PSYCHOPHYSIOLOGICAL RESPONSES TO DISTRESS AND EUSTRESS

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Doctoral Dissertation

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ABBREVIATIONS

AIC	Akaike Information Criterion
ACTH	Adrenocorticotropic hormone
ANS	Autonomous nervous system
AUC	Area under the curve
AVP	Arginine-vasopressin
BIC	Bayesian Information Criterion
BP	Blood pressure
C	Cortisol
CAR	Cortisol awakening response
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CPT	Cold pressor task
CRH	Corticotrophin releasing hormone
DBP	Diastolic blood pressure
E	Epinephrine
GAS	General adaptation syndrome
GC	Glucocorticoid
GnRH	Gonadotropine-releasing hormone
HPA	Hypothalamus-pituitary-adrenal
HPG	Hypothalamus-pituitary-gonadal
HR	Heart rate
HRV	Heart rate variability
IBI	Inter beat interval
ICC	Intermediolateral cell column
MT	Memory-search task
NE	Norepinephrine
PEP	Pre-ejection period
PNS	Parasympathetic nervous system
RMSSD	Root mean square of successive heart beat differences
sAA	Salivary alpha-amylase
SAM	Sympatho-adreno medullary
SBP	Systolic blood pressure
SNS	Sympathetic nervous system
T	Testosterone

1 GENERAL INTRODUCTION

1.1 THE PHENOMENON OF STRESS

Living organisms are in a constant struggle of adaptation with various environmental and homeostatic[1] challenges, known as stressors. Stress, in a broad sense, is the physiological and psychological adaptation to these factors. The physiological response to stress is orchestrated by the allostatic system, and affects the whole body (McEwen and Wingfield, 2003).

The first short article on stress by Selye (1936) reported structural changes in rats in response to various harmful interventions. Selye found that prolonged exposure to toxic chemicals, cold, heat, or frequent administration of electric shocks provoked the same internal alterations in the body: enlargement of the adrenal glands, shrinking of the lymph nodes, and development of gastric ulcers. It was also discovered that elevated levels of glucocorticoid (GC) secretion was associated with these changes (Selye, 1956). Selye described the so-called general adaptation syndrome (GAS) that he observed in rats in response to prolonged exposure to various stressors. The GAS consists of three phases. 1) Alarm, 2) Resistance, and 3) Exhaustion (Selye, 1956).

More recently, Selye's model has been challenged, particularly the notion that the determinants of stress are non-specific, and that the stress response is physiologically uniform. Evidence from several studies suggested that different stressors can elicit various patterns of physiological responses, moreover, stress reactivity also shows large inter-individual variation (Lovallo and Thomas, 2000; Meaney et al., 1993). Furthermore, Mason (1968) argued that in humans, the psychological factors can mediate stress responses. This concept became predominant over time, and nowadays the role of psychological factors in stress response are rarely debated (Lazarus, 1993).

Another important milestone in the history of stress research was the concept of "allostasis". McEwen advocated the use of this term instead of homeostasis to express the dynamism of the process to maintain stability through change (McEwen and Wingfield, 2003). McEwen also introduced the term 'allostatic load,' which refers to damage caused by the over-activity or under-activity of allostatic systems (McEwen, 1998).

1 Homeostasis is "the ability of an organism to maintain the internal environment of the body within limits that allow it to survive" (Fink, 2007, p. 347, vol 2))

As more and more questions were raised in the stress field, definitions became less solid. For example, stress can be defined formally as "an actual or anticipated disruption of homeostasis or an anticipated threat to well-being" (Ulrich-Lai and Herman, 2009, p. 397). However, there has never been a complete consensus among researchers about the exact definition of stress (e.g., see a detailed account in Selye, 1975). The most widely cited quote about this debate is from Selye himself, who in his later years told reporters, "everyone knows what stress is, but nobody really knows". Scientific debate did not subside over the years, making some scientists conclude that attempts to define stress are an "exercise in futility" (Levine et al., 1989, p. 341). The debate has continued to this date (c.f. the introduction chapter of the "The Handbook of Stress Science" Contrada and Baum, 2011).

1.2 THE STRESS RESPONSE

In response to an imminent or anticipated stressor, the body reacts with several changes in order to aid survival. These changes are collectively known as the stress response, and contain metabolic, autonomic and central nervous, neuroendocrine, and immune components (Chrousos, 1997). The changes are mediated by the allostatic system that has central and peripheral parts. Heart rate and blood pressure increase rapidly in response to sympathetic activation and elevated catecholamine levels. This way, glucose and oxygen can be transported faster to the muscles and to the brain, which is also aided by hemodynamic redistribution. To provide sufficient amount of energy for survival, the liver releases glucose and lipids to the bloodstream, while gluconeogenesis and lipolysis are facilitated. Breathing quickens in order to promote oxygen intake. The coagulability of the blood increases as a preventive measure against overt blood loss in case of an injury. Parts of the immune system that deal with imminent pathogens become more active. Other immune functions – such as those responsible for inflammation – are inhibited to conserve resources. Body functions that are not essential for coping with the imminent threat – such as digestion, growth, and reproduction – are subdued (Sapolsky et al., 2000).

Glucocorticoids (GCs) – the end products of the HPA axis – also affect the central nervous system, triggering mental consequences. For example vigilance increases and the attention scope narrows down in order to aid focusing on the immediate threat (Lovallo and Thomas, 2000). Moreover, enhanced memory encoding aids the individual to cope with future encounters with the stressor, and on the other hand, GCs inhibit the retrieval of memories (Wolf, 2008). Figure 1.1 shows the most important acute responses to a perceived stressor.

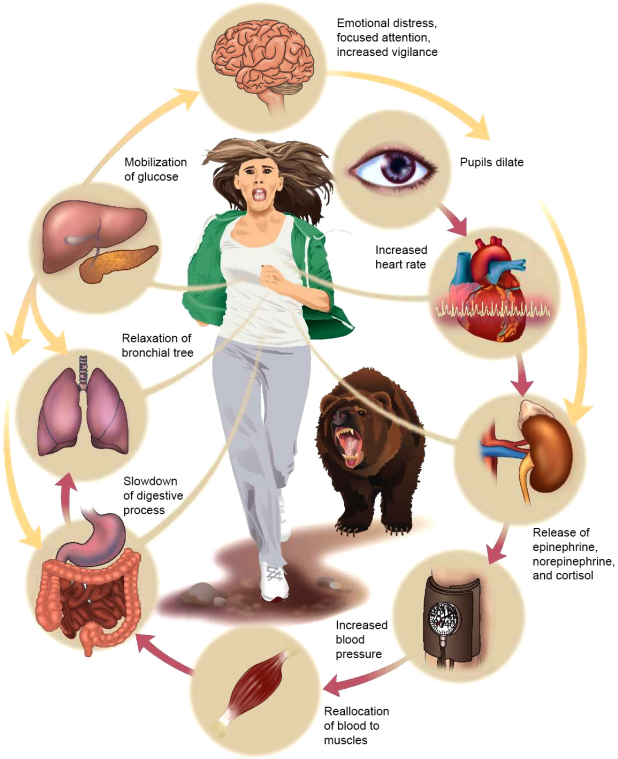

Figure 1.1: **Acute changes in the body in response to an imminent stressor.**

1.3 ALLOSTATIC SYSTEM

The allostatic system has several components, from which the HPA axis and the ANS are the most important.[2] The two systems have several overlapping functions that can strengthen the stress response, and have different response latency to stressors. The ANS response develops in seconds, but diminishes in minutes after the stressor cessation, while the HPA axis response takes $10-20$ minutes to develop from stressor onset, and one or several hours to diminish.

1.3.1 *Hypothalamus-pituitary-adrenocortical axis*

The HPA system has parts in the central nervous system as well as in peripheral organs (Chrousos, 2009). The perception of a stressor originates in the sensory systems of the brain that activate neural and neuroendocrine systems through limbic pathways (Lovallo

2 Inflammatory cytokines and metabolic hormones also play role in allostasis. As this present dissertation has a limited scope, we are unable to provide a full overview of the whole allostatic system, and only present the functioning of the ANS and the HPA axis in stress in more detail. Interested readers should refer to the review by McEwen (2003).

and Thomas, 2000). The limbic system is involved in emotional and memory functions, and participates in the evaluation of the stressor through accessing previous emotional recollections (Ulrich-Lai and Herman, 2009). The experience of stress is process through the interaction of the prefrontal cortex, the hippocampus, and the amygdala (McEwen, 2007). The stress system also receives neural input from the brainstem that sends information about potentially life threatening homeostatic events, such as perturbations in volume and electrolyte balance, blood oxygen and glucose levels. Homeostatic stressors are outside of the scope of this current dissertation – as we focus on psychological stressors – and we are not going to discuss this issue further. Interested readers should refer to the excellent review of Ulrich-Lai and Herman (2009).

As a result of perceived stress, the paraventricular nucleus of the hypothalamus releases the neuropeptides CRH (corticotropin-releasing hormone) and arginine-vasopressin (AVP). These peptides transfer to the pituitary gland to stimulate the secretion of the adrenocorticotropic hormone (ACTH)[3]. ACTH gets into the bloodstream and reaches the adrenal cortex, facilitating the production of glucocorticoids (GC), such as cortisol, which is the predominant GC in humans (Lovallo and Thomas, 2000). Figure 1.2 shows the HPA axis activity in response to a stressor.

Cortisol is a major stress and metabolic hormone in humans, and affects virtually every cell in the body. The affinity of a tissue to cortisol depends on the number of expressed intracellular corticosteroid receptors: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). As cortisol can cross the blood-brain barrier, it also affects the central nervous system. Brain regions implicated in stress response – e.g. the hippocampus, hypothalamus, amygdala, and frontal cortex – abundantly express MR and GR, thus these brain regions are especially sensitive to cortisol. This increased affinity forms the basis of a negative feedback loop that can terminate the stress response. Once the stressor have diminished, the brain regions implicated in the stress response inhibit the release of intermediary hormones – CRH and ACTH – (Lupien et al., 2009).

Apart from the negative feedback loop there are other mechanisms that modify the access of GCs in the systemic circulation to their receptors in their various target cells. For example approximately 95% of the circulating cortisol is protein bound – such as corticosteroid binding globulin and albumin – and cannot bind to corticosteroid receptors. Some target tissues have local mechanisms to release the cortisol from the binding proteins (e.g. in inflamed tissues; Bucking-

3 ACTH is produced by cleavage from pro-opio-melanocortin (POMC), which is a polypeptide and a precursor of several peptide hormones (besides ACTH, POMC is the precursor of β-endorphins that play a role in pain sensitivity, and melanocyte-stimulating hormones (MSH) that regulate appetite and sexual behavior (Chrousos, 1997).

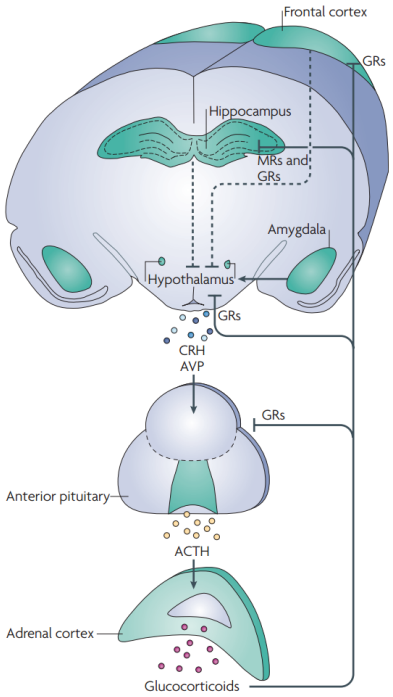

Figure 1.2: **The HPA axis** (adapted from Lupien et al., 2009)

ham, 2006). Moreover, the access of cortisol to the receptors is further regulated by 11β-hydroxysteroid dehydrogenase (HSD-11β) enzymes within the target cells. HSD-11β2 converts the biologically active cortisol to the inactive cortisone, that can no longer bind to the MR. Conversely, HSD-11β1 converts cortisone to cortisol (Buckingham, 2006).

Actions of glucocorticoids

Cortisol is not just a stress hormone, but also an important metabolic hormone that regulates energy distribution and consumption. Under natural, unstimulated conditions, the secretion of glucocorticoids follow a circadian rhythm (see Fig. 1.3). After awakening, cortisol level increases to the daily maximum, followed by declining concentrations throughout the day, and lowest levels in the late evening hours. In the afternoon, cortisol levels stay relatively stable. This rhythm is influenced by altered sleep patterns and exposure to daily life stressors (Smyth et al., 1997).

Stress induced GCs (e.g. cortisol) mediate several actions in the body to aid survival in the short term, but can be damaging if maintained (McEwen, 1998). GCs enhance cardiovascular function in acute stress partly by increasing sensitivity to catecholamines. Metabolic functions are altered to increase blood glucose concentration through several ways. Gluconeogenesis, lipolysis, and proteolysis are facilitated. At the same time GCs counteract insulin, and promote appetite and food seeking behaviors (mainly in chronic stress). GCs control

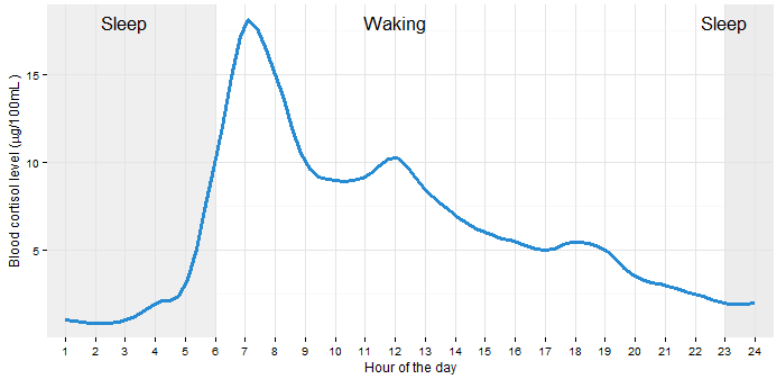

Figure 1.3: **Blood cortisol level over the day** (data source from Lovallo and Thomas, 2000)

fluid volume by suppressing local edema that occurs in response to injury. GCs alter the functioning of the immune system by increasing the activity of the faster natural immune system and decreasing the activity of the slower adaptive immune system. Immune cells are mobilized to the sites of infection or injury – if any –, and the immune response in general changes in favor of humoral immunity instead of cellular (Glaser and Kiecolt-Glaser, 2005; Segerstrom and Miller, 2004). GCs also prevent inflammation and wound healing. Through the interactions with other hormonal systems, GCs inhibit the reproductive function and growth (Lovallo and Thomas, 2000).

Cortisol also affects the CNS, and the memory and attention processes in particular. These actions are also mediated through the control over cerebral blood flow and glucose distribution (McEwen, 2003). It facilitates the consolidation, while inhibits the retrieval of declarative, long-term memories, and also impairs the functioning of the working memory (Wolf, 2008).

Recurring or lasting stressors can cause elevated GC levels that increase the allostatic load – that is the "wear and tear that results from over-activity or under-activity of allostatic systems" (McEwen, 1998, p. 171). Chronically high GC levels can cause several medical conditions that include hypertension, insulin resistance, abdominal obesity, loss of muscle and bone mass, suppression of immune responses, atrophy of brain structures like the hippocampus, and memory impairment (McEwen, 1998).

On the other hand, chronically low GC levels also have detrimental effects on the functioning of the body. Insufficient amount of GCs can cause inflammatory and autoimmune symptoms, and contribute to cytokine imbalance. It can promote the formation of chronic pain and fibromyalgia and chronic fatigue syndrome (Chrousos, 2009; Generaal et al., 2014; McEwen, 2003).

Measurement of the HPA system activity

In recent years, salivary cortisol has become the routine[4] measurement method of HPA axis activity (Hellhammer et al., 2009). Sampling saliva is non-invasive, and the amount of salivary cortisol is highly correlated with unbound – thus biologically active – serum cortisol level. Cortisol is freely transported into saliva from blood by passive diffusion, and this renders salivary cortisol to be unaffected by salivary flow rate (Bosch, 2014). This attribute makes salivary cortisol measurement even more applicable in various research settings.

Although single measurements of cortisol can be useful in acute stress studies, this method is less suitable for estimating the general state of the HPA axis. However, there are methods to assess the general reactivity of the HPA axis. Daily cortisol amount can be measured by the area under the curve method (Pruessner et al., 2003), and shows the total cortisol outflow during a day. Ideally multiple measurements (at least four) should be conducted on a number of days (Nicolson, 2008). The diurnal cortisol slope – i.e. the slope of the regression line of the daily cortisol values – has also been used as a diurnal variation of cortisol level (Lupien et al., 1996). The problem with daily cortisol output and cortisol slope is that many factors influence daily cortisol production, and these metrics can hardly reflect basal cortisol levels.

One of the most widespread measures of HPA axis function is the cortisol awakening response (CAR) (Schmidt-Reinwald et al., 1999). The CAR is operationalized as the change in cortisol levels from awakening to either a later time point (e.g., 30 or 60 minutes) or the highest value of several assessments over the first hour (Nicolson, 2008). The CAR has been suggested to serve a preparatory function to help the individual to cope with daily stressors (Fries et al., 2009). In a meta-analysis CAR was positively related to general life stress, while negatively associated with fatigue, burnout, exhaustion, and posttraumatic stress syndrome (Chida and Steptoe, 2009).

1.3.2 *Autonomic regulation of the stress response*

The autonomic response to stress was first described by Cannon (1915), who also coined the terms ”homeostasis” and ”fight-or-flight”. The ANS response is responsible for the rapid adaptation of the body to the stressor. The ANS consists of a sympathetic (SNS) and a parasympathetic (PNS) branches[5]. Simply put, the SNS is responsible for the

4 Other methods include measurement from blood, urine, and hair (Nicolson, 2008; Stalder and Kirschbaum, 2012).

5 Some consider the ”enteric nervous system” (ENS) as a third branch of the ANS. The ENS innerves the gastrointestinal tract and has its own independent reflex activity. Others consider the ENS as part of the PNS on basis of functionality (Lovallo and Sollers III, 2007). With regard to stress responses, this differentiation between the

excitatory responses, while the PNS for the energy conserving responses.

As Figure 1.4 shows, most of the organs have a dual innervation as they receive neural input from both the SNS and the PNS. In most cases, the sympathetic and parasympathetic arms of the ANS have opposite effects on the organs they innervate, and the balance of sympathetic to parasympathetic outflow determines the ultimate level of activity in the particular organ (Lovallo and Sollers III, 2007). This balance changes constantly as the environmental demands change. During states of fight or flight, this balance is predominantly sympathetic. Some stressors – e.g. those associated with passive coping – can evoke a co-activation of SNS and PNS, whereby both branches are activated (Berntson et al., 1991; Koolhaas et al., 1999). This matter of stress response specificity is discussed in detail later in this chapter.

Due to differences in triggering mechanisms, the activity of the two autonomic branches is asynchronous over the course of an acute stressor. The PNS tends to exhibit a faster off and onset than the SNS (Berntson et al., 1997, 2007; Somsen et al., 2004). For example PNS withdrawal during acute stress almost immediately restores post-stress, when sympathetic activation still lingers (see Berntson et al., 2007).

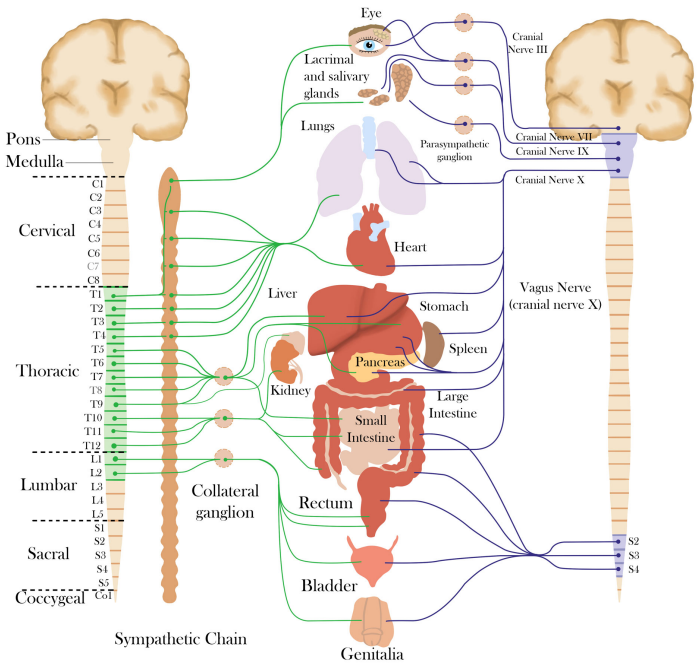

Figure 1.4: **The innervations of organs by the sympathetic and the parasympathetic branches of the autonomous nervous system** (source: anatomybodypart.com)

PNS and ENS might not be necessary, that is why this dissertation will only discuss the SNS and the PNS.

Sympathetic nervous system

As part of the stress response, projections of PVN neurons reach the brainstem, with three important destinations that modulate the ANS response (Lovallo and Thomas, 2000). 1) Through the nucleus paragigantocellularis to the locus coeruleus (LC), that sends noradrenergic fibers to the entire CNS resulting an ascending cerebral activation; 2) to the nucleus of the solitary tract (NTS), where cardiovascular sympathetic reflexes are organized; 3) to the intermediolateral cell column (ICC) of the spinal cord, which is the pathway for all sympathetic preganglionic fibers.

he ICC mediates the functions of the SNS, altering cardiac, pulmonary, hepatic, and gastrointestinal activities – among others – to prepare the body for emergency situations. As Figure 1.4 shows, neurons in the upper and middle thoracic segments of the ICC control sympathetic activity in organs in the head and thorax, while neurons in the lower thoracic and upper lumbar segments control abdominal and pelvic organs and targets in the lower extremities. In addition to nervous input, the SNS activity increases the circulating level of epinephrine (E; primarily from the adrenal medulla), and norepinephrine (NE; primarily from sympathetic nerve terminals) through facilitating the adrenal medulla (Ulrich-Lai and Herman, 2009). This mechanism is also called the sympathetic adrenal medullary system (SAM) or sympatho-adrenal system. Catecholamines do not cross the blood-brain barrier – however NE is present as a neurotransmitter in the CNS –, and only exert their effects peripherally by binding to adrenergic receptors. Many cells throughout the whole body express these receptors, and the binding of a catecholamine to the receptor will generally stimulate the sympathetic nervous system or the fight-or-flight response (Lovallo and Sollers III, 2007).

Adrenergic receptors and the actions of catecholamines

Catecholamines can produce diverse physiological effects by acting on different types of adrenergic receptors. The two main types of these receptors are alpha (α) and (β) receptors, with further sub-types (Leonard, 2003; Lovallo and Sollers III, 2007).

There are two types of α receptors. Both types are present in the brain as well as in vascular and intestinal smooth muscle. Common functions include vasoconstriction in the veins, and decreased motility of smooth muscle in the gastrointestinal tract. Moreover, α_1 receptor actions include specific vasoconstriction in the skin, kidney and brain, and specific smooth muscle contraction in the skin, hair muscles, sweat and mucous glands, and the urinary bladder. Further effects include gluconeogenesis from adipose tissue and liver. α_2 receptors cause a negative feedback on norepinephrine, inhibits insulin

and glucagon release from the pancreas, and activates platelets to aid blood coagulation.

Three subtypes of β receptors are currently known, differing in their distribution in the body. β_1 and β_2 receptors also appear in the brain, mediating general excitatory effects. β_1 receptor actions include increased cardiac output by increasing heart rate and stroke volume, facilitate renin secretion in the kidney (elevating arterial blood pressure) and ghrelin secretion from the stomach (regulating energy distribution and hunger). β_2 receptor actions include smooth muscle relaxation in the bronchi of the lung to increase oxygen input, and smooth muscles of the gastrointestinal tract to inhibiting motility, it dilates arteries in the skeletal muscle to redirect blood flow. Further actions include facilitation of lipolysis and gluconeogenesis, increase in renin secretion in the kidneys, protein secretion in the salivary glands (e.g. alpha-amylase), while inhibition of insulin secretion in the pancreas and histamine secretion in mast cells. β_3 receptor actions include enhanced lipolysis in the brown adipose tissue and relaxation of the smooth muscles of the bladder.

Parasympathetic nervous system

The PNS also reacts to stressors. As discussed previously, in most cases the activity of the PNS decreases during acute stress, however some stressors facilitate both the SNS and PNS (Bosch et al., 2001, 2003). The parasympathetic preganglionic fibers exit the central nervous system either from the pons and medulla as cranial nerves or from the sacral level of the spinal cord. The cranial nerves – specifically cranial nerves III, VII, IX, and X – innervate the head, neck, cardiovascular system, and gut. The lower preganglionic fibers exit the sacral segments of the spinal column, and innervate the intestines, the bladder, and the genitalia. PNS nerves travel longer distances to their ganglia, located near the target organs, while the postganglionic fibers then travel short distances to their target tissues (see Figure 1.4).

The PNS uses acetylcholine (ACh) as its neurotransmitter that acts on two types of receptors, the muscarinic and nicotinic cholinergic receptors. When stimulated, the preganglionic neuron releases ACh, which acts on nicotinic receptors of postganglionic neurons. The postganglionic neuron then releases ACh to stimulate the muscarinic receptors of the target organ (Leonard, 2003). The PNS exercises control over the organs exclusively through nervous input contrary to the SNS that also has an endocrine component through the SAM system's catecholamine secretion. That is why, the neurotransmitters for the PNS are less important to understand the stress response.

Measuring the ANS response to stress

As most of the organs receive neural input from both the SNS and PNS, the psychophysiological measurement of stressful experiences is not always clear-cut. For example the heart rate is affected by both arms of the ANS, and also has its own pacemaker. In the following section, we present the most widely used measurement methods of ANS activity.

Blood E and NE levels: Obviously, the most straightforward metric of SNS is the assessment of catecholamines. However catecholamines cannot be easily measured from saliva, thus researchers prefer proxy measures of sympathetic activation that can be assessed using non-invasive methods.

Blood pressure: Blood pressure (BP) is controlled by the SNS and is widely used as a sympathetic marker. Both systolic and diastolic BP increases in response to sympathetic activation, which is mainly driven by α adrenergic actions (Reid, 1986).

Electrodermal reponse: Electrodermal reponse, or skin conductibility has been a frequently used measure of SNS activity, as the opening of pores of the skin that secrete sweat are innervated by the sympathetic sudomotor nerves. The firings of sudomotor nerves correspond to observable increases in skin conductance (Bach et al., 2010). Skin conductibility can be decomposed to phasic and tonic components, that show sudden excitatory effects, and general arousal, respectively (Benedek and Kaernbach, 2010).

Cardiac measures: Among the cardiac measures, there are few that are considered as purely sympathetic markers. The time interval from the beginning of electrical stimulation of the ventricles to the opening of the aortic valve – also known as the pre-ejection period (PEP) – is regarded as such. PEP is inversely related to myocardial contractility, and is interpreted as β adrenergic influences on the heart (Sherwood et al., 1990).

Salivary alpha-amylase: Lately, salivary alpha-amylase (sAA) has gained rapid popularity as a noninvasive marker of sympathetic nervous system (SNS) activity, because its salivary concentration rapidly increases during acute stress (Nater and Rohleder, 2009). However there are concerns about the interpretations of sAA as a purely sympathetic marker, and there are also a number of methodological issues regarding the assessment (Bosch et al., 2011). The second chapter of this dissertation deals with the interpretative and methodological caveats regarding this marker in length.

Vagal tone: The normal rhythm of the heart is controlled by the cardiac sinus node, which receives neural input from both PNS (vagal nerve) and SNS – that decrease and increase heart rate, respectively –, and is also influenced by circulating catecholamines and GCs. The activity of the PNS is often measured by estimating the vagal tone, as the vagus nerve innerves the majority of the body's internal organs.

The vagal tone cannot be measured directly, but the phenomenon of respiratory sinus arrhythmia (RSA)[6] makes it possible to approximate it. The slower dynamics of SNS actions at the sinus node limit sympathetic contributions to respiratory modulations of HRV, thus high frequency (0.15 Hz to 0.4 Hz) changes in heart rate are attributed to the PNS (Berntson et al., 1997). An increase in vagal tone both slows the heart and makes heart rate more variable. There are several methods for measuring heart rate variability (HRV), and time domain and frequency domain metrics exist. The most frequently used HRV measures are the root mean square of successive differences (rMSSD) and high frequency (HF) changes in heart rate (Task Force et al., 1996).

1.3.3 *Interplay between the HPA axis and other hormonal systems*

The stress system is highly interconnected with other hormonal systems. The inhibition of reproduction and growth axes serves energy conserving purposes during stress. The changes in the immune system fine tune the body's defense systems to be able to deal with imminent stressors instead of longer term protective effects. The connections of the HPA axis and other neuroendocrine systems are discussed briefly below, and shown in Figure 1.5. For a more comprehensive overview, see the review by Chrousos (1997).

The reproductive axis is inhibited at all levels by various components of the HPA axis (see Figure 1.5, panel A). CRH suppresses the production of gonadotropin-releasing hormone (GnRH) production in the hypothalamus both directly and indirectly (through facilitating β-endorphin production). GCs also inhibit GnRH production, and also affects the pituitary gland by suppressing the luteinizing hormone, and follicle-stimulating hormone production in the pituitary, and renders the gonads less sensitive to these latter hormones, thus preventing the secretion of sex steroids (Chrousos, 1997).

Prolonged HPA axis activation leads to the suppression of thyroid and growth axes (see Figure 1.5, panel B). That is why children with chronic stress often do not reach their final growth potential (Chrousos and Gold, 1992). CRH indirectly inhibits growth hormone and thyroid-stimulating hormone production, while GCs suppress them directly, and also interferes with hormonal actions in the target tissues. Besides facilitating growth and playing role in metabolism, the growth hormone also helps to maintain competence of the immune cells (Glaser and Kiecolt-Glaser, 2005).

The most widely researched system that interacts with the HPA axis is the immune system. It has long been discovered that long term

6 RSA optimizes distribution of oxygen through coordinating the pulmonary and circulatory systems. During inhalation, vagal activity is temporarily suppressed causing an immediate increase in heart rate, while during exhalation heart rate decreases as vagal activity resumes (Berntson et al., 1993).

stress can impair health, and subdue immune functioning (McEwen, 1998). Although the effects of stress are more complex than just general suppression, and numerous immune functions are actually enhanced in response to acute stress (Segerstrom and Miller, 2004). During acute stress, both SNS and HPA systems are activated and they both alter immune functioning (see Figure 1.5, panel C). The SNS facilitates several immune components by endocrine (through the SAM) pathways, and direct nerve connections to the lymph nodes (Glaser and Kiecolt-Glaser, 2005). On the other hand, the HPA system – GCs in particular – suppresses leukocyte traffic and effectiveness, proinflammatory cytokine production, and inhibits inflammatory effects in the target tissues. Various immune components – such as cytokines, like interleukin 1, IL-1β – facilitate both the SAM axis, and CRH production. A more comprehensive summary of the interplay between the stress and the immune systems is presented by Glaser and Kiecolt-Glaser (2005).

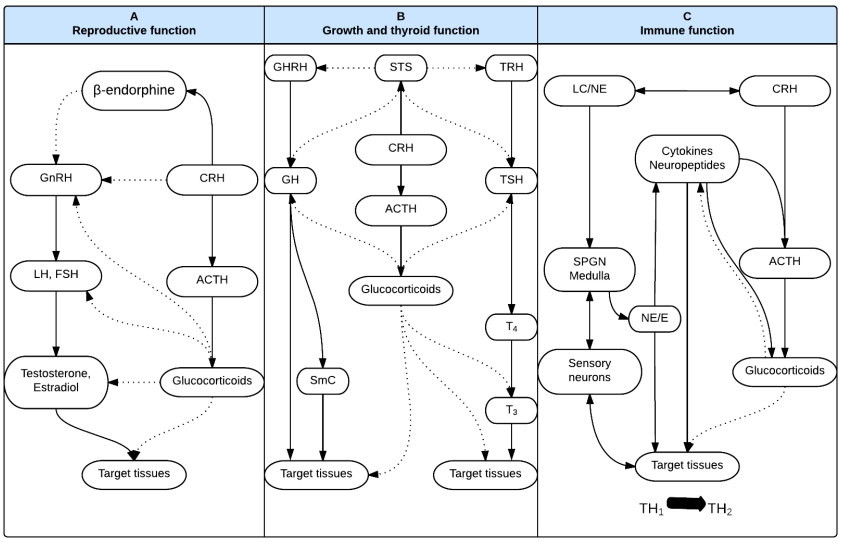

Figure 1.5: **Simplified representation of the interactions of the HPA axis and other neuroendocrine systems.** Solid lines represent facilitatory, while dotted lines represent inhibitory effects. Abbreviations: GnRH: gonadotropin-releasing hormone, CRH: corticotropin releasing hormone, LS: luteinizing hormone, FSH: follicle-stimulating hormone, GHRH: growth-hormone-releasing hormone, STS: somatostatin, TRH: thyrotropin releasing hormone, GH: growth hormone, TSH: thyroid-stimulating hormone, T4: thyroxine, T3: triiodothyronine, SmC: somatomedin C, LC/NE: Locus coeruleus, SPGN: sympathetic postganglionic neurons, NE/E: norepinephrine/epinephrine, TH: T-helper. For details, see text. (modified from Chrousos and Gold, 1992; Chrousos, 1997).

1.3.4 *Individual differences in stress reactivity*

Although everybody experiences stress in life, stress reactions can differ across individuals even if the stressor is the same. Extensive research has been conducted to discover the sources of this variation, and both environmental and genetic factors have been identified. Among the environmental factors, early life experiences and parental care proved to be the most important. Animal models demonstrated that parental care can affect the development of neural systems that mediate stress reactivity. Increased level of hypothalamic and amygdaloid CRF gene expression, decreased capacity of hippocampal GC receptor system (that normally down-regulates stress response), and hippocampal underdevelopment were seen as a result of stressful environmental factors in early life (see Meaney, 2001; Meaney et al., 1993). It is suggested that these changes serve a preparatory role to cope with environmental demands, thus ultimately aiding survival as dangerous surroundings require fast responses and constant vigilance. Early life hardships can alter physiological stress reactivity in humans, and this might contribute to the formation of psychiatric conditions. Several human studies have shown the association of childhood adversities and susceptibility to various stress-related mental disorders, such as anxiety and mood disorders (McEwen, 2007; Sapolsky, 1994).

The genetic basis of stress reactivity and resiliency has been a hot topic in recent years. Multiple polymorphisms have been discovered as potential mediators between stressors and stress reactivity including HPA axis related genes, serotonin transporter genes, and genes implicated in catecholamine metabolism (for an overview see Feder et al., 2009). Dopamine neurotransmission plays a key role in the regulation of neural circuits supporting cognitive and affective behavioral processes. By augmenting excitatory sensory input and attenuating inhibitory prefrontal input to the amygdala and hippocampus, dopamine activity can alter affective responses and emotional regulation in stress (Drabant et al., 2006). Genetic polymorphisms that impact dopamine activity can explain biological mechanisms of individual differences in stress reactivity. In this regard, the polymorphism of the catechol-O-methyltransferase (COMT) is one of the most widely studied and relatively frequent polymorphism (Axelrod and Tomchick, 1958). COMT is responsible for degrading catecholamines – dopamine and norepinephrine in particular – in the post-synaptic neurons. COMT-induced dopamine degradation is of particular importance in brain regions with low expression of the presynaptic dopamine transporter, such as the prefrontal cortex where COMT clears out sixty percent of the dopamine. If dopamine is not degraded quickly, neurons can remain excited for a longer time.

COMT can be built either of valine or methionine amino acids, depending on a common functional polymorphism (val^{158}met). The variant with valine degrade dopamine four times faster than that of methionine (Axelrod and Tomchick, 1958). Approximately 50% of humans have a combination of both slow and fast enzymes (val/met); 25% have only fast enzymes (val/val); and 25% have only slow enzymes(met/met) (DeMille et al., 2002). Individuals with only val phenotype were nicknamed "warriors", while individuals with only met phenotype were named "worriers" (Goldman et al., 2005). "Warriors" have increased COMT activity and lower prefrontal extracellular dopamine compared to "worriers". The val^{158}met polymorphism is associated with several cognitive-affective phenomena, and even differences in personality. It affects prefrontal function and working memory capacity and has also been associated with anxiety and emotional dysregulation. Further, "warriors" can handle stress better than "worriers", because the latter group of people can get over excited and overwhelmed by potent stressors. On the other hand, "warriors" need stress for optimal mental functioning, and might not perform well in under-stressed circumstances (Drabant et al., 2006; He et al., 2012).

The Yerkes-Dodson Law suggests that performance ability is in an inverted u-shaped relationship with arousal. In other words, too low or too high arousal can lead to underachievement, while the optimal level of arousal can contribute to good performance (Yerkes and Dodson, 1908). As discussed earlier in this section, there can be substantial individual differences in the optimal level of arousal, and these differences can be rooted in genetic factors or personal life experiences. As arousal is closely related to stress, it was suggested that the Yerkes-Dodson Law applies to stress as well, i.e. stress and performance are related in a similar, inverted u-shaped way (Chrousos, 1997). Accordingly, GCs levels and cognitive performance were reported to be in an inverted u-shaped relationship (Lupien et al., 2007). Moreover, a recent study reported an inverted u-shaped relationship between optimal performance ability and physiological arousal and cortisol level during a performance task (Peifer et al., 2014).

1.3.5 *Stressor specific responses*

Although Selye originally defined stress response as a general adaptation pattern, it became clear over time that not all stressors elicit the same physiological reaction. For example different laboratory stressors can elicit dissimilar ANS response patterns. Tasks that require active coping – such as arithmetic tests, memory search tasks, public speaking, etc. – regularly provoke a classical fight-or-flight response, meaning increased SNS and decreased PNS activation. In contrast, passive laboratory tasks – e.g. watching a gruesome surgical video or enduring pain – can elicit a sympathetic and parasympathetic co-

activation (Bosch et al., 2001, 2003). This latter response pattern is called "aversive vigilance", and can aid survival in dangerous situations where neither fighting nor escaping is possible temporarily. The PNS can be modulated faster than the SNS, and the quick release of vagal tone can almost immediately shift the body from aversive vigilance to flight-or-flight state.

Furthermore, previous studies showed that mental stress is a potent stimulator of epinephrine output, whereas norepinephrine is more closely associated with physical activity (Lovallo and Thomas, 2000). Catecholamine production in general was shown to reflect the intensity of stress rather than its emotional valence. Pleasant stimulation, such as watching a funny movie, induced elevated epinephrine levels as well as did an unpleasant stimulation – films that elicited fear and anger (Levi, 1972).

While the response specificity of catecholamines has not always been consistent in studies, the production of GCs have been rather reliably associated to negative emotions and distress (Denson et al., 2009; Dickerson and Kemeny, 2004). A study found that the same reaction time task can provoke cortisol increase when participants were punished for bad performance, while cortisol level did not change when participants were rewarded for good performance (Lovallo and Pincomb, 1990). In another research, thirty participants were subjected to three different 30-minute mood inductions on separate days: a) humorous video, b) public speaking task, and c) rest. Although both the video and speaking task caused psychological arousal, cortisol levels were only elevated in response to public speaking, whereby negative affect was also elevated (Buchanan et al., 1999). Other studies showed that a task perceived as a challenge – rather than a threat – only increase catecholamine output, and not cortisol (Frankenhaeuser et al., 1980). Frankenhaeuser showed that this physiological pattern consistently emerges in response to tasks of "effort without distress" (Frankenhaeuser, 1986). The meta-analysis of Dickerson and Kemeny (2004) summarized the findings of 208 human acute stress experiments, and concluded that performance tasks that induced novelty, uncontrollability, or social threat elicited the largest cortisol responses. A further meta-analysis estimated the role of specific affective states in cortisol responses of to various laboratory stressors. The analysis confirmed that cortisol increases related to negative emotional states; tasks without negative emotions elicited no significant cortisol response (Denson et al., 2009). This observation means that eustress – or positive stress – elicits a different physiological reaction than distress.

Not just negative emotions, but recently positive emotions have been proposed to influence stress responses (Dockray and Steptoe, 2010). However, experimental evidence about the effects of positive emotions on stress hormones is rather scarce. One experimental study

showed that women with higher positive emotional style showed a faster blood pressure recovery and smaller cortisol responses to acute laboratory stressors (Bostock et al., 2011). A cross-sectional study showed associations between positive emotions and decreased cortisol levels in middle aged men and women (Steptoe et al., 2005). Another study with pregnant women showed that positive life events predicted lower baseline awakening cortisol levels, independently of negative life events (Pluess et al., 2012). The mechanisms that mediate between positive affect and mitigated stress hormone output are largely unknown.

A particularly neglected area of stress research is the investigation of eustress (or positive stress). Selye suggested that eustress is similar to distress, but it is associated with positive emotions, instead of negative ones (Selye, 1976). As Selye believed that stress responses are uniform, he thought that eustress elicits similar psychophysiological reactions to distress (Selye, 1975). This assumption however has never been tested. To this day, there are virtually no studies deliberately investigating the physiological correlates of eustressors. Nevertheless some studies in emotion research might be considered as such, particularly the investigation of psychophysiological correlates of "flow experience". Flow is considered to be a mental state where individuals are completely immersed in a high performance, joyful activity, constantly maintaining an energized focus (Csikszentmihalyi, 1990). A study that examined flow in professional piano players found that flow is associated with increased SNS and decreased PNS activation, similarly to active coping (de Manzano et al., 2010). Another study using an experimental video gaming paradigm reported decreased PNS activity, and showed that cortisol was elevated in the flow inducing task compared to a too easy (and boring) task (Keller et al., 2011). However, the authors noted that the experimental task did not elicit positive emotions, therefore it is difficult to interpret it as an eustressor. Another studies found that sympathetic arousal and production of cortisol are in an inverted U shape relationship with flow, i.e. the formation of flow experience requires some, but not too much sympathetic or HPA activity (Peifer et al., 2014). The findings of flow research can certainly contribute to the investigation of eustress, but more research is clearly needed in this area.

1.4 PSYCHOLOGY OF STRESS

As one of the first researchers to emphasize the role of psychological factors in the stress response, Mason (1968) reported that cognitive-emotional influences are among the most potent natural stimuli known to affect the HPA axis. Mason's work showed the importance of situational characteristics, such as novelty, unpredictability, and uncontrollability in activating the stress system. According to this obser-

vation, numerous, purely psychological stressors were shown to elicit stress response. For example, the HPA axis is activated in response to natural stressors in life such as bereavement (Irwin et al., 1988), academic examination stress (Malarkey et al., 1995), public speaking (Bassett et al., 1987), and anticipation of a competitive event (Alix-Sy et al., 2008). Psychological stress tasks in laboratory research have also been found to stimulate the HPA axis. Cognitive tasks, public speaking or verbal interactions were associated with increased cortisol levels (Dickerson and Kemeny, 2004). Moreover, cortisol responses were associated with psychological states and feelings. Challenge, novelty, threat, brooding, submission, and perception of social threat were all significant predictors of cortisol increase in acute stress tasks (Denson et al., 2009).

Psychological stressors have been sorted into three broad categories. Harm refers to psychological damage that had already been done (e.g. loss of a beloved, failure, etc.). Threat is the anticipation of harm that has not happened yet. Challenge results from difficult demands that can be possibly overcame (Lazarus, 1993).

1.4.1 *Social threat as a stressor*

Group membership has become a key to survival for social animals, and this could be the reason that humans are exceedingly sensitive to social stressors (Eisenberger and Lieberman, 2004). It has been recognized that the perception of threat extends not only to physical, but also to the social self (e.g. Rohleder et al., 2007). The social self is formed by constant social comparison, and individuals inherently compare themselves to their peers (Festinger, 1954). It appears that the social evaluation is often perceived as a threat, and threats to the social self can provoke marked stress responses (Gruenewald et al., 2004). Several studies have shown that social threat is among the most potent stressors (Dickerson and Kemeny, 2004; Mason, 1968). For example, in a research women had to give speeches alone, or either in front of a single person or an audience of four people. Participants who performed the task alone did not produce a significant stress response, but those who spoke in front of four people showed increased cortisol level and fight-or-flight reaction. Psychological distress and physiological stress response were correlated with the size of the audience (Bosch et al., 2009).

1.4.2 *Competition as a stressor*

A special case of stress is competitive stress. The competitive context incorporates several elements that had been previously identified as stressors: anticipation, social evaluation, performance pressure, and eventually, the adversaries also have to deal with the out-

come of the competition (Alix-Sy et al., 2008; Fülöp, 2009; Tauer and Harackiewicz, 2004). As some of the studies in this dissertation deal with competitive contexts, we briefly summarize how competition can elicit stress responses.

The most evident field of competition is sports, and many studies have examined the psychophysiological effects of sport competitions. For example a study showed that a marathon running competition increased cortisol levels more than a non-competitive event with comparable physical strain (Cook et al., 1987). Another study examined competitive dancers, who showed higher levels of cortisol on the competition day, compared to a training day. What is more, cortisol response to the competition was much larger than the response to a potent laboratory stressor (Rohleder et al., 2007).

Physiological responses to sport competitions can be confounded by the effects of physical exercise – that also increases cortisol level (Skoluda et al., 2015). That is why attempts have been made to examine competitions without exercise. Studies that used sedentary competitions – competitive attention task, toy car racing, chess, video games, etc. – also showed increased cardiovascular responses, compared to individual gameplay (Harrison et al., 2001; Ricarte et al., 2001; Veldhuijzen Van Zanten et al., 2002). However, these competitions did not consistently elicit HPA response. Losers often experience negative emotions (Fülöp, 2009), which can contribute to physiological stress responses. Accordingly, some studies reported dissimilar HPA response for different competitive outcomes, with the losers showing elevated cortisol levels compared to the winners (e.g. Costa and Salvador, 2012; Filaire et al., 2009).

However, response differences between winners and losers do not necessarily mean these are caused by victory or defeat. Salvador and Costa (2009) proposed a psychophysiological model of coping with competition (see Fig. 1.6). They proposed that appraisal of the situation creates either an active (proactive) or passive (reactive) coping response. Active coping more likely leads to victory, and passive to defeat. Therefore different response patterns might not be the causes, but the effects of winning and losing (Salvador, 2005). As part of this response pattern, testosterone increases during active coping – thus improving performance –, and decreases in passive. This difference can play a crucial role in competition, because testosterone can facilitate competitive performance (Archer, 2006; Salvador and Costa, 2009). Also, the PNS activation in passive coping can reduce cardiac output, which may impair performance (Koolhaas et al., 1999).

A recent theory proposes that in some special cases, losing can also lead to elevated testosterone levels (Zilioli et al., 2014). This can happen when the competition takes place in an unstable status hierarchy – for example when opponents regard each other as equals or the competition is even. Individual differences in the perception of status

instability can play a pivotal role in this process. It has been shown that power motivation, as a trait characteristic, can alter the response to competitive challenge. Those who rank high on power motivation scale are more determined to win, and they often show testosterone increases after losing (Schultheiss and Rohde, 2002; Schultheiss et al., 2005; Stanton and Schultheiss, 2009; Wirth et al., 2006). This observation is in line with the previously presented model of coping with competition. More detailed explanation of the psychobiological responses to competition – and especially the role of testosterone – can be found in a later chapter.

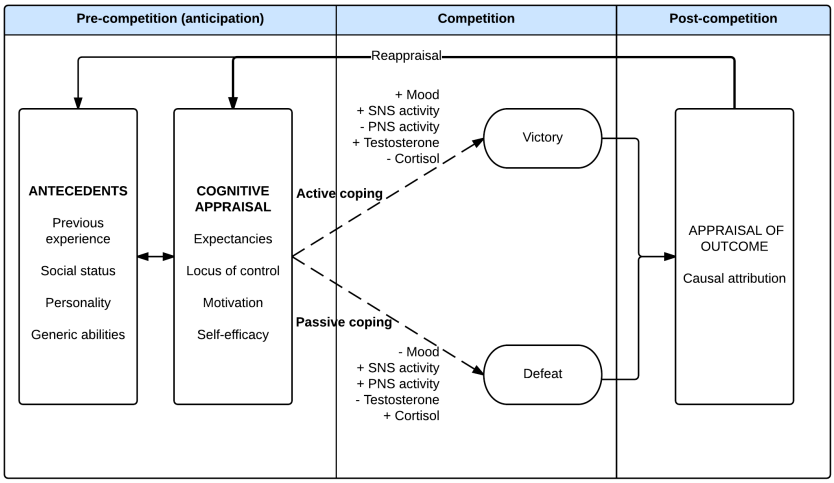

Figure 1.6: **A model of coping with competition**. For explanation, see text (modified from Salvador and Costa, 2009)

1.4.3 *Coping with stress*

The psychological models of coping have been extensively studied. One of the most popular models of coping – called the transactional model – originates from Lazarus and Folkman (1984). According to this model, stressors are evaluated as part of a two-step appraisal process. In the first step it is assessed whether the stressor is potentially harmful; in the second step, it is estimated if the person's capabilities are adequate to cope with the threat. Therefore the psychological stress is the imbalance of environmental demands and coping abilities, and previous experience and competence can be as important as the stressor itself in the formation of stress (see Figure1.7).

Studies identified numerous coping strategies, and attempts have been made to establish the main categories of coping behavior (Carver and Connor-Smith, 2010). One of the first distinctions had been made between problem-focused and emotion-focused coping. Problem-focused coping is directed at the stressor itself: taking action to elim-

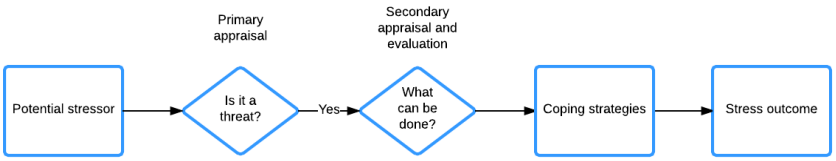

Figure 1.7: **Transactional model of stress and coping** (based on Lazarus and Folkman, 1984)

inate or to avoid it. Contrarily, emotion-focused coping is aimed at minimizing emotional distress triggered by stressors (Lazarus and Folkman, 1984). Others distinguish between engagement and approach coping. Engagement coping is aimed at dealing with the stressor, and disengagement coping is aimed at escaping the threat using avoidance, denial, and wishful thinking (Skinner et al., 2003). Within engagement coping, differences have been made between attempts to control the stressor itself (primary- control coping), and attempts to adapt to the stressor (accommodative coping). This latter approach includes strategies such as acceptance, cognitive restructuring, and goal adjustment. Moreover, proactive coping refers to the strategy that prevents threatening and harmful events to unfold before they happen (Carver and Connor-Smith, 2010).

Individual differences in stress resiliency has been long evident, however several views exist about the sources of this variability. Theories suggest that stress resiliency is a trait-like feature, although it might be developed. Among others hope (Snyder et al., 1991), optimism (Scheier and Carver, 1987), hardiness (Kobasa, 1979), constructive thinking (Epstein and Meier, 1989), learned resourcefulness (Rosenbaum, 1989), self-efficacy (Bandura, 1982), and sense of coherence (Antonovsky, 1993) were named as the most important factors in stress resiliency. Efforts have been made to integrate factors of stress resiliency into a single model. For example Oláh (2005) proposed that a "psychological immune system" can help coping through three subsystems: 1) approaching, monitoring; 2) mobilizing, creating, executing; 3) self-regulatory.

1.5 OVERVIEW OF THE DISSERTATION

The present dissertation consists of six sections. The four central chapters present different studies, while the general introduction and discussion chapters provide a background and a summary. The dissertation is centered on the concept of stress, and in particular the phenomenon of stress response specificity and individual response stereotypy (Stern and Sison, 1990). In other words, the dissertation investigates how different stressors can elicit different physiological

responses, and how individual characteristics affect the way we respond to stressors.

The four central chapters of the dissertation contain two experimental studies, a meta-analysis, and a cross-sectional study. Despite the topics of these chapters are not being closely related to each other, they share several characteristics. All of these studies use biological stress markers and psychophysiological methods to investigate the stress response, thus mapping the interconnections between biological and psychological factors in stress. The second, third, and fourth chapters investigate stressors that differ in the way they activate the stress system, i.e. showing stress response specificity. Moreover, these studies all deal with acute stress. The third and fifth chapters both deal with individual differences in stress responses.

The second chapter presents how two common acute laboratory stressors – the cold pressor task and memory-search task – affect the autonomic nervous system and glandular secretion of salivary alpha-amylase. This enzyme is considered a promising candidate of sympathetic nervous system activity, and therefore receives a lot of attention in stress research nowadays.

The third chapter investigates how a challenging laboratory competition affects the autonomic nervous system, the stress system and the adrenal-pituitary-gonadal system that has a well-known role in competitive behavior. We furthermore investigated how competitive attitudes can be related to testosterone responses and performance.

The fourth chapter is a meta-analysis about acute cortisol responses to a popular eustressor: video gaming. As video gaming is sometimes regarded as stressful experience and researchers has been using them occasionally as acute stressors in studies, a meta-analysis was long overdue to clarify if video games can elicit stress responses.

The fifth chapter presents findings about how the functioning of the HPA system – as indexed by the cortisol awakening response – can be associated with recurring nightmares. This study is the most different from the others as it measures longer term effects, uses a cross-sectional design, and a non-laboratory ecological setting. Nevertheless, this study can bring a broader understanding about how the stress system can be associated with psychological processes.

The last chapter summarizes results and draws conclusions about the findings.

2

A FLUID RESPONSE: ALPHA-AMYLASE REACTIONS TO ACUTE LABORATORY STRESS ARE RELATED TO SAMPLE TIMING AND SALIVA FLOW RATE

Background: Salivary alpha-amylase (sAA) is used as a sympathetic (SNS) stress marker, though its release is likely co-determined by SNS and parasympathetic (PNS) activation. The SNS and PNS show asynchronous changes during acute stressors, and sAA responses may thus vary with sample timing. **Method**: Thirty-four participants underwent an 8-minute memory task (MT) and cold pressor task (CPT). Cardiovascular SNS (pre-ejection period, blood pressure) and PNS (heart rate variability) activity was monitored continuously. Unstimulated saliva was collected repeatedly during and after each laboratory stressor. **Results**: Both stressors increased anxiety. The MT caused an immediate and continued cardiac SNS activation, but sAA concentration increased at task cessation only (+54%); i.e., when there was SNS-PNS co-activation. During MT sAA secretion even decreased (−35%). CPT robustly increased blood pressure but not sAA. **Discussion**: In summary, sAA fluctuations did not parallel changes in cardiac SNS activity or anxiety. sAA responses seem contingent on sample timing, likely involving both SNS and PNS influences. Verification in other stressors and contexts seems warranted.[1]

2.1 INTRODUCTION

The discovery that the adrenal stress hormone cortisol can be measured reliably and non-invasively from saliva was a methodological breakthrough in stress research, and much effort has since been dedicated to determine if the assessment of other neuro-endocrine markers may benefit from the ease of saliva collection. As a promising candidate, salivary alpha-amylase (sAA) has gained rapid popularity as a noninvasive marker of sympathetic nervous system (SNS) activity (Granger et al., 2007; Nater and Rohleder, 2009). sAA is a digestive enzyme that breaks down starch into glucose and maltose, and enzymatic activity (in Units/ml) is used as a proxy for sAA concentration[2]. The use of of sAA as a marker of SNS activity seems justified: sAA release from the salivary glands is under strong control of local sympathetic nerves (Proctor and Carpenter, 2007), its salivary concentra-

1 This chapter was published previously as: Nagy, T., van Lien, R., Willemsen, G., Proctor, G.,Efting, M., Fülöp, M., Bárdos, G., Veerman, E. C. I., Bosch, J. A. (2015). A fluid response: alpha-amylase reactions to acute laboratory stress are related to sample timing and saliva flow rate. *Biological Psychology.* doi:10.1016/j.biopsycho.2015.04.012

2 sAA concentration is derived from the amount of enzyme that catalyzes the conversion of 1µmol of substrate (i.e., starch) per minute.

tion rapidly increases during acute stress, and its use as a marker of sympathetic activation is also validated by pharmacological studies (Bosch et al., 1998, 2003; Ehlert et al., 2006; van Stegeren et al., 2006, 2008).

Whereas it is undisputed that sAA release is under sympathetic control, the inference that increases in sAA *therefore* signify sympathetic activation is nonetheless problematic. The inference is logically flawed (i.e., affirming the consequent), and there are also strong empirical arguments to question this inference (Bosch et al., 2011). Most of these arguments center around the fact that the parasympathetic nerves also play a significant role in sAA release. For example, several sAA-rich saliva glands, like the sublingual and minor glands, are almost exclusively under parasympathetic nervous system (PNS) control (Bosch et al., 2011). Further, experimental studies show that the sympathetic effects on sAA release are strongly moderated by concurrent PNS activity, a phenomenon denoted as 'augmented secretion' (Proctor and Carpenter, 2007).

In order to better understand the differential contribution of the PNS and SNS to sAA responses during stress, we have previously compared sAA secretion in response to stressors that elicit distinct patterns of autonomic activity (Bosch et al., 2003). It was found that a stressor eliciting sympathetic-parasympathetic co-activation (i.e., viewing a surgical video) caused a marked sAA release (+65%), whereas a cognitive stressor causing a sympathetic activation in conjunction with parasympathetic inhibition (i.e., a memory search task) showed no significant change in sAA release (+10%). Importantly, the latter stressor caused a much stronger sympathetic activation (as measured by cardiac PEP, LVET, and blood pressure responses) than the stressful video (Bosch et al., 2003). These findings therefore are inconsistent with the idea that sAA reliably represents SNS activity, and consistent with a moderating effect of parasympathetic activity (Berntson et al., 1991; Proctor and Carpenter, 2007).

On the basis that sAA release is orchestrated by joint activity of the two autonomic branches, we predicted that sample timing may be critical to the observed sAA responses during stress. This prediction builds on knowledge that activity in the autonomic branches is asynchronous over the course of an acute stressor, whereby the PNS tends to exhibit a faster off and onset than the SNS (Berntson et al., 1997, 2007; Somsen et al., 2004). Studies have shown, for example, that the PNS withdrawal during acute stress rapidly restores immediately post-stress, at which time sympathetic activation still lingers (Berntson et al., 2007). Some have even reported a parasympathetic rebound immediate post-stress, whereby PNS activity overshoots baseline levels, causing a transient sympathetic-parasympathetic co-activation (Mezzacappa et al., 2001; Rottenberg et al., 2003). Hence, we predicted that the largest sAA increase will be observed immediately

post stress, when the PNS will have little effect or possibly even an augmenting effect on sAA, and we further predicted that the smallest sAA changes will be observed during stress, when SNS effects on sAA may be attenuated by a PNS withdrawal. It is noteworthy that nearly all published studies have only sampled sAA at stressor termination, and the study by Bosch et al. (2003) – which found no effect of a cognitive stressor on sAA release – collected saliva *during* the stressor.

The present study had one further aim: to address the role of salivary flow rate as a factor relevant to sAA studies. The use of sAA as a SNS marker is based on the fact that sAA secretion (U/min) is under SNS control. However, most stress studies have instead measured sAA concentration (U/ml) (Bosch et al., 2011). The implicit assumption that these two parameters yield identical results has remained largely untested (Beltzer et al., 2010; Proctor and Carpenter, 2001; Nater and Rohleder, 2009). As shown in the formula below[3], saliva flow rate (ml/min) is the sole determinant of the relation between sAA secretion and concentration, and flow rate is almost exclusively under parasympathetic control (Garrett, 1987; Proctor and Carpenter, 2007). Accordingly, sAA concentration may provide an overestimation of sAA secretion when salivary flow rate decreases – reflecting reduced PNS activation of the saliva glands – but may provide an underestimation when saliva flow rate increases. This aspect of glandular physiology may also have clear implications to sample timing: during acute stress, when PNS activity shows a strong withdrawal, also the largest effects on flow rate may be anticipated and hereby the largest discrepancy between sAA concentration and secretion (Bosch et al., 2002, 2011).

In light of the preceding discussion, the present study examined the temporal dynamics of sAA during two acute laboratory stressors known to elicit distinct autonomic nervous system responses: i.e., a memory-search task (MT) and a cold pressor task (CPT) (Bosch et al., 2001, 2003; Willemsen et al., 1998, 2002). The MT elicits a prototypical 'fight or flight' cardiac autonomic response pattern, characterized by a vagal withdrawal and enhanced sympathetic drive. In contrast, the CPT primarily elicits a localized vascular sympathetic activation characterized by a robust blood pressure response, but elicits little cardiac autonomic changes (Allen et al., 1992; Willemsen et al., 1998, 2002; Winzer et al., 1999; Ring et al., 2000). We anticipated the largest sAA increase at stressor off-set, when autonomic balance is shifted towards SNS-PNS co-activation, and we expected the smallest sAA changes during the stressor, when parasympathetic withdrawal may attenuate sympathetic effects on sAA secretion. We expected sAA during CPT to increase in parallel with pain, anxiety and pressor responses. Autonomic responses during CPT have rarely been deter-

3 sAA secretion (U/min) = sAA concentration (U/ml) × salivary flow rate (ml/min).

mined beyond three minutes (Mourot et al., 2009) and this is the first study to investigate the temporal dynamics of sAA release during CPT. Correlational analyses were performed to explore associations between glandular responses and cardiovascular autonomic indices.

2.2 METHOD

2.2.1 *Participants*

Thirty-four university undergraduates (of which 18 were males) volunteered to take part in the study (Mean age $= 22.1$, SD $= 3.2$; Mean BMI $= 21.7$, range: $17.7 - 28.3$). Participants received study credits for their participation. Inclusion criteria were: (a) no current medical treatment or prescribed medication, (b) no signs of colds or upper respiratory tract infection in the past two weeks. Participants signed informed consent, and the research protocol was approved by the local ethics committee of the Vrije Universiteit.

2.2.2 *Procedure*

In preparation, participants were instructed to refrain from using alcohol or nonprescription drugs 24 hours before testing. Participants were asked not to deviate from their usual sleeping habits on the previous night, avoid vigorous exercise on the day of the experiment, and to abstain from smoking (five participants reported to be smokers), drinking caffeinated beverages, eating, and brushing teeth (to prevent gingival bleeding) one hour prior to the experiment. Women were scheduled within the 7 days after their menses. Compliance with instructions was verified by a detailed health behavior questionnaire. Experiments were set between 13:30 and 16:00 to minimize circadian effects (Nater et al., 2007).

On arrival, the experimental procedure was explained to the participant and electrodes for electrocardiography (ECG) and impedance cardiography (ICG) were attached. After rinsing the mouth with tap water, participants were familiarized with the saliva-collection procedure and filled out questionnaires, followed by a 20-minute resting baseline during which they engaged in leisurely reading. This was followed by one of the two experimental manipulations, counterbalanced across participants, each lasting for 8.5 minutes: i.e., (1) a computerized memory-search test (MT); (2) cold pressor task (CPT). Each task was followed by a 16 minute recovery period, after which the other stressor was presented. With each stressor, six saliva samples were collected (collection procedure is detailed further below): a pre-task baseline saliva sample, followed by a sample at 2 and 5.5 minutes into the task, and a fourth sample was collected immediately upon termination of the task at 8.5 minutes (task cessation or stres-

sor offset). The latter corresponds with the usual stress-sample time point in sAA studies. During recovery two further saliva samples were taken at 4 minutes and 10 minutes post-task, when the participants were again engaged in quiet reading. Between the last recovery measure of the first task and the baseline measurement of the second task the participants rested for 20 minutes. Participants filled out a stress questionnaire in conjunction with the baseline, the stressor offset, and the 10-minute recovery sample. Electrocardiogram (ECG) and an impedance cardiogram (ICG) were recorded continuously and blood pressure readings were taken at regular intervals that coincided with saliva sampling.

Memory-Search Task (MT)

For this task participants were instructed to memorize a set of four characters (letters and numbers) and to respond by pressing a lever (yes/no) within a set time ($<$ 2000ms) if one of the memorized characters appeared on a screen among six random characters (Bosch et al., 2001, 2003; de Geus et al., 1993). The memory set was changed every minute. Participants received instantaneous feedback on correct or wrong responses, and a personal score as well as a group average was presented. False performance feedback was provided at random intervals. In order to sustain effort, available reaction time was automatically adjusted depending on performance.

Cold Pressor Task (CPT)

Participants were instructed to immerse one arm until below the elbow in 8°C water for eight minutes (Willemsen et al., 1998). While CPT traditionally involves lower temperatures ($2-4$°C) and shorter immersion ($1-3$ min), the 8°C was chosen as it allows exposure that matched the timing of the MT while still eliciting distress and a robust pressor response (Willemsen et al., 1998). This longer CPT protocol has been validated using pharmacological blockade studies (Ring et al., 2000; Winzer et al., 1999), demonstrating that it induces a robust and sustained pressor response that is alpha-adrenergic dependent. CPT seemed relevant to include for two reasons: First, the highly localized (i.e., vascular) sympathetic activation that characterizes this task poses the question whether sAA will primarily follow a vascular or a cardiac activation pattern. Second, the evidence for an effect of CPT on sAA is mixed (with some studies increases increase and some observing no effect) and these studies lack independent verification of sympathetic activation other than blood pressure responses (Mourot et al., 2009; Willemsen et al., 2002).

2.2.3 *Questionnaires*

The state subscale of the Spielberger State-Trait Anxiety Inventory (STAI-Y) was used as an indicator of distress (van der Ploeg, 1988). The state scale of STAI is among the most commonly used measures of anxiety and distress used in clinical and stress research, and also frequently used in laboratory stress studies (Campbell and Ehlert, 2012). The scale consists of 20 brief statements, rated on a 4-point Likert scale (from "Very much" to "Not at all"), reflecting cognitions and feelings of nervousness, tension, and apprehension, or the lack thereof ("I am tense; I am worried" and "I feel calm; I feel secure."). The state scale of STAI boasts excellent psychometric qualities (Cronbach's alpha state subscale = .94 (van der Ploeg, 1988).

Following the CPT, participants were asked to rate the pain caused by the CPT on a 6-grade Likert scale (1: not at all painful, 6: extremely painful). Participants also filled out a detailed self-report questionnaire on health (perceived health, use of medication, other medical treatment, use of contraceptives), habitual health behavior (smoking; alcohol, tea, and coffee consumption; physical exercise, sleep duration and quality), and health behaviors in the 24 hours preceding the experiment. Height and weight were assessed using standard methods.

2.2.4 *Saliva collection*

Saliva was collected using the 'spitting method', described in detail by Navazesh (1993). This method exhibits a good retest reliability and is generally accepted as the preferred method in saliva research (Bosch et al., 2011; Bosch, 2014; Navazesh and Kumar, 2008; Rohleder and Nater, 2009). The saliva colleting method was practiced before the experiment to familiarize participants with the procedure. Participants were instructed to empty their mouth of saliva by swallowing and to let the saliva accumulate at the floor of the mouth without stimulating flow by oro-facial movements (i.e., 'unstimulated' saliva).

Saliva was expectorated into a pre-weighed, ice-chilled polypropylene test tube every 60 seconds, for a total of two minutes. Momentary salivary flow rate was determined gravimetrically, dividing the amount of saliva (grams corresponding to milliliters) by two, yielding ml/minute. After collection, saliva was homogenized using a vortex mixer and clarified by centrifugation (at $10,000\times$ g, 4 min) to eliminate buccal cells and oral microorganisms. The clear supernatant was divided into 500 µl aliquots and stored at $-20°$C until analysis.

2.2.5 *Amylase determination*

Amylase activity was determined using the quantitative kinetic determination kit (from Roche; Mannheim, Germany), as described by Bosch et al. (1996, 1998). In short, saliva (10 µl, starting dilution 1:60 in PBS) was mixed with 190 µl of amylase reagent and incubated for 2 minutes at 37°C. The increase in absorption (at 410 nm) over the subsequent 2 minutes was measured and compared with the activity of a multienzyme standard (Sigma Diagnostics). Amylase activity was expressed in units per milliliter (U/ml). All samples from each participant were assayed in quadruplicate in the same assay run. The intra-assay reliability (CV%) was 4%.

2.2.6 *Cardiovascular assessment*

Assessment of cardiovascular response focused on blood pressure and cardiac autonomic parameters. Blood pressure was measured with a Dinamap Vital Signs Monitor (Critikon model 845 XT). Blood pressure was assessed right after saliva collections except for the first stressor reading, which was obtained in the first minute of each task.

Indices of sympathetic and parasympathetic drive were obtained by analysis of Impedance Cardiography (ICG) and ECG signals (Berntson et al., 1997; de Geus and van Doornen, 1996), recorded continuously from six Ag/AgCl spot electrodes (AMI type 1650-005, Medtronic) using the Vrije Universiteit Ambulatory Monitoring System (VU-AMS) device (de Geus and van Doornen, 1996; Willemsen et al., 1996). The ECG and ICG complexes were ensemble averaged with reference to the ECG R wave across 30-second periods. From these ensambles 2 minute average levels were computed for heart rate (HR), root mean square of successive differences (RMSSD) and pre-ejection period (PEP), corresponding to each saliva collection (again with exception of the first task-reading, with corresponds to minutes $0-2$). Changes in PEP were used to index changes in cardiac sympathetic drive (Sherwood et al., 1990), and RMSSD was used to index changes in cardiac parasympathetic/vagal tone (Berntson et al., 1997).

2.2.7 *Data reduction and analysis*

Data were analyzed using full-within subject Task $\times$ Time ANCOVAs. Partial eta-squared (η_p^2) was used as a measure of effect size, which is comparable to adjusted R2 obtained in regression analyses (Tabachnick and Fidell, 2001), whereby values of 0.02, 0.13 and 0.26 indicate small, medium, and large effect sizes, respectively (Cohen, 1992). Analyses were checked for possible confounding effects of task order and other potential confounders (sex, BMI, and smoking status). As inclusion of these covariates had little effect on the results, the de-

tailed overview of these results is presented in the online supplement. Simple repeated-measures ANOVAs were conducted for follow-up analyses, and paired t-tests were used for post-hoc comparisons, using Bonferroni correction. Untransformed values are presented in figures, but for analyses data were transformed to restore normality (square root transformation (sqrt[x]): salivary flow rate, sAA concentration, and sAA secretion data; log transformation ($\ln[1+x]$) for RMSSD). Blood pressure readings were incomplete for five participants due to a technical fault. Outliers were defined as ± 3.5SD and removed from analyses ($n = 1$ for PEP). When the sphericity assumption was violated (as determined by Mauchli's test), the p value was corrected using the Greenhouse-Geisser correction. In that case, respective epsilon (ε) values are presented along with corrected significance levels. For correlational analyses we used the percentage change values ($\Delta\%$, relative to baseline). Significance for these exploratory analyses was set at $p < .01$, to account for multiple comparisons. Data were analyzed using IBM SPSS Statistics 20.0 for Windows.

2.3 RESULTS

2.3.1 *Anxiety, pain and autonomic and cardiovascular responses*

Verifying that both tasks were perceived as stressful, Figure 2.1 shows that both tasks significantly increased state anxiety (time effect: $F(2, 54) = 52.50$, $\varepsilon = .59$, $p < .001$, $\eta_p^2 = .66$), whereby slightly higher increases were reported during the MT than during CPT ($t(30) = 3.35$, $p = .002$). There was no significant effect of task order or sex ($p > .20$).

Participants rated the subjective pain caused by the CPT to be 3.6 on a 6-grade scale, which was not significantly different ($t(46) = 0.21$, $p = .835$) from pain rating from published data derived from a more conventional brief CPT protocol (immersing the hand in 4°C water for 90 seconds) (Allen et al., 1992).

Next, analyses aimed at verifying if the tasks exhibited the expected patterns of cardiovascular and autonomic activity. As shown in Table 2.1 and Figure 2.2, the MT evoked the expected pattern of increased sympathetic activation (evidenced by reduced PEP and increased blood pressure) and a vagal withdrawal (reduced RMSSD). At MT offset, RMSSD was restored to baseline values whereas PEP was still significantly shorter, indicated ongoing sympathetic activation. In line with prior research, the CPT showed little effect on heart rate and cardiac autonomic measures (See Table 2.1), but exhibited a robust increase in blood pressure that was similar to the blood pressure increase during the MT. For both tasks, cardiovascular parameters returned to baseline levels within 5 minutes after stressor

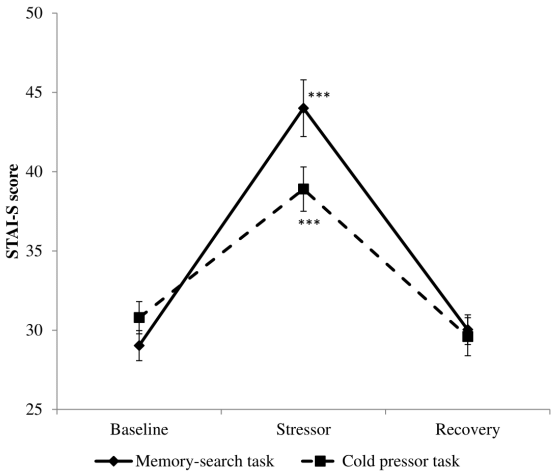

Figure 2.1: **Anxiety scores (Mean ± SE) during baseline, task, and recovery phases of Memory-search Task and Cold Pressor Task.** ***p < .001, representing difference from baseline.

termination. Adjusting for task order, gender, BMI, or smoking did not alter any of the reported results (see online supplement).

Table 2.1: Statistical results for cardiovascular responses

	Task × Time interaction		**Memory Task Time effect**		**Cold Pressor Task Time effect**	
Measure	**Statistic**	η_p^2	**Statistic**	η_p^2	**Statistic**	η_p^2
SBP	F (6,156) = 2.46*	.09	F (6,168) = 6.79***	.20	F (6,180) = 7.88***	.21
DBP	F (6,156) = 1.20, ε = .66	.04	F (6,168) = 11.60***, ε = .67	.29	F (6,180) = 5.52***, ε = .72	.16
HR	F (6,192) = 16.91***, ε = .45	.35	F (6,192) = 5.32***, ε = .33	.36	F (6,192) = 2.23, ε = .69	.07
RMSSD	F (6,192) = 17.31***, ε = .63	.35	F (6,192) = 15.96***, ε = .66	.33	F (6,192) = 1.82, ε = .63	.05
PEP	F (6,186) = 4.93**, ε = .55	.14	F (6,186) = 8.44***, ε = .64	.21	F (6,186) = 1.81, ε = .51	.06

Note: MT: Memory-search Task, CPT: Cold Pressor Task, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HR: Heart Rate, RMSSD: Root Mean Square of Successive Differences, PEP: Pre-Ejection Period. F values represent within-subject effects with adjustment for task order; *p < .05, **p < .01, ***p < .001.

2.3.2 *Salivary parameters*

Salivary flow rate

Salivary flow rate responses are presented in Figure 2.3 Repeated measures ANOVA yielded a significant Task × Time interaction

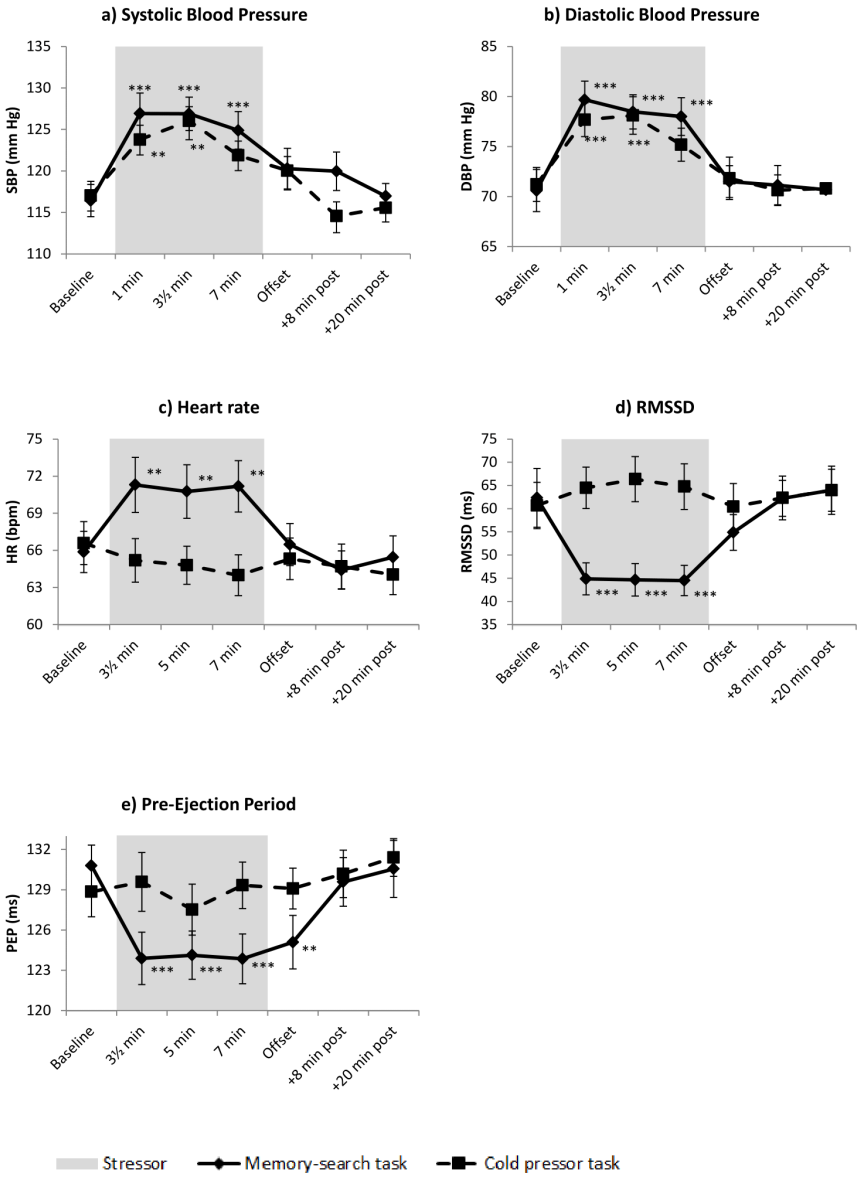

Figure 2.2: **Cardiovascular parameters (Mean ± SE) during Memory-search Task and Cold Pressor Task.** Asterisks indicate significant pair-wise differences from baseline values respectively, using Sidak correction; the timings on the x-axis indicate the end of each 2-minute ECG/ICG epoch. **p < .01, ***p < .001

($F(5, 145) = 16.23$, $p < .001$, $\eta_p^2 = .35$) showing a different pattern of change for the two stressors. Separate analyses of each task showed that MT clearly altered flow rate ($F(5, 160) = 45.77$, $p < .001$, $\varepsilon = .76$, $\eta_p^2 = .59$). Flow rate decreased from baseline during the MT (−55% and −56% at 3.5 and 7 minutes respectively; $t(33) = 7.99$ and 6.99, respectively, $ps < .001$) which was followed by a modest rebound above baseline levels at stressor offset (+22%, $t(33) = -3.88$, $p < .001$). The CPT did not significantly affect salivary flow rate ($F(5, 160) = 1.00$

, $p = .418$, $\eta_p^2 = .03$). Males had a higher average salivary flow rate than females ($F(1, 29) = 9.27$, $p = .005$, $\eta_p^2 = .24$), but adjusting for sex did not alter the results. Exploratory analyses did not identify other potential confounders (see online supplement).

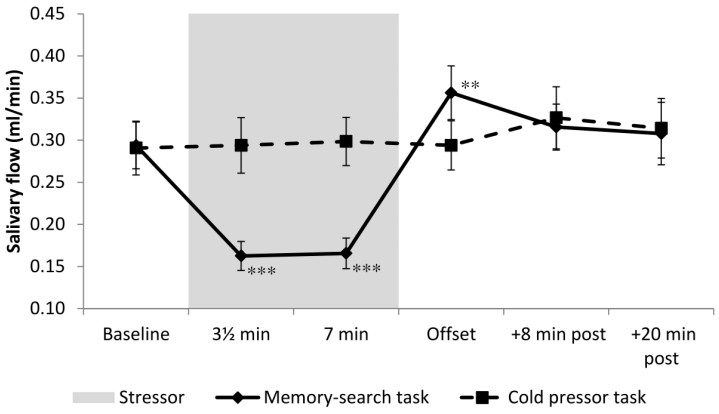

Figure 2.3: **Salivary flow rate (Mean ± SE) during Memory-search Task and Cold Pressor Task.** **$p < .01$, *** $p < .001$, representing Sidak corrected difference from baseline values. The timings on the x-axis indicate when each 2-minute saliva collection finished.

Salivary α-amylase concentration

As can be seen on Figure 2.4, the effect of the two tasks was different on sAA concentration over time, yielding a significant Task × Time interaction ($F(5, 150) = 5.90$, $\varepsilon = .67$, $p = .001$, $\eta_p^2 = .16$). Separate analyses of each task yielded a significant increase from baseline of sAA concentration in MT ($F(5, 155) = 10.60$, $p < .001$, $\varepsilon = .61$, $\eta_p^2 = .26$). Post-hoc analyses showed that during the MT sAA concentration did not change ($ps > .900$), and showed significant increase at stressor offset (+54%, $t(33) = -6.28$ $p < .001$), and subsequently returned to baseline levels ($ts < .81$, $ps > .35$ for recovery).The CPT did not significantly affect sAA concentration ($F(5, 155) = 1.01$, $\varepsilon = .68$, $p = .396$, $\eta_p^2 = .03$).Exploratory analyses showed no response differences related to covariates (see online supplement).

Salivary α-amylase secretion

As can be seen in Figure 2.5, the two tasks elicited different sAA secretion patterns, yielding a significant Task × Time interaction ($F(5, 145) = 22.18$, $p < .001$, $\eta_p^2 = .43$). Separate analyses of each task response showed that MT elicited significant changes in sAA secretion ($F(5, 150) = 35.63$, $p < .001$, $\varepsilon = .69$, $\eta_p^2 = .54$), whereby sAA secretion was decreased from baseline during MT (−43% and -31%, $t(31) = 5.81$ and 5.95, respectively, $ps < .001$), followed by a rapid increase above baseline values at stressor offset (+60%, $t(33) = -5.92$,

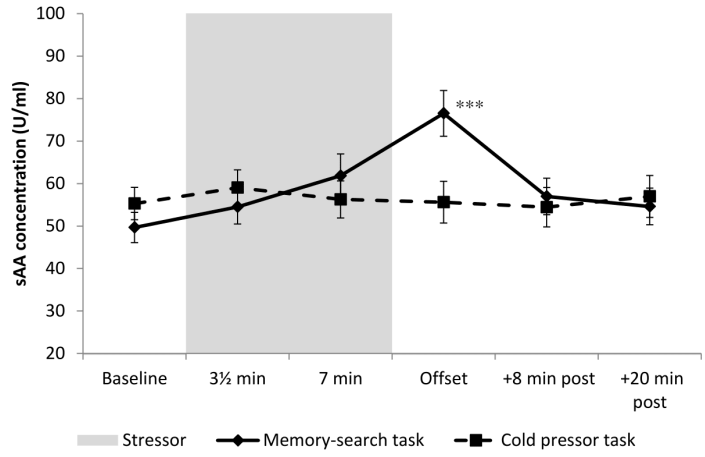

Figure 2.4: **Salivary alpha-amylase concentration (Mean ± SE) during Memory-search Task and Cold Pressor Task.** ***$p < .001$, representing Sidak corrected difference from baseline values for each task. The timings on the x-axis indicate when each 2-minute saliva collection finished.

$p < .001$); during recovery sAA secretion returned to baseline values

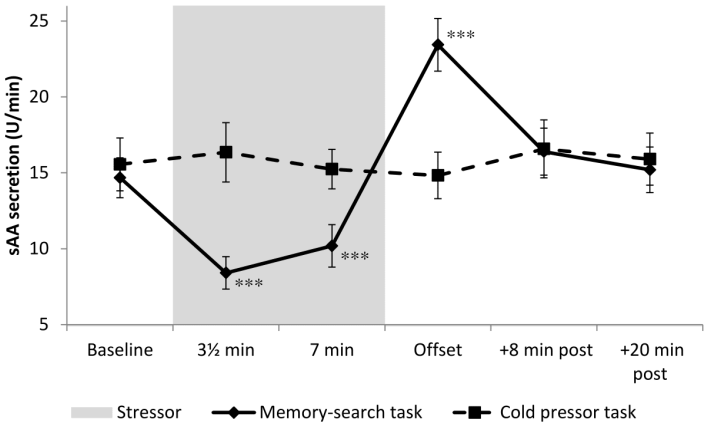

Figure 2.5: **Salivary alpha-amylase secretion (Mean ± SE) during Memory-search Task and Cold Pressor Task.** ***$p < .001$, representing Sidak corrected difference from baseline values. The timings on the x-axis indicate when each 2-minute saliva collection finished.

($ts = -1.36$ and -0.63, $ps > .180$). The CPT did not significantly affect sAA secretion ($F(5, 155) = 0.51$, $p = .770$, $\eta_p^2 = .02$).

Men showed a higher sAA secretion than women ($F(1, 27) = 9.87$, $p = .004$, $\eta_p^2 = .27$), which is explained by higher salivary flow rates in men. However, adjusting for sex did not alter the results (see online supplement).

2.3.3 *Correlational analyses*

The increase in salivary flow rate at MT offset correlated positively with RMSSD change ($r(33) = .56$, $p = .001$), supporting the idea that enhanced parasympathetic tone was associated with increased flow rate. This finding was replicated for the association between RMSSD and sAA secretion ($r(33) = .43$, $p = .01$), but not for RMSSD and sAA concentration. No associations were observed between PEP changes and changes in salivary measures at any of the time points.

Relative changes in sAA concentration and sAA secretion were significantly correlated at all time points; correlation coefficients were .65, .76, .66, .71, and .56 during CPT, and .59, .61, .63, .57, and .55 during MT (all $ps \leqslant .001$ for 3.5, 7, 8.5 (offset), +8, and +20 minutes, respectively).

2.4 DISCUSSION

For the present study it was predicted that sample timing may be an important determinant of sAA responses to stress, and this idea was tested by comparing sAA responses during two stressors known to elicit distinct patterns of cardiac autonomic activity. The results appeared consistent with expectations: over the course of a cognitive stressor (MT) an increase in sAA was evident at stressor offset only. Moreover, during this stressor sAA concentration was virtually unaffected and sAA secretion even decreased, despite evidence of consistent and robust sympathetic activation (i.e., decreased PEP, increased BP). These findings indicate that sAA increases upon stressor termination – the measurement time point used in most studies – may not be representative of what is happening during stress. Also notable is that the CPT had little effect on sAA levels, although participants reported pain and showed a marked increase in anxiety as well as vascular sympathetic activation (i.e., elevated blood pressure). Further, the change of sAA and PEP did not correlate at any time point. Overall, these results suggest that the interpretation of sAA as a marker of stress/arousal or sympathetic activity is less straightforward than perhaps assumed, and the role of concurrent parasympathetic activity (related to aspects like measurement timing and saliva flow rate) warrant more attention in future studies.

The present study also aimed to address the ongoing debate on the possible confounding role of salivary flow rate in determining sAA concentration. The empirical literature has been somewhat inconclusive on this issue, with some reports indicating that the influence of flow rate is fairly modest (Bosch et al., 1996; Rohleder et al., 2006; Sánchez-Navarro et al., 2012). However these cited studies found very little effect on flow rate to begin with, which makes substantial confounding unlikely. The relation between sAA concentration and sAA

secretion is mathematically determined by flow rate, and a different conclusion should, and indeed does, emerge when looking at studies in which flow rate is more strongly affected: in those instances sAA concentration and sAA secretion clearly diverge (Arhakis et al., 2013; Beltzer et al., 2010; Bosch et al., 2003; Proctor and Carpenter, 2001). This observation is reinforced by the present study, in which sAA concentration and (flow-rate adjusted) sAA secretion showed a strong discrepancy, i.e., sAA secretion showed a decrease during the MT while concentration did not change. Moreover, changes in sAA concentration and secretion showed only modest shared variance. We conclude therefore that sAA concentration cannot be confidently used as a proxy for sAA secretion and requires adjustment for salivary flow rate under some conditions (Beltzer et al., 2010).

Replicating prior research, salivary flow rate showed a modest rebound immediately post-MT, overshooting baseline levels, which suggests enhanced glandular parasympathetic activation (Bakke et al., 2004; Rohleder et al., 2006). This observation is somewhat reminiscent of a phenomenon known as vagal rebound (Blascovich and Mendes, 2010; Mezzacappa et al., 2001; Rottenberg et al., 2003), and it is therefore interesting that the increase in salivary flow rate positively correlated with concurrent vagal activity (RMSSD) at stressor offset. As outlined in the introduction, a PNS rebound in combination with a lingering sympathetic activation was predicted to augment sAA secretion as a result of autonomic co-activation of salivary glands (Proctor and Carpenter, 2007). On a speculative note, this observation made us wonder if the typical sAA increase post-stress might reflect a relief response, rather than a stress response. Admittedly a similar relief could be anticipated (but was not seen) with the CPT. Perhaps somewhat reassuring is that whereas the samples collected during stress showed a strong discrepancy between sAA concentration and secretion, at MT offset this discrepancy seemed more modest.

To ascertain that MT and CPT were comparable on a temporal level, we used an extended 8-minute CPT protocol which has been validated elsewhere (Winzer et al., 1999; Ring et al., 2000). Reassuringly our data, and that of others, showed that this CPT protocol faithfully replicated what has been shown for more conventional short CPT protocols: i.e., it induced a robust elevation in blood pressure that was sustained for the full duration of the immersion, it increased anxious distress (as measured by the Spielberger state anxiety questionnaire), and the average pain ratings were comparable to that of a brief CPT protocol (Allen et al., 1992). Importantly, likewise replicating prior reports was the observation that the CPT had no effect on sAA levels (Felmingham et al., 2012; Giles et al., 2014; O'Donnell et al., 2009; Skoluda et al., 2015), although increases have been reported as well (Lord et al., 2011; van Stegeren et al., 2008). It has been speculated that these inconsistencies might, in part, be explained by confound-

ing effects of inadvertent stimulation of flow rate, e.g., clenching or chewing during this painful stimulus (Arhakis et al., 2013; Bosch et al., 2011). Further studies using standardized collection of unstimulated saliva may be able to refute or confirm this possibility.

The lack of an sAA response during CPT also illustrates a more general point about the use of SNS markers in psychophysiology; i.e., SNS responses tend to show a high level of anatomical specificity (Folkow, 2000). Just as the CPT induced a strong vascular activation without a comparably strong sympathetic activation on a cardiac level (Allen et al., 1992; Willemsen et al., 1998, 2002), the CPT also did not induce significant SNS activation at the level of the salivary glands. Such anatomical specificity has been observed even within organs, including the salivary glands. For example, the secreto-motor sympathetic nerve fibers – responsible for the glandular secretion of sAA – are activated independently of the vasoactive sympathetic nerve fibers that regulate vasoconstriction in glandular tissue (Proctor and Carpenter, 2007). By implication, such specificity would suggest that it is better to denote sAA as a marker of *glandular* autonomic activity, which would be consistent with how other SNS markers are typically described (e.g., PEP as a marker of cardiac sympathetic activity).

2.4.1 *Limitations*

Several limitations of this study should be noted. It is possible that the non-response of sAA to stress might reflect that, different from cardiac and pressure responses, sAA possibly needs several minutes to develop. However, this possibility appears to be contradicted by available evidence. For example, electrical stimulation of the glandular nerves as well as reflex activation of these nerves (e.g., by chewing) increases sAA secretion within seconds (Garrett, 1987; Proctor and Carpenter, 2001). Further, sAA has been shown to increase within 3 minutes or less in response to psychological stressors, such as a stressful video (Engert et al., 2011; Takai et al., 2004) or a brief cold pressor test (Lord et al., 2011; van Stegeren et al., 2008). However, to date only a few studies have investigated temporal dynamics of sAA release and even fewer studied such dynamics during psychological stimuli, and clearly more research is needed to characterize the dynamics of sAA and determine optimal sampling strategies.

Another limitation is that the correlational analyses – linking cardiovascular autonomic measures with salivary responses – were exploratory and of modest statistical power, and replication in larger samples is needed to provide more conclusive data. The results of these exploratory analyses seemed consistent to other studies correlating measures of sympathetic activity with sAA, in that the associations observed here were relatively small (Bosch et al., 2003; El-Sheikh et al., 2008; Granger, 2006; Nater et al., 2006; Thoma et al.,

2012). This low correspondence with other measures of sympathetic activity also appears consistent the phenomenon of anatomical specificity discussed earlier, and low correspondence is the general observation from studies investigating correlations among various markers of SNS activity (e.g., skin conductance, catecholamines, PEP, pupil dilation) (Bosch et al., 2011). Future studies may therefore consider pharmacological manipulations or direct nerve stimulation instead of correlations for validation of sAA (Kuebler et al., 2014; Mills et al., 2000; Ring et al., 2000; van Stegeren et al., 2006; Winzer et al., 1999). However, we note that a recent study using a sophisticated statistical approach observed strong association between catecolamines and sAA (Ditzen et al., 2014). Another possible limitation is the lack of a resting control condition. Reassuringly, prior studies have systematically failed to demonstrate salivary changes during no-manipulation control conditions (Bosch et al., 2001, 2003; Willemsen et al., 2002), suggesting that repeated measurement by itself is unlikely to account for the observed sAA changes. Participants had to undergo the two stress task separated by 20 minutes, and therefore carry-over effects could be a concern. This possibility seems, however, less likely in light of the facts that: : 1) Autonomic/cardiovascular variables had returned to baselines values for at least 15 minutes; 2) The baseline levels did not significantly differ between tasks; 3) The order of the tasks was counterbalanced across participants. Importantly, the inclusion of task order as a covariate did not change any of the results (see online supplement).

Finally, it would be relevant to determine how the present findings generalize to other types of stressors, such as those involving social evaluation. For example, a recent study which compared psychological and physiological responses to several commonly used laboratory stressors (Skoluda et al., 2015), observed that the TSST elicits a more potent sAA response than CPT or a cognitive stressor. As most studies, this study collected saliva immediately upon completion of the stressor, but not during the stressor, which are the time points where we observed a zero or negative sAA response.

2.4.2 *Strengths*

A notable strength of the present study is the collection of unstimulated saliva using the spitting method. This method is generally accepted as a 'gold standard', and prevents the noise associated with absorbent materials, such as incomplete of sAA, inducing flow rate by accidental chewing, and poor quantification of saliva production (Beltzer et al., 2010; Bosch et al., 2011; Bosch, 2014; Proctor and Carpenter, 2001; Rohleder et al., 2006). There is also evidence that the use of absorbent materials may attenuate stress effects. For example, Rohleder et al. (2006) found that the stress-induced sAA increases

measured with salivettes were much smaller than those of whole saliva (respectively, +70% versus +130% for sAA concentration, and +80% versus +320% for secretion). However, it might be speculated that the nonresponse of sAA concentration during the tasks was caused by saliva collection causing some sort of interruption to the task, e.g., by being distracting. This interference appears less likely as we did not observe significant differences in cardiovascular and autonomic responses between time points with and without saliva collection. This speculation also seems inconsistent with the fact that sAA increased only when the cognitive task had actually stopped. We should note that because of the specific sampling method used, our results might not generalize to studies that use absorbent materials for saliva collection or collect stimulated saliva. Future research may therefore further compare different saliva sampling techniques.

2.4.3 *Conclusion*

In conclusion, the present study demonstrated timing-dependent and stressor-specific changes in sAA in response to acute laboratory stressors. Although the MT caused a clear and continuous elevation of cardiac sympathetic activity, sAA increases were only observed immediately post-stress. Moreover, although the CPT was perceived as stressful, painful, and robustly elevated blood pressure, no changes in sAA were seen. Together these observations lead us to conclude that the interpretation of sAA as a measure of SNS activity, or as a physiological marker of stress, is less solid than often assumed. Lastly, our results indicate that, depending on changes in saliva flow rate, sAA concentration and secretion may diverge substantially and that adjustment of sAA for salivary flow rate is warranted in future studies.

3

THE EFFECT OF REWARD ON SALIVARY HORMONAL LEVELS AND PERFORMANCE DURING COMPETITION IN YOUNG MEN

Background: Prior research has shown that in the context of competition, larger rewards are associated with amplified physiological arousal. Yet, few studies examined the effect of reward on testosterone (T) level, although this hormone has been shown to respond to competitive situations and status shifts. Cortisol (C) was also implicated in the competitive process, but few studies have been able to verify the hypothesized relationship between C and performance in competition. **Method**: The present study investigated salivary T and C level, physiological arousal (heart rate and heart rate variability), subjective arousal, and mood changes during a face-to-face video game competition in young males. Moreover, competitive attitudes were also assessed. Participants were either rewarded equally, or the winner gained more money than the loser (unequal reward group). **Results**: Subjective and cardiac arousal was increased in both conditions, and participants in the unequally rewarded condition were more aroused in the beginning of competition. In the unequally rewarded group, losers showed higher C levels, and C was associated with competitive performance in both winners and losers. T only increased in hypercompetitive losers. Competitive performance however was correlated with both winners' and losers' T values, supporting the hypothesis that T level and the competitive performance are likely to be in a reciprocal relationship. **Conclusion:**: Results demonstrate that reward conditions can be related to changes in subjective and cardiac arousal and C levels. Moreover T changes were determined by the interaction of outcome and hypercompetitive attitude. Our findings support the status instability hypothesis, and provide novel insights to competitive psychoendocrinology.[1]

3.1 INTRODUCTION

Biopsychological responses to competition have been extensively studied in the last decades. In particular the androgen steroid hormone testosterone (T) received considerable attention. Numerous studies reported increased T levels in preparation for, or in response to various types of competitions (Bateup et al., 2002; Booth et al., 1989; Edwards et al., 2006; Elias, 1981; Jiménez et al., 2012; Kivlighan et al., 2005; Mazur and Lamb, 1980; Mazur et al., 1997; Steiner et al., 2010;

1 The following chapter is the extended version of a previously published article: Nagy,T., Kovács, K. J., Polyák, Á., Harmat, L., Bárdos, G., Fülöp, M. (2015). A versengés jutalmazásának hatása a nyáltesztoszteronszintre és a teljesítményre fiatal felnőtt férfiakban: A hiperversengés szerepe [The effect of reward on salivary testosterone level and performance in young adult males during competition: The role of hypercompetitiveness]. Magyar Pszichológiai Szemle 70(1), 121–141. doi:10.1556/0016.2015.70.1.8

Suay et al., 1999; van der Meij et al., 2010) , and several reported that winners have higher T levels after a competition than losers (Costa and Salvador, 2012; Elias, 1981; Gladue et al., 1989; Jiménez et al., 2012; Mazur et al., 1992; McCaul et al., 1992; Zilioli and Watson, 2012). These findings are explained by two distinct theories. 1) The biosocial theory of status argues that increased T level after winning a competition is an adaptive response that helps to consolidate social status (Mazur and Booth, 1998; Mazur, 1985) . 2) The complementary challenge hypothesis emphasizes the T increase in preparation for, and during competitive situations as T is supposed to facilitate competitive effort and performance (Archer, 2006; Wingfield et al., 1990, 2001) .

Notwithstanding, T response to a competition has not been consistently found in all studies (Filaire et al., 2001; Gonzalez-Bono et al., 2000, 1999; Salvador et al., 1987, 2003; Schultheiss et al., 1999; Schultheiss and Rohde, 2002; Serrano et al., 2000). Edwards (2006) argued that T changes in a competition are likely moderated by psychological and contextual factors. For example mood (McCaul et al., 1992; Salvador and Costa, 2009; Salvador, 2005) , internal attribution of the outcome (Gonzalez-Bono et al., 2000, 1999; Serrano et al., 2000) , personality (Schultheiss et al., 1999; Schultheiss and Rohde, 2002; Suay et al., 1999) , or the subjective importance of the competition (Costa and Salvador, 2012; Steiner et al., 2010; van der Meij et al., 2010) were proposed as psychological mediators. Possible contextual factors include home territory (Carré et al., 2006; Neave and Wolfson, 2003) , the opponent's mindset (van der Meij et al., 2010) , and reward conditions (McCaul et al., 1992) . The latter might be of particular importance, as real life competitions often involve monetary or other incentives, which may partly determine the intensity of the competition (McCaul et al., 1992) . Moreover, reward is often associated with status change (promotions, awards, prizes, etc.), therefore reward might be a potential moderator of T response to a competition.

Yet, to this date there is only one study that directly manipulated reward conditions and assessed T changes in a competitive situation. In the experiment of McCaul et al. (1992; second experiment) participants had to take part in a coin flipping contest where they could win or lose. Furthermore, they were either told that they played for money or not (neutral condition). Although participants were aware of their lack of control on the outcome, after the contest winners showed higher T levels than losers. The neutral group however did not show significant T change. Thus, this result demonstrated that reward might moderate T response to competition. However, in the aforementioned study the outcome of the competition did not depend on the participants' effort. This is rare in real life situations, and therefore limits interpretation and generalizability of the results.

Previous studies suggest that individual differences in competitiveness can also play a role in T response to competition. For example power motivation was shown to moderate the T reaction to losing, and high power motivation individuals showed increases, rather than decreases to losing (see Schultheiss and Rohde, 2002) . This line of research needs more evidence however, as studies are still scarce.

The cardiovascular system is likewise responsive to competition (Harrison et al., 2001; Veldhuijzen Van Zanten et al., 2002) . Furthermore, reward was shown to moderate cardiac responses in competitive settings. For example, a study randomly assigned participants to groups which varied in the prospective reward in a horse-race gambling task (Wulfert et al., 2008) . The results showed a strong positive association between the size of the reward and heart rate. Importantly, this finding was replicated in settings that required effort instead of luck (Richter and Gendolla, 2007, 2009) . These findings suggest that reward size might moderate cardiac variables in an effort based competition.

The competitive context incorporates several elements that had been previously identified as stressors: anticipation, social evaluation, performance pressure, and eventually, the adversaries also have to deal with the outcome of the competition (Booth et al., 1989; Denson et al., 2009; Dickerson and Kemeny, 2004) . Cortisol (C) increase to competition has been also shown in several studies (e.g. Cook et al., 1987; Filaire et al., 2009; Rohleder et al., 2007) . This C increase was observed to be more salient for competitors with lower status (i.e. less experience), probably because of lower self-confidence (Filaire et al., 2009; Zilioli and Watson, 2013) . It was also reported that losers often have higher C levels than winners (Booth et al., 1989) . Nevertheless, response differences between winners and losers do not necessarily mean these are caused by victory or defeat. Salvador and Costa (2009) proposed that appraisal of the situation might create either an active (proactive) or passive (reactive) coping response. Active coping more likely leads to victory, and passive to defeat. Therefore different response patterns might not be the causes, but the effects of winning and losing – at least in competitions that require effort (Salvador, 2005) . As part of this response pattern, T might increase during active coping – thus improving performance –, and decreases in passive coping, whereby C increases. This difference can play a crucial role in competition, because testosterone can facilitate competitive performance (Archer, 2006; Salvador and Costa, 2009) .

In overall, research is needed to elucidate the effects of reward on T and C levels in a competitive context. Using a face-to-face competition which requires effort and skill rather than luck can improve generalizability through ecological validity. Also, assessing competitive performance could clarify the presumed connection between T and performance (Archer, 2006; Mazur, 1985; Wingfield et al., 1990)

. Interestingly, only a few studies volunteered to investigate this relationship using objective measures of performance (Gladue et al., 1989; Mazur and Lamb, 1980) . Moreover it should be investigated if prospective performance pressure and reward can increase anticipatory C response in a laboratory context (Alix-Sy et al., 2008) .

To address the aforementioned questions regarding reward conditions, performance, and T, in the present study forty young adult males competed in a 30-min video game whereby they could win money. They were randomly assigned to two different reward conditions in pairs: in the "unequal reward" condition the winner gained three times as much money as the loser; whilst in the "equal reward" condition the winner was awarded the same amount of money as the loser. T and C levels were determined from saliva, cardiac variables were assessed continuously, and mood was assessed several times throughout the competition. We predicted that physiological arousal (HR) and T level will be higher during the competition compared to baseline, and higher even in the "unequal condition" than in the "equal condition". We also anticipated higher C levels in the unequal condition than in the equal. Furthermore, we expected the competitive performance to correlate with T and C levels, i.e. higher T level will be associated with better competitive performance and larger difference between competitors' final scores.

3.2 METHOD

3.2.1 *Participants*

Based on a priori power analysis[2], forty healthy young adult nonsmoker males took part in the study. The age of participants ranged between 18 and 28 years (mean age = 21.7 years; SD = 2.3; mean BMI = 23.0 kg/m^2, SD = 3.5). Every participant had at least brief previous experience with first person shooter video games. Participants received monetary reward according to the research design (see details further below). The study was approved by the research ethics committee of Eötvös Loránd University, Budapest.

3.2.2 *Procedure*

Volunteers were recruited via university mailing lists, where they were provided a link to a short online survey. The survey contained

2 To estimate sample size, a priori power analysis was conducted using GPower 3.1 (Faul et al., 2007) . Parameters for the analysis were based on a similar study (McCaul et al., 1992, second experiment) . Power was estimated for ANOVA between-within interaction (expected effect size (f) = .25 (medium), $\alpha = .05$, $1 - \beta = .80$, number of between subject groups = 4, number of measurements = 2 (number of T samples), correlation between measurements = .60).

questions about demographic information (age, gender), general health behaviors (e.g. smoking), and participants' proficiency in various games (ranging from 1: "very bad" to 5: "excellent") like card games, video games, etc., and the regularity of playing those games (ranging from 1: "never" to 5: "daily"). Eligible respondents ($18-35$ years old male, non-smokers who had previous video gaming experience) were contacted via phone to set an appointment. Participants with similar video gaming usage frequency and skills were paired. Pairs were randomly distributed into two research conditions ("equal reward" and "unequal reward") by computer generated random numbers. Participants in the "unequal condition" were told that they will get money according to their performance (the winner would receive 3000, and the loser 1000 Hungarian Forints), while the "equal group" was informed that they will get paid regardless of performance ($1500-1500$ HUF). This information was communicated one day before the session and also at the start of the session. Participants were also informed previously about the perquisites for testing. They were asked to refrain from smoking, alcohol consumption and caffeinated beverages, and to avoid strenuous exercise on the day of the session. Furthermore, they were requested not to eat two hours before the session, and not to brush teeth one hour before the session to prevent gingival bleeding (that might affect salivary hormonal levels).

Research sessions were conducted by a male experimenter between 1200h and 1700h to minimize the effects of diurnal changes on T and C levels (Dabbs, 1990; Kudielka et al., 2012) . The participants arrived separately, and rested for $10-15$ minutes after arrival. Then they were escorted to an air-conditioned laboratory room. Following a short briefing, participants signed an informed consent form. Electrodes for heart rate monitoring were set up and tested. Participants were familiarized with the research task, and were allowed to practice for $2-3$ minutes. Subsequently, baseline measures were taken and the first saliva sample was collected. Then participants played a competitive video game against each other in six, 5-minute long game levels. The game levels did not differ in difficulty, and were used to prevent boredom. Perceived arousal and valence were assessed several times before, during, and after the competition. During the game, the experimenter repeatedly informed the participants about their actual scores. After the competitive task, the winner was announced, and recovery assessments were made, second saliva sample was taken, and participants filled a questionnaire about competitive attitudes.

3.2.3 *Competitive task*

Participants played a popular first person shooter game (Call of Duty™: Modern Warfare™ 2; Activision; Santa Monica, USA) in a

competitive one vs. one setting. In the game, players had to search and eliminate each other's avatar from a first person perspective. The one who managed to eliminate the opponent scored one point. The destroyed avatar returned to the game instantly until the time for the level expired. Participants played the game for 30 minutes, in six different levels, each lasting for five minutes. The player who scored more points by the end of the sixth level won the game. The game scores were recorded for further analysis, along with the final game score difference (score difference = winner's score – loser's score). To avoid uneventful gameplay, the adversary's position was continuously shown on a map on the corner of the screen.

The game was ran by an Xbox 360^{TM} console (Microsoft; Redmond, USA); the screen was projected on the wall by an XGA VPL-CX10 projector (Sony; Tokyo, Japan) in a vertical split screen mode, while SP-S350 active speakers (Genius; Taipei, Taiwan) provided sound. The participants controlled the game using two wireless Xbox 360^{TM} controllers (Microsoft; Redmond, USA).

3.2.4 *Questionnaires*

Participants evaluated their arousal and valence on a 9×9 *affect grid* (Russell et al., 1989) . In this grid, arousal was the vertical axis, ranging from -4 (very sleepy) to $+4$ (very aroused), while horizontal axis represented valence, from -4 (very unpleasant) to $+4$ (very pleasant).

Hypercompetitive attitude (HCA) and personal development competitive attitude (PDCA) were assessed by standard scales (Ryckman et al., 1990, 1996) . Hypercompetitiveness refers to a competitive attitude that seeks competition in every area of life and favors winning above everything. On the other hand, individuals with PDCA utilize competition for self-development, therefore regards the process of competition more important than the outcome. Both scales contain statements, and participants can report agreement or disagreement with the statements on a five-point Likert scale (1: completely disagree . . . 5: completely agree). HCA scale contains 26, and the PDCA scale 15. Both scales were used in their previously used Hungarian version (Fülöp et al., 1999) , and the scales showed acceptable reliability ($\alpha = .82$ for both scales).

3.2.5 *Cardiac measurement*

For monitoring heart rate, two Actiheart devices were used (Camntech; Cambridge, UK), attached by disposable self-adherent electrodes (Fiab; Vicchio, Italy). One electrode was fixed just below the apex of the sternum while the other under the nipple. The reliability and validity of the Actiheart device was verified elsewhere (Brage et al., 2005) . An Actiheart reader (Camntech; Cambridge, UK) was

used to retrieve recorded inter-beat-interval (IBI) data after the sessions. Heart rate (HR) and root mean square differences of successive heartbeat intervals (RMSSD) were averaged for 5-minute time periods. RMSSD reflects vagal tone and is considered as a proxy for parasympathetic activity (Task Force et al., 1996) .

3.2.6 *Hormonal assessment and analysis*

Saliva samples were collected using polypropylene salivettes (Sarstedt; Nümbrecht, Germany)[3] five minutes before, and five minutes after the competitive task. Saliva was centrifuged and stored frozen at $-20°C$ until analysis. Two participants did not produce sufficient amount of saliva, leaving 38 participants with complete salivary data. Moreover, one participant's C values were identified as outliers (> 3.5 SDs from mean) and were excluded from analysis.

Salivary T and C levels were measured in duplicate by commercially available EIA kit (T: 1-2402; C: 1-3002; both from Salimetrics, State College, USA) according to the manufacturer's instructions. The sensitivity of the T assay was 1 pg/ml. Intra- and interassay coefficients (CV) were $< 6.7\%$ and $< 15.9\%$, respectively. The sensitivity of the C assay is 0.003 µg/dL. Intra- and interassay coefficients (CV) were 3.4% and $< 6.4\%$ respectively.

3.2.7 *Data reduction and analysis*

For the preliminary analysis 2×2 ANOVAs were used with Reward condition (unequal vs. equal reward) $\times$ Outcome (winner vs. loser) as between subjects variables. Non-normally distributed variables were analyzed using non-parametric tests.

As participants competed in pairs, the data can be considered to be clustered, therefore the use of mixed effect modeling was used for analyzing arousal, valence, HR, RMSSD, C, and T changes. The null model contained only the random intercepts as predictors. This null model was compared to further models – based on the hypotheses – that contained linear and quadratic time effects, reward condition (unequal vs. equal), outcome (winner vs. loser), and the interactions of these variables. For T only, HCA and PDCA and their interactions with time, condition, and outcome were also added as predictors.

Model estimates were calculated using restricted maximum likelihood method (REML), but were recalculated for model comparisons using the maximum likelihood (ML) method. Model comparisons

3 Using synthetic absorbent materials for saliva sampling has been suspected to deflate T levels (Atkinson et al., 2008; Granger et al., 2004; Shirtcliff et al., 2001) . Although the biggest study to date found no significant difference from whole saliva T levels in men, using the same collection device we used in the present study (Celec and Ostatníková, 2012) .

were made based on the Bayesian information criterion (BIC) and the Akaike information criterion (AIC). A model with the smaller BIC and AIC values was preferred. In this chapter, we only present the final accepted models, but investigated models and model comparisons can be found in appendix B. To provide a measure of effect size, R^2 statistics for the mixed models were calculated using the method of Nakagawa and Schielzeth (2013), that yields two types of R^2s: marginal R^2 (R^2_m), that represents the variance explained by the fixed factors, whereas conditional R^2 (R^2_c) shows the variance explained by the entire model with random and fixed effects.

Data analysis was conducted in R 3.2.0 (R Core Team, 2014) , and mixed models were built using the lme4 package (Bates et al., 2012) .

3.3 RESULTS

3.3.1 *Preliminary analysis*

Table 3.1 shows the descriptive statistics for attributes and gaming performance in each group. There was no significant difference in subgroups by age ($U < 223.50$; $p > .517$), BMI ($U < 171.50$; $p > .399$), and gaming skill ($U < 256.50$; $p > .507$). Participants in the equal reward condition generally played more video games ($U = 285.50, p = .014$). Therefore this factor was used as a covariate in later analyses to eliminate confounding effects. There was no difference between the groups in hypercompetitive attitude scale ($F < 0.89$, $p > .350$), while

Table 3.1: Means and standard deviations of participant attributes and competitive performance in the subgroups

	Unequal reward		**Equal reward**	
	Winner	**Loser**	**Winner**	**Loser**
Attributes of participants				
Age (years)	21.4 (2.5)	21.8 (2.7)	21.4 (2.2)	22.0 (1.9)
BMI (kg/m^2)	23.6 (3.3)	23.6 (3.7)	21.4 (3.8)	23.4 (3.0)
Gaming skill	3.6 (0.8)	3.6 (1.0)	4.0 (0.8)	4.1 (1.0)
Gaming frequency	3.2 (0.6)	3.2 (0.8)	4.0 (1.1)	3.9 (1.1)
Hypercompetitivene attitude (HCA)	3.0 (0.4)	2.6 (0.6)	2.9 (0.5)	3.1 (0.5)
Personal development competitive attitude (PDCA)	3.4 (0.6)	3.7 (0.4)	4.0 (0.4)	3.9 (0.3)
Competitive performance				
Final score	43.2 (18.0)	22.4 (13.4)	41.7 (16.2)	29.9 (7.7)
Score difference	21.1 (17.8)		11.5 (14.7)	

Note: The sample size was 10 in all subgroups.

the difference was significant for personality development attitude scale, whereby the equal reward group had higher average ($F = 7.19$; $p = .011$; $\eta^2_p = .17$).

As for the competitive performance, the final score was obviously larger for winners than for losers ($U = 316.00$, $p = .002$). Score difference between winners and losers was also higher in the unequal reward condition, although this difference did not reach statistical significance ($U = 26.00$, $p = .069$).

3.3.2 *Subjective arousal*

The final accepted mixed model of the predictors of subjective arousal yielded that participants in the unequal reward condition had a higher pre-competition reported arousal than those in the equal reward condition, as shown in Table 3.2, by the significant interaction between

Table 3.2: Final mixed-effects model of subjective arousal

Random effects	**Var**	**SD**			
Participant (Intercept)	0.33	0.58			
Pair (Intercept)	0.03	0.16			
Fixed effects	**b**	**SE**	**df**	**t**	**p**
(Intercept)	0.112	0.291	146.7	0.38	0.701
Time	1.067	0.117	277.0	9.13	<0.001
$Time^2$	-0.130	0.012	277.0	-10.49	<0.001
Condition (ER)	-0.300	0.318	65.7	-0.94	0.348
Condition (ER) *Time	0.112	0.050	277.0	2.27	0.024

Model summary: AIC = 981.7, BIC = 1011.8, $R^2_m = .22$, $R^2_c = .42$

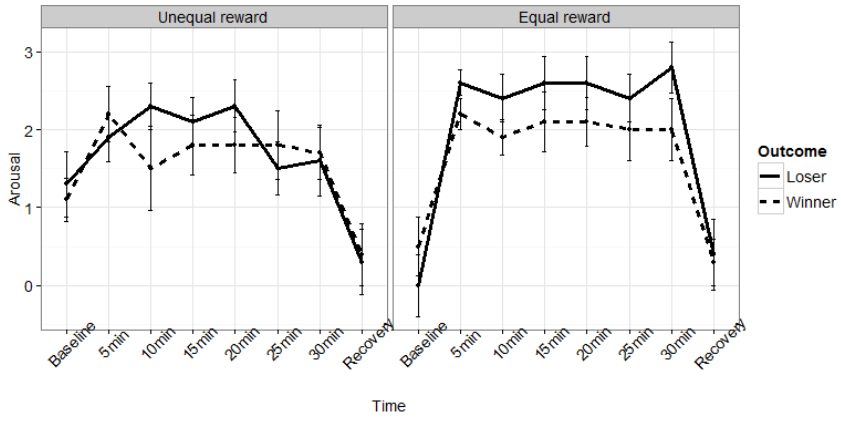

Figure 3.1: **Changes of subjective arousal through the competition in winners and losers by reward conditions.** Error bars represent standard error.

condition and time. Otherwise, subjective arousal increased during the competition in both conditions i.e. a quadratic time trend was observable (inverted u-shape). The outcome of the competition did not

affect the subjective arousal, and was not included in the final model (see Fig 3.1).

3.3.3 *Valence*

As shown on Figure 3.2, winners reported more positive feelings over time than losers, as demonstrated in Table 3.3 by the significant interaction between outcome and time, according to the final accepted mixed model. Post-hoc analysis revealed that this difference became significant when the final result was announced ($t(38) = -4.17$, $p < .001$). Valence was not affected by reward conditions, and participants in different reward conditions did not react differently to the competition outcome, thus these effect were not included in the final model.

Table 3.3: Final mixed-effects model of valence

Random effects	**Var**	**SD**			
Participant (Intercept)	0.83	0.91			
Pair (Intercept)	0.07	0.26			
Fixed effects	**b**	**SE**	**df**	**t**	**p**
(Intercept)	1.159	0.298	96.6	3.89	<0.001
Time	-0.034	0.041	278.0	-0.82	0.413
Outcome (W)	-0.148	0.413	52.3	-0.36	0.721
Outcome (W) *Time	0.186	0.059	278.0	3.17	0.002
Condition (ER) *Time	0.112	0.050	277.0	2.27	0.024

Model summary: AIC = 1106.1, BIC = 1132.5, $R^2_m = .22$, $R^2_c = .42$

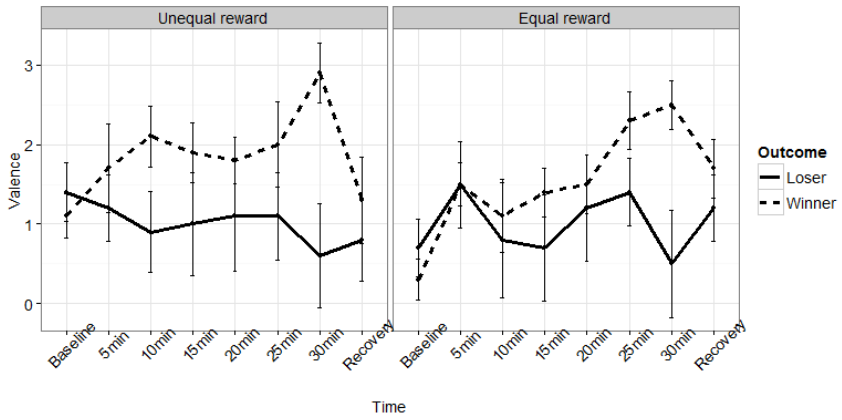

Figure 3.2: **Changes of valence through the competition in winners and losers by reward conditions.** Error bars represent standard error.

3.3.4 *Heart rate*

As shown on Figure 3.3, in the final accepted mixed model heart rate showed a different pattern during the competition in the unequal condition than in the equal reward condition. Although HR increased in both groups during the competition (showing a quadratic time trend), in the unequal reward group HR was higher in the baseline and during the first part of the competition, as evidenced by the significant interaction between condition and (linear) time effect (see Table 3.4). Outcome of the competition or BMI did not change the results and were not included in the final model.

Table 3.4: Final mixed-effects model of heart rate

Random effects	**Var**	**SD**			
Participant (Intercept)	206.63	14.38			
Pair (Intercept)	0.00	0.00			
Fixed effects	**b**	**SE**	**df**	**t**	**p**
(Intercept)	81.09	3.52	52.5	23.06	<0.001
Time	6.85	0.66	277.0	10.46	<0.001
$Time^2$	-0.81	0.07	277.0	-11.71	<0.001
Condition (ER)	-7.12	4.75	43.8	-1.50	0.141
Condition (ER) *Time	0.70	0.28	277.0	2.50	0.013

Model summary: AIC = 2190.1, BIC = 2220.2, $R^2_m = 0.07$, $R^2_c = 0.87$

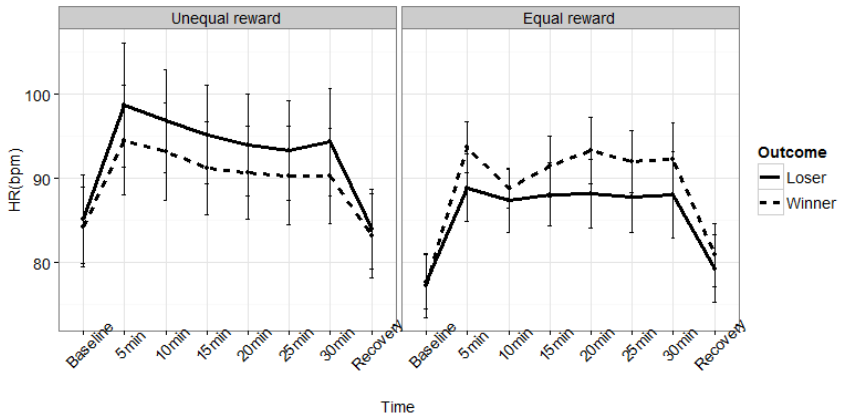

Figure 3.3: **Changes of heart rate through the competition in winners and losers by reward condition.** Error bars represent standard error.

3.3.5 *Heart rate variability (RMSSD)*

Outliers were identified for RMSSD (> ±3.0SD) and removed from the analyses (N = 2). Similarly to HR, RMSSD exhibited a different response pattern in the unequal condition than in the equal reward con-

dition over time (see Figure 3.4), as the final accepted mixed model yielded. Although RMSSD decreased in both conditions during the competition, it started off as lower in the unequal reward condition than in the equal reward condition. However, this difference became less pregnant over time (see Table 3.5). Outcome of the competition or BMI did not change the results and were not included in the final model.

Table 3.5: Final mixed-effects model of heart rate variability (RMSSD)

Random effects	**Var**	**SD**			
Participant (Intercept)	65.76	8.11			
Pair (Intercept)	27.48	5.24			
Fixed effects	**b**	**SE**	**df**	**t**	**p**
(Intercept)	34.95	3.09	35.1	11.30	<0.001
Time	-4.51	0.79	263.0	-5.71	<0.001
$Time^2$	0.54	0.08	263.0	6.46	<0.001
Condition (ER)	3.13	3.92	23.4	0.80	0.433
Condition (ER) *Time	-0.68	0.33	263.0	-2.02	0.045

Model summary: AIC= 2135.3, BIC = 2165.0, $R^2_m = 0.05$, $R^2_c = 0.69$

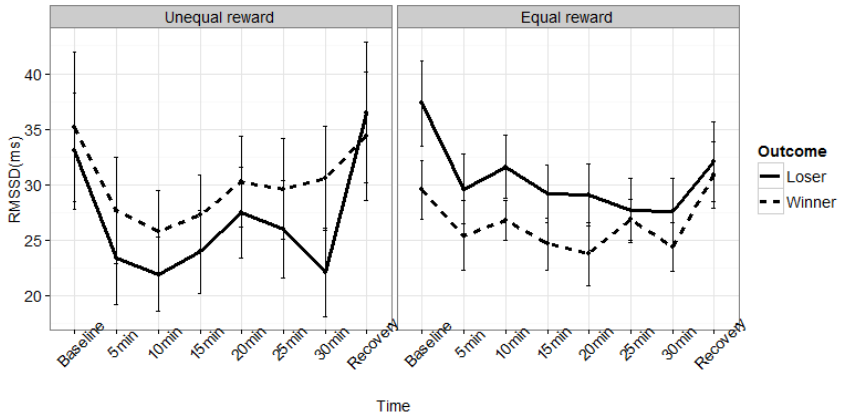

Figure 3.4: **Changes of heart rate variability (RMSSD) through the competition in winners and losers by reward condition.** Error bars represent standard error.

3.3.6 *Testosterone*

Testosterone changes were examined in a two-step process. First, we investigated the time effect on T level, and the interaction of outcome and condition with time, to investigate the effect of these factors on T change. These models yielded that T did not significantly change over time, and condition and outcome were not significant predictors

of T, as the AIC and BIC values of these models were not smaller than that of the null model (see Figure 3.5 and appendix B).

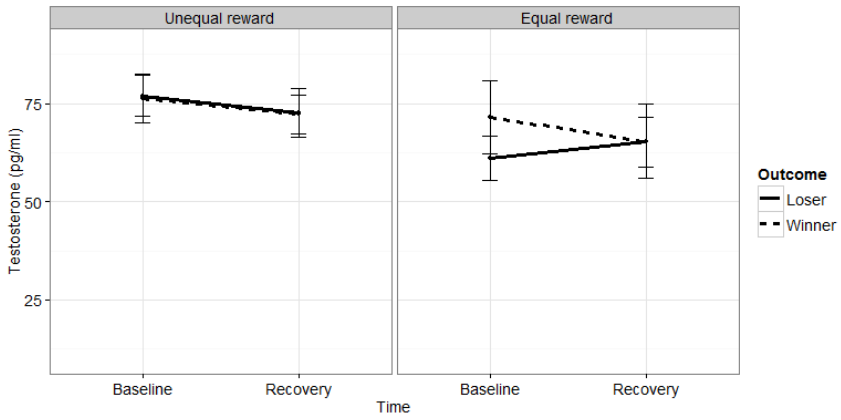

Figure 3.5: **Changes of testosterone through the competition in winners and losers by reward condition.** Error bars represent standard error.

To investigate the effects of the competitive attitudes, we added the competitive attitudes to the model. Hypercompetitiveness significantly predicted T change in interaction with outcome (see the final model in Table 3.6), and produced better AIC and BIC values than the null model. More specifically, as Figure XX show, more hypercompetitive losers showed a T increase, rather than a decrease in both conditions, compared to less hypercompetitive losers and winners (see Fig 3.6). The personality development competitive attitude did not prove to be a significant predictor.

Table 3.6: Final mixed-effects model of testosterone

Random effects	**Var**	**SD**			
Participant (Intercept)	310.94	17.63			
Pair (Intercept)	70.05	8.37			
Fixed effects	**b**	**SE**	**df**	**t**	**p**
(Intercept)	123.34	31.36	63.4	3.93	<0.001
Time	-43.83	13.01	34.0	-3.37	0.002
Outcome (W)	-91.18	51.60	63.8	-1.77	0.082
Hypercompetition	-19.02	10.93	63.3	-1.74	0.087
Time*Outcome(W)	53.94	21.37	34.0	2.52	0.016
Time*Hypercompetition	15.45	4.54	34.0	3.40	0.002
Outcome (W)*Hypercompetition	34.91	17.61	64.0	1.98	0.052
Time*Outcome (W)*Hypercompetition	-20.57	7.28	34.0	-2.82	0.008

Model summary: AIC= 636.40, BIC = 662.04, $R^2_m = 0.05$, $R^2_c = 0.88$

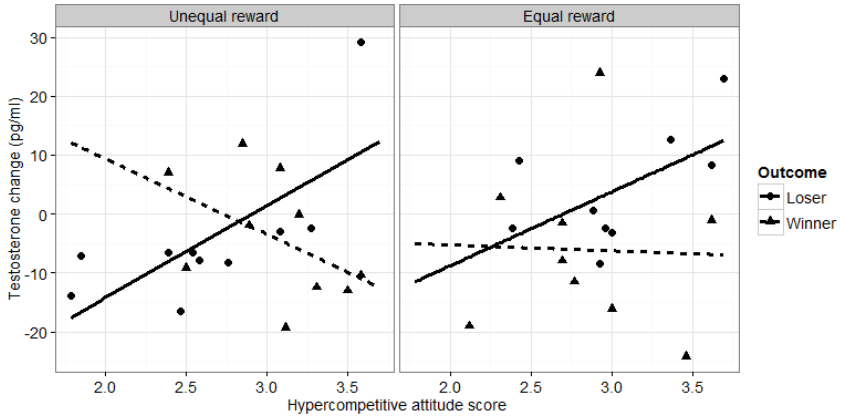

Figure 3.6: **Association between hypercompetitive attitude and testosterone change in winners and losers by reward condition.** Error bars represent standard error.

3.3.7 *Cortisol*

As it can be seen on Figure 3.5, cortisol decreased during the competition in all subgroups. However, the significant interaction between outcome and condition revealed that the losers in the unequal reward condition had significantly higher cortisol levels both at baseline and post-competition than the other subgroups (see Table 3.7).

Table 3.7: Final mixed-effects model of cortisol

Random effects	**Var**	**SD**			
Participant (Intercept)	8.95	2.99			
Pair (Intercept)	<0.01	<0.01			
Fixed effects	**b**	**SE**	**df**	**t**	**p**
(Intercept)	8.20	1.37	59.4	6.01	<0.001
Time	-2.41	0.52	36.0	-4.67	<0.001
Condition (UR)*Outcome (L)	3.69	1.55	33.0	2.38	0.023
Condition (ER)*Outcome (L)	-0.92	1.59	33.0	-0.58	0.568
Condition (UR)*Outcome (W)	0.93	1.59	33.0	0.59	0.561

Model summary: AIC= 395.33, BIC= 413.76, $R^2_m = 0.25$, $R^2_c = 0.73$

3.3.8 *Competitive performance and hormonal responses*

Associations between performance and testosterone levels were explored using Spearman correlations. Table 3.8 shows that winners' pre- and post-competition T levels were positively correlated both with the final score, and the score difference. Thus, those winners who had higher testosterone levels achieved more points in the competition, and were more likely to increase the score difference during

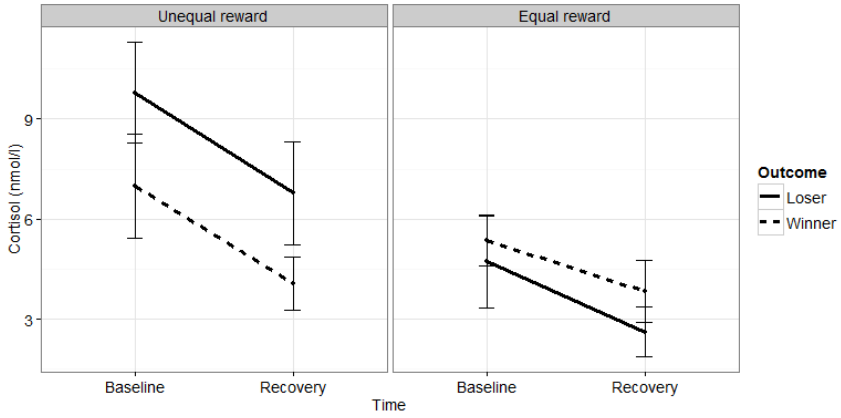

Figure 3.7: **Changes of cortisol through the competition in winners and losers by reward condition.** Error bars represent standard error.

the competition. In losers, pre- and post-competition T levels were not significantly correlated with the final score, although the final score was positively correlated with T change. Thus, those losers who achieved higher score at the end of the competition had a bigger increase (or smaller decrease) in T during the competition. Moreover, losers' T change was negatively correlated with score difference, e.g. a more decisive losing was associated with larger T decrease.

Table 3.8: Correlations between competitive performance and testosterone (Spearman rho coefficients)

	Final score	**Score difference**	**Pre-competition T**	**Post-competition T**	**T change**
Final score		.61**	.60**	.57*	.02
Score difference	-.39		.63**	.52*	-.06
Pre-competition T	.00	.41		.90**	-.22
Post-competition T	.45	.07	.75**		.12
T change	.56*	-.47*	-.43	.20	

Note: Values above the diagonals show the coefficients for winners (N = 19), values below the diagonal represent coefficients for losers (N = 19). $* : p < .05, ** : p < .01$.

Similarly to T, C levels were also associated with competitive performance. As Table 3.9 show, winners' pre- competition C level was associated with the final score, and the score difference between winners and losers. Moreover, post-competition C was associated with the final score. This can mean that the higher C levels were associated with better performance for winners. On the other hand, losers' C change was negatively correlated with the score difference. This finding suggests that C increase in response to the competition was associated with smaller score difference in losers. In other words, those losers

who remained close to winning showed a smaller C decrease than those who suffered a decisive defeat.

Table 3.9: Correlations between competitive performance and cortisol (Spearman rho coefficients)

	Final score	**Score difference**	**Pre-competition C**	**Post-competition C**	**C change**
Final score		0.62**	0.56*	0.73**	-0.10
Score difference	-0.39		0.57*	0.45	-0.27
Pre-competition C	-0.20	0.32		0.64**	-0.77**
Post-competition C	-0.16	0.27	0.88**		-0.09
C change	0.26	-0.46*	-0.55*	-0.24	

Note: Values above the diagonals show the coefficients for winners (N = 18), values below the diagonal represent coefficients for losers (N = 19). $* : p < .05, ** : p < .01$.

3.4 DISCUSSION

The present study investigated whether different reward conditions affect psychobiological reactions and performance in a video gaming competition in young men. We found that subjective arousal and heart rate increased, while heart rate variability decreased during the competition. Reward conditions determined these responses, as the unequally rewarded group showed greater subjective arousal and cardiac effort in the beginning of the competition. As expected, winners scored more points and felt more pleasant at the end of the game than losers. T levels did not change in overall during the competition, although more hypercompetitive participants showed increased T level to losing. C levels were the highest in the losers of the unequally rewarded competition, but decreased during the competition in all subgroups. Winners' pre- and post-competition T and C level was positively correlated with the score they achieved in the competition and the score difference between the winner and the loser. Losers' T and C change were negatively correlated with the game score difference.

To our best knowledge, this was the first attempt to connect the change in T level to competitive attitude. Moreover, the correlations between T and C level and performance provide evidence for theoretical models.

3.4.1 *Overall response to competition*

Competition increased subjective arousal and heart rate, while decreased heart rate variability compared to pre- and post-competition resting values. Our results confirm previous findings that competi-

tion increases heart rate and decreases parasympathetic nervous system activity (e.g. heart rate variability) (Costa and Salvador, 2012; Harrison et al., 2001; Ricarte et al., 2001; van der Meij et al., 2010; Veldhuijzen Van Zanten et al., 2002)

In overall, T levels did not increase during the competitive situation. Although not in line with the challenge hypothesis, several previous studies yielded similar results (Filaire et al., 2001; Gonzalez-Bono et al., 2000, 1999; Mazur et al., 1997; Salvador et al., 1987, 2003; Schultheiss et al., 1999; Schultheiss and Rohde, 2002; Serrano et al., 2000) . It is possible that T levels elevated before the first measurement (anticipatory rise) (Booth et al., 1989; Mazur et al., 1997; Suay et al., 1999) , making it difficult to observe additional increases. Alternatively, the lack of T change may be attributed to the absence of physical extortion. Archer's (2006) meta-analysis indicated that studies involving physical competition caused more accentuated rises in T levels than laboratory studies. In line with this assumption, fatigue might have a moderating role in the changes of T levels (Edwards, 2006) .

3.4.2 *Effect of reward conditions*

Subjective arousal and cardiac effort was larger in the beginning of the competition in the unequally rewarded group. This finding supports previous observations about the arousal facilitating effects of reward, and suggests that participants were aware of the difference in reward conditions and were more aroused by the thought of higher prospective reward (Richter and Gendolla, 2007, 2009) . Notwithstanding, the reward conditions caused no clear differences in T levels during the competition. The only previous study on T and reward found that T response is more pronounced when the reward is higher in a competition (McCaul et al., 1992) . Our results did not support this finding.

3.4.3 *Effect of competitive outcome*

Winners reported better mood than losers at the end of the competition. Nonetheless, post-competition T levels were similar in winners and losers. This finding is not unprecedented as several other studies were not able to show T differences between winners and losers (Gonzalez-Bono et al., 1999; Mazur et al., 1997; Salvador et al., 1987; Serrano et al., 2000; Suay et al., 1999; van der Meij et al., 2010) . Nevertheless, competitive outcome did play a role in determining T response in interaction with hypercompetitiveness. Hypercomompetitive participants showed a smaller decrease or even an increase to losing than less hypercompetitive losers and winners. Mehta and Josephs (2006) have found that T increase after losing a competition

was related to a desire for a rematch. They concluded that those losers who showed T increase might have tried to regain status with a rematch, while those losers with T decrease wanted to withdraw from further stats loss. A recent study proposes, that in unstable hierarchies, the reaction of winners and losers might be the opposite of what the biosocial status theory suggests (Zilioli et al., 2014) . This "status instability hypothesis" builds on the connection between T and risk taking behavior (Stanton et al., 2011) . A competitor with elevated T level might be more willing to take another challenge to boost status, while a competitor with diminished T level is more likely avoid further challenge to consolidate status (Mehta and Josephs, 2006) .

Hypercompetitive individuals were shown to seek higher status, and may ignore status hierarchies (e.g. difficulty to accept losing) (Ryckman et al., 1994) . These attributes can enhance the perception of unstable hierarchy, resulting in T increases, as suggested by the status instability hypothesis. In line with these assumptions, the present study found higher T levels in hypercompetitive losers – compared to less hypercompetitive losers and winners.

Cortisol showed a normal diurnal decreasing trend through the competition, suggesting that participants were not distressed by the competition in general (Smyth et al., 1997) . Baseline and post-competition C levels were higher in the unequal rewarded losers than in the other subgroups. In other words, reward condition and competitive outcome jointly predicted C levels. As this difference was already present before competition, it should be regarded as an anticipatory reaction to the upcoming challenge (Alix-Sy et al., 2008) . Unequal reward condition might have posed a bigger competitive pressure, as indicated by higher pre-competition subjective arousal, heart rate, and decreased parasympathetic activity in this group, compared to the equal reward condition group.

3.4.4 *Competitive performance and hormonal levels*

Although the biosocial theory of status relies on the assumption that higher T level increases competitive success, only a handful of earlier studies attempted to use objective performance markers while assessing T levels in face-to-face competition (Gladue et al., 1989; Mazur et al., 1992; van Anders and Watson, 2007) . Addressing this research gap, the present study operationalized competitive performance as final score and score difference at the end of the game. Results yielded that winners with higher T levels (both pre- and post-competition) reached higher score difference during the game. In other words high T winners were more likely to achieve a decisive victory instead of a close one. Furthermore, score difference was negatively correlated to loser's T change. This result corresponds to earlier suggestions that

the quality of the winning or losing (i.e. decisive or close) can moderate T change (Gladue et al., 1989; Mazur and Lamb, 1980) . These results are in line with the previously mentioned status instability hypothesis, as a close defeat can be perceived as an unstable hierarchy can be associated with T increase (Zilioli and Watson, 2014) .

The dose–response relation of the activity of the stress system (represented by C level) and performance ability is suggested to follow an inverted u-shaped curve (Chrousos, 1997) . Too low or too high stress level can lead to underachievement, while the optimal level of stress can facilitate performance. It has been speculated that performance might be related to stress in a similar way as arousal – as the Yerkes-Dodson Law suggests (Yerkes and Dodson, 1908) , which states the relationship between arousal and performance follows an inverted u-shaped curve. Individual differences can determine the optimal level of stress, that might be rooted in stress resiliency (Meaney et al., 1993) . Optimal performance experience – aka psychological flow – has been reported to be associated with cortisol levels, following an inverted u-shaped curve during performance tasks (Peifer et al., 2014) . It is important to note that participants did not feel distressed during the competition that is detailed here – according to self-reported valence –, therefore only one leg of this inverted u-shaped association might be observable in the present study, ultimately resulting in a linear relationships between cortisol and performance.

Salvador and Costa (2009) suggested, that along with cortisol, other physiological responses can also conjointly determine performance. They predicted that a physiological response pattern that resembles passive coping (Koolhaas et al., 1999) can impede performance and might contribute to losing. This response is characterized by increased C and parasympathetic activation, decreased T, and negative mood (Salvador and Costa, 2009) . In the present study, participants were not distressed by the competition, as they did not report negative feelings, and they did not show cortisol increase during the competition. Thus the model of Salvador and Costa cannot be fully supported or refuted.

3.4.5 *Limitations*

The findings of the present study should be handled with caution because of a number of limitations. Some variables were not experimentally manipulated (e.g. competitive outcome, competitive performance), preventing to draw causal relationships. Furthermore, although we have conducted an a priori power analysis, it is possible that the study lacked power to show the psychophysiological and hormonal effects of competition. Also, the difference between winners' and losers' monetary reward might have been too small to elicit clear differences in psychophysiological variables. Additionally, one might

argue that the aggressive content in the competitive game could have influenced T levels. However, a meta-analysis of several studies have found no clear connection between T level and aggression in humans (Archer, 2006) . Furthermore, a recent study has shown – using a relatively large sample (N = 237) – that aggressive content (boxing) in a computer game did not cause higher T level than a non-aggressive video game (volleyball) (Carré et al., 2013) .

3.4.6 *Conclusion*

To our best knowledge, this is the first study to investigate the effects of reward conditions on testosterone and cortisol levels in a skill and effort based, face-to-face, laboratory competition. A major accomplishment of this study was to elucidate the moderating role of competitive attitudes in hormonal reactions to competition. Findings regarding cortisol suggest that anticipatory response might contribute to losing under bigger competitive pressure. Furthermore, the objective assessment of performance supported the claim that hormonal levels and the competitive performance can be in a reciprocal relationship. In particular, our results showed a positive association between testosterone and cortisol levels with performance in winners, while competitive performance was linked to testosterone and cortisol changes in losers. Our findings support the status instability hypothesis, demonstrating that when the winner's status is unstable – either because of the hypercompetitive attitude of the loser or because of the closeness of the match – the loser can show elevated testosterone level.

4

THE PURSUIT OF EUSTRESS: META-ANALYSIS OF THE EFFECTS OF VIDEO GAMING ON CORTISOL LEVEL

Background: Only a fraction of the thousands of stress studies investigated eustress – a challenging experience that is associated with positive emotions. Moreover, the results of these studies have not been summarized in a meta-analysis yet. Video gaming is considered to be a form of eustress, and its sedentary nature makes it suitable for investigating the effects of eustress on the HPA system. **Method**:Studies were collected that measured the effects of video gaming on cortisol level. Literature search was conducted on online databases, and by screening article references and contacting researchers. Cortisol levels at different time points were extracted to calculate effect sizes. Time, methodological parameters, participant, and game characteristics were extracted to be assessed as moderators. **Results**: Twenty-four studies were included in the analysis. A multi-level mixed-effects meta-analysis revealed that during video gaming, cortisol levels decreased over time ($g = -0.53$, 95% CI $[-0.68, -0.38]$, $p < .001$). Moreover, studies conducted in the morning were more likely to find cortisol increases than afternoon studies. Individual characteristics, game attributes, violence, and competition in the game did not predict effect size. **Conclusion**: Findings suggest that video gaming did not activate the HPA axis, and cortisol level followed the normal diurnal decline. Violence level of the games or competitiveness of the gameplay did not change this pattern. Results indicate that eustress does not elicit the same HPA reaction as distress. To our best knowledge, this is the first meta-analysis to investigate psychoendocrine effects of eustress. [1]

4.1 INTRODUCTION

Selye regarded the stress response generic, and dismissed the idea of response specificity. Nevertheless, he distinguished distress from eustress[2], but believed that the physiological responses would be uniform. In other words he predicted that cortisol level would increase similarly to an equally intense eustress and distress (Selye, 1975). As an early critic of Selye, Mason advocated that psychological factors have a decisive role in physiological stress reaction. He suggested that emotional distress is the main reason for the activation of the HPA axis (Mason, 1968). To summarize findings about distress, several excellent meta-analyses were conducted. They found that unpleasant situations, like the loss of control, performance pressure, and social threat were consistently associated with cortisol increases (Denson

1 This chapter has not been published yet.

2 Eustress was defined as a "positive psychological response to a stressor, as indicated by the presence of positive psychological states" (Simmons and Nelson, 2001, p. 9).

et al., 2009; Dickerson and Kemeny, 2004). On the other hand, there has been little research on the physiological correlates of eustress, and there are no meta-analyses to summarize findings.

Eustress can develop if a stressor is perceived as a challenge and the individual has the abilities to overcome the demands (McGowan et al., 2006). In these situations, the activity can become a source of positive feelings (Lazarus, 1993). Similarly to distress, some activities should be more effective in eliciting eustress than others. For example in situations that are more easily perceived as challenges, and where the demands of the task match the abilities of the individual. One branch of eustress studies focuses on the individual differences in the perception of stressors, such as high-demand work environments (Gibbons et al., 2008; McGowan et al., 2006; Simmons et al., 2003; Simmons and Nelson, 2001). It was found that personality characteristics as hope and optimism can predict work eustress in nurses (Simmons et al., 2003; Simmons and Nelson, 2001). Others found that task focused coping was correlated with eustress experiences during work (McGowan et al., 2006).

A different branch of research that might help to understand eustress concerns a related concept, the "flow experience" (Hargrove et al., 2013). Flow is defined as a mental state associated with immersion in a high performance, joyful activity, and constantly maintained energized focus (Csikszentmihalyi, 1990). Similarly to eustress, flow requires a match between task demand and personal skill. A study that examined psychophysiological reactions to flow found that sympathetic arousal and production of cortisol are in an inverted U shape relationship with subjective flow, i.e. the formation of flow experience requires some, but not too much sympathetic or HPA activity (Peifer et al., 2014).

Another problem in the research of eustress is the lack of standard situations and research paradigms that elicit eustress. Previous research used dancing, yoga, video watching, and video gaming to elicit eustress (Berk et al., 1989; Buchanan et al., 1999; Peifer, 2012; West et al., 2004). These situations differ in several regards, such as the level of required physical activity, controllability, and the ability to elicit immersion. Not surprisingly, these interventions yielded rather mixed results, with decreasing, increasing, and unchanging cortisol levels.

We propose that video gaming could be used as a task that invokes eustress based of two reasons. First, video games are purposefully designed to match demand to skill in order to make players feel control, which should elicit eustress in a performance task (Sherry, 2004). Second, as video gaming is sedentary, physiological data would not be confounded by physical activity (Skoluda et al., 2015). Consequently, video gaming might be a good candidate as a model situation for eustress.

There are some factors that were suggested to disrupt the potential of video games to elicit eustress. One such factor is media violence. Some studies suggested that violent content is associated with increased arousal that might alter psychological and physiological reactions to video gaming (Anderson et al., 2010). Another potential confounder is competition, that was suggested to enhance negative emotional reactions in players that might alter HPA reactions (Adachi and Willoughby, 2011). Indeed, competition was found to affect cortisol and other hormonal levels outside of the video gaming context (see Salvador and Costa, 2009), that should be taken into consideration. Taken together, prior research on the effects of video games on cortisol level should be evaluated, as findings are not evident. Therefore a meta-analysis is needed to summarize findings and identify potential confounders.

The purpose of the present study is to investigate the effects of eustress on HPA activity. In order to fulfill this aim, we collected data on cortisol changes over time from studies that exposed participants to video gaming. To examine moderator effects, data was extracted about study characteristics, participant attributes, and the games and gaming contexts that were used in the studies. Based on previous research on stress, we hypothesized that cortisol level will not increase in response to video gaming; instead, it will exhibit a gradual decrease over time. Previous research have shown that session timing can affect cortisol levels, thus we expected morning studies to yield larger effect sizes than afternoon studies. To control individual differences, we included sex and age as predictors. Although prior findings are not consistent, some studies suggest sex and age differences in cortisol response (Takai et al., 2007; van Stegeren et al., 2008; Yim et al., 2010). Moreover, different game preferences of males and females may cause dissimilar engagement levels, and ultimately different physiological response (Lucas and Sherry, 2004). Based on previous research differences in game characteristics were not expected to affect cortisol changes. The level of violence in the game was not proven to be stressful in previous research (Ivarsson et al., 2009; Maass et al., 2010b,a), therefore we did not expect this factor to be a significant predictor of cortisol change. Based on previous research, we hypothesized that competitive gameplay will be more stressful – therefore eliciting increases in cortisol – compared to non-competitive gaming (Salvador and Costa, 2009).

4.2 METHODS

4.2.1 *Data sources and search strategy*

Literature search was conducted on major online databases (PsychNet, Science Direct, PubMed, JSTOR, Ebsco, ProQuest Dissertations

and Theses database) were searched for studies published until January of 2015. Keywords were "video game", "television game", "computer game", and "cortisol". References of included articles were also screened for additional studies, and corresponding authors of all articles were contacted about unknown studies.

4.2.2 *Selection criteria*

Study attributes

Experimental studies were considered for inclusion that used video games and assessed cortisol at least two times: before video gaming and at a later time point. Studies that used other experimental manipulations (e.g. distressing task, orally administered cortisol, etc.), along with video gaming were considered, but only those subgroups and time points were included that could not have been affected by additional manipulations. Peer-reviewed journal articles, book chapters, and accepted theses and dissertations were eligible for inclusion. In case of a double publication of the same study (e.g. publication of a thesis research in a journal) we only included data from the latest source.

Participant characteristics

No restrictions were made for study participants' sex and age. We calculated effect sizes for males and females separately – when possible – and handled these gender groups as subsets of the study. Because the majority of the studies involved adult participants, and there was only one late adolescent sample (mean age = 17 years), we used two age categories: children between $6-13$ years, and adults above 17 years.

Video games

We used the Merriam-Webster Dictionary's definition of video game: "an electronic game played by means of images on a video screen and often emphasizing fast action" (Merriam-Webster, 2015).We included electronic games regardless of the device the games can be played on – i.e. console games and computer games were both included. Exergames[3] were not included as they involve physical movement that can confound cortisol measurement (Skoluda et al., 2015). Similarly, computerized versions of regular games (e.g. chess, scrabble, quiz) were not included as this analysis was intended to investigate the effects of video games that fit the previous definition – therefore involved fast action. Further, we only included studies that used video

3 The "exergame" term refers to video games that involve elements of exercise and physical movement, and aim to induce physical exertion (Mellecker et al., 2013).

games that were designed for entertainment purposes, and have been marketed commercially. We excluded studies that used computerized laboratory tasks (e.g. ultimatum games, game-like mental workload tasks, social exclusion simulations, etc.), as these are not meant for entertainment purposes.

It was also categorized if a game was violent or not, based on the categorization of the Electronic Entertainment Rating Board's content descriptors (ESRB, 2015). A game was considered violent if the 'violence' descriptor was present in the games summary. If a game was described as containing 'mild violence' (e.g. pac-man), it was not considered violent. Some studies used competitive settings, when the participants had to compete against each other or a confederate. This information was also extracted to use as a moderator.

4.2.3 *Data extraction*

Data extraction was performed by two independent researchers, and disagreements were later resolved by consensus. Number, age group, and sex of the study participants, length of gameplay, timing of the experiment (AM vs. PM), video game name and type, cortisol sampling method (saliva vs. plasma), and cortisol sampling times were extracted.

The outcome variable was the cortisol response at different time intervals. Studies varied in the number of cortisol assessments, and the time points of the samplings. Effect sizes were calculated based on the difference between baseline measurements and later time points (see details below).

Data extraction method was also coded by two independent researchers, and considered to be precise if effect sizes could be calculated using exact data (e.g. means and standard deviations). Data extraction was considered imprecise if data was based on estimations (e.g. read from figures or using estimated standard deviations). The method of data extraction was used as a moderator in later analyses.

4.2.4 *Calculating effect sizes*

To calculate the within-subject effect of the change in cortisol level from baseline to later time points, we used Equation 4.1, presented by Morris and DeShon (2002). The effect size is corrected for dependence by using a correlation between repeated samples. This term was estimated as 0.7 from studies that reported correlations of multiple cortisol measurements. Note that this value represents a conservative approach, i.e. results in smaller absolute effect size.

$$d = \frac{\overline{X}_{post} - \overline{X}_{pre}}{\frac{SD_{post}+SD_{pre}}{2}} \cdot \frac{1}{\sqrt{2(1-r)}} \tag{4.1}$$

Equation 4.1: $\overline{X}_{post}$ and $\overline{X}_{pre}$ represent post and pre task means, and SD_{post} and SD_{pre} the standard deviations, respectively. A correction term (r) was used to account for the repeated measurement, that represents the correlation between the repeated measurements (Morris and DeShon, 2002).

The within-subject d value was converted to sample size corrected Hedges g using Equation 4.2 (Morris and DeShon, 2002).

$$g = d\left(1 - \frac{3}{4N-9}\right) \tag{4.2}$$

Equation 4.2: d is the within-subject Cohen's d effect size, and N is the sample size. The effect size is positive if there was a cortisol increase compared to the baseline, and negative if there was a decrease.

For calculating the variance of Hedges' g, we used Equation 4.3 (Morris, 2000).

$$var_g = \frac{g^2}{2(N-1)} + \frac{2(1-r)}{N} \tag{4.3}$$

Equation 4.3: g is the sample size corrected Hedges' g effect size, N is the sample size, and r represents the correlation between the repeated measurements.

4.2.5 *Data analysis*

The multi-level approach was used to analyze the effect sizes. This method accounts for the clustering in the data and allows studies to contribute multiple effect sizes, which maximizes the information that can be obtained from each study. As a further advantage, multilevel models treat the individual studies as random effects, permitting better generalization of the findings to the population than fixed effect models (Hox and Leeuw, 2003). Data analysis was conducted using R 3.2.0 (R Core Team, 2014), with the metafor package providing functions for conducting meta-analysis (Viechtbauer, 2010).

4.3 RESULTS

4.3.1 *Study selection process*

Three data sources were used to obtain studies. Searches in electronic databases yielded 727 results, 14 studies were identified from references, and one study was suggested by an author of a relevant article. From these sources, 52 studies were selected for full text review. 28 of these studies were excluded based on selection criteria (detailed in the methods section). In the end of the selection process, twenty-four studies were included in the meta-analysis. Figure 4.1 shows the flow diagram of the study selection process.

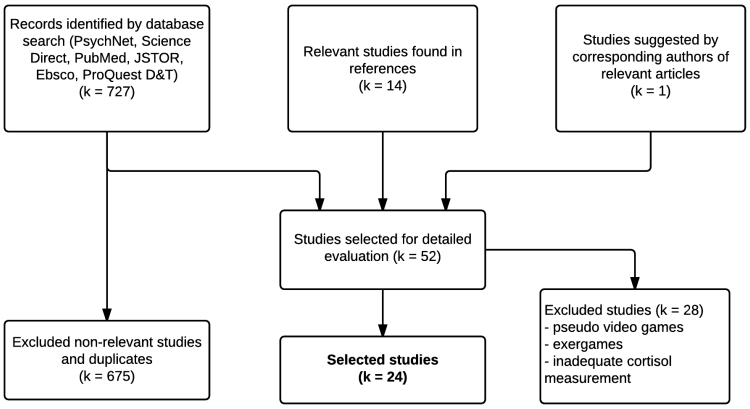

Figure 4.1: **Flow diagram of the study selection process**

4.3.2 *Publication bias*

Publication bias was investigated by examination of the funnel plot and asymmetry tests. The funnel plot was generated using the average effect sizes of the studies, because methods that investigate publication bias operate on the study level. Neither visual inspection, nor the rank correlation, Kendall's $\tau = -0.18$, $p = 0.227$ (Begg and Mazumdar, 1994) and the Egger regression test, $z = -0.90$, $p = 0.367$ (Sterne et al., 2001) indicated asymmetry in the funnel plot (see Figure 4.2). Moreover, the Duval & Tweedie trim and fill method did not predict any missing studies (Duval and Tweedie, 2000). These results suggest that the collected studies were not significantly affected by publication bias.

4.3.3 *Study characteristics*

In total, 1276 individuals participated in 47 subsamples in the 24 studies included in the meta-analysis. The average number of effect sizes

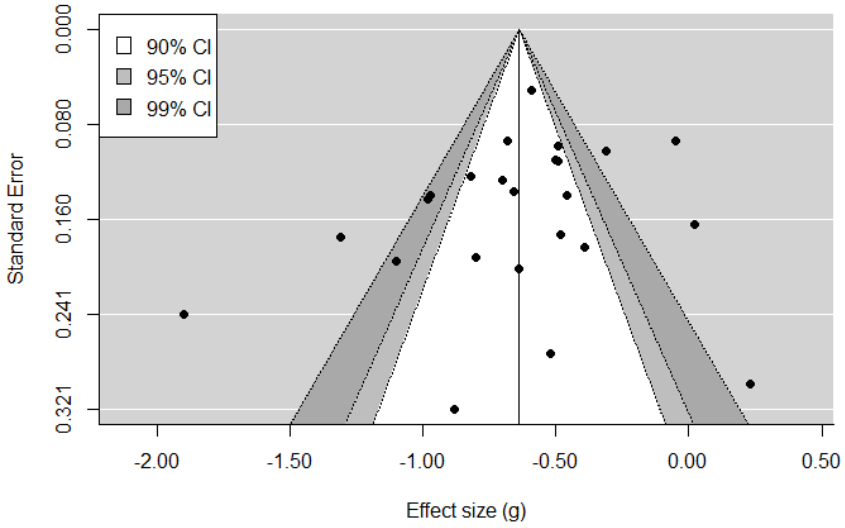

Figure 4.2: **Funnel plot showing the mean effect sizes (g) of the studies against standard errors.**

per study was 4.3 ($sd = 4.0$, range: $1 - 16$). There were 30 subsamples with only males ($N = 685$), and 5 subsamples with only females ($N = 107$), while 11 subsamples contained both males and females ($N = 484$). 19 studies (40 subsamples) were investigating adolescents and adults (between $17 - 27$ years of age, weighted mean = 21.4 years), while 6 studies (7 subsamples) examined children (between 6 and 13 years of age, weighted mean = 10.8 years).

The majority of studies ($k = 19$) assessed salivary cortisol, and 5 assessed plasma cortisol. The median number of saliva samples per study was 3 (range: $2 - 13$). The average sampling time was at 43 min ($sd = 28$ min) after stressor onset (not counting the baseline assessment; range: $8 - 120$ min). Four studies were conducted in the morning, and seventeen studies were conducted in the afternoon; three studies did not report when the study sessions were held.

We categorized video games into four ad hoc categories. Arcade games (e.g. Pong, Pac-man, Mutant storm), first person shooter games (FPS; e.g. Unreal tournament, Killzone, Quake III Arena), Tetris (as this was the most frequently used single game), and other games that did not fit previous categories (e.g. Fifa 09, Manhunt, Mario Kart). In four studies, it was either not possible to acquire which game was used or several games were used in the same subsamples. The average length of the gameplay was 32 min ($sd = 32$ min, range: $10 - 120$ min). In 11 studies, participants had to compete with each other, and in 13 studies, they played individually. 13 studies used games that did not contain violence, six studies used violent games, and four either did not report the game, or used multiple, dissimi-

Table 4.1: Characteristics of the included studies.

Study	Average g	No. of gs	N	Sex	Age group	Cortisol sample	Session timing	Game type, Competition, Violence	Game length (min)
Abdulla et al., 1985	-0.88	10	10	M	Adult	Plasma	AM	Arcade, C, NV	60
Hubert & de Jong-Meyer, 1992	-1.31	16	48	-	Adult	Saliva	PM	-, C, -	20
Mazur et al., 1997	-0.50	6	60	M, F	Adult	Saliva	PM	Arcade, C, NV	15
Denot-Ledunois et al., 1998	-0.52	2	10	-	Children	Saliva	PM	Tetris, NC, NV	42
Skosnik et al., 2000	-0.39	2	20	-	Adult	Saliva	PM	FPS, NC, V	15
Kapuku et al., 2002	-0.48	1	24	M	Adult	Plasma	-	Arcade, NC, NV	10
Fulgham, 2003	-0.46	1	36	M	Children	Saliva	PM	Other, C, NV	20
Hebert et al., 2005	-0.70	6	52	M	Adult	Saliva	PM	FPS, NC, V	10
Sharma et al., 2006	-0.64	1	20	-	Adult	Plasma	PM	Other, NC, -	60
Ivarsson et al., 2009	-1.90	2	42	M	Children	Saliva	PM	Other, NC, V, NV	120
Steptoe et al, 2009	-0.59	2	300	-	Children	Saliva	PM	Arcade, NC, NV	10
Beaven et al., 2010	0.23	12	7	M	Adult	Saliva	AM	FPS, C, V	10
Maass et al., 2010	-0.80	2	25	-	Children	Saliva	PM	FPS, NC, V	45
Oxford et al., 2010	-0.49	8	74	M	Adult	Saliva	PM	FPS, C, V	120
Oxford, 2010	-0.68	5	94	M	Adult	Saliva	PM	FPS, C, V	25
Auer, 2011	-0.66	8	44	M, F	Adult	Saliva	-	Arcade, C, NV	20
Chaput & Visby, 2011	0.02	6	22	M	Adult	Plasma	AM	Other, NC, NV	60
Keller et al., 2011	-0.82	3	61	M	Adult	Saliva	PM	Tetris, NC, NV	15
Mohan et al., 2011	-1.10	2	32	M	Adult	Plasma	PM	Other, NC, -	20
Singh et al., 2012	-0.05	2	68	M	Adult	Saliva	AM	Other, NC, -	10
Zilioli & Watson, 2012	-0.49	2	70	M	Adult	Saliva	PM	Tetris, NC, NV	15
Zilioli et al., 2013	-0.97	2	55	M	Adult	Saliva	PM	Tetris, NC, NV	15
Peifer et al., 2015	-0.31	1	61	-	Adult	Saliva	PM	Arcade, NC, NV	25
Zilioli & Watson, 2014	-0.98	2	53	F	Adult	Saliva	PM	Tetris, NC, NV	15

Note: Of the twenty-four included studies, there were twenty peer-reviewed journal articles, three theses, and one book chapter. Abbreviations: M: males, F: female, C: competitive, NC: non-competitive, V: violent, NV: non-violent. Study references are abbreviated. Dash in the Sex column means that it was not possible to create a distinct male or female subsample.Study references in the table are abbreviated, the full references are: Abdulla et al. (1985); Hubert and de Jong-Meyer (1992); Mazur et al. (1997); Denot-Ledunois et al. (1998); Skosnik et al. (2000); Kapuku et al. (2002); Fulgham (2003); Hébert et al. (2005); Sharma et al. (2006); Ivarsson et al. (2009); Steptoe et al. (2009); Beaven et al. (2010); Maass et al. (2010b); Oxford et al. (2010); Oxford (2010); Auer (2011); Chaput et al. (2011); Keller et al. (2011); Mohan et al. (2011); Singh et al. (2012); Zilioli and Watson (2012, 2013); Zilioli et al. (2014); Peifer et al. (2015)

larly violent games in the same subsets. One study used violent and non-violent games in different subsamples. Table 4.1 summarizes the study characteristics.

4.3.4 *Overall effect of video gaming on cortisol level*

The average effect size across all assessments in all studies was -0.53 ($se = 0.08$), which was significantly different from zero, $z(103) = -7.03, 95\%$ CI $[-0.68, -0.38]$, $p < .001$. This means that during video gaming, cortisol level decreased in average by $-.53$ standard deviations below baseline values. The test of heterogeneity was also significant $Q(103) = 557.92$, $p < .001$, suggesting a variance in cortisol changes that could be predicted by other factors.

4.3.5 *Moderator analyses*

Several moderator effects were tested, including within-study effects (linear and quadratic effects of sample timing), study characteristics (data collection method, session timing of the study sessions, sample

type), participant characteristics (sex, age group), game characteristics (type, length, violence, competition). Table 4.2 shows the results of moderator analyses.

Table 4.2: Results of linear mixed-effects moderator analyses.

Predictor	**b**	**SE**	**95% CI**	**z**	**p**
Within-study effects					
Time	-0.0101	0.0013	-0.0126; -0.0075	-7.79	<.001
Time^2	-0.0001	<0.0001	-0.0001; -0.0000	-5.61	<.001
Study characteristics					
Data extraction (imprecise vs. precise)	0.0501	0.1649	-0.2730; 0.3732	0.304	0.761
Session timing (AM vs. PM)	-0.6239	0.1483	-0.9145; -0.3333	-4.21	<.001
Sample (plasma vs. saliva)	-0.1839	0.1917	-0.5596; 0.1918	-0.96	0.337
Participant characteristics					
Sex (female vs. male)	-0.0154	0.1037	-0.2187; 0.1879	-0.15	0.882
Age group (adult vs. children)	-0.2864	0.1876	-0.6541; 0.0812	-1.53	0.127
Game characteristics					
Game type (arcade vs. Tetris)[a]	-0.2622	0.2295	-0.7121; 0.1877	-1.14	0.253
Game type (arcade vs. FPS)	0.0425	0.2144	-0.3778; 0.4628	0.20	0.843
Game type (arcade vs. other)	-0.2013	0.2711	-0.7327; 0.3300	-0.74	0.458
Game length	-0.0029	0.0024	-0.0076; 0.0018	-1.20	0.229
Violence (non-violent vs. violent)	0.1112	0.1702	-0.2224; 0.4448	0.65	0.514
Competition (competitive vs. non-competitive)	0.0107	0.1558	-0.2946; 0.3160	0.07	0.945

Note: Study was used as a random effect in all analyses to account for the multiple effect sizes associated with individual studies. Predictors were added separately, and restricted likelihood (REML) method was used to estimate fixed effects. Estimates are expressed as differences from the intercept; in categorical variables the first category represents the intercept. [a]: game type remained non-significant predictor when used different game types as default level.

Effects of sampling time

We tested whether the timing of cortisol assessment predicted differences in effect sizes. Sampling time was a significant predictor of cortisol change. According to the significant linear effect of time, assessments obtained later in time yielded lower cortisol values. Moreover, the quadratic effect of time (time^2) was also a significant, suggesting that cortisol decrease showed a local negative peak between $30-45$ minutes (see Figure 4.3). A further plot in Appendix C shows the effect sizes against in each study separately.

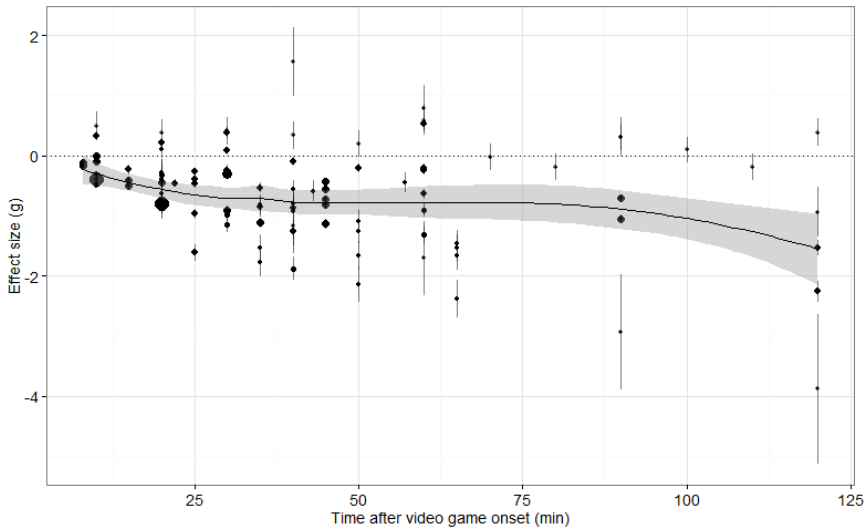

Figure 4.3: **Effect sizes against sampling times.**The solid line represents a smoothed spline function weighed by study size that predicts the average effect over time. Point size corresponds to base 10 logarithm of the sample size. Error bars represent 95% CI. Gray area represents the 95% CI of the function.

Study characteristics

Some studies contained only incomplete statistics, and effect sizes were estimated based on figures or pooled statistics. However, precise effect sizes were not significantly different from imprecise estimations. Some studies used salivary while others plasma cortisol. Effect sizes were not affected by the difference in the sampling method. There was a difference according to the timing of the study sessions. Those studies that were conducted in the morning produced higher effect sizes than those that were conducted in the afternoon.

Participant characteristics

Subsamples that contained only females did not yield significantly different effect sizes from those that were conducted with only male subsamples. Similarly, studies that investigated children provided similar effect sizes to the adult studies.

Game characteristics

The games were categorized into four categories by genre. These game categories were associated with similar effect sizes, so there was no game category that consistently elicited higher or lower cortisol levels. The length of the game was not a significant predictor of the changes of effect size.

The games were also categorized by the level of violence; however the moderator analysis did not find a significant dissimilarity in the

effect sizes associated with violent games, compared to non-violent games. Moreover, some studies used competitive settings where participants were competing against each other. This study setting did not yield different effect sizes than the studies that utilized individual gameplay.

4.3.6 *Model comparison*

Individually significant predictors (linear time, quadratic time, and the timing of the study sessions) were included in a common model additively. As the comparison statistics show in Table 4.3, all three predictors remained significant in the common model, showing that each of them can explain unique variance. Moreover, the most complex model had the best model fit, i.e. the lowest AICc and BIC values.

Table 4.3: Comparisons of models containing significant fixed effects

Model	**df**	**AICc**	**BIC**	**logLik**	**p**
Null model	2	230.0	234.9	-112.9	
Time	3	178.5	185.8	-86.1	<.001
Time + Time^2	4	154.0	163.6	-72.8	<.001
Time + Time^2 + Session timing	5	146.3	158.2	-67.8	.002

Note: Comparisons were made between the null model that contains only the random effect of study, and subsequent models. Models were recalculated using the maximum likelihood (ML) method for model comparison. AICc: Akaike Information Criterion corrected for small sample sizes, BIC: Bayesian Information Criterion, logLik: log likelihood statistic of the model comparison

4.4 DISCUSSION

yielded lower levels of cortisol in general. However, cortisol seemed to show a local peek between 45 and 60 minutes. Moreover, studies conducted in the morning were more likely to find cortisol increases than afternoon studies. Study participants' age group and sex were not associated with cortisol changes. Similarly, the length and type of the game or the level of violence in the game did not affect cortisol responses. Despite our expectations, cortisol level was not increased in competitive gameplay, compared to solitary gameplay.

Cortisol is not just a stress hormone, but also an important metabolic hormone that regulates energy distribution and consumption (Lovallo and Thomas, 2000). Under natural, unstimulated conditions, the secretion of cortisol follows a circadian rhythm. After awakening, cortisol level increases to the daily maximum, followed by declining concentrations throughout the day, and lowest levels in the late night hours (Smyth et al., 1997). This diurnal cycle is the

most parsimonious explanation for the decrease of cortisol to video gaming.

Besides the linear decrease, cortisol levels also showed a significant quadratic trend over time, driven by a local negative peak at $30-45$ minutes, and a relatively higher cortisol level between 45 and 60 minutes (see Figure 4.4). Cortisol has been known to exhibit response latency (Kirschbaum and Hellhammer, 1994), and it is possible that this cortisol pattern is a manifestation of a delayed HPA activity increase at 45-60 minutes, superimposed on the normative diurnal decrease. The other speculative possibility is that video gaming inhibited the activation of the HPA axis at $30-45$ minutes more effectively. Nevertheless, it is difficult to support these assumptions as very few of the studies used a control condition; therefore causal interpretations are limited.

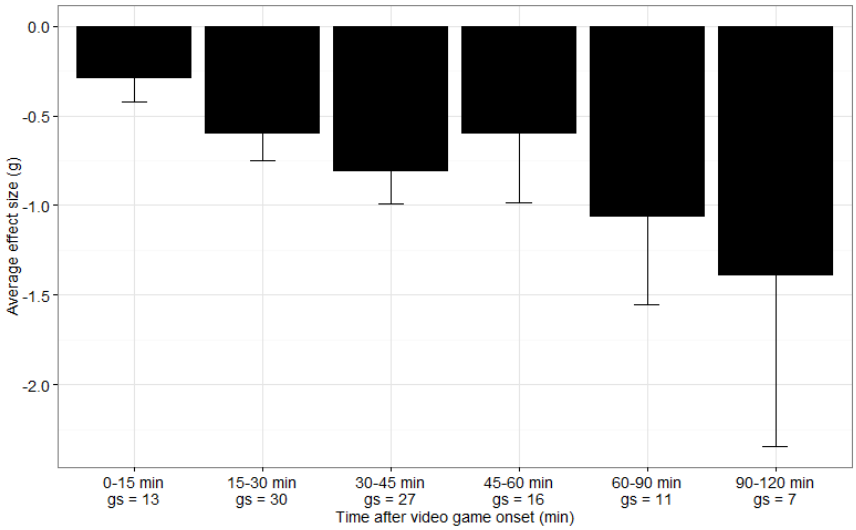

Figure 4.4: **Means of the effects sizes calculated for six time intervals, weighted by study sample size.**The numbers of effect sizes that were used to calculate means are reported on the x axis. Error bars represent 95% CI.

Studies that assessed autonomic nervous system activity such as electrodermal response, heart rate, heart rate variability, and blood pressure reported increased physiological arousal during video gaming (e.g. Anderson et al., 2010; Kapuku et al., 2002; Keller et al., 2011; Maass et al., 2010b; Singh et al., 2012). These findings show an increased sympathetic, and a decreased parasympathetic activity that resembles the fight-or-flight response pattern (Koolhaas et al., 1999). On the other hand, the present meta-analysis confirmed that video gaming does not increase HPA activity. Therefore video games show the same response pattern that was termed as "challenge without distress" (Frankenhaeuser, 1986). Video games are purposefully designed to be entertaining, and this usually means an optimal fit of

skill and demand (Boyle et al., 2011). This optimal fit can result in the feeling of control over the game, and can invoke the experience of eustress. A well known motive for video gaming is the feature of video games to provide the feeling of control for players (Yee, 2006). Consequently, playing video games is often used as a form of emotional coping to recover from stress (Reinecke, 2009). Immersion in a video game also means that the player can temporarily ignore everyday stressors (Hilgard et al., 2013). This might be an indirect way of decreasing stress and HPA activity, i.e. by getting away from stressful stimuli.

Violence level of the video games did not affect cortisol response. It appears that virtual participation in violent events is not as stressful for participants to elicit a significant HPA response. However, the games that were used in the twenty-four included studies obviously do not represent the entirety of video games and findings are limited to games and genres that were used in these studies. It would be interesting to investigate cortisol reactions to games that were designed to be stressful, for example horror games. Research that investigated cortisol reactions to gruesome and highly stressful videos were able to elicit increased cortisol levels (Nejtek, 2002; Takai et al., 2004), but there are no studies currently that measured the effects of video gaming on cortisol response to truly stressful games.

Contrary to the hypothesis, competitive game setting did not elicit increased HPA response compared to the solitary gameplay. One plausible explanation is that players in both conditions were equally stressed or relaxed. It is also possible that the lack of difference can be attributed to the observation that winners and losers can demonstrate a dissimilar cortisol responses in competition (Salvador and Costa, 2009). However, currently there are too few studies to verify the effects of the competitive outcome on cortisol level in response to video game competition.

4.4.1 *Strengths and limitations*

A notable strength of the current meta-analysis is the application of mixed-effects modeling. This method allowed the use of multiple effects sizes per study, and maximized the information that could be obtained from each study. By extracting several variables from the studies, we were able to investigate potential mediator effects. Furthermore, we only included sedentary video games, so the physical movement could not have confounded our results. Moreover, we used studies that were not primed for the investigation of eustress. In fact, some included studies attempted to use video games as sources of stressor tasks, aiming to elicit a HPA axis response (e.g. Hébert et al., 2005; Kapuku et al., 2002; Sharma et al., 2006; Steptoe et al., 2009).

Some limitations of this meta-analysis should also be noted. First, almost none of the studies used control groups when assessing the effects of video gaming on cortisol. This makes causal interpretations difficult, and findings should be regarded as correlational. Moreover, the vast majority of studies used young adult male participants, therefore findings about sex and age may not be generalizable. Most of the included studies reported on laboratory research, where participants were made to play with designated games in a specific timeframe, under a constant surveillance. This unnatural setting might have confounded the results and may limit the ecological validity of the findings.

4.4.2 *Conclusion*

To our knowledge, this is the first meta-analysis to aggregate results about HPA reactions to a commonly used sedentary eustressor. Findings suggest that video gaming did not activate the HPA axis, and cortisol level followed the normal diurnal decline. Moreover, violence level of the games or competitiveness of the gameplay did not change this pattern. Results indicate that eustress elicits a different HPA reaction compared to distress. This means that our meta-analysis supports Mason's hypothesis about stress response specificity, contrary to Selye's hypothesis of general adaptation. On a methodological level, this implies that video gaming should not be used as a stressor in stress-reactivity studies. Further research is needed to be able to generalize the results to other forms of eustress.

5

FREQUENT NIGHTMARES ARE ASSOCIATED WITH BLUNTED CORTISOL AWAKENING RESPONSE IN WOMEN

Background: Nightmares are relatively common sleep complaints that seem to be associated with affective distress. To date, few attempts have been made to link nightmares to the biological markers of the stress response, and the HPA response in particular. **Method**: The present study examined the relationship between frequent nightmares and the cortisol awakening response (CAR) in a cross-sectional study of working women (N = 188). **Results**: Analysis revealed that those who reported frequent nightmares (N = 13) showed a blunted CAR on a working day, compared to those who did not report nightmares. This result was independent of psychiatric symptoms, demographic variables, and lifestyle. **Discussion**: Our preliminary findings suggest that decreased HPA reactivity might be a trait-like feature of women with frequent nightmares.[1]

5.1 INTRODUCTION

Approximately five percent of the adult population suffers regularly from nightmares – vivid and terrifying dreams that lead to abrupt awakenings (Spoormaker et al., 2006). Still, relatively little is known about the pathogenesis of nightmares and its relation to sleep and mental disorders(Levin and Nielsen, 2007). Within the frames of the "continuity hypothesis", Schredl suggested an association between stressful life experiences and nightmares (Schredl, 2003). Whereas, questionnaire-based studies emphasise the relevance of state-like effects (such as increased emotional pressure) leading to frequent nightmares (Schredl, 2003, 2013), theoretical models (Levin and Nielsen, 2007) as well as longitudinal (Van Liempt et al., 2013) and twin studies (Coolidge et al., 2010) point to the influence of trait-like vulnerability factors for the development of frequent nightmares. In their multilevel, integrative model Levin and Nielsen (Levin and Nielsen, 2007) proposed that state-like *affect load* as well as trait-like *affect distress* might contribute to the frequent occurrence of terrifying dream experiences. Both factors seem to be related to increased emotional reactivity underlain by impaired fronto-limbic circuitry (Levin and Nielsen, 2007; Simor et al., 2012b), and presumably abnormal stress responses.

1 This chapter was published previously as: Nagy, T., Salavecz, G., Simor, P., Purebl, G.,Bódizs, R., Dockray, S., Steptoe, A. (2015). Frequent nightmares are associated with blunted cortisol awakening response in women. *Physiology & Behaviour.* doi:10.1016/j.physbeh.2015.05.001

Unfortunately, previous studies have mostly relied on reports of subjective stress, and few nightmare studies used biological stress markers. Nonetheless, indirect evidence suggests an association between the HPA axis activity and nightmares. For instance, some of the brain regions implicated in nightmare formation (Nielsen and Levin, 2007) – the hippocampus, the amygdala and the medial prefrontal cortex in particular – abundantly express glucocorticoid receptors, influence HPA axis activity and regulate stress responses (Herman et al., 2005). Additionally, the cortisol awakening response (CAR) – which refers to the sharp increase in cortisol following awakening and indicates the reactivity of the HPA system – has been negatively correlated with impaired sleep quality (Backhaus et al., 2004). On the other hand, frequent nightmares have also been associated with impaired sleep quality (Spoormaker et al., 2006), reduced sleep efficiency, increased nocturnal awakenings, relatively decreased slow wave sleep (SWS) and increased REM pressure (Simor et al., 2012a). Moreover, several studies have shown that PTSD – a severe psychiatric condition in which affected individuals often experience vivid nightmares – is related to a blunted CAR (Levin and Nielsen, 2007; Rohleder et al., 2004; de Kloet et al., 2007; Wessa et al., 2006).

The above findings led us to the assumption that the altered functioning of the HPA system might contribute to the pathogenesis of frequent nightmares. In particular, in light of the previously reported associations between sleep disturbances, PTSD symptoms and reduced CAR we expected that frequent nightmares would be associated with a blunted CAR. This hypothesis was examined within a non-clinical sample of women who provided seven cortisol samples through a working and a leisure day.

5.2 METHODS

This article reports on analyses performed on the Hungarian subset of the Daytracker Study, an investigation of the relationship between well-being and health in working women. Only women were included in this study, as there are very few studies that address the population of working women (Steptoe et al., 2009). The study was approved by the Research Ethics Committees of Semmelweis University and University College London, and all participants signed an informed consent form. Participants received a small honorarium at the end of the study.

5.2.1 *Participants*

Participants were recruited from full-time female employees of Semmelweis University in Budapest via emails and flyers. The inclusion criteria were explicitly declared during recruitment, thus volunteers

applied only if not 1) pregnant; 2) suffering from acute or chronic illness such as cardiovascular disease, diabetes, cancer, endocrine disorder; 3) diagnosed with mental disorder (e.g. major depression, PTSD, bipolar disorder, etc.); 4) taking steroid, hypertensive or anti-inflammatory medication or beta- blockers. From the initial 202 included participants, we excluded those with missing data on nightmares (N = 6), and morning cortisol level (N = 8). Thus, the final size of the study sample was 188.

5.2.2 *Procedure and assessment*

After a briefing about the study protocol, participants completed two 24h assessments on a working day and on a leisure day. The 24-hour periods started at 17h and ended next day. To avoid any sequence effects, assessment randomly started on a working or a leisure day. During the assessments, participants provided seven saliva samples each day – using Salivettes (Sarstedt, Leicester, UK). Participants were instructed not to eat, drink or brush their teeth until after the 30 min post-waking sample, and 15 minutes before later saliva samplings. Saliva samples were timed immediately at waking, 30 minutes after awakening, at 10h, 12h, 15h, 17h, and at bedtime. Saliva samples were stored in a cold place or refrigerator until they were transported to the university lab within 1-2 days. Subsequently, the samples were kept frozen at $-20°C$ until analysis. Analysis was carried out using a high sensitivity chemiluminescence assay at the Technical University in Dresden (Germany). Inter- and intra-assay coefficients of variance (CVs) were $< 8\%$. CAR was calculated using the area-under-the-curve with respect to ground (AUCG), using the waking and the 30 min post-awakening cortisol values (Chida and Steptoe, 2009). We chose this method as it is relatively robust and after natural log transformation it followed normal distribution (Shapiro-Wilk normality tests: $W = 0.99$, $p = .520$, and $W = 0.99$, $p = .423$ for working and leisure day CAR, respectively).

Standardized survey questions and questionnaires were used to assess demographic data, mental and somatic health, lifestyle, and sleep quality (Haraszti et al., 2014). These factors were used as covariates to exclude known confounders of the CAR (Chida and Steptoe, 2009). Tests relevant to the current study included depressive symptoms (CES-D, Cronbach's $\alpha = .88$), trait anxiety (STAI-T, $\alpha = .92$), perceived health (PHQ-15, $\alpha = .80$), Jenkins sleep problems questionnaire ($\alpha = .75$), and morningness-eveningness scale ($\alpha = .89$). The items of these questionnaires were rated on four- or five-point Likert scales.

Participants also answered single questions – that were dichotomized later – about alcohol consumption (non-drinker/drinker), smoking status (non-smoker/smoker), and number of children. Stress was

assessed using ecological momentary assessment (EMA) (Shiffman et al., 2008), whereby participants were answered the question *"On a scale of 1 to 5, please rate how stressed you are at this moment"* seven times over one workday and leisure day at the same time when saliva samples were taken (see below). Mean stress scores were calculated for each day. Participants were asked if they had nightmares frequently using a single binary question: *"Do you frequently have nightmares that wake you up?"*. This measure has been used commonly in nightmare studies (Levin and Nielsen, 2007). Moreover, participants reported the emotional quality of dreams on days of the measurement using single choice questions (*"How would you describe the emotional quality of your dreams?"*; options: very unpleasant, unpleasant, neutral, pleasant, very pleasant, don't remember).

5.2.3 *Statistical analysis*

Welch's t-tests and chi-square tests were used to compare the characteristics of the nightmare and non-nightmare groups. To investigate the effect of frequent nightmares on CAR, we carried out separate ANCOVAs on working and leisure days controlling for age, BMI, morningness, education, depression, anxiety, physical symptoms, sleep quality, alcohol consumption, smoking, physical exercise, sleeping time. Data analysis was performed with R 3.1.2 (R Core Team, 2014).

5.3 RESULTS

Preliminary analysis showed that participants with frequent nightmares had a significantly lower BMI, were more depressed and anxious, reported more sleep problems and somatic symptoms, and showed a lower morningness score. The frequent nightmare group also reported more stress then participants without frequent nightmares on both days, although this difference was on the threshold of statistical significance. Differences were not significant for sleep duration, demographic, and lifestyle related variables (see Table 5.1).

Figure 5.1 shows the profiles of cortisol output over the work and leisure days. We found typical diurnal profiles, with relatively high cortisol on waking, an increase over the first 30 minutes of the day, and progressive decreases in output across the day. Those participants who experienced nightmares frequently showed a significantly smaller CAR than those who did not experience nightmares frequently $F(1, 186) = 6.98$, $p = .009$, $\eta_p^2 = .04$ (values without covariates). The relationship remained significant after controlling for all covariates ($F(1, 153) = 4.72$, $p = .030$, $\eta_p^2 = .026$), however due to the list-wise deletion of missing values, the sample size changed to 155 (nightmare group = 11, non-nightmare group = 144). Moreover, given that the

Table 5.1: Differences between participants with and without frequent nightmares

	Nightmare group N=13	**Non-nightmare group N=175**	**Statistic**	***p***
Demographic variables and lifestyle				
Age (yrs)	33.8 (11.4)	37.5 (10.5)	t (13.6) = 1.12	.281
BMI (kg/m^2)	21.7 (3.4)	24.3 (4.6)	t (16.2) = 3.13	.006
Has university degree	69.2%	62.1%	$\chi^2 = 0.05$	.828
Has children	23.1%	40.6%	$\chi^2 = 0.91$	.341
Drinks alcohol occasionally	84.6%	87.9%	$\chi^2 = 0.00$	1.000
Smokes	0%	15.9%	$\chi^2 = 1.2$	.270
Morningness score	30.7 (7.9)	37.8 (7.4)	t (13.6) = 3.10	.008
Total exercise (hours per week)	3.2 (2.0)	2.3 (1.5)	t (13.0) = -1.63	.126
Health and mental health				
Depression (CES-D)	22.9 (10.9)	10.7 (8.4)	t (13.0) = -3.94	.002
Trait anxiety (STAI-T)	53.3 (11.8)	39.6 (10.1)	t (13.3) = -4.08	.001
Physical symptoms (PHQ-15)	35.8 (6.1)	24.8 (5.8)	t (12.5) = -6.03	<.001
Sleep problems (Jenkins scale)	20.3 (6.1)	11.9 (5.0)	t (13.3) = -4.86	<.001
Daily activities				
Wake time (working day) h:m	6:05 (1:11)	6:00 (1:00)	t (13.3) = -0.24	.811
Wake time (leisure day) h:m	7:29 (1:13)	7:02 (1:16)	t (14.1) = -1.25	.233
Total sleep duration (working day) h:m	7:02 (1:10)	7:20 (1:08)	t (13.8) = 0.90	.382
Total sleep duration (leisure day) h:m	8:03 (2:06)	8:11 (1:26)	t (12.9) = 0.25	.810
Daily stress (working day)	2.5 (0.9)	1.9 (0.7)	t (13.1) = -2.15	.051
Daily stress (leisure day)	2.1 (0.8)	1.6 (0.6)	t (13.1) = -2.05	.061

inclusion of covariates only controls for linear relationships between the outcome and the predictors, we conducted an additional analysis on a subsample of non-nightmare sufferers matched to the subgroup of nightmare sufferers by age, BMI, sleep quality (Jenkins score), and morningness scores. The difference in CAR between the nightmare group and the matched control was significant on the working day $F(1, 24) = 4.30$, $p = .049$, $\eta_p^2 = .15$ (for details of the matched sample, please refer to the online supplement).

The difference in CAR between the nightmare and non-nightmare group was not significant on the leisure day $F(1, 183) = 0.78$, $p = .379$, $\eta_p^2 = .004$. There were no significant differences between the two groups at the other time points of either day ($ts < 1.53$, $ps > .15$). Moreover, the working day CAR and the leisure day CAR of the nightmare group were not different $t(12) = -0.06$, $p = .952$, while in the non-nightmare group the CAR was significantly higher on the working day than on the leisure day $t(171) = 6.77$, $p < .001$.

Significantly more participants could recall the emotional quality of their dreams in the nightmare group than in the non-nightmare group on the working day (92% vs. 48%, respectively; $\chi^2(1) = 7.71$, $N = 181$, $p = .005$) as well as on the leisure day (84% vs. 51%, respectively;

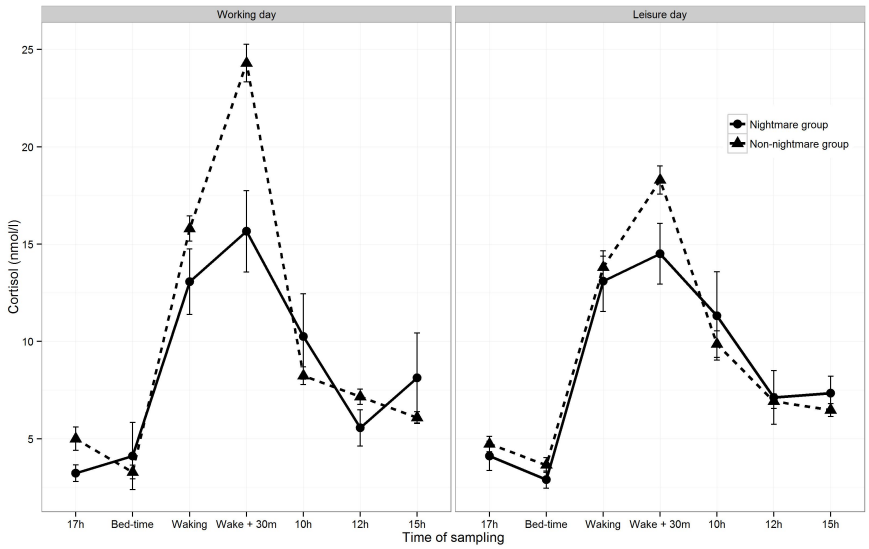

Figure 5.1: **Diurnal cortisol levels of women having frequent nightmares (N=13), and without frequent nightmares (N=175) on a working and a leisure day.** Data points represent untransformed values, error bars represent SEM.

$\chi^2(1) = 4.33$, N = 183, p = .037). The emotional quality of dreams was not significantly different between the groups on either day (working day $\chi^2(4) = 6.45$, N = 95, p = .168; leisure day ($\chi^2(4) = 5.81$, N = 99, p = .214). Figure 5.2 shows the percentage of participants reporting a particular emotional quality of dreams on the two days.

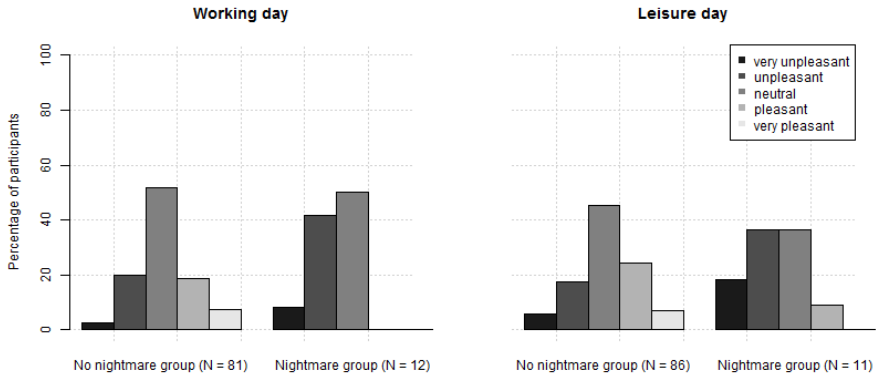

Figure 5.2: **Percentage of participants reporting a particular emotional quality on the days of the assessment.**

The emotional quality of the actual morning's dream report was not associated with CAR (working day: $F(4, 88) = 0.61$, p = .659; leisure day: $F(4, 90) = 1.35$, p = .258). Recall of a bad dream on the day of the measurement added to the model as a dichotomous covariate (0: Neutral or pleasant dream, 1: unpleasant and very unpleasant

dream) was not significant (working day: $F(1,90) = 0.67$, $p = .414$; leisure day: $F(1,92) = 0.04$, $p = .852$).

5.4 DISCUSSION

In a non-clinical sample of working women, we found that frequent nightmares were associated with a blunted CAR on a working day. This finding was independent of age, BMI, education, depression, anxiety, sleep time and quality, chronotype, physical symptoms, and health behaviors (physical activity, smoking, and alcohol consumption). To the best of our knowledge, this is the first study to demonstrate an association between CAR and the condition of experiencing nightmares frequently. In coherence with earlier studies, participants with frequent nightmares rated themselves as more depressed, anxious, were characterized by more sleep problems and lower morningness score, and experienced increased daily stress (Levin and Nielsen, 2007; Schredl, 2003; Nielsen, 2010). This co-morbidity of psychological complaints and nightmares is often attributed to heightened emotional reactivity (Schredl, 2003).

One possible function of the CAR is to facilitate coping with the upcoming daily stresses (Fries et al., 2009). Accordingly, the present study has shown that participants without frequent nightmares had a smaller CAR on the leisure day compared to the working day. Similar findings have been reported in other studies (Kunz-Ebrecht et al., 2004). Interestingly, participants with frequent nightmares did not show a CAR difference between the two days. We might speculate that nightmare sufferers have a limited physiological adaptability to anticipated stressors. Nightmare sufferers recalled more dreams during the study period, however, flattened CAR was not related to the emotional quality of reported dreams reported, regardless the type of the day (working or leisure). This suggests, that reduced CAR in the nightmare group during the working days reflects a trait-like vulnerability of the HPA axis. This finding argues against a state-like dependency between CAR and negative dream experiences and is in coherence with earlier findings showing altered physiological sleep parameters in nightmare sufferers regardless of having nightmares on the time of the measurement (Simor et al., 2013, 2014).

As previous studies reported, nightmare disorder is usually associated with poor sleep quality, including subjective reports and objective sleep indices (Simor et al., 2012a; Steiger, 2002; Simor et al., 2014). It would be plausible to assume that spontaneous, brief awakenings or hyperarousal during sleep (previously reported among nightmare sufferers; Simor et al., 2013) might also increase nocturnal cortisol secretion and lead to attenuated CAR through the exhaustion of HPA reactivity (Chida and Steptoe, 2009; Wilhelm et al., 2007). To investigate this option, Dettenborn and her collegues studied the CAR

after waking participants up several times during three consecutive nights (Dettenborn et al., 2007). They found no evidence for the presumed effect of the repeated forced awakenings on the morning CAR. However, it is possible that the exhaustion of the CAR develops only in the long term. This possibility is also supported by the findings of the present study, whereby bad dreams on the measurement day were not associated with diminished CAR while frequent nightmares were. Persisting alterations in HPA function might reflect structural changes, as in the case of PTSD (Villarreal et al., 2002).

Our findings of blunted CAR in subjects reporting frequent nightmares, resemble the data regarding the association between PTSD symptoms and flattened CAR (Levin and Nielsen, 2007; Rohleder et al., 2004; de Kloet et al., 2007; Wessa et al., 2006). Atypical HPA axis activation, sleep disturbances and frequent nightmares are prevalent features of PTSD (Germain et al., 2008). Increased arousal-promoting neural activity reflected by increased noradrenergic tone during sleep seem to contribute to poor sleep quality and nightmarish experiences in these patients (Ahmadpanah et al., 2014). Our data suggests that an association between blunted CAR and frequent nightmares might be present among non-clinical populations as well. However, the clinical relevance of this association will need to be addressed by further studies.

Frequent nightmares may be the result of dysfunctional autonomic regulation in sleep. Nielsen and Levin (Nielsen and Levin, 2007) proposed that during dreaming, distressing memories are reconstructed, recombined, and re-contextualized in order to change the associated emotions, eventually leading to the extinction of fearful memories. An important element during this process is the desynchronization of the emotional content and the autonomic reaction in REM sleep (i.e. an increased emotional distress coupled with low autonomic response). During nightmares, this fear extinction mechanism is supposed to be impaired, because the autonomic response in REM sleep is too intense (Levin and Nielsen, 2007; Nielsen and Levin, 2007). Frequent nightmares might reflect a recurring dysfunction in the autonomic regulation during the REM phase. It may be also possible that this autonomic arousal is reflected in the HPA response. Unfortunately, we are not aware of any studies that measured HPA activity during actual nightmares. The present findings might serve as a preliminary indication that the HPA system is involved in the production of nightmares.

5.4.1 *Strengths and limitations*

It is important to emphasise that measures were taken in a general population sample that was not primed to the study of nightmares. Another strength of our study is that we measured psychosocial fac-

tors with standard questionnaires, and employed EMA to assess stress over the day. The limitations of the study should also be noted. Categorization of participants to nightmare and control groups was done by using a single question. Although this method is prevalent in the literature, it can limit the findings (Levin and Nielsen, 2007). As nightmares, by definition, have a negative influence on sleep quality, it is difficult to completely rule out the possibility that the blunted CAR was the result of poor sleep and not the nightmares per se – even though this possibility was accounted for statistically. Moreover, the study involved university-based working women which may limit the generalizability of the results. Further, the effect size for the CAR difference between the nightmare and non-nightmare groups on the working day is modest, and studies with larger group sizes are needed to verify the association of frequent nightmares and diminished CAR.

5.4.2 *Conclusion*

The association between blunted CAR suggests altered HPA functioning in nightmare sufferers. This altered functioning might result from reduced physiological adaptability to stressors in individuals with frequent nightmares. Further studies are needed to explore the association between the HPA system and the production of nightmares.

6 GENERAL DISCUSSION

The main objective of this dissertation was to demonstrate how different stressors can invoke dissimilar psychophysiological responses, and how individual differences can moderate these reactions. Moreover, we endeavored to explore the differences in physiological reactions between distress and eustress. To this end, we conducted four studies to investigate these topics.

6.1 SUMMARY OF THE STUDIES

The first study (Chapter 2) was a laboratory experiment that showed how the sympathetic and parasympathetic nervous system activity can be modulated differently by two common acute laboratory stressors – a cold pressor task and memory-search task in a sample of thirty-four young adults. We found that the two tasks increased anxiety, but the physiological responses were different. The memory-search task elicited a classic fight-or-flight response, while the cold pressor task only increased blood pressure; thus effects on both the sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) were dissimilar for the two laboratory stressors. As it was demonstrated, the two branches of autonomic nervous system affect glandular secretion differently. While the PNS is responsible for controlling salivary flow, the SNS controls protein production. Although salivary alpha-amylase (sAA) production is supposed to reflect SNS activity, this research demonstrated that the PNS can indirectly influence sAA secretion through salivary flow. Moreover, as the SNS and PNS show asynchronous changes during acute stressors, sAA response may thus vary with sample timing. Our conclusion is that that sAA changes can be timing-dependent and stressor-specific, thus researchers should take caution when interpreting sAA responses as markers of SNS activity.

The second laboratory experiment (Chapter 3) investigated the effects of reward in a competition on the autonomic nervous system (ANS), the hypothalamus-pituitary-adrenal (HPA) and hypothalamus-pituitary-gonadal (HPG) axes in a sample of forty young males. During a face-to-face, 30-minute long video game competition subjective arousal, mood, heart rate, salivary testosterone and cortisol level were assessed, along with competitive attitudes. Participants were either rewarded equally, or the winner gained more money than the loser. We found that subjective and cardiac arousal increased during competition, and participants in the unequally rewarded condition (whereby

the winners gained more money than losers) were more aroused in the beginning of competition. In the unequally rewarded group, losers showed higher cortisol (C) levels, and C was associated with competitive performance in both winners and losers. Testosterone (T) level only increased in hypercompetitive losers. Competitive performance however was correlated with both winners' and losers' T values, supporting the hypothesis that T level and the competitive performance are likely to be in a reciprocal relationship. Our findings supported the status instability hypothesis, and provided novel insights to competitive psychoendocrinology.

In the second study, we used a video gaming competition that elicited positive mood. Prior research had suggested that mood might influence C response; therefore we wanted to investigate if eustress exerts a different HPA reaction than distress. That is why in the third research (Chapter 4), we wanted to summarize findings about the effects of eustress on the HPA axis in a meta-analysis. However, we had to realize that there is a lack of studies regarding eustress. Based on earlier research, we concluded that eustress can be induced in performance tasks that can optimally fit the task demands to personal skills, and elicit positive emotions. As video games are purposefully designed to attain these characteristics, we chose this activity as a model situation for eustress. We conducted a multi-level mixed-effects meta-analysis on twenty-four studies. Results yielded that during video gaming, cortisol levels decreased over time. Individual characteristics, game attributes, violence, and competition in the game did not predict effect sizes. These findings suggest that video gaming did not activate the HPA axis, and cortisol level followed the normal diurnal decline. Therefore we concluded that eustress is not likely to elicit the same HPA reaction as distress.

The fourth study (Chapter 5) was a cross-sectional research in an ecological setting that aimed to explore the associations of HPA functioning with emotional processes. In this study, we investigated the relationship between the cortisol awakening response (CAR) – that is used as an index of general responsiveness of the HPA system – and recurring nightmares. Nightmares are relatively common sleep complaints that seem to be associated with affective distress, but few attempts had been made to link nightmares to the biological markers of stress, and the HPA response in particular. 188 women provided seven cortisol samples over the course of a working and a leisure day. Analysis revealed that those who reported frequent nightmares (N = 13) showed a blunted CAR on a working day, compared to those who did not report nightmares, independent of psychiatric symptoms, demographic variables, and lifestyle. This finding suggested that decreased HPA reactivity might be a trait-like feature of women with frequent nightmares. We believe that this study can help to bring a broader understanding about how individual differences in HPA

functioning can be associated with psychological processes and emotional experiences. In the context of the dissertation, these findings suggest that individual differences in HPA functioning might be associated with alterations in emotional states. This can remind us that the correlation between emotions and stress responses can go both ways. In other words, not just emotional responses can trigger physiological responses but physiological responses can also influence affective states.

6.2 MAIN FINDINGS OF THE DISSERTATION

In this section, we highlight the main findings of the dissertation, focusing on the implications. Besides the general aims of the dissertation to further our understanding about stress response specificity and individual response stereotypy, the included studies had other, less firmly connecting objectives. Naturally, not all of the findings are completely novel, but all of them provide insights that might help to improve our knowledge about the human physiology and psychology of stress.

6.2.1 *Different stressors elicit dissimilar physiological responses*

Contrary to Selye's claim about the generality of the stress response, it was found that stress can show a high degree of response specificity to different stressors (Lovallo and Pincomb, 1990; Mason, 1968; Skoluda et al., 2015). Even distress can elicit different stress response patterns, such as those found during active and passive coping (Bosch et al., 2001; Koolhaas et al., 1999). Moreover, the SNS itself can demonstrate a high level of anatomical specificity (Folkow, 2000), causing dissociated SNS reactions in different organs. For example in our first study, blood pressure was increased during the cold pressor task, but neither cardiac, nor glandular physiology changed significantly. This result had been reported in other studies, and often referred to as a predominantly alpha adrenergic activation of the SNS (Allen et al., 1987; Willemsen et al., 1998). On the other hand, the memory-search task elicited a full-fledged fight-or-flight response that extended to vascular, cardiac, and glandular SNS and PNS responses.

6.2.2 *Interpretation of sAA as a measure of SNS activity is uncertain*

SAA has gained rapid popularity as a noninvasive marker of SNS activity (Nater and Rohleder, 2009). The second study investigated the potential confounders of this marker. It was found that sAA fluctuations did not parallel changes in cardiac SNS activity or anxiety. Moreover, sAA responses seemed to be contingent on sample timing, likely involving both SNS and PNS influences. These observations

lead us to conclude that the interpretation of sAA as a measure of SNS activity is less solid than often assumed. Furthermore, results indicate that sAA concentration and secretion may diverge substantially and future studies should use the latter as it is adjusted for salivary flow rate.

6.2.3 *Eustress is associated with a different HPA activity compared to distress*

Previous studies had demonstrated that distress increases cortisol level, and particularly those stressors can boost cortisol level that feature uncontrollability or uncertainty (Dickerson and Kemeny, 2004). On the other hand, research has been scarce about the physiological correlates of eustress (Nelson and Cooper, 2005). Some findings suggested that eustress is a response to a performance task that is perceived as controllable and enjoyable (Simmons and Nelson, 2001). In our second study, we used a performance task (video gaming) that elicited positive mood, and had been designed to provide the feeling of control – therefore had a high potential to elicit eustress. We found that even though physiological arousal was increased during video gaming, cortisol level followed the normal diurnal decline. This suggests that cortisol decrease might occur during an exciting, competitive task, if the feeling of control is maintained.

To verify this result, we aggregated findings in a meta-analysis about the effects of video gaming on HPA axis activity. The analysis – based on twenty-four studies – confirmed our previous result, and showed that cortisol levels decrease over time during video gaming. We interpret this finding as an indication that eustress is likely elicit a different HPA response than distress.

6.2.4 *Competitive attitude can moderate testosterone response to competition*

Testosterone level was shown to react to competitive environment, but this observation has not been consistent. It was proposed that individual and situational factors can moderate this association (Edwards et al., 2006). Competitive attitudes have not been investigated as moderators previously. In the second study, we found that hypercompetitive losers were more likely to show a testosterone increase than less hypercompetitive losers and winners. This finding supports the status instability hypothesis, demonstrating that when the winner's status is unstable – either because of the loser doesn't accept it or because of the closeness of the match – the loser can show elevated testosterone level (Zilioli et al., 2014). This finding can help to explain why certain individuals react to losing with increased com-

petitive motivation, and others with withdrawal (Fülöp, 2009, 2013; Fülöp and Nagy, 2015; Mehta and Josephs, 2006)

6.2.5 *Competitive performance is associated with cortisol and testosterone levels*

Theories about competition rely on the assumption that performance is associated with testosterone and cortisol levels (Archer, 2006; Mazur and Lamb, 1980; Wingfield et al., 1990). Nevertheless, few human studies have verified this association. In our second study, we found correlations between performance and hormonal levels. There was a positive association between pre- and post-competition testosterone and cortisol levels with performance in winners, while competitive performance was linked to testosterone and cortisol changes in losers. This provides further evidence that in some situations, testosterone and cortisol might be in a reciprocal relationship with competitive performance.

6.2.6 *Functioning of the HPA axis is implicated in the production of nightmares*

The association between the stress system and nightmares has long been hypothesized, but empirical support for this relationship was not available. Our study demonstrated, that frequent nightmares can be associated with increased everyday stress, and altered HPA functioning. This altered functioning might result from reduced physiological adaptability to stressors in individuals with frequent nightmares (Simor et al., 2012b). With this preliminary finding, we hope to contribute to the neurocognitive model of nightmares (Nielsen and Levin, 2007).

6.3 FUTURE DIRECTIONS

Stress research has come a long way, but our understanding about stress is far from complete. Although the positive psychology movement urged researchers to adopt the health centered (salutogenic) viewpoint instead of the predominant disease-centered (pathogenic) perspective, stress research has not yet accommodated this idea (Seligman and Csikszentmihalyi, 2000). By investigating the positive outcomes of stress, we could gain novel insights about stress as a whole (Nelson and Cooper, 2005).

In the last decade, the interest in the health consequences of positive emotions has grown rapidly (Cohen et al., 2006; Dockray and Steptoe, 2010; Seligman, 2008). Findings suggest that positive emotional states exert a protective effect on health, but the mediators are currently not well understood. Physiological, behavioral, socioe-

conomic, and lifestyle related factors can all be implicated in this relationship (Cohen et al., 2006; Marsland et al., 2007; Pressman and Cohen, 2005; Steptoe et al., 2005). Stress and coping processes should also partly explain the connection between positive affect and health. It seems like stress reactions may differ in the presence of positive emotions, and this response might be independent of negative emotions (Bostock et al., 2011; Dockray and Steptoe, 2010).

These findings are promising, and demonstrate that the investigation of positive emotions can yield important insights for human health and longevity (Danner et al., 2001). However, currently we lack solid evidence that positive emotions are truly the causes of beneficial health outcomes, as most of the findings are based on correlational studies (Cohen et al., 2006; Marsland et al., 2007). Clearly, more experimental research is needed, whereby emotional states are systematically manipulated to verify the effects of positive affect on health outcomes. For example it would be interesting to see how participants of different stress level can profit from the short term effects of eustress. Furthermore, identifying the key characteristics of eustress inducing activities would help us to construct more effective interventions. In this dissertation, we hope to have provided new findings and methodologies to facilitate the research of stress, and particularly the differences in the ways we respond to distress and eustress.

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APPENDIX

A

APPENDIX A: ANALYSIS AND DISCUSSION OF THE COVARIATES FOR CHAPTER 2

Table A.1: P values and effect sizes (η_p^2) of the covariates in the repeated-measures ANCOVAs

	Task order	**Sex**	**BMI**	**Smoking**
Cardiovascular parameters				
SBP	.524 (.02)	.762 (.00)	.001 (.36)	.730 (.01)
DBP	.279 (.05)	.424 (.03)	.034 (.16)	.508 (.02)
HR	.274 (.04)	.061 (.11)	.097 (.08)	.218 (.05)
RMSSD	.331 (.03)	.843 (.00)	.273 (.04)	.306 (.03)
PEP	.302 (.03)	<.001 (.36)	.037 (.13)	.055 (.11)
Salivary parameters				
Salivary flow rate	.640 (.00)	.004 (.24)	.530 (.01)	.640 (.01)
sAA concentration	.055 (.11)	.628 (.01)	.129 (.08)	.135 (.07)
sAA secretion	.115 (.09)	.008 (.22)	.183 (.06)	.414 (.02)

Note: SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, HR: Heart Rate, RMSSD: Root Mean Square of Successive Differences, PEP: Pre-Ejection Period.

Table A.1 shows the p values for the covariates of the ANCOVAs analyzing cardiovascular and salivary parameters. As it can be seen in the table, task order and smoking were not significant covariates for any of the outcomes. Sex was significantly associated with PEP, salivary flow rate, and sAA secretion: i.e., males had significantly longer PEP ($F(1,31) = 17.56$, $p < .001$, $\eta_p^2 = .36$), a higher average salivary flow rate ($F(1,29) = 9.27$, $p = .005$, $\eta_p^2 = .24$), and a higher sAA secretion ($F(1,27) = 9.87$, $p = .004$, $\eta_p^2 = .27$). However, including sex as a covariate did not decrease the significance of the time and Task $\times$ Time effect on salivary flow, sAA secretion, and PEP.

BMI was associated with several cardiovascular parameters: those with higher BMI had a higher SBP ($F(1,26) = 14.40$, $p = .001$, $\eta_p^2 = .36$) , DBP ($F(1,26) = 5.00$, $p = .034$, $\eta_p^2 = .16$), and a longer PEP ($F(1,31) = 4.73$, $p = .037$, $\eta_p^2 = .13$). However, adding BMI as a covariate did not decrease the significance of the time effect for SBP, DBP and PEP, nor the Task $\times$ Time interaction for PEP.

B

APPENDIX B: MODEL COMPARISON TABLES FOR CHAPTER 3

Abbreviations for all tables: ER: Equal reward, UR: Unequal reward, W: winner, L: Loser, AIC: Akaike Information Criteria, ICC: Intra-class correlation. Best models were selected based on smallest AIC values, that were calculated using maximum likelihood estimation

AROUSAL

	Model 1			Model 2			Model 3			Model 4		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts												
(Intercept)	1.70	1.48 - 1.92	**<.001**	1.91	1.57 - 2.25	**<.001**	-0.04	-0.52 - 0.44	.877	0.11	-0.46 - 0.68	.701
Time				-0.05	-0.10 - 0.01	.117	1.12	0.90 - 1.35	**<.001**	1.07	0.84 - 1.30	**<.001**
Time^2							-0.13	-0.15 - -0.11	**<.001**	-0.13	-0.15 - -0.11	**<.001**
Condition (ER)										-0.30	-0.92 - 0.32	.348
Time * Condition (ER)										0.11	0.02 - 0.21	**.024**
Random Parts												
N_{Id}	40			40			40			40		
N_{Pair}	20			20			20			20		
ICC_{Id}	0.159			0.160			0.237			0.240		
ICC_{Pair}	0.013			0.013			0.016			0.018		
Observations	320			320			320			320		
AIC	1074.45			1073.98			983.73			981.66		

Table B.1: Model comparison for arousal

	Model 1			Model 2			Model 3			Model 4			Model 5		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts															
(Intercept)	1.35	1.01 - 1.69	**<.001**	1.08	0.66 - 1.51	**<.001**	1.22	0.85 - 1.59	**<.001**	1.16	0.58 - 1.74	**<.001**	1.36	0.53 - 2.20	**.002**
Time				0.06	0.00 - 0.12	**.048**				-0.03	-0.12 - 0.05	.413	-0.08	-0.19 - 0.04	.186
Time^2							0.01	-0.00 - 0.01	.116						
Outcome (W)										-0.15	-0.96 - 0.66	.721	0.11	-1.04 - 1.27	.847
Time * Outcome (W)										0.19	0.07 - 0.30	**.002**	0.16	-0.00 - 0.32	.052
Condition (ER)													-0.40	-1.59 - 0.78	.505
Time * Condition (ER)													0.09	-0.07 - 0.25	.293
Condition (ER) * Outcome (W)													-0.52	-2.16 - 1.11	.533
Time*Condition (ER) * Outcome (W)													0.05	-0.18 - 0.28	.669
Random Parts															
N_{Id}		40			40			40			40			40	
N_{Pair}		20			20			20			20			20	
ICC_{Id}		0.397			0.400			0.398			0.356			0.369	
ICC_{Pair}		0.000			0.000			0.000			0.029			0.030	
Observations		320			320			320			320			320	
AIC		1118.4			1116.5			1117.9			1106.1			1109.7	

Table B.2: Model comparison for valence

	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts																		
(Intercept)	89.19	84.72 - 93.67	**<.001**	89.73	85.01 - 94.46	**<.001**	90.49	85.92 - 95.06	**<.001**	90.68	84.13 - 97.22	**<.001**	93.98	84.75 - 103.20	**<.001**	81.09	74.20 - 87.99	**<.001**
Time				-0.12	-0.45 - 0.21	.481										6.85	5.57 - 8.14	**<.001**
Time^2							-0.05	-0.09 - -0.02	**.006**	-0.06	-0.11 - -0.01	**.024**	-0.05	-0.09 - -0.02	**.006**	-0.81	-0.95 - -0.68	**<.001**
Outcome (W)										-0.36	-9.62 - 8.89	.939	-2.98	-15.95 - 10.00	.656			
Time * Outcome (W)										0.02	-0.06 - 0.09	.676						
Condition (ER)													-6.99	-19.97 - 5.99	.298	-7.12	-16.43 - 2.20	.141
Time * Condition (ER)													6.00	-12.35 - 24.35	.526			
Condition (ER) * Outcome (W)																0.70	0.15 - 1.24	**.013**
Random Parts																		
N_{Id}	40			40			40			40			40			40		
N_{Pair}	20			20			20			20			20			20		
ICC_{Id}	0.806			0.806			0.810			0.814			0.818			0.864		
ICC_{Pair}	0.000			0.000			0.000			0.000			0.000			0.000		
Observations	320			320			320			320			320			320		
AIC	2300.2			2301.7			2294.5			2298.3			2299.2			2190.1		

Table B.3: Model comparison for heart rate

	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts																		
(Intercept)	28.45	25.00 - 31.89	**<.001**	28.51	24.71 - 32.30	**<.001**	27.84	24.25 - 31.42	**<.001**	28.34	23.70 - 32.97	**<.001**	34.95	28.89 - 41.01	**<.001**	24.15	17.36 - 30.94	**<.001**
Time				-0.01	-0.37 - 0.34	.940							-4.51	-6.06 - -2.96	**<.001**			
Time^2							0.02	-0.01 - 0.06	.220	0.01	-0.04 - 0.06	.737	0.54	0.38 - 0.70	**<.001**	0.08	0.00 - 0.16	**.047**
Outcome (W)										-1.00	-6.90 - 4.91	.744				5.05	-3.09 - 13.20	.237
Time^2 * Outcome (W)										0.03	-0.05 - 0.11	.453				-0.02	-0.13 - 0.09	.668
Condition (ER)													3.13	-4.56 - 10.81	.433	7.71	-1.68 - 17.10	.116
Time * Condition (ER)													-0.68	-1.33 - -0.02	**.045**			
Time^2 * Condition (ER)																-0.13	-0.24 - -0.03	**.016**
Condition (ER) * Outcome (W)																-11.03	-22.17 - 0.11	.067
Time^2 * Condition (ER) * Outcome (W)																0.10	-0.05 - 0.25	.191
Random Parts																		
N_{Id}		38			38			38			38			38			38	
N_{Pair}		20			20			20			20			20			20	
ICC_{Id}		0.463			0.462			0.463			0.487			0.477			0.412	
ICC_{Pair}		0.169			0.169			0.170			0.150			0.200			0.237	
Observations		304			304			304			304			304			304	
AIC		2169.9			2171.9			2170.4			2173.9			2135.3			2173.2	

Table B.4: Model comparison for RMSSD

	Model 1			Model 2			Model 3			Model 4			Model 5			Model 6		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts																		
(Intercept)	5.59	4.42 - 6.77	**<.001**	9.20	7.30 - 11.10	**<.001**	12.80	9.36 - 16.24	**<.001**	11.43	8.88 - 13.97	**<.001**	9.98	7.27 - 12.69	**<.001**	8.20	5.64 - 10.77	**<.001**
Time				-2.41	-3.40 - -1.41	**<.001**	-3.01	-4.89 - -1.14	**.003**	-2.97	-4.33 - -1.60	**<.001**	-2.59	-4.01 - -1.16	**.001**	-2.41	-3.40 - -1.41	**<.001**
Outcome (W)							-2.90	-7.90 - 2.10	.259				-1.60	-5.48 - 2.29	.424			
Condition (ER)							-5.96	-10.96 - -0.96	**.022**	-4.57	-8.22 - -0.93	**.016**						
Time * Outcome (W)							0.10	-2.63 - 2.83	.943				0.37	-1.67 - 2.42	.724			
Time * Condition (ER)							0.90	-1.83 - 3.63	.521	1.15	-0.80 - 3.11	.255						
Outcome (W) * Condition (ER)							2.93	-4.24 - 10.09	.426									
Time * Condition (ER) * Outcome (W)							0.50	-3.41 - 4.41	.805									
Condition (UR) * Outcome (L)																3.69	0.82 - 6.56	**.016**
Condition (ER) * Outcome (L)																-0.92	-3.86 - 2.03	.545
Condition (UR) * Outcome (W)																0.93	-2.01 - 3.88	.538
Random Parts																		
N_{Id}	37			37			37			37			37			37		
N_{Pair}	19			19			19			19			19			19		
ICC_{Id}	0.551			0.695			0.632			0.660			0.690			0.620		
ICC_{Pair}	0.000			0.000			0.000			0.000			0.000			0.000		
Observations	74			74			74			74			74			74		
AIC	414.670			399.152			399.837			395.729			396.830			395.328		

Table B.5: Model comparison for cortisol

	Model 1			Model 2			Model 3			Model 4			Model 5		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts															
(Intercept)	70.40	63.59 - 77.22	**<.001**	74.37	65.50 - 83.24	**<.001**	81.30	65.59 - 97.01	**<.001**	80.76	68.88 - 92.64	**<.001**	69.78	58.01 - 81.54	**<.001**
Time				-2.65	-6.43 - 1.14	.179	-4.33	-11.32 - 2.65	.231	-4.15	-9.33 - 1.02	.124	-0.32	-5.58 - 4.93	.904
Outcome (W)							-1.09	-22.16 - 19.98	.920				9.19	-6.43 - 24.81	.254
Condition (ER)							-24.33	-47.16 - -1.51	**.040**	-13.48	-30.74 - 3.78	.133			
Time * Outcome (W)							0.36	-9.52 - 10.24	.943				-4.64	-12.07 - 2.79	.228
Time * Condition (ER)							8.46	-1.69 - 18.61	.110	3.18	-4.33 - 10.70	.412			
Outcome (W) * Condition (ER)							21.70	-8.92 - 52.32	.171						
Time * Condition (ER) * Outcome (W)							-10.56	-24.91 - 3.79	.157						
Random Parts															
N_{Id}		38			38			38			38			38	
N_{Pair}		19			19			19			19			19	
ICC_{Id}		0.629			0.636			0.671			0.669			0.637	
ICC_{Pair}		0.192			0.193			0.166			0.155			0.197	
Observations		76			76			76			76			76	
AIC		638.542			638.711			644.436			640.395			641.081	

Table B.6: Model comparison for testosterone (first step)

	Model 1			Model 2			Model 3			Model 4		
	B	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*	*B*	*CI*	*p*
Fixed Parts												
(Intercept)	69.78	58.01 - 81.54	**<.001**	129.45	58.37 - 200.53	**.001**	123.62	65.52 - 181.72	**<.001**	90.69	28.06 - 153.33	**.006**
Time	-0.32	-5.58 - 4.93	.904	-45.48	-75.26 - -15.70	**.005**	-43.83	-67.96 - -19.70	**.001**	-20.13	-48.32 - 8.05	.170
Outcome (W)	9.19	-6.43 - 24.81	.254	-141.20	-278.97 - -3.44	**.049**	-91.38	-187.00 - 4.24	.065			
Time * Outcome (W)	-4.64	-12.07 - 2.79	.228	80.49	22.60 - 138.37	**.010**	53.94	14.32 - 93.56	**.011**			
Condition (ER)				-80.95	-208.92 - 47.01	.219				-20.61	-117.98 - 76.76	.679
Hypercompetition				-18.32	-44.82 - 8.17	.179	-19.12	-39.37 - 1.14	.069	-3.51	-25.25 - 18.24	.753
Time * Condition (ER)				11.64	-41.98 - 65.25	.673				-2.91	-47.24 - 41.42	.898
Outcome (W) * Condition (ER)				152.54	-46.40 - 351.48	.137						
Time * Hypercompetition				15.66	4.56 - 26.76	**.009**	15.45	7.03 - 23.86	**.001**	5.64	-4.15 - 15.43	.266
Outcome (W) * Hypercompetition				48.59	1.83 - 95.36	**.046**	34.98	2.35 - 67.62	**.039**			
Condition (ER) * Hypercompetition				21.13	-22.60 - 64.85	.347				2.55	-30.64 - 35.74	.881
Time * Outcome (W) * Condition (ER)				-49.94	-133.28 - 33.39	.247						
Time * Outcome (W) * Hypercompetition				-28.49	-48.14 - -8.84	**.007**	-20.57	-34.07 - -7.06	**.005**			
Time * Condition (ER) * Hypercompetition				-3.12	-21.44 - 15.20	.741				1.88	-13.24 - 17.00	.809
Outcome (W) * Condition (ER) * Hypercompetition				-45.15	-112.35 - 22.06	.192						
Time * Outcome (W) * Condition (ER)* Hypercompetition				14.97	-13.18 - 43.12	.304						
Random Parts												
N_{Id}	38			38			38			38		
N_{Pair}	19			19			19			19		
ICC_{Id}	0.637			0.845			0.702			0.733		
ICC_{Pair}	0.197			0.020			0.170			0.094		
Observations	76			76			76			76		
AIC	641.081			646.601			636.397			644.100		

Table B.7: Model comparison for testosterone by adding hypercompetition (second step)

C

APPENDIX C: DISTRIBUTION OF EFFECT SIZES IN THE INCLUDED STUDIES FOR CHAPTER 4

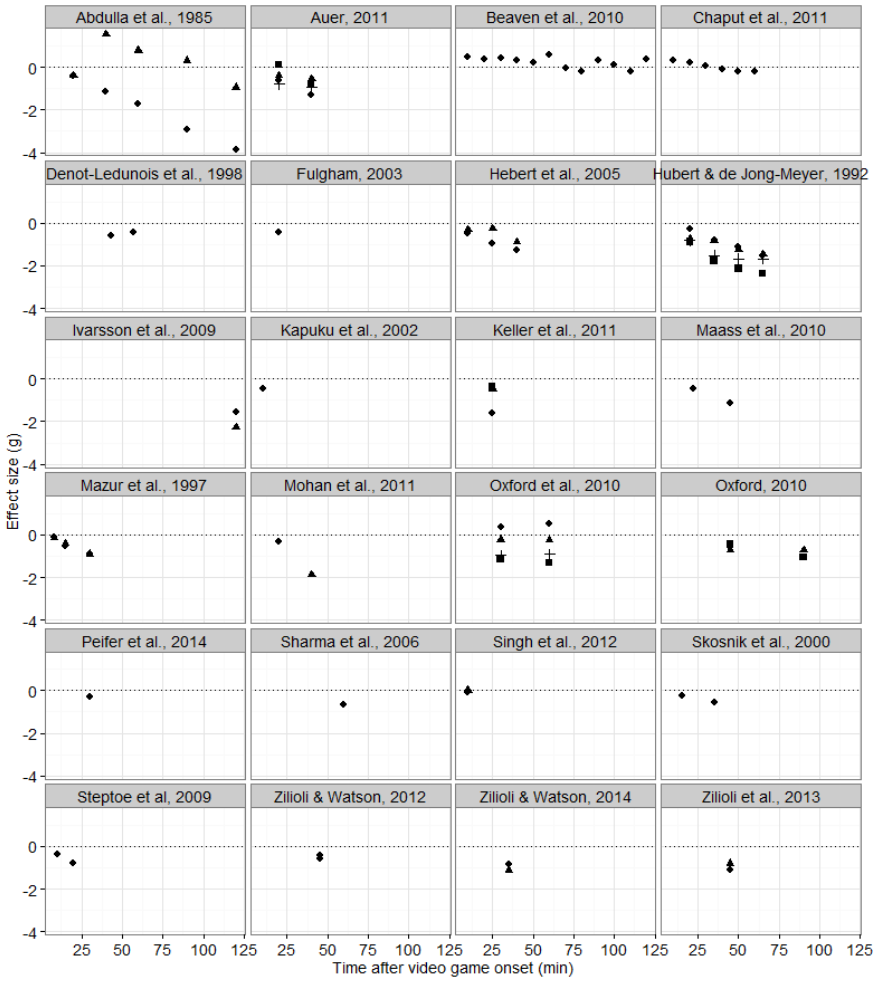

Figure C.1: **Effect sizes against time by study.** The plot shows all effect sizes arranged by studies, presented against sampling time. Different shapes represent different subsamples in the study.

D

APPENDIX D: CHARACTERISTICS OF THE MATCHED SAMPLE FOR CHAPTER 5

Table D.1: Differences in demographic, lifestyle, health, and daily activity characteristics between participants with frequent nightmares and a matched non-nightmare sample

	Nightmare group (N=13)	Matched non-nightmare group (N=13)	Statistic	p
Demographic variables and lifestyle				
Age (yrs)	33.8 (11.4)	35.2 (11.5)	t (24.0) = 0.29	.773
BMI (kg/m2)	21.7 (3.4)	24.15 (5.2)	t (19.6) = 1.64	.116
Has university degree	69.2%	85.0%	χ^2 = 0.22	.642
Has children	23.1%	38.0%	χ^2 = 0.18	.671
Drinks alcohol occasionally	84.6%	100.0%	χ^2 = 0.46	.497
Smokes	0.0%	0.0%	χ^2 = 0.00	1.000
Morningness score	30.7 (7.9)	35.0 (7.1)	t (23.7) = 1.46	.157
Total exercise (hours per week)	3.2 (2.0)	2.9 (1.3)	t (20.4) = -0.58	.566
Health and mental health				
Depression (CES-D)	22.9 (10.9)	10.8 (6.2)	t (19.0) = -3.50	.002
Trait anxiety (STAI-T)	53.3 (11.8)	42.9 (10.2)	t (23.5) = -2.40	.025
Physical symptoms (PHQ-15)	35.8 (6.1)	27.2 (6.0)	t (22.8) = -3.56	.002
Sleep problems (Jenkins scale)	20.3 (6.1)	18.5 (7.1)	t (22.4) = -0.87	.394
Daily activities				
Wake time (working day) h:m	6:05 (1:11)	5:51 (0:57)	t (21.6) = 0.50	.619
Wake time (leisure day) h:m	7:09 (1:13)	6:32 (1:46)	t (21.3) = -1.04	.311
Total sleep duration (working day) h:m	7:02 (1:10)	7:13 (1:21)	t (23.6) = 0.35	.726
Total sleep duration (leisure day) h:m	8:03 (2:06)	7:43 (1:28)	t (23.3) = -0.44	.664
Daily stress (working day)	2.5 (0.9)	2.4 (0.9)	t (24.0) = -0.17	.867
Daily stress (leisure day)	2.1 (0.8)	1.9 (0.8)	t (23.9) = -0.59	.559

Note: Values represent means (SD) or percentages. Statistics were calculated using Welch's t tests and Pearson's Chi-square tests with Yates continuity correction.

COLOPHON

This document was typeset using the typographical look-and-feel `classicthesis` developed by André Miede. The style was inspired by Robert Bringhurst's seminal book on typography "*The Elements of Typographic Style*". `classicthesis` is available for both LaTeX and LyX:

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