been encoded correctly.

2.2.2.3 Memory Testing

Recognition memory for the faces was tested at two time points, 30 minutes after acquisition and on the next day. In each test 26 of the old faces were presented together with 26 new lures, so different faces were used in each test. Half of the old faces had been shown with an angry and the other half with a happy expression during acquisition. Contrary to acquisition, during the memory tests all faces were presented with a neutral expression. Participants were required

to not only indicate whether the face was old or new but also state which expression it had when presented the first time. There was no time limit for making a choice; the face was presented until the decision was placed.

2.2.2.4 Cold Pressor Test

The CPT consisted of a procedure in which participants had to immerse their right hand for 3 minutes into ice water (2-3 °C) or warm water (36 -37 °C) as control procedure. Moreover, in the cold water condition a camera was being directed to the participant to add a social evaluative element. Previous research has shown that the addition of a social evaluative component can enhance the stress response to the CPT (Schwabe et al., 2008). Participants were sitting comfortably in a chair. Before the start of the CPT, they provided a saliva sample and rated their current subjective arousal and stress levels. When they had finished an experimenter came in, informed them that the cold water procedure was now about to start and then set the water bath to the right side of the test person. The participants were instructed to put their right hand including the wrist into the water and take it out when the experimenter told so. During the stress procedure there was no interaction between investigator and participant, they were not informed about the time left. After the end of the stress procedure, participants were given a towel to dry themselves. After that, they provided another rating of their subjective stress and arousal levels.

A total of seven participants terminated the CPT procedure before 3 minutes had passed. Those were excluded from all further analysis to ensure standardization of the intervention.

2.2.2.5 Physiological measurements

Stress values for heart rate and blood pressure during the CPT were measured at 0.5 and 2.5 minutes after hand immersion. Baseline values were obtained from three measurements taken in 5 minute intervals during a ten minute resting period before the start of the experiment as well as a five minute resting period after the CPT. Saliva samples were collected after the first resting period, before the CPT as well as 10, 20 and 35 minutes after the CPT.

2.2.3 Stimuli and Apparatus

2.2.3.1 Stimuli

Stimuli consisted of 104 male faces half of which served as lures (neutral expression) in the memory tests. The remaining 52 faces composed the learning lists and were each available with neutral, happy and angry expressions. The order as well as the expression in which participants saw a specific face was pseudorandomized. There were six such pseudorandomized learning lists consisting of 26 happy and 26 angry faces each. Participants were randomly assigned to one of the six learning lists.

Every learning list had two corresponding test lists. Test lists consisted of 52 neutral faces half of them were presented before the others were new.

2.2.3.2 Heart Rate and Blood Pressure

Heart rate and blood pressure were assessed using ECG electrodes (Tyco Healthcare H34SG Ag/AgCl electrodes) placed in lead II configuration and the Dinamap system (Critikon; Tampa, Florida, USA). The cuff was placed on the right upper arm. The ECG signal was stored to disk with a sampling rate of 1 kHz at 16 bit resolution. Beat detection was performed offline by WinCPRS (Absolute Aliens Oy, Turku, Finland) as was artifact control.

2.2.3.3 Cortisol

Saliva was collected using Salivettes (Saarstedt, Germany). Samples were kept at room temperature until the end of the session and then stored at -20 °C, until analysis. The fraction of free cortisol in saliva was determined using a time-resolved immunoassay with fluorescence detection, as described in detail elsewhere (Dressendorfer et al., 1992).

2.2.3.4 Stress and Arousal Ratings

Subjective stress and arousal were assessed before and after the CPT. Participants were asked to rate how stressed and how aroused they felt on visual analog scales ranging from 0 to 100.

2.2.4 Data Preparation and Statistical Analysis

Baseline and stress values for heart rate and blood pressure were averaged separately and then subtracted (mean stress – mean baseline) yielding a difference score for each participant. Cortisol measurements were integrated by calculating the area under the curve with respect to increase (AUCi) as described by Pruessner (Pruessner et al., 2003). To quantify the increase in experienced stress and arousal a difference score was calculated subtracting pre CPT values from post CPT values.

Subjects in the stress condition were divided into equal groups by median-split over their heart rate difference score (median Δ HR: 3.5 bpm), resulting in the factor GROUP (High HR Responders N = 67, 37 female; Low HR Responders N = 68, 33 female and Controls N = 71, 36 female). Separate univariate analyses of variance were used to assess whether the three groups differed in heart rate, blood pressure, cortisol and subjective ratings on stress and arousal. Welch's correction (Welch, 1951) was applied if the assumption of homogeneity of variances was violated.

Memory performance was analyzed applying Signal Detection Theory. The discriminability index $d' = \varphi^{-1}(HR) - \varphi^{-1}(FAR)$ and $\ln(\beta) = \frac{[\varphi^{-1}(FAR)]^2 - [\varphi^{-1}(HR)]^2}{2}$ were calculated as measure of recognition memory performance for facial identity and response bias, probabilities of 0 or 1 were replaced by 0.5/*n* or (*n*-0.5)/*n*, respectively (Wickens, 2002). Expression memory performance was quantified as percentage correct according to a two alternative forced choice model (Stanislaw and Todorov, 1999).

Hypotheses were tested with Analysis of Variance; two separate 3 (GROUP) *2 (SEX) *2 (TIME) *2 (VALENCE) ANOVAs with TIME and VALENCE as within and GROUP and SEX as between subjects factors were used to assess the influence of stress on identity and expression memory. All statistical analyses were done with IBM SPSS 20 Statistics (IBM Corp.; Armonk, New York, USA).

2.3. Results

2.3.1 Response to the CPT

2.3.1.1 Heart Rate and Blood Pressure

Separate univariate Analyses of Variance conducted on the deltas of heart rate, systolic, diastolic blood pressure and mean arterial pressure with the between subjects factors GROUP and SEX revealed a significant main effect of GROUP for all dependent variables (Δ HR: $F(2,131) = 90.31$ $p < 0.001$; Δ SBP: $F(2,133) = 72,88$ $p < 0.001$; Δ DBP: $F(2,133) = 72,81$ $p < 0.001$; Δ MAP: $F(2,128) = 86,88$ $p < 0.001$). Heart rate differed between high and low responders ($t(114) = 12.93$ $p < 0.001$) as well as high responders and controls ($t(136) = 11.68$ $p < 0.001$) but not between low responders and controls ($t(137) = 1.06$ $p = 0.293$). There was no difference between high and low responders in blood pressure (Δ SBP $t(133) = 1.1$ $p = 0.264$; Δ DBP $t(133) = 1.2$ $p = 0.244$; Δ MAP t(133) = 1.5 $p = 0.14$), but both groups differed significantly from controls (low responders: Δ SBP $t(126) = 8.9$ $p < 0.001$; Δ DBP $t(131) = 9.8$ $p < 0.001$; Δ MAP $t(114) = 9.4$ $p < 0.001$; high responders: Δ SBP $t(136) = 11.1$ $p < 0.001$; Δ DBP $t(136) = 11.1$ $p < 0.001$; Δ MAP $t(118) = 11.7$ $p < 0.001$). SEX did not show any significant main effects nor was it involved in any interactions with GROUP. Heart rate and blood pressure profiles for the different groups are shown in Figure 1.

2.3.1.2 Cortisol

Cortisol data from two subjects, one from the control and one from the stress group, got lost and therefore those had to be excluded from analysis on cortisol values.

Analysis of Variance with cortisol AUCi as dependent and GROUP and SEX as between subject factors resulted in a main effect of GROUP ($F(2,123) = 18.79$ $p < 0.001$). Cortisol was elevated with respect to control in both high ($t(104) = 5.77$ $p < 0.001$) and low responders ($t(111) = 3.74$ $p = 0.003$) but there was no significant difference in cortisol rise between high and low responders ($t(132) = 1.81$ $p = 0.255$). The factor SEX did not produce a significant main effect nor did it interact with GROUP. See Figure 1 for a depiction of cortisol profiles of the different groups.

2.3.1.3 Subjective Stress and Arousal

Rating data of ten participants was missing; therefore those subjects had to be excluded from analysis on subjective ratings. There was a main effect of GROUP for both, arousal ($F(2,121) = 48.06$ $p < 0.001$) and stress ratings ($F(2,191) = 25.05$ $p < 0.001$); high responders and low responders had higher stress (high responders: $t(113) = 6.55$ $p < 0.001$; low responders: $t(127) = 5.98$ $p < 0.001$) and arousal (high responders: $t(105) = 8.68$ $p < 0.001$; low responders: $t(114) = 7.03$ $p < 0.001$) ratings than controls. There was no difference between high and low responders in subjective stress ($t(126) = 1.14$ $p = 0.255$) but high responders showed increased subjective arousal as compared to low responders ($t(126) = 2.26$ $p = 0.025$).

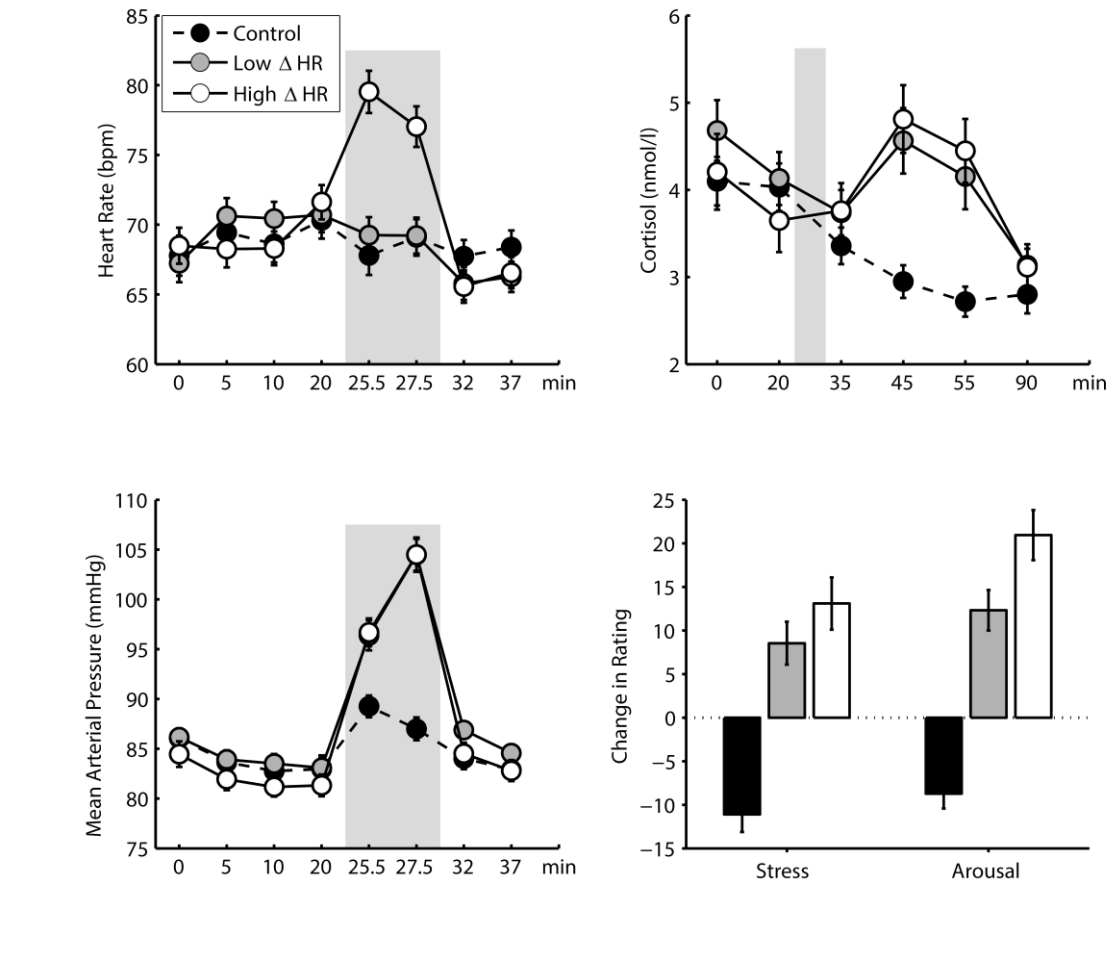

Figure 1: Heart rate, blood pressure and cortisol profiles of the control, low and high HR response groups during the course of the experiment. The grey area indicates the time of the CPT. Lower right panel: Change in ratings of subjective stress and arousal from pre- to post-CPT values between groups. Error bars represent standard errors.

2.3.2 Memory Performance

2.3.2.1 Identity Memory

A 3 (GROUP) *2 (SEX) *2 (TIME) *2 (VALENCE) ANOVA resulted in a significant main effect of TIME ($F(1,200) = 13.28$ $p < 0.001$) and VALENCE ($F(1,200) = 49.87$ $p < 0.001$), indicating better recognition memory performance in the immediate test and for positive faces. Additionally, a significant interaction emerged between the factor GROUP and TIME ($F(2,200) = 4.64$ $p = 0.011$). Whereas groups did not differ in the first recognition test, in the delayed test the High Delta HR group significantly outperformed both the Low Delta HR ($t(114) = 2.50$ $p = 0.013$) and the control group ($t(136) = 3.40$ $p = 0.001$). There was no significant difference between the Low Delta HR and the control group ($t(137) = 0.85$ $p = 0.392$). There were no significant interactions involving VALENCE. Also SEX had no significant main effect nor did it interact with any of the other variables.

An additional ANOVA conducted on response biases ($\ln(\beta)$) with GROUP and SEX as between subjects factors confirmed that there were no significant group differences in response bias (GROUP: $F(2,200) = 0.89$ $p = 0.412$; SEX: $F(1,200) = 3.01$ $p = 0.084$; GROUP*SEX: $F(2,200) = 0.86$ $p = 0.227$).

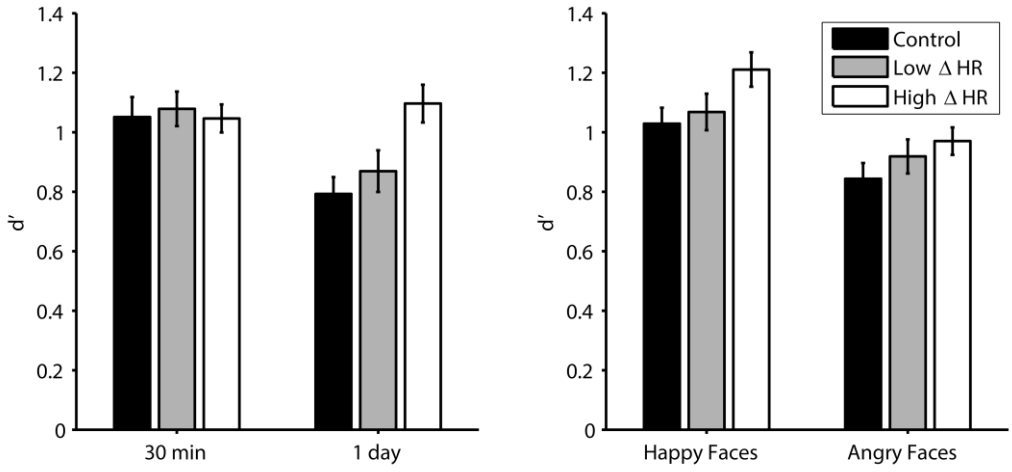

Figure 2: Identity memory performance of controls, low and high HR responders as function of testing timepoint (left panel) and valence (right panel). Error bars represent standard errors.

2.3.2.2 Expression Memory

A 3 (GROUP) *2 (SEX) *2 (TIME) *2 (VALENCE) ANOVA on memory for the expression of the faces revealed a significant main effect of VALENCE ($F(1,200) = 26.66$ $p < 0.001$), and a marginally significant main effect of TIME ($F(1,200) = 3.85$ $p = 0.051$) indicating higher performance for positive expressions and in the immediate test. There were no significant interactions including VALENCE (all F <0.94 all p >0.335) or TIME (all F <0.91all p >0.340). The factors GROUP and SEX did not produce significant main effects nor were they involved in any interactions (GROUP: all F <0.83 all p >0.450; SEX: all F <2.06 all p >0.131).

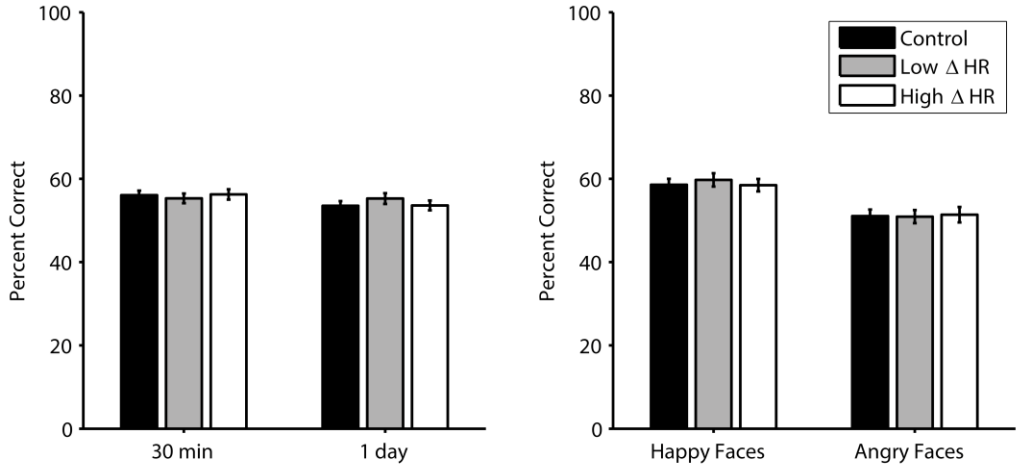

Figure 3: Expression memory performance of controls, low and high HR responders as function of testing timepoint (left panel) and valence (right panel). Error bars represent standard errors.

2.4 Discussion

The aim of the present study was to investigate whether post-learning stress-induced HR responses predict memory consolidation. 206 participants were exposed to a CPT or a non-stressful control procedure immediately after watching male emotion-expressing face portraits. Recognition memory was tested after 30 minutes and one day later. The CPT group was divided into two groups according to the median stress-induced HR change. High HR responders (in comparison to low HR responders as well as to the non-stressful control group) showed enhanced recognition memory one day after learning. Cortisol, sex, and the emotional

expression of the face portraits did not play a role in this effect.

The CPT is a predominantly adrenergic stressor (Pascualy et al., 2000; Ward et al., 1983) inducing alpha- and beta-adrenergic activation. It is often employed in psychophysiological stress research. This test also induces HPA axis activation, but this effect is less pronounced (Dickerson and Kemeny, 2004; McRae et al., 2006). Our findings are in line with these previously published results. We observed strong stress-induced increases in blood pressure, a consequence of alpha-adrenergic activation, but only mild (0.4 nmol/l on average) albeit significant increases in cortisol. The heart rate response to the CPT showed a much higher variability between subjects. This is a common finding in CPT studies (Glenn, 2003; Jauregui-Renaud et al., 2001; Mourot et al., 2009) suggesting individual differences in stress-induced beta-adrenergic arousal. Beta-adrenergic activation induces symptoms (e.g. palpitations) which may easily be perceived. Indeed, our results show that while having comparable increases in blood pressure, HR high and low responders significantly differed in their reported levels of subjective arousal.

Importantly, all subjects who terminated the CPT prematurely were excluded from final statistical analyses. In other studies such participants were often included in the analysis (Buchanan et al., 2006; Cahill et al., 2003; Schwabe and Wolf, 2010), leading to variable stress exposure times which might contribute to the variability in individual stress responses. Here, we assured a constant exposure time of three minutes, thus the observed differences cannot be attributed to unstandardized conditions in the intervention protocol. The ECG signal was manually controlled for artifacts, thus assuring that a normal sine rhythm was present in all participants. Hence, the observed changes in HR were solely driven by autonomic nervous system regulation. Still, an increase in HR can theoretically be induced by both, vagal withdrawal and sympathetic activation. Nonetheless, it was previously shown that administration of the beta-blocker propanolol completely blocks the CPT stress induced HR response (Houben et al., 1982; Victor et al., 1987), indicating that during the CPT HR is under predominantly beta-adrenergic control.

Earlier studies enhanced post-learning beta-adrenergic signal transmission pharmacologically by administration of epinephrine or yohimbine (Cahill and Alkire, 2003; Southwick et al., 2002). These studies have tested memory after an interval of 7 days. Our results corroborate their findings and show that (endogenous) beta-adrenergic stimulation may affect memory

consolidation already after a considerably shorter interval of only one day. However, such an effect was not detectable on the first test, 30 minutes after learning. This difference suggests that only long term consolidation is affected by beta-adrenergic activation, probably depending on processes initiated during sleep. It is well established that sleep has a critical function in the consolidation of recently acquired procedural and declarative memories of different types (Diekelmann and Born, 2010; Marshall and Born, 2007; Stickgold, 2005) extending to recognition memory for emotional faces as well (Wagner et al 2007). Furthermore, emotional memories, which are characterized by sympathetic arousal during and shortly after their initial formation, seem to be particularly sensitive to the effects of sleep (Groch et al., 2011; Payne et al., 2008; Wagner et al., 2001; Wagner et al., 2006). Thus, the temporal pattern in our results might reflect the necessity of a sleeping period for the effects of beta-adrenergic activation on consolidation to become apparent. Nevertheless, we did not employ neutral stimuli or a no sleep control group and are thus not able to conclude on this issue. Also, it should be noted that although we allowed for a minimum time window of 20 minutes between stress exposure and memory testing, retrieval might have been impaired during the immediate post-stress period. Elevated cortisol levels are known to impair memory retrieval (Buchanan et al., 2006). Moreover, beta-blockade has been shown to abolish impairing stress effects on memory retrieval (Schwabe et al., 2009), although in another study endogenous autonomic arousal indexed by heart rate did not affect retrieval performance (Buchanan et al., 2006). However, we cannot fully exclude that a rapid consolidation effect compensated by stress induced retrieval inhibition had been present already in the first test.

Cortisol has been shown to impact on human memory consolidation (Andreano and Cahill, 2006; Kuhlmann and Wolf, 2006). Cortisol crosses the blood brain barrier to act on glucocorticoid and mineralocorticoid receptors located in brain structures responsible for memory regulation i.e. the amygdala, hippocampus and prefrontal cortex (Roozendaal, 2002; Roozendaal and McGaugh, 2011). However, high and low HR responders did not differ in baseline and stress-induced cortisol levels indicating that the observed memory effects cannot be attributed to cortisol.

A rise in blood pressure leads to activation of peripheral baroreceptors and it could be shown that such baroafferent stimulation facilitates memory processes (Moor et al., 2005). However, high and low HR responders did not differ in stress-induced blood pressure increases, and thus the observed memory effects cannot be attributed to memory modulation through changes in

blood pressure.

In the present study we did not observe any sex effects. This is surprising given the prominence of sexually dimorphic results in the literature on stress (Bangasser and Valentino, 2012; Ordaz and Luna, 2012; Regitz-Zagrosek et al., 2013) and stress effects on memory (Andreano and Cahill, 2009; ter Horst et al., 2012). The comparatively high sample size in our study with sexually balanced experimental groups makes it unlikely that a lack of statistical power is responsible for this negative finding. Nevertheless, although the sample was balanced for sex we did not take the use of hormonal contraceptives or the current phase of the menstrual cycle into account. Previous research could demonstrate that the presence of sex differences in stress responses crucially depends on these factors (Kajantie and Phillips, 2006). Therefore, potential sex effects might have been cancelled out by differences in menstrual cycle.

Our results seem contrary to reports on the missing of an association between the non-invasive, salivary marker of sympathetic activity, sAA, and memory consolidation (Bryant et al., 2013; Felmingham et al., 2012; Preuss and Wolf, 2009; Smeets et al., 2009). A possible explanation for this discrepancy is power differences due to the relatively small sample sizes in studies of sAA. Here, we used a substantial sample size and avoided loss of power by doubling the size of the experimental group. However, since sAA levels were not assessed, we are not able conclude on this issue.

The memory paradigm employed in this study used pictures of happy and angry faces as stimuli. Importantly, different faces were used in each of the two tests. We thereby excluded carry-over effects in retrieval performance from the first to the second test. Moreover, all faces were presented with a neutral expression at test. This allowed us to not only assess memory for the faces but also for their respective expression. Additionally, this test composition (presentation of neutral stimuli during recognition testing) ensured that stimulus induced arousal and valence effects were isolated from the retrieval episode. Previous studies reporting beta-adrenergic modulation of memory consolidation used exclusively free (Cahill and Alkire, 2003; Southwick et al., 2002) or cued (Smeets et al., 2008) recall paradigms to assess memory performance. Although in our experiment expression of the faces varied between acquisition and testing it should be considered a test of recognition memory since recognition of facial identity does not depend on variant features as perspective, gaze or expression of a specific face (Bruce and Young, 1986). Measuring recognition memory requires assessment of discrimination

performance and response bias as both might differentially reflect experimental manipulations. However, our results show that for the case of facial identity recognition, beta-adrenergic activation enhances consolidation without affecting response bias.

We found a strong effect of stimulus valence. Happy faces were generally better remembered than angry ones. This valence effect is frequently observed in studies concerning memory for faces (D'Argembeau and Van der Linden, 2007; 2011; D'Argembeau et al., 2003; Putman et al., 2004; Verde et al., 2010) and probably due to attention processes during encoding (D'Argembeau and Van der Linden, 2007). Importantly, valence did not interact with time of testing nor HR response, suggesting that both positive and negative stimuli benefitted equally from an enhancement of consolidation by beta-adrenergic activation. This is in line with previous studies showing that stress and arousal effects on memory depend on the arousal properties of the to-be-remembered stimuli rather than their valence (Kuhlmann and Wolf, 2006; Nielson and Lorber, 2009). Yet, not all studies observe such an independence of arousal effects from stimulus valence (Wang, 2012). The design of the present study allows us to further elaborate on this topic. Since we presented all faces with a neutral expression in the memory tests, we can exclude valence effects on retrieval processes that might overshadow valence specific stress effects on consolidation. Furthermore, we found that not only were the stress effects on identity memory independent from valence, also the memory *for* the valence a specific face had previously been presented in (i.e. expression memory) was unaffected by stress. Therefore, our results add further evidence to the notion that stimulus valence is not a modulating factor concerning stress effects on memory consolidation.

In summary, we conclude that beta-adrenergic activation elicited endogenously after learning enhances memory consolidation irrespective of stimulus valence, and that the stress induced heart rate response might be an adequate predictor for this effect.

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2.i Author Notes

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Chapter III: Enhanced stress response by a bilateral feet compared to a unilateral hand Cold Pressor Test

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3.0 Abstract

The Cold Pressor Test (CPT) is a frequently employed laboratory stress protocol. However, with many experimental designs the application in its classic form (immersion of the dominant hand into ice-water) is problematic as unilateral stimulation may need to be avoided and/or hands are required for further measurements. Here, we describe a simple modification of the classic CPT in which both feet are immersed into ice-water and compare the evoked neuroendocrine stress response to the classic CPT in a within-subjects design. Twenty-four healthy participants were exposed to each of both CPT versions on two subsequent days in randomized order. Heart rate, blood pressure, salivary alpha-amylase and cortisol were measured at baseline and during or after CPT exposition, respectively, along with subjective ratings of pain and stress. The bilateral feet CPT induced marked increases in all measured stress parameters. Moreover, with the exception of blood pressure, autonomic and endocrine responses were enhanced compared to the classic CPT. The bilateral feet CPT thus is a valid and simple modification and may be useful when application of the classic CPT is unfeasible or a stronger neuroendocrine stress response is of interest.

Keywords: cold pressor test modification, feet, hand, salivary alpha-amylase, cortisol, heart rate, blood pressure

3.1 Introduction

The Cold Pressor Test (CPT) has become a widely used tool in experimental research of different areas. First described by Hines and Brown (1932) it consists of a procedure in which the dominant hand is immersed into ice-water for a short period of time. The test was originally designed as a standard stimulus to increase blood pressure under laboratory settings. As participants experience the CPT as a stressful procedure it is also frequently employed as a physical laboratory stressor. Its ease of use, the possibility of exact timing, and the short application duration are advantages that distinguish the CPT from other popular stress protocols as the Trier Social Stress Test (Kirschbaum et al., 1993). However, these advantages are opposed by some practical disadvantages due to the typical unilateral hand immersion. As such, unilateral CPT's induce laterality specific effects (Harper et al., 2000; McGinley and Friedman, 2014) that may create unwanted interference in all studies that require unilateral stimulus presentation or lateralized responses (e.g. somatic motor activation) in some form. Unwanted laterality effects may be avoided by bilateral instead of unilateral hand immersion (Suter et al., 2007). However, this further hampers the assessments of stress response parameters during the CPT (e.g. Finapres-type beat-to-beat blood pressure, manual button pushes, or manual report). The necessity of rendering both hands free during CPT exposure may be addressed by changing the stimulation site from hand to forehead (Saab et al., 1993) or foot (Previnaire et al., 2012). Indeed, a bilateral CPT feet immersion procedure was shown (Frings et al., 2013) to elicit a neuroendocrine stress response (e.g. salivary cortisol and heart rate increases). So far, this new CPT version has only been compared to a control condition with warm water (Frings et al., 2013), but not to the classic unilateral hand immersion procedure, which may represent a weaker stressor than the bilateral feet CPT. The current study was conducted to compare the neuroendocrine stress responses elicited by bilateral feet CPT and classical dominant hand CPT versions. Avoiding confounding effects of interindividual response heterogeneity we exposed participants to both stressors in randomized order and assessed responses in heart rate, blood pressure, salivary alpha-amylase and cortisol along with subjective ratings of pain and stress.

3.2 Methods

3.2.1 Sample

Twenty-four healthy male (N=12) and female (N=12) students (mean age: 22.5 years, SD: 2.5 years, mean BMI: 22.6, SD: 2.2) participated in the study. Participation was limited to right handed, healthy Caucasians with normal weight (Body Mass Index between 19 and 25) and age between 18 and 35 years. Six of the female participants were using oral contraceptives. Applicants were not included if they showed any evidence of acute or chronic diseases of the circulatory system (deviations from sine rhythm, glaucoma, Raynaud's disease, history of fainting, resting blood pressure above 140/90 mmHg), history of psychiatric disease or family history of arterial hypertension, and cerebral or aortic aneurisms. Furthermore, the following exclusion criteria were applied: smoking of more than five cigarettes per day, drug intake or current use of medication, increased objective or subjective sensitivity to cold.
A personal screening interview determined if all criteria for inclusion in the study were met. Blood pressure was measured and normal sine rhythm confirmed during a 10 minute resting period. All participants were informed about their right to stop the experiment at any time and gave written informed consent. They were compensated with 50.00 € after completion of the study. All procedures were approved by the ethical committee of the state's medical association (Landesärztekammer Rheinland-Pfalz) and were in accordance with the Declaration of Helsinki.

3.2.2 General procedure

Experiments were carried out in the afternoon between 2 p.m. and 6 p.m. Participants reported to the lab on two subsequent days at exactly the same time of day. The study protocol was the same for both days but differed in the type of CPT being employed (hand CPT or feet CPT). Participants were informed beforehand that the experiment on both days would consist of multiple resting phases and a hand or feet cold water bath and that cardiovascular and saliva measurements would be taken. They were not aware which CPT version they would be subjected to nor that it would be alternated between sessions. Both experimental sessions were carried out in the same room. Upon arrival on the first day, participants were randomly assigned to one of two groups determining the sequential order in which they were exposed to feet and

hand CPTs. They were sitting comfortably in a chair and after electrodes and cuffs were placed provided a first saliva sample. The protocol then started with a ten minute resting period during which heart rate and blood pressure were assessed. Then, the participants provided a saliva sample and a rating of their current stress level. After that, they were exposed to either the hand or feet CPT. Hereafter, participants again rated their current stress levels and provided two saliva samples. The stress procedure was then followed by a 10 minute resting period during which heart rate and blood pressure were assessed. After the resting period participants gave another saliva sample before electrodes and cuffs were removed. They were then led into a separate room and stayed there alone for another hour during which an experimenter came in every 15 minutes and asked for a saliva sample. Some magazines were provided. A timeline of the experiment is shown in Figure 4.

3.2.3 Cold Pressor Test

The CPT consisted of a procedure in which participants had to immerse their right hand or both feet into ice-water (water temperature 2-3°C) for 3 minutes. The waterbath was prepared in a 18×30×13 cm (40×30×25 cm for the feet CPT) sized rectangular tub filled with 2.5 liters (10 liters for the feet CPT) of water. Ice was added and the waterbath stirred until 2 °C were reached. The procedure for both feet and hand CPT followed the same protocol. Participants were sitting comfortably in a chair and after having provided a saliva sample and a rating of their current stress level were first asked to take off their shoes and socks (feet condition). When they had finished a same sex experimenter came in, informed them that the cold water procedure was now about to start and then set the water bath to the right side of or to the ground in front (feet condition) of the test person. The participants were instructed to put their right hand including the wrist or both feet including the ankles, respectively, into the water and take it out when the experimenter told so. They were informed beforehand that during the experiment they would have to immerse their hand or feet into icewater for three minutes but that they might terminate it at their discretion. During the CPT they were not informed about the time left. The experimenter stayed in the room and asked participants to orally rate the strength of pain and stress experienced in one minute intervals, noting down the results. Otherwise, there was no interaction between investigator and participant. Blood pressure and heart rate were measured at 0.5 and 2.5 minutes after hand or feet immersion. After the end of the stress procedure, participants were given a towel to dry themselves and asked to put their socks but not their

shoes back on (feet condition). After that, they provided another saliva sample and rated their current stress levels.

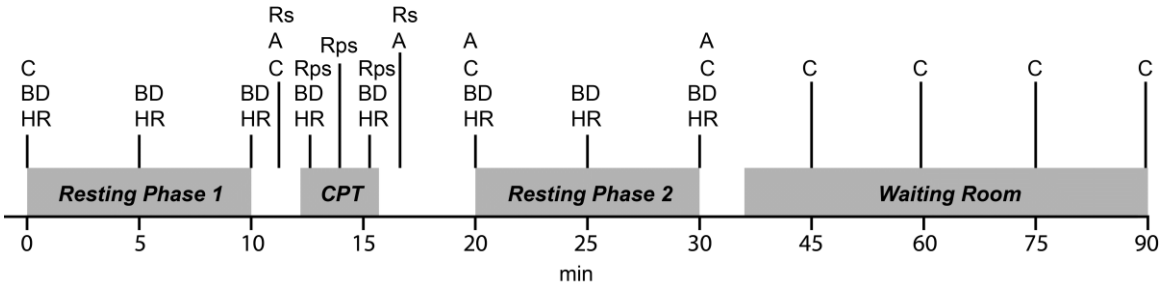

Figure 4: Timeline of the experimental procedure on one day depicting the timing of all measurements. C= cortisol; A= alpha-amylase; BD= blood pressure; HR= heart rate; Rs= stress rating; Rps= pain and stress rating.

3.2.4 Physiological measurements

3.2.4.1 Cortisol

Saliva was collected using Salivettes (Saarstedt; Nümbrecht, Germany) and sampled at the start of the experiment, immediately before the CPT as well as 5, 15, 30, 45, 60 and 75 minutes after the end of the CPT. Participants were asked to refrain from eating and drinking anything but water from two hours before until the end of the experiment. Samples were kept at room temperature until the end of the session and then stored at -20 °C, until thawing before analysis. The fraction of free cortisol in saliva was determined using a time-resolved immunoassay with fluorescence detection (Dressendorfer et al., 1992). Inter- and intra-assay coefficients of variation were between 7% to 9% and 4% to 7%, respectively.

3.2.4.2 Salivary alpha-amylase (sAA)

Saliva was collected using standard Eppendorf tubes (1.5 ml, Eppendorf; Hamburg, Germany) and sampled immediately before the CPT, immediately after the CPT as well as 5 and 15 minutes after the end of the CPT (always before cortisol sampling at times were sAA and cortisol were assessed). Participants were instructed to wait until saliva accumulated under their tongue and then spit it into the tube through a straw. Samples were kept at room temperature

until the end of the session and then stored at -20 °C, until thawing before analysis. sAA concentrations were determined using a quantitative enzyme kinetic method (Lorentz et al., 1999). Inter- and intra-assay coefficients of variation were between 6% to 8% and 3% to 6%, respectively. Cortisol and alpha-amylase were analyzed in the Biochemical Laboratory (University of Trier, Trier, Germany).

3.2.4.3 Heart rate and blood pressure

Heart rate and blood pressure were assessed using the Dinamap system (Critikon; Tampa, Florida, USA). ECG data was recorded in parallel and manually checked for artifacts and extrasystoles with the software WinCPRS (Absolute Aliens Oy; Turku, Finland). ECG electrodes (Tyco Healthcare H34SG Ag/AgCl electrodes) were placed in lead II configuration. The ECG signal was stored to disk with a sampling rate of 1 kHz at 16 bit resolution. Stress values for heart rate and blood pressure during the CPT were measured at 0.5 and 2.5 minutes after hand or feet immersion. Baseline values were obtained from three measurements taken in 5 minute intervals during the ten minute resting period before and after the CPT.

3.2.5 Subjective ratings

Subjective stress levels were assessed before, during and after the CPT along with pain ratings assessed during the CPT and Likert data are reported. During the CPT participants were asked in 1 minute intervals to orally rate how intense they experienced pain and stress on a scale from one (“not at all stressed/painful”) to ten (“extremely stressed/painful”) at 0.5, 1.5 and 2.5 minutes after hand or feet immersion.

3.2.6 Data preparation and statistical analysis

Data was analyzed with mixed-model ANOVAs conducted on the respective dependent variables and incorporating the between subjects factors SEX (“male” vs. “female”) and SEQUENCE (“hand CPT first” vs. “feet CPT first”) and the within subject factors STRESSOR (“hand CPT” vs. “feet CPT”) and TIME (measurement timepoint, depending on the respective measure). Baseline and stress values for heart rate and blood pressure were averaged separately

and resulting values entered into the ANOVA with the factor TIME being "baseline" vs. "stress". For sAA and cortisol baseline and post-stress values at which a significant change could be expected (CPT end +0 min. and +5 min. for sAA; CPT end +15 min and + 30 min for Cortisol) were entered. Significant TIME by STRESSOR interactions were followed up by apriori planned contrasts to assess whether there was a) a difference in baseline values between both stressors, b) a significant change from baseline to post-stress values within each level of stressor and c) whether these changes differed significantly between both levels of stressor. One participant (hand CPT first group) terminated the feet CPT prematurely and was excluded from all analysis. Another participant (feet CPT first group) was excluded from statistics on cortisol values as the baseline sample could not be analyzed. The final sample size then was N=23 (N=22 for cortisol analyses). Effects with an alpha-error probability below 5% were deemed significant. Huynh-Feldt correction was applied where sphericity assumptions were violated. All analyses were realized with IBM SPSS Statistics 20. (IBM Corp.; Armonk, New York, USA).

3.3 Results

3.3.1 Heart rate

A SEQUENCE*SEX*STRESSOR*TIME mixed-model ANOVA on heart rate values revealed a significant main effect of TIME ($F(1, 19) = 14.99$, $p = .001$, $\eta_p^2 = .441$) as well as a significant interaction of STRESSOR*TIME ($F(1, 19) = 34.97$, $p < .001$, $\eta_p^2 = .648$). Only the feet CPT version led to a significant increase in heart rate compared to baseline ($F(1, 19) = 32.01$, $p < .001$, $\eta_p^2 = .628$), whereas there was no significant effect on heart rate in the hand CPT ($F(1, 19) = .98$, $p > .05$, $\eta_p^2 = .049$). Both stressors did not differ in heart rate at baseline ($F(1, 19) = .44$, $p > .05$, $\eta_p^2 = .022$). Also, there were no main effects or interactions involving SEQUENCE or SEX (all Fs < 1.45, all ps> .05, all η_p^2s < .071).

3.3.2 Blood pressure

Separate SEQUENCE*SEX*STRESSOR*TIME mixed-model ANOVAs conducted on systolic (SYS), diastolic (DIA) and mean arterial blood pressure (MAP) values revealed a significant main effect of TIME for SYS ($F(1, 19) = 139.93$, $p < .001$, $\eta_p^2 = .88$), DIA ($F(1, 19) = 135.52$, $p < .001$, $\eta_p^2 = .877$) and MAP ($F(1, 19) = 87.69$, $p < .001$, $\eta_p^2 = .822$) indicating a significant increase for all dependent variables. There were no differences in baseline or stress level blood pressure between both stressors as both the main effect of STRESSOR (SYS: $F(1, 19) = .43$, $p > .05$, $\eta_p^2 = .002$; DIA: $F(1, 19) = 1.69$, $p > .05$, $\eta_p^2 = .082$; MAP: $F(1, 19) = .9$, $p > .05$, $\eta_p^2 = .045$) as well as the interaction of STRESSOR*TIME (SYS: $F(1, 19) = .23$, $p > .05$, $\eta_p^2 = .012$; DIA: $F(1, 19) = .48$, $p > .05$, $\eta_p^2 = .025$; MAP: $F(1, 19) = 1.65$, $p > .05$, $\eta_p^2 = .08$) did not reach significance. Also, there were no main effects or interactions involving SEQUENCE or SEX (all Fs < 2.3, all ps> .05, all η_p^2s < .101). Heart rate and blood pressure profiles for both stressors are depicted in Figure 5.

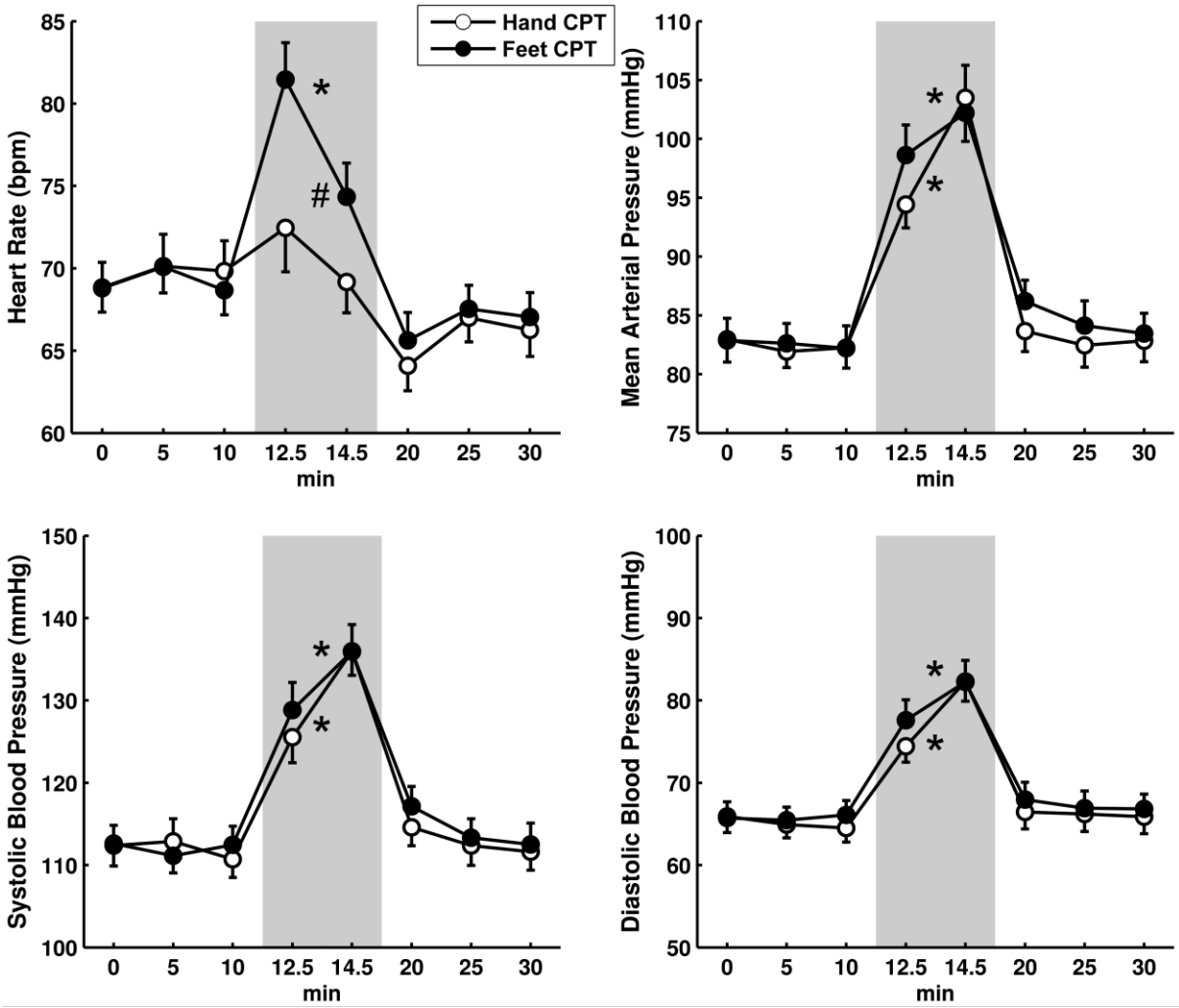

Figure 5: Heart rate (upper left panel), mean arterial (upper right panel), systolic (lower left panel) and diastolic (lower right panel) blood pressure for hand CPT and feet CPT over the course of the experiment. Times on the x-axis refer to the start of the experiment, the grey area indicates the time of the CPT. Error bars represent standard errors. *Significant difference mean CPT vs. baseline values ($p < .05$); #Significant difference in mean CPT vs. baseline values between hand and feet CPT ($p < .05$).

3.3.3 Cortisol

A SEQUENCE*SEX*STRESSOR*TIME mixed-model ANOVA resulted in a significant interaction of STRESSOR*TIME ($F(2, 36) = 6.68$, $p = .006$, $\eta_p^2 = .264$, HF-$\varepsilon = .848$), the main effects of STRESSOR ($F(1, 18) = 2.2$, $p > .05$, $\eta_p^2 = .109$) and TIME ($F(2, 36) = 3.38$, $p > .05$, $\eta_p^2 = .158$, HF-$\varepsilon = .688$) did not reach significance. As shown in Figure 3 cortisol values after the hand CPT did not change significantly with respect to baseline at 15 minutes post stress ($F(1, 18) = 3.27$, $p > .05$, $\eta_p^2 = .158$) and decreased at 30 minutes post stress ($F(1, 18) = 11.84$, $p = .004$, $\eta_p^2 = .397$), whereas there was a significant rise in cortisol values after the feet CPT at 15 ($F(1, 18) = 6.16$, $p = .023$, $\eta_p^2 = .255$) but not 30 ($F(1, 18) = 2.61$, $p > .05$, $\eta_p^2 = .127$) minutes post stress. A-priori planned contrasts further revealed that the two stressors differed in their change from baseline to post stress values at 15 minutes ($F(1, 18) = 10.63$, $p = .004$, $\eta_p^2 = .371$) and 30 minutes ($F(1, 18) = 6.13$, $p = .023$, $\eta_p^2 = .254$) whereas there was no significant difference between both stressors at baseline ($F(1, 18) = 3.0$, $p > .05$, $\eta_p^2 = .143$). There were no significant main effects of SEQUENCE or SEX nor did they interact with STRESSOR and TIME (all Fs < 2.1, all ps> .05, all η_p^2s < .105) indicating that the effects were not moderated by participants' sex or sequential order of exposure.

3.3.4 Salivary alpha-amylase

There was a significant main effect of TIME ($F(2, 38) = 7.19$, $p = .002$, $\eta_p^2 = .275$) and a significant STRESSOR*TIME interaction ($F(2, 38) = 3.51$, $p = .044$, $\eta_p^2 = .156$, HF-$\varepsilon = .934$). Both the feet and the hand CPT led to an increase in sAA concentrations with respect to baseline immediately (hand CPT: ($F(1, 19) = 4.61$, $p = .045$, $\eta_p^2 = .195$), feet CPT: ($F(1, 19) = 9.43$, $p =$

.006, η_p^2 = .332)) as well as 5 minutes (hand CPT: (F(1, 19) = 7.61, p = .013, η_p^2 = .286), feet CPT: (F(1, 19) = 11.23, p = .003, η_p^2 = .372) after the CPT. sAA levels did not differ between both stressors at baseline (F(1, 19) = .61, p > .05, η_p^2 = .031) but the increase from baseline to sAA concentrations assessed immediately (F(1, 19) = 4.32, p = .05, η_p^2 = .185) and 5 minutes (F(1, 19) = 3.98, p = .06, η_p^2 = .173) after the CPT was marginally greater in the feet than in the hand version. Furthermore, a significant interaction of SEQUENCE*STRESSOR (F(1, 19) = 9.84, p = .005, η_p^2 = .341) arose indicating higher overall sAA concentrations during the day of the hand CPT versus the day of the feet CPT when the hand CPT was performed first (t(10) = 2.11, p = .049, d = .638), and a similar effect (i.e. higher values for the feet vs. hand CPT day) when the feet CPT was performed first (t(11) = 2.34, p = .031, d = .745). Thus, overall sAA concentrations were higher during the first than during the second day of the experiment. Otherwise, there were no significant main or interaction effects involving SEQUENCE or SEX (all Fs < 2.1, all ps> .05, all η_p^2s= .101). sAA profiles for both stressors are depicted in Figure 6.

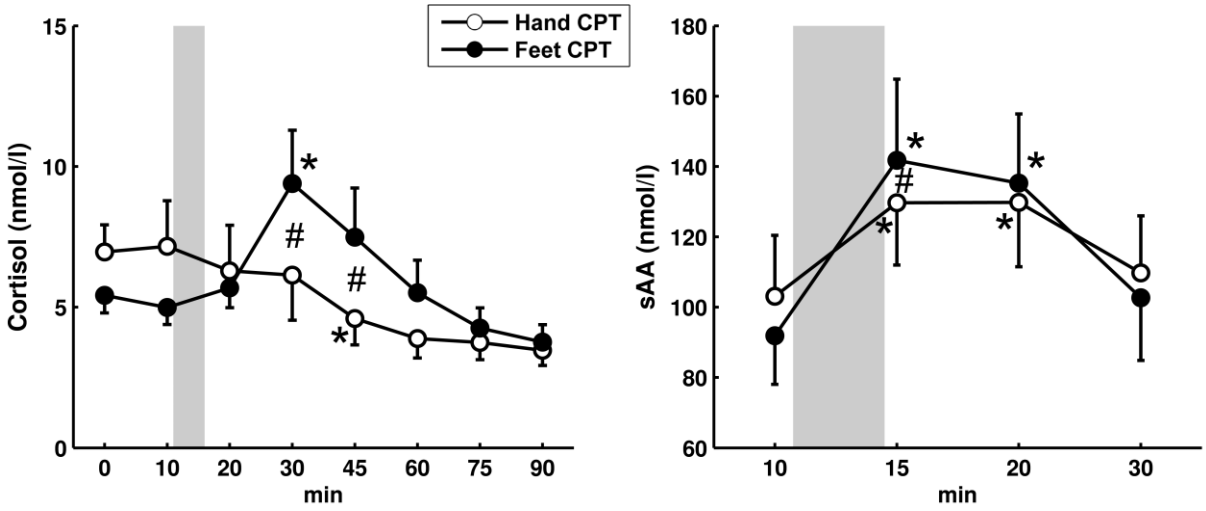

Figure 6: Cortisol (left panel) and sAA (right panel) concentrations for hand CPT and feet CPT over the course of the experiment. Times on the x-axis refer to the start of the experiment, the grey area indicates the time of the CPT. Error bars represent standard errors. *Significant difference to baseline (p < .05); #Significant difference in change to baseline between hand and feet CPT (p < .05).

3.3.5 Subjective Ratings

3.3.5.1 Stress

There was a significant main effect of STRESSOR ($F(1, 19) = 4.32$, $p = .05$, $\eta_p^2 = .185$), TIME ($F(4, 76) = 39.18$, $p < .001$, $\eta_p^2 = .673$, HF-$\varepsilon = .884$) and a significant TIME*STRESSOR interaction ($F(4, 76) = 4.34$, $p = .004$, $\eta_p^2 = .186$, HF-$\varepsilon = .975$). Stress ratings were significantly increased during and immediately after the CPT compared to baseline values (all *p*s< .001, all *d*s > .744). There was no difference in stress ratings between stressors at baseline ($t(22) = 1.06$, $p > .05$, $d = .223$) or after the CPT ($t(22) = .75$, $p > .05$, $d = .176$) but participants reported to be more stressed during the feet CPT than during the hand CPT at the first ($t(22) = 3.11$, $p = .005$, $d = .648$) and second measurement ($t(22) = 3.19$, $p = .004$, $d = .664$), at the last measurement the difference was not significant ($t(22) = 1.86$, $p > .05$, $d = .388$). There were no significant main effects or interactions involving SEQUENCE or SEX (all *F*s < 2.24, all *p*s> .05, all η_p^2s < .106).

3.3.5.2 Pain

There was a significant main effect of TIME ($F(1, 38) = 3.82$, $p = .042$, $\eta_p^2 = .168$, HF-$\varepsilon = .787$). Pain ratings increased from the first to the second measurement ($t(22) = 3.25$, $p = .003$, $d = .715$) and decreased again to the end of the CPT ($t(22) = 2.74$, $p = .012$, $d = .583$). There was no difference between feet and hand CPT in overall pain ratings (main effect STRESSOR: $F(1, 19) = 1.6$, $p > .05$, $\eta_p^2 = .078$) but reported pain levels differed depending on measurement timepoint as indicated by a significant STRESSOR*TIME interaction ($F(1,38) = 5.71$, $p = .007$, $\eta_p^2 = .231$, HF-$\varepsilon = .971$). Whereas pain ratings were higher for the feet CPT at the first ($t(22) = 2.6$, $p = .017$, $d = .557$) assessment they did not differ at the second ($t(22) = 1.08$, $p > .05$, $d = .228$) and last measurement ($t(22) = 0.11$, $p > .05$, $d = .05$). Again, there were no significant main effects or interactions involving SEQUENCE or SEX (all *F*s < 3.43, all *p*s> .05, all η_p^2s < .153). Mean stress and pain ratings for the feet and hand CPT are presented in Table 1.

Table 1: Mean subjective pain and stress ratings (± SEM) for the hand and feet CPT versions. p-values refer to the hand vs. feet CPT comparisons.

	Hand CPT	*Feet CPT*	*p*
Stress			
Before CPT	2.21±.39	1.77±.33	.297
CPT start +0.5 min	4.05±.40	5.39±.47	.005
CPT start +1.5 min	4.62±.47	5.92±.46	.004
CPT start +2.5 min	4.49±.47	5.20±.39	.076
After CPT	3.70±.47	4.17±.51	.458
Pain			
CPT start +0.5 min	5.23±.42	6.18±.38	.021
CPT start +1.5 min	6.35±.36	6.70±.42	.316
CPT start +2.5 min	5.96±.38	5.87±.42	.848

3.3.6 Correlations between hand and feet CPT responses

All dependent variables but cortisol responses (15 min. post stress: $r = .29$, $p > .05$; 30 min. post stress: $r = -.53$, $p = .01$) were positively correlated between hand and feet CPT versions. Blood pressure responses correlated positively (SYS: $r = .45$, $p = .031$; DIA: $r = .5$, $p = .015$; MAP: $r = .352$, $p > .05$), as did heart rate ($r = .637$ $p = .001$) and sAA responses (immediately post stress: $r = .432$, $p = .04$; 5 min. post stress: $r = .497$, $p = .016$). Also, mean stress ($r = .65$, $p = .001$) and pain ($r = .62$, $p = .002$) ratings during the CPT were positively correlated between hand and feet versions as was the increase in stress ratings from baseline to mean CPT values ($r = .5$, $p = .015$).

3.4 Discussion

The aim of the current study is to prove feasibility of a bilateral feet CPT version as a laboratory stress protocol by comparing it to the well validated and widely used classic unilateral hand

CPT. The bilateral feet CPT version was developed to avoid several practical limitations inherent to the classic unilateral hand CPT, such as asymmetric stimulation. According to a within-subject design participants were studied twice, in randomized order, one day apart: once they received the bilateral feet CPT, the other time the unilateral hand CPT version. Several cardinal markers of the human stress response were assessed at baseline, during, and after CPT stress. Clearly, the bilateral feet CPT induced marked increases in all measured stress parameters. Moreover, with the exception of blood pressure, autonomic and endocrine responses were enhanced compared to the classic CPT.

Variations of stimulation site have so far only been tested and compared when the CPT was used according to its original purpose as vasoconstrictor stimulus and were thus restricted to cardiovascular responses. Our results support the existing findings in that substantial and comparable increases in blood pressure may be achieved irrespective of stimulation site whereas heart rate responses seem to be more sensitive to such modifications (Durel et al., 1993; Saab et al., 1993). To the best of our knowledge this is the first study to also compare sAA and cortisol reactions between unilateral hand and the bilateral feet CPT providing a comprehensive account on its qualities as a laboratory stress protocol.

We found a significant increase in salivary cortisol 15 minutes after application of the bilateral feet CPT, replicating earlier findings (Frings et al., 2013). By contrast, no significant increases in cortisol could be observed after unilateral hand immersion. This fits in well with previous research reporting only mild (Larra et al., 2014) or absent (Duncko et al., 2009; McRae et al., 2006) cortisol responses to the classic CPT procedure. In fact, another modification of the standard CPT, the socially evaluated CPT (SECPT), has been proposed to selectively enhance cortisol responses (Minkley et al., 2014; Schwabe et al., 2008). The cortisol reactions produced by the bilateral feet CPT are of similar magnitude. Therefore, it might also represent such a cortisol enhancing modification of the classic CPT. Moreover, unlike the SECPT the bilateral feet CPT does not selectively promote cortisol reactions, as was shown in the original SECPT report by Schwabe et al. (2008), but seems to enhance all components of the stress response including autonomic and subjective reactions. It might thus be a useful alternative in situations where a stronger neuroendocrine stress response is of interest. Note that every participant underwent both protocols at exactly the same time of day and thus the differences in cortisol responses cannot be attributed to diurnal variations in cortisol concentrations.

Blood pressure responses did not differ between the two versions. Conversely, we found substantially higher reactions in heart rate and marginally enhanced sAA concentrations with the bilateral feet CPT. Increases in heart rate during the CPT are beta-adrenergically mediated (Houben et al., 1982; Victor et al., 1987) whereas the blood pressure response to the CPT primarily stems from alpha-adrenergically mediated peripheral vasoconstriction (Frank and Raja, 1994; Lovallo, 1975). sAA concentrations, on the other hand, have been shown to be sensitive to both, alpha- and beta-adrenoceptor activation (Nater and Rohleder, 2009). Thus, the pattern of differences in these markers of sympathetic activity might suggest a selective enhancement of beta-adrenergic response components in the bilateral feet CPT while representing a similar alpha-adrenergic challenge. An alternative explanation may be enhanced vagal withdrawal in the bilateral feet CPT. It might seem surprising that the standard CPT did not lead to significant increases in heart rate. This, however, is a common finding in studies using the CPT. While the standard CPT has been shown to be capable of producing a full neuroendocrine stress response it is primarily an alpha-adrenergic task (Allen et al., 1992; Frank and Raja, 1994) and not very reliable in activating beta-adrenergic response components. Accordingly, sAA and heart rate have been reported to be significantly increased by CPT exposure in some studies (Duncko et al., 2009; Smeets et al., 2008) whereas others could not detect any change in these parameters (Felmingham et al., 2012; Schulz et al., 2011; Schwabe et al., 2008) or only in a subgroup of participants (Larra et al., 2014).

Both, the feet and hand CPT induced pain and increased subjective stress ratings. Participants reported more pain and stress during the first half of the feet CPT compared to the hand CPT, however, this difference vanished to the end of the CPT. Immediately after the CPT stress ratings for both versions were similar. This is interesting to note as in CPT studies subjective ratings are often only gathered before and immediately after the intervention. Especially in comparative designs it might thus be recommendable to also assess ratings during the CPT as they might reveal additional information that is not reflected in pre/post measurements.

Stress is a multifaceted phenomenon comprised of changes in multiple neuronal and endocrinological variables. Still, cortisol and indices of beta-adrenergic activation can be considered key components of the stress response as they are primarily involved in most known stress effects on the brain (Erickson et al., 2003; McEwen, 2007). Therefore, heart rate, sAA and cortisol are of crucial interest when the CPT is used as a laboratory stressor. Our finding that bilateral feet immersion produces higher responses in all of these measures makes the

bilateral feet CPT a highly valuable tool within experimental stress research. Its benefits lie further in combining the ease of use and time efficiency of the classic CPT with the additional advantages that laterality effects are avoided and both hands are rendered free. Nevertheless, as stress responses are enhanced, conclusions from studies using different CPT protocols should be drawn with care as the results might not necessarily be comparable.

The design of this study does not allow for a clear attribution whether variation of the stimulation site or bilateral stimulation is responsible for the observed response enhancements by the bilateral feet version. Sendowski et al. (1997) found enhanced heart rate reactions when stimulation surface was increased from finger to hand and arm. Similarly, bilateral hand immersion leads to higher sympathetic responses than unilateral hand immersion (Seals, 1990). On the other hand, unilateral feet immersion has been shown to elicit comparable cardiovascular responses compared to unilateral hand immersion (Saab et al., 1993). It thus seems likely, that the increase of stimulation surface might be responsible for our findings. However, further studies are needed to conclude on this issue.

In the present study we did not observe any sex effects. While this is in line with previous research showing that cardiovascular responses to the CPT are independent of participants' sex (Jones et al., 1996) cortisol responses to stress in general are likely to differ between the sexes (Kudielka et al., 2009). However, it should be noted that we did not aim at investigating sex effects and our sample size might have been too small to detect these. Also, sex differences in response to stressors have been shown to be crucially depending on menstrual cycle phase and use of oral contraceptives (Kajantie and Phillips, 2006; Tersman et al., 1991), which we did not control in the present study. Therefore, we cannot exclude that sex specific effects might become apparent if these factors are taken into account.

Exposure to both CPT versions was varied within subjects. Given the high interindividual variability in stress responses to the CPT this is an advantage of this study as it allows for a clear attribution to the influence of the stressor modification rather than the response characteristics of the participant. Nevertheless, it also raises the possibility that our results simply reflect an effect of measurement repetition. To control for possible effects of multiple exposure we varied the order in which both versions were applied between subjects. Regardless of the stressor administered, sAA concentrations were higher on the first day of the experiment probably reflecting lower arousal levels on the second day due to habituation to the

experimental setting. This could be owed to testing on two subsequent days and might have been avoided with a longer test interval. Nevertheless, we found that all observed differences between feet and hand immersion where apparent regardless of the sequence in which they were administered. Therefore, our findings clearly result from the modification of the protocol rather than its repetition.

In summary, we conclude that the bilateral feet CPT represents a valid alternative to the classic CPT as it is capable of producing a full neuroendocrine stress response. It may therefore be employed if practical concerns hinder the use of the one hand CPT and/or unilateral stimulation needs to be avoided. Even without these practical concerns, it might still be given preference as it represents a simple and feasible modification that produces enhanced responses in parameters that are of crucial interest for most psychobiological stress studies.

References Chapter III

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3.i Author Notes

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Chapter IV: Stress disrupts distractor-based retrieval of SR episodes

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4.0 Abstract

The binding of stimulus and response features into S-R episodes or "event files" is a basic process for the efficient control of behavior. However, relevant information is usually accompanied by information that is irrelevant for the selection of action and recent studies showed that this irrelevant information is also bound into event files. In this study, we investigated the possible modulation of distractor-response binding due to stress. To this end, participants were treated with a variant of the cold pressure stress test and worked through a binding experiment before and directly after the stress treatment. Physiological and subjective stress measures were surveyed and did predict the change in binding effects: Binding in stressed participants ceased as compared to a non-stressed control group. Increases in cortisol and blood pressure are discussed as main reasons for decreased S-R retrieval.

Keywords: stress; cortisol; stimulus-response binding; distractor processing

4.1 Introduction

The complex world we live in offers us infinite possibilities to behave. Thus, in order to cope with the world's complexity, our cognitive system has to rely on a limited number of simple and efficient processes and mechanisms. The binding of stimulus features and response features into object files or event files is one such basic process that is essential for an efficient control of behavior (Hommel, 1998; Kahneman and Treisman, 1984). While object files denote the integration of different stimulus features into a perceptual object, event files denote the integration or association of stimulus and response information in a unitary mental representation. In particular, it is assumed that these files are stored in memory and are automatically retrieved by subsequently encountered stimuli that match features of the previous episode. This episodic retrieval process is a core feature of automatization in perception and action (Logan, 1988; Treisman, 1992) and it is assumed that this retrieval of previous actions operates fast and automatically, exerting efficient bottom-up control of behavior by establishing stimulus-driven behavioral routines.

Interestingly, binding and retrieval processes incorporate not only those stimulus features that are relevant for determining a response but also include irrelevant or distracting information (Rothermund, Wentura, and De Houwer, 2005; see also Frings, 2011; Frings, Rothermund, and Wentura, 2007; Hommel, 1998, 2005, 2007; Mayrand Buchner, 2006; Mayr, Buchner, and Dentale, 2009). In particular, even a distractor that competes with a target stimulus (like, for example, a flanking stimulus in a classical interference task, the Eriksen flanker task, Eriksen and Eriksen, 1974) can become integrated with the response that has been elicited by the simultaneously presented target into an event file and upon the next encounter can retrieve the last response that was given in its presence; a phenomenon that has been labeled distractor-response binding (cf. Figure 7; we will explain how we measure distractor-response binding at the end of the introduction). Distractor-response binding and retrieval have been observed with a variety of tasks and stimuli; in particular, a distractor-based retrieval of previous responses has been demonstrated with visual (Frings, 2011), auditory (Moeller, Rothermund, and Frings, in press; Mayr and Buchner, 2006), and tactile stimuli (Moeller and Frings, 2011), across modalities (Frings, Moeller, and Rothermund, submitted), with emotional material (Giesen and Rothermund, 2011), with location tasks (Frings and Moeller, 2010), and even across different tasks (i.e., in a task switching context; Forstmann, Brass, and Koch, 2007; Rothermund et al., 2005); these findings attest to the generality of basic binding and retrieval mechanisms in

perception and action.

Integrating irrelevant information into event files can be seen as an adaptive default configuration of the cognitive system because it allows for redundancy gains and implicit learning: Irrelevant features of stimuli can often be assumed to be informative with regard to correct behavior in natural settings because they correlate with relevant features due to their co-occurrence within certain objects. For example, a potential predator may be identified by the shape of its body that elicits a flight response. The color of the predator's fur then also becomes associated with the flight response, which further enhances the activation of the flight response during subsequent encounters with the predator due to some kind of redundancy gain or Garner effect (Garner and Felfoldy, 1970).

In the present article we explore the effects of stress on distractor-response binding. Stress typically induces several endocrine responses. In particular, stress increases the amount of circulating glucocorticoids (i.e. cortisol in humans) due to activation of the hypothalamic-pituitary-adrenal axis; cortisol affects the dopaminergic neurotransmission (Rothschild et al., 1985). There is also evidence that stress can affect the dopamine level in a more direct fashion, as stress increases dopaminergic activity particularly in the prefrontal cortex (e.g., Arnsten and Goldman-Rakic, 1998). Yet, these endocrine responses are known to influence the cognitive processing of information in general as there is evidence for the effects of stress responses on perception (e.g., sensorimotor gating; Richter et al., 2011), action (e.g., effect on automatic motor responses; Deuter, Kuehl, Blumenthal, Schulz, Oitzl, and, Schachinger, in press), attention (e.g., better selection; Aston-Jones, Rajkowski, and Cohen,, 1999), memory (e.g., increasing consolidation but decreasing retrieval; Roozendaal, and McGaugh, 2011), and learning (e.g., enhanced habit learning; Schwabe et al.;2007). More specifically we discuss what one can expect – against the background of the findings on stress and cognition – for the relationship between stress and distractor-response binding.

On the one hand, one may assume that stress increases distractor-response binding effects as higher levels of dopamine usually enhance learning (e.g., Schulz, 2000; Law and Gold, 2009). The encoding of a response together with the relevant and irrelevant sensory features of this episode may be interpreted as single-trial learning (e.g., Standing, Conezio, and Haber, 1970; Rutishauser, Mamelak, and Schuman, 2006; Frings and Rothermund, 2011). In a typical paradigm testing for distractor-response binding effects, the distractor of trial n-1 is repeated as

the distractor in trial n. Thus, one may argue that the association between the response and the stimulus in trial n-1 is better learned in a condition with high dopaminergic activity as compared to a condition with low dopaminergic activity which should ultimately increase the impact of repeated distractors. In the same vein, presenting positive pictures has been shown to enhance the binding of visual and action features (Colzato, van Wouwe, and Hommel 2007) presumably due to a stimulation of the dopamine system (Ashby, Isen and Turken, 1999; Suri, 2002). Yet, it should be noted that interindividual differences govern the sensitivity of learning processes in positive and negative situations (e.g., Frank et al., 2005). Therefore, although similar effects of stress and positive affect on learning are not impossible (because stress as well as positive affect may affect learning via an increased arousal), it remains speculative to argue that reward and positive affect contexts actually shape learning through the same pathways as compared to negative affect and stressful situations.

In addition, stress can have a differential impact on cognitive processes. In fact, effect of stress intensity and duration on cognitive functions may be non-monotonous (e.g., Young, Drevets, Schulkin, and Erickson, 2011) and may be different for selective cognitive processes (e.g., impaired declarative memory retrieval, but enhanced emotional memory consolidation; Roozendaal, and McGaugh, 2011). Plessow and colleagues (2011), too, found only specific cognitive functions to be reduced under stress. In particular, they argued that cognitive processes operating at an abstract level (e.g., controlling task sets or rules) will be impaired whereas processes operating at the level of stimulus-features will not or be even facilitated (see Arnsten, 2009 for the same argument as well). With respect to distractor-response binding (a phenomenon assuming binding on the level of perceptual and action features) one may thus conclude that under stress this kind of process may actually be boosted.

On the other hand, one may argue that stress decreases distractor-response binding effects. In particular, many studies showed that selection is affected by stress in that humans seem to focus only on the relevant features while stressed leading to the paradox finding that interference due to irrelevant information is reduced under stress (e.g., Chajut and Algom, 2003; Steinhauser, Maier, and Hübner, 2007). Yet, when one does not process distractors (or at least process them to a smaller degree) any distractor-based retrieval would be reduced. In the same vein, Colzato, Kool and Hommel (2008) observed reduced binding of relevant features and response features under presumably high stress levels. Finally, cortisol is inversely related to memory retrieval in explicit (de Quervain et al., 2000) and implicit (Grillon et al., 2004; Nees et al., 2008; Roemer

et al., 2011) associative learning, thus, one may argue that distractor-based retrieval effects are generally impaired under high stress as compared to low stress.

We explored the effects of stress on distractor-response binding with a sequential priming paradigm, in which the distractor stimuli of the prime trial are sometimes repeated as distractors in the probe trial (distractor-to-distractor priming). In this paradigm, an integration of the irrelevant prime distractor into an event file is reflected in an interaction of distractor repetition effects with response repetition (Frings et al., 2007; Frings, 2011; Frings and Moeller, 2012; Giesen and Rothermund, 2011; Rothermund et al., 2005). In particular, in the case of a distractor repetition between a prime and a probe display, a distractor will facilitate the response to the probe target if the prime response is also repeated as the probe response. The binding of the prime response and the prime distractor leads to the retrieval of a response which is *compatible* to the to-be-executed probe response, hereby facilitating responding in the probe. In contrast, a distractor repetition between a prime and a probe display impedes responding to the probe target if the response changes between the prime and probe. The binding of the prime distractor and response in the prime trial leads to the retrieval of a response that is *incompatible* to the to-be-executed probe response hereby slowing responding in the probe due to response interference (see Figure 7). Participants worked through two blocks of a sequential priming paradigm while physiological parameters were measured. Between the blocks, a variant of the cold pressure test was applied. We were particularly interested in the effects of the stress-treatment on distractor-response binding.

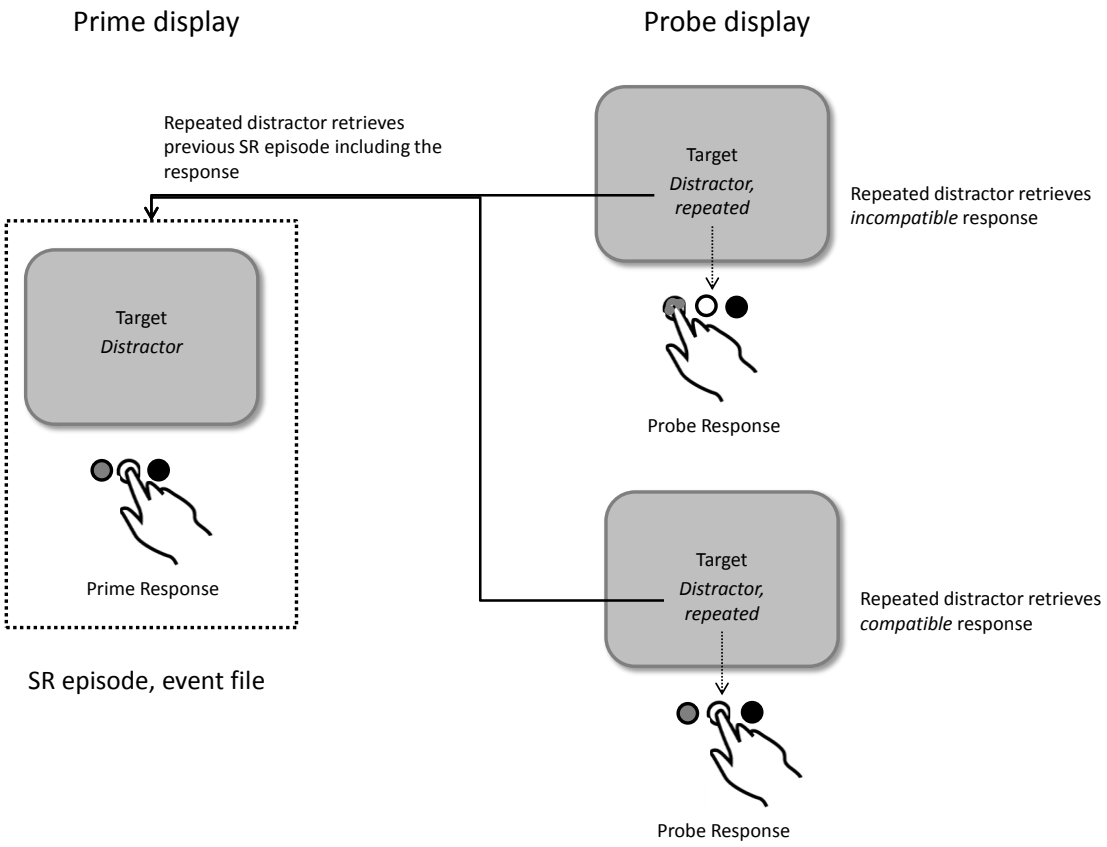

Figure 7: Schematic display of distractor-response binding in a prime-probe sequence. The prime display is encoded together with the response, the target, and the distractor. A repetition of the distractor in the probe will retrieve the whole prime episode including the prime response. In dependence of the to-be-executed probe response, the retrieved response can be compatible or incompatible.

4.2 Methods

4.2.1 Participants

Twenty-two healthy students from the University of Trier were recruited. They received a monetary reward for participation. Exclusion criteria were any acute or chronic somatic or psychiatric illness, any history of psychiatric disorders, any family history of aneurysms, a BMI lower than 20 or greater than 25 kg/m2, smoking, or any illicit drug intake in the last six months. Volunteers gave their informed written consent. Study procedures were approved by the Ethical Committee of the State's Medical Association (Landesärztekammer Rheinland-Pfalz) and was in accordance with the latest revision of the declaration of Helsinki.

4.2.2 Stress test

The Cold Pressor Test (CPT) is a widely used tool in psychophysiological research. First described by Hines and Brown (1932) it consists of a procedure in which participants have to immerse a limb into ice water for several (usually 2-3) minutes. The CPT reliably triggers activation of the sympathetic nervous system, as expressed in elevated blood pressure, heart rate and increased skin conductance (Lovallo, 1975). It also leads to a rise in cortisol (al'Absi, Petersen, and Wittmers, 2002; Bullinger et al., 1984), a stress hormone released by the hypothalamus-pituitary-adrenal (HPA) axis, which qualifies it as a valid laboratory stressor. Within experimental research the CPT has therefore often been used as stress protocol and found to be capable of modulating a range of psychophysiological phenomena as startle (Schulz, Plein, Richter, Blumenthal, and Schächinger, 2011), learning (Duncko, Cornwell, Cui, Merikangas, and Grillon, 2007) and memory processes (Schwabe, Bohringer, Chatterjee, and Schächinger, 2008).

In the standard version of the CPT subjects are asked to place one hand (often the non-dominant hand) into ice water. However, bilateral two hand water immersion tests have been used (Suter, Huggenberger, and Schächinger, 2007) to avoid potential effects of unilateral stimulation. Furthermore, local cold of the hands may impact on the speed of manual button presses in the post-CPT period. Therefore, a bilateral foot cold pressor test version was used, in which participants had to immerse both feet for 3 minutes into ice water (2-3 °C) or warm water as control procedure. They were sitting comfortable in a chair and first asked to take off their shoes and socks. After that a same-sex experimenter came in, set the water bath on the ground in front of the test person and said that the cold water stress procedure would now start. The participants were instructed to put both feet including the ankles into the water and take them out when the experimenter told so. Directly at the beginning of the CPT as well as one and two minutes after the start subjective ratings of pain and stress intensity were gathered. Blood pressure and heart rate were measured at 0.5 and 2.5 minutes after feet immersion, baseline values were obtained from two measurements during a 5 minute resting period before the start of the experiment. Saliva samples were collected using Salivette tubes (Sarstedt, Germany), after the resting period, before the CPT as well as 15 and 30 minutes after the CPT. When the stress procedure had finished, participants were given a towel to dry themselves and asked to put their socks but not their shoes back on. During the stress procedure there was no interaction between investigator and participant, they were not informed about the time left.

4.2.3 Physiological measurements

Heart rate was derived from ECG. Standard Ag/AgCl electrodes (ECG Tyco Healthcare H34SG Ag/AgCl electrodes of 45 mm diameter) were used for ECG (standard lead II configuration) recording by Biopac MP150 system and ECG100C amplifier modules. Systolic, diastolic, and mean blood pressure was measured with standard cuff oscillometric Dinamap monitor (Dinamap SX 1846, Critikon, US). Cortisol concentration was determined by immunoassay with fluorescence detection (Dressendorfer, Kirschbaum, Rohde, Stahl, and Strasburger, 1992).

4.2.4 Materials and Apparatus

The experiment was conducted using the E-prime software (E-prime 1.2). Stimuli were shown on a standard color monitor. The stimuli were the letters D, F, J, and K in the Courier New font type. Each letter was about 0.9 cm high and 0.4 cm wide. Target stimuli were shown in red color, while distractor stimuli were shown in green color. The background was black. Three letters (two identical distractors and one target) were presented in a row forming a letter string at the screen center (e.g. DFD).

4.2.5 Procedure

Each participant was tested individually. Participants were randomly assigned to one group after they entered the laboratory with the restriction that at the end of the experiment both groups had the same number of participants. Instructions were given on the screen and summarized by an experimenter. Participants were instructed to place the index and middle fingers of both hands on the keys D, F, J, and K of the computer keyboard. Participants' task was to classify the identity of red target letters. A typical trial consisted of the following events: Participants started each trial by pressing the space bar. After pressing the space bar a fixation marker ('*') appeared at the screen center for 500 ms. Then the prime display was presented. One red target letter was flanked by two, identical distractors; all stimuli were presented adjacent at the screen center. Participants' task was to press the corresponding key to the target letter identity. After the response to the prime display a blank screen was shown for 500 ms before the probe display appeared. Again participants had to categorize the identity of the red

target letter by pressing the corresponding key. Both prime and probe displays remained on the screen until participants responded.

Assignment of stimuli to the different roles as distractor or target in prime and probe displays was randomly selected. All possible incompatible pairings of distractors and targets were run for both prime and probe displays with equal frequency of these pairings in the probe displays. Participants worked through two blocks of 336 trials each. Between the blocks the CPT was applied. Before the experimental trials, participants practiced the task for 40 trials. For analyzing the effect of distractor-response binding, only four types of trials were analyzed. In particular, trials can be classified as response change trials (RC; prime target and probe target had different identities) and response repetition trials (RR; prime targets were also presented as probe targets). In addition, the distractor can be repeated between the prime and probe (DR; distractor repetition trials) or it can change between the prime and probe (DC; distractor change trials). Note that in these trials no distractor-to target- or target-to-distractor-repetition was possible. In each block 48 trials for each of the four conditions (RRDR, RRDC, RCDR, and RCDC) were conducted. Distractor-response binding effects are measured only within this subset of trials (192 trials per block). In particular, distractor-response binding effects would be indicated by an interaction of response repetition and distractor repetition (see Figure 1).

4.2.6 Design

The design comprised three within-subjects factors, namely response relation (repeated versus unrepeated) and distractor relation (repeated versus unrepeated) and time (the experimental block before the CPT versus the experimental block after the CPT). In addition, one factor was varied between subjects, namely the variant of the CPT (stressed versus non-stressed).

4.3 Results

4.3.1 Stress test

Repeated measures ANOVA revealed significant interactions between the within factor „time“ (cardiovascular baseline values at 5 and 3 min before, and stress values 0.5 and 2.5 min after CP start; saliva cortisol values 20 and 5 min before, and 15 and 30 min after CP start) and the between factor “intervention group” (stress versus control) for mean arterial blood pressure (MAP; $F(3,60) = 7.63$, GG/HF-adj. $p < .001$), heart rate (HR; $F(3,60) = 4.12$, GG/HF-adj. $p < .025$), and saliva cortisol ($F(3,60) = 4.21$, GG/HF-adj. $p < .04$). Subsequent contrasts of stress values against the second baseline value revealed significant group effects for MAP (1st stress value: $F(1,20) = 8.34$, $p < .01$; 2nd stress value: $F(1,20) = 13.82$, $p < .002$), HR (1st stress value: $F(1,20) = 8.1$, $p < .01$; 2nd stress value: $F(1,20) = 4.64$, $p < .05$), and saliva cortisol (1st stress value: $F(1,20) = 8.56$, $p < .01$; 2nd stress value: $F(1,20) = 4.35$, $p < .05$).

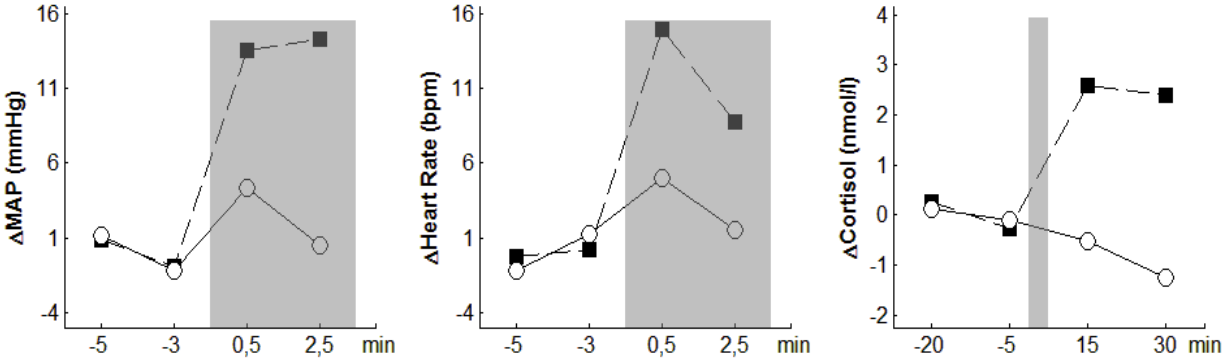

Figure 8: Changes in Blood Pressure, Heart Rate and Salivary Cortisol in response to the CPT (gray area). Filled squares represent the stress empty circles the control group.

4.3.2 Subjective ratings

Wilcoxon's rank-sum test was used to test for group differences in subjective stress and pain ratings (mean ratings are depicted in Table 2). The stress group showed significantly higher ratings for stress at 0.5 ($Ws = 83.5$, $p = .003$), 1.5 ($Ws = 73.0$, $p < .001$) and 2.5 minutes ($Ws =$

69.0, $p < .001$) and for pain (all Ws = 66.0, all ps $< .001$). Within the stress group, an ANOVA revealed significant differences in pain ratings between measurements ($p = .026$). Follow-up Wilcoxon signed-rank tests showed that pain ratings differed significantly between the 0.5 and the 1.5 minute ($z = -1.97$, $p = .007$) as well as the 0.5 and 2.5 rating ($z = -2.70$, $p = 0.048$), but did not change from the 1.5 to the 2.5 minute rating ($z = -0.05$, $p = 0.96$).

Table 2: Mean subjective ratings (SEM) for pain and stress during the CPT and control procedure.

	Stress			Pain		
	0.5 min	1.5 min	2.5 min	0.5 min	1.5 min	2.5 min
Control	1.82 (0.26)	2.00 (0.38)	1.55 (0.28)	1.00 (0.00)	1.09 (0.09)	1.00 (0.00)
CPT	5.00 (0.78)	6.18 (0.77)	5.82 (0.66)	4.91 (0.77)	6.64 (0.47)	6.64 (0.34)

4.3.3 Binding effects

Only the reaction times (RTs) from prime-probe sequences with two correct responses were considered. Moreover, only RTs above 200 ms and below 2000 ms were further analyzed. According to these constraints, 5.5 % of the trials were discarded (prime error rate 2.9 %, probe error rate 2.4 %). Mean RTs are depicted in Table 3.

Table 3: Mean reaction times in ms as a function of response repetition (repeated versus changed), distractor repetition (repeated versus changed), time (block 1 versus block2), and stress (stressed versus non-stressed group) with standard deviations in parenthesis.

		Stressed		*Non-stressed*	
		Block 1	Block 2	Block 1	Block 2
Response Repetition	Distractor Repetition	528 (79)	533 (65)	576 (114)	564 (95)
	Distractor Change	591 (122)	655 (81)	586 (75)	710 (133)
Response change	Distractor Repetition	714 (128)	640 (124)	778 (177)	718 (151)
	Distractor Change	696 (92)	662 (107)	775 (191)	710 (141)
DR binding		40 (85)	50 (55)	7 (60)	77 (59)

Note. Distractor-Response binding was computed as the interaction of response repetition x distractor repetitions ((RRDR+RCDC)/2) – ((RRDC+RCDR)/2).

RTs from correct trials were submitted to a 2 (response repetition) x 2 (distractor repetition) x 2 (time) x 2 (stress) MANOVA. The main effects for response relation and distractor relation were significant, $F(1,20) = 43.99$, $p < .001$, $\eta_p^2 = .69$, $F(1,20) = 30.12$, $p < .001$, $\eta_p^2 = .60$, for response repetition and distractor repetition, respectively. The main effects for time and stress-manipulation were not significant. Overall, a significant distractor-response binding effect was observed, as revealed by the interaction of response and distractor repetition, $F(1,20) = 12.76$, $p = .002$, $\eta_p^2 = .39$. Yet, this binding effect was modulated by the time factor, namely, binding was generally larger in block 1 as compared to block 2, $F(1,20) = 8.59$, $p = .008$, $\eta_p^2 = .30$. Most important, however, this three-way interaction was further specified by the factor stress, i.e., the change in the binding effect from block 1 to block 2 was different as a function of whether participants were stressed or not, as shown in the four-way interaction $F(1,20) = 4.81$, $p = .040$, $\eta_p^2 = .19$ (see Figure 9a).

To better grasp this finding, we also computed a regression analysis in which we used the change in binding from block 1 to block 2 as the dependent variable and entered the stress factor

as a dummy-coded predictor. This regression model was significant, $F(1,20) = 4.81$, $p = .040$ (in fact, it reflects exactly the same analysis as the four-way interaction reported above) and explained 19% variance in the change of the binding effect. In addition, we also entered the changes in the physiological/endocrine measures as further predictors into the model, but none could significantly improve the model (due to the fact, that all these predictors are highly correlated with each other and the dummy-coded stress variable).

Note that both groups showed numerically different binding effects in block 1 (cf. Table 1) that, however, were statistically not significantly different, $t(20) = 1.08$, $p = .295$; nevertheless, one might speculate that the change in binding is a mere result of different levels of binding in block 1. To hedge against such an interpretation, we entered binding effects of block 1 and the dummy-coded stress-factor as predictors in a multiple regression analysis with the change in binding from block 1 to block 2 as the dependent variable. Stress remained a marginally significant predictor for the change in binding independently of binding levels in block 1, $\beta = .296$, $p = .076$ while binding in block 1 was a significant predictor, too $\beta = .617$, $p = .001$.

In addition, we also computed a multiple regression analysis with binding effects of block 1 and the dummy-coded stress-factor as predictors and the binding effect in block 2 as the dependent variable. Again, the stress factor remained a marginally significant predictor for the binding effects in block 2 (in which the control group showed 77 ms versus the stressed group showing 50 ms), $\beta = .365$, $p = .074$ while binding in block 1 also predicted the binding in block 2, $\beta = .531$, $p = .013$.

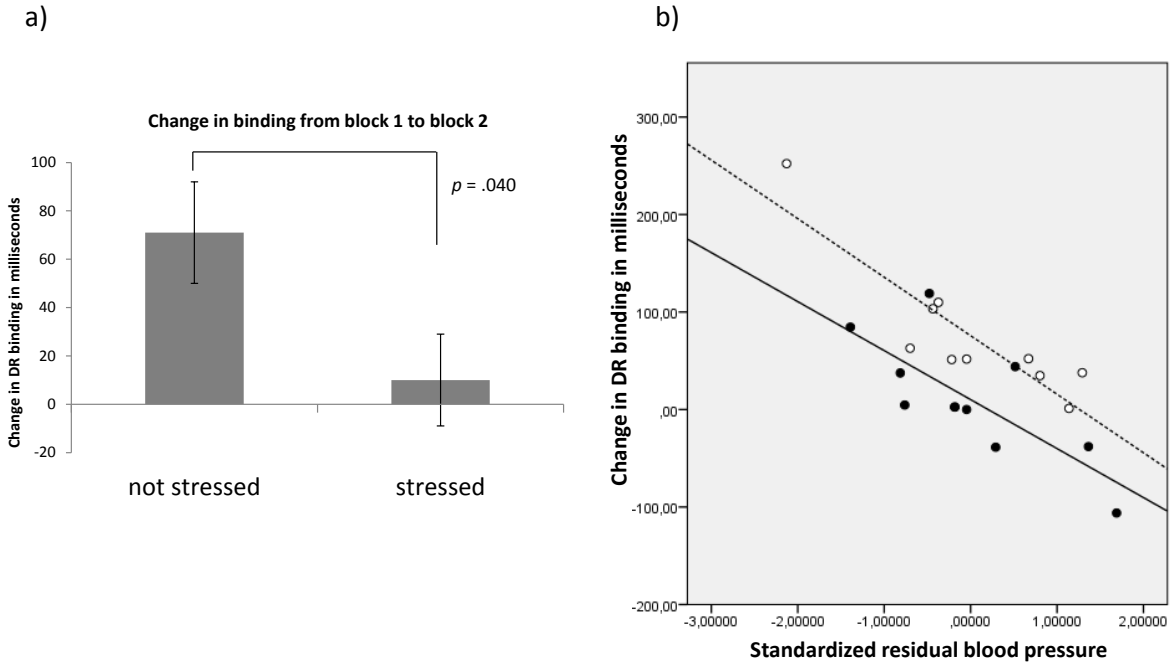

Figure 9: Changes in the binding effect (the interaction of response repetition x distractor repetition) as a function of stress. Figure 9a depicts the mean effect of change in binding in milliseconds as a function of stress. Error bars depict standard errors of the mean. Figure 9b depicts a multiple regression of the change in the binding effect from block 1 to block 2 as a function of stress (dummy-coded; the filled circles depict the stressed group, the not-filled circles depict the control group) and stress-independent change in blood pressure (z-standardized; individual average of both stress blood pressure readings).

Interestingly, in a multiple regression model, the change in blood pressure that was unrelated to the stress manipulation (the residuals after a regression of group on blood pressure) added significantly to the model. In fact, adding the stress-unrelated change in blood pressure increases the explained variance to 47% (this change was significant, $p = .009$) and the dummy-coded stress variable as well as the blood pressure were both significant predictors, both ps< .02 (see Figure 9b).

4.4 Discussion

We explored the effects of stress on the phenomenon of distractor-based retrieval of SR episodes. To this end, we applied a variant of the CPT on participants' feet and compared their distractor-response binding effects before and directly after the stress manipulation. The stress manipulation was successful as reflected in the significant change in the stress-group in blood pressure, heart rate and salivary cortisol as compared to the control group; this pattern was also reflected in the subjective stress and pain ratings. In addition, both groups showed significant binding effects. However, the control group showed a significant change in binding between block 1 and block 2 – in other words, during the experiment the distractor-response binding effects got larger for this group. In contrast, the stressed group showed no such enhancement of distractor-response binding.

Distractor-response binding is a specific phenomenon caused by a general mechanism that helps humans to effectively deal with the demands of their environment. In particular, binding of stimulus and action features helps to establish nearly automatic SR routines. Thus, participants will rely on binding effects as to effectively work through the experiment – at least under normal circumstances (this is what the control group showed as their binding effect increased from block 1 to block 2). Under stress, however, the increase in binding along time is impaired (see, Colzato et al., 2008, for a similar finding concerning the binding between target features and responses). Stress elicits a complex pattern of endocrine responses and hence it is here impossible to pinpoint the exact parameter which reduces binding effects. However, the increase in cortisol seems to be a likely candidate. Cortisol is known to impair retrieval and associative learning (Grillon et al., 2004; Nees et al., 2008; Roemer et al., 2011). In addition, rapid disruptions of cognitive processes by cortisol have recently been shown (Richter et al., 2011); thus, cortisol can impact cognition much earlier than it is measurable in the saliva. Distractor-response binding effects hinge on the encoding and the retrieval of the prime episode; with higher levels of cortisol, the prime-retrieval will be hampered even if participants rely on the binding effects during the course of the experiment. The net effect of impaired retrieval due to cortisol and the enhancement of binding will result in no change in the amount of binding between block 1 and block 2 for the stressed group.

As outlined in the introduction, stress also influences the amount of DA, particularly in the PFC (Arnsten, 2009). Yet, typically an inverted U-shaped function between DA and performance is

suggested and hence it is problematic to analyze the exact effects of DA on performance with an IV with only two-factor levels (stressed versus not stressed) because any pattern would be in line with such a quadratic relationship. In addition, we did not measure the levels of DA; thus, despite the undoubted effects of DA on learning (although in a reward context and not a stress context), we cannot pinpoint the impact of DA on distractor-binding yet.

However, another interesting result was that the change in blood pressure was – independently of the stress-modulation – related to the change in the binding effect. In particular, the more the blood pressure increased from block 1 to block 2, the worse the binding became. In other words, high blood pressure decreases binding effects. We can only speculate about the mechanisms responsible for this finding. However, a blood pressure rise will activate arterial baroreceptors, and their firing has been shown to affect cognitive-motor processes to induce prolonged reaction times (Edwards et al., 2007), reduced pain perception (Dworkin et al.,1994), and impair brainstem-relayed reflexes, such as the startle response (Nyklicek, Wijnen, and Rau, 2005; Schulz et al., 2009). Our data suggest that baroreflex activation by stress-induced blood pressure increases may specifically impair binding, but this question should be revisited in future studies.

Taken together, we conclude that acute stress as elicited by the CPT reduces the impact of distractor-based SR retrieval. This finding is in line with previous findings on feature binding in action and perception (Colzato et al., 2008). However, further research is clearly needed as to analyze which particular parameter of the stress response influences binding, and whether the here reported stress effects are in monotonous dependency to stress intensity and duration. For example, administering different doses of cortisol and or blood pressure elevating substances via infusions should make it possible to explore the exact relationship between stress, stress hormones and binding.

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4.i Author Notes

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