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Virtually Stressed? A Psychobiological Investigation of the Trier Social Stress Test VR as a Standardized and Economic Paradigm for Laboratory Stress Induction

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Abstract

In order to investigate the psychobiological consequences of acute stress under laboratory conditions, a wide range of methods for socially evaluative stress induction have been developed. The present dissertation is concerned with evaluating a virtual reality (VR)-based adaptation of one of the most widely used of those methods, the Trier Social Stress Test (TSST). In the three empirical studies collected in this dissertation, we aimed to examine the efficacy and possible areas of application of the adaptation of this well-established psychosocial stressor in a virtual environment. We found that the TSST-VR reliably incites the activation of the major stress effector systems in the human body, albeit in a slightly less pronounced way than the original paradigm. Moreover, the experience of presence is discussed as one potential factor of influence in the origin of the psychophysiological stress response. Lastly, we present a use scenario for the TSST-VR in which we employed the method to investigate the effects of acute stress on emotion recognition performance. We conclude that, due to its advantages concerning versatility, standardization and economic administration, the paradigm harbors enormous potential not only for psychobiological research, but other applications such as clinical practice as well. Future studies should further explore the underlying effect mechanisms of stress in the virtual realm and the implementation of VR-based paradigms in different fields of application.

Keywords: Stress; Hypothalamic-pituitary-adrenal axis; sympathetic-adrenal medullary system; Trier Social Stress Test; virtual reality; TSST-VR

1. General Introduction

“Every stress leaves an indelible scar, and the organism pays for its survival after a stressful situation by becoming a little older.”

(Selye, 1956)

As the second decade of the twenty-first century is drawing to a close, the words by pioneering stress researcher Hans Selye seem as timely as when they were conceived for his seminal work *The stress of life* in 1956. In a time of rapid technological and societal developments that confront us with ever-changing demands, being able to quickly adapt to novel circumstances while maintaining one’s psychological and physiological equilibrium might be one of the most crucial capabilities of healthy individuals. In fact, the process of recognizing a stressful external event and putting up resistance via adaptations in bodily functions usually followed by a period of exhaustion was outlined early on by Selye in the General Adaptation Syndrome framework (G.A.S.; Selye, 1976).

According to this influential model, the advent of a stressor is generally followed by a myriad of physiological processes as part of homeostatic systems that enable the organism to produce compensatory and anticipatory adjustments to maintain an ideal set of steady-states. This concept can be seen as the starting point for our understanding of the physiological mechanisms of stress coping and since then it has been further developed to incorporate the specific stress effector systems of the human body (which will be delineated in detail in section 2.1.; Goldstein & McEwen, 2002). In this fashion, a healthy organism is able to adapt to adverse events in an effective manner and avoid detrimental consequences in the short term.

In the event that stressors persist and the physiological and psychological strain does not abate for an extended period of time, however, overactivity of these stress effector systems can eventually favor the development of numerous pathologies. As these pathologies encompass a wide range from decreased immune resistance to viral infections (McEwen et al., 1997) through psychiatric disorders such as major depression or anxiety disorders (Leistner & Menke, 2018; Smoller, 2016) to the formation of tumors and increased metastatic spread (Reiche, Nunes, & Morimoto, 2004) stress has now widely been recognized to be one of the most impactful noxious agents of our time (Hassard, Teoh, Visockaite, Dewe, & Cox, 2018).

The importance of understanding the psychophysiological effects of acute and chronic stressors under highly controlled laboratory conditions can thus not be overstated. In the past decades, numerous paradigms of laboratory stress induction have been developed with the goal of simulating conditions akin to the ones perceived during real-life stressors. While the focus initially lay mostly on creating physically adverse circumstances in order to elicit physiological responses (e.g. via the immersion of body parts in painfully cold water; Hines, 1932), meta-analytic assessment shows that a more pronounced stress response can be expected following paradigms that encompass adverse cognitive aspects such as social evaluation (Dickerson & Kemeny, 2004).

One of the most commonly used stress induction paradigms that is based on this effect mechanism is the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993). This method incorporates two of the key components that Dickerson and Kemeny deemed to be essential for the onset of a psychobiological stress response: Presenting participants with a sequence of ego-threatening socially evaluative tasks that largely unfold beyond their control.

Over the years, this paradigm, often considered the gold standard of laboratory stress induction (Allen et al., 2017), has been adapted to fit the particular requirements of specific settings (e.g. group experiments; von Dawans, Kirschbaum, & Heinrichs, 2011) and specific research populations (e.g. TSST for children, Buske-Kirschbaum et al., 1997). A common factor of all previous adaptations of the TSST, however, is their reliance on a panel of human judges in order to elicit socially evaluative stress, thus limiting standardization and making the paradigm somewhat resource-consuming.

This might be changing with the advent of Virtual Reality (VR)-based research methods in psychology. As technical advancement in this field has brought forth sophisticated and affordable head-mounted displays, paradigms that immerse participants in computer-generated environments are becoming increasingly attractive for psychological research and application. In the clinical domain, Virtual Reality Exposure Therapy (VRET) has been found to show great promise for the treatment of specific phobias (Morina, Ijntema, Meyerbröker, & Emmelkamp, 2015) and social anxiety disorders (Powers & Emmelkamp, 2008).

Based on this empirical evidence that demonstrates the potential of simulating anxiety-inducing scenarios in VR, Kelly, Matheson, Martinez, Merali, and Anisman (2007) were the first to utilize a VR-adaptation of the TSST that relied entirely on virtual avatars in order to elicit a psychobiological stress response. Although the technical implementation in this first study has to be considered comparatively limited, the results nevertheless demonstrate a tendency for successful neuroendocrine activation. In the following years, several research groups developed their own virtual adaptations of the TSST and tested their potential for laboratory stress induction (Hartanto et al., 2014; Jönsson et al., 2010; Kothgassner, Felnhofer, et al., 2016; Kotlyar et al., 2008). While most of the empirical evidence points towards a high effectiveness of the TSST-VR concerning the activation of one of the main stress effector systems, the sympatho-adrenal-medullary (SAM) system, results seem to be less conclusive regarding the response of the other, the hypothalamus-pituitary-adrenal (HPA) axis (Annerstedt et al., 2013; Shiban et al., 2016). Considering that stress reactivity to a virtual TSST will presumably be a function of several additional factors that have no relevance in the original TSST such as graphical fidelity (Ocasio-De Jesús, Kennedy, & Whittinghill, 2013) or the subjective sense of presence (Gromer, Reinke, Christner, & Pauli, 2019), it can be assumed that not all relevant effect mechanisms of the TSST-VR have yet been elucidated.

The present dissertation was composed with the aim of extending the existing research in this field with a systematic evaluation of the paradigm using a state of the art adaptation of the TSST in an immersive virtual environment. For this purpose, a study was carried out to compare the effects of the TSST-VR and the TSST *in vivo* to their respective control conditions in terms of the activation of both major physiological stress effector systems. In a second study, the influence of the subjective experience of presence in the virtual situation on stress reactivity was evaluated. The last study of this dissertation was meant to bridge the gap between methodological evaluation of the paradigm and application for research purposes and investigated the effects of acute psychosocial stress in VR on emotion recognition, one of the most crucial abilities needed to navigate our social environment.

2. The Psychobiology of Virtual Stress

The following chapter gives an overview of the theoretical framework that is needed to understand the merits of developing a standardized method for socially evaluative stress induction in a virtual environment. For this purpose, several time-honored stress induction paradigms will be reflected upon. Subsequently, the benefits of utilizing virtual environments in psychological research will be described. The last section of this chapter provides an overview of the development history of the particular stress induction paradigm under investigation in this dissertation.

2.1. Laboratory Stress Induction and Associated Physiological Parameters

In order to achieve high external validity, a laboratory stress induction paradigm should fulfill the essential criterion of activating the two main stress effector systems in the human body, the HPA axis and the SAM system. As numerous studies have shown a link between psychological distress and physiological alterations attributed to the activation pattern of these two systems (Chrousos, 2009), they can be utilized as reliable indicators for the psychobiological stress response and thus provide scientists with a wide range of suitable physiological measures. To understand the role of the two stress effector systems in maintaining homeostasis, their main functioning pathways shall be briefly delineated, starting with the hypothalamic-pituitary-adrenal pathway.

Following the onset of a perceived stressful event, the hypothalamus releases corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) into a specialized portal blood system which stimulates the secretion of adrenocorticotropic hormone (ACTH; Tsigos & Chrousos, 2002) from the anterior pituitary. The secretion of ACTH in turn results in a heightened production and release of glucocorticoids from the adrenal cortex. Glucocorticoids can be understood as vital agents of the HPA axis that play a major part in the modulation of a multitude of metabolic, cardiovascular, immune, and behavioral functions

(Bamberger, Schulte, & Chrousos, 1996). Since it has been shown that the concentration of the glucocorticoid cortisol can be reliably assessed not only in blood plasma, but also in saliva (Vining, McGinley, Maksvytis, & Ho, 1983) it has proven to be a practical indicator of HPA axis activity for psychobiological science (Kirschbaum & Hellhammer, 1994). While there are other markers that are associated with HPA axis activity and well suited to laboratory assessment (such as ACTH or cytokines that give an indication of HPA axis reactivity like tumor necrosis factor-α, Interleukin-1β, or Interleukin-6; Tsigos & Chrousos, 2002), salivary cortisol is one of the most frequently measured compounds due to its high validity and economic sampling procedure (Hellhammer, Wüst, & Kudielka, 2009).

In addition to the HPA axis, the SAM system plays a crucial part in maintaining body homeostasis due to its role in the regulation of the autonomic nervous system (ANS). The SAM consists of the sympathetic nervous system and the adrenal medulla and mainly acts through the catecholamines epinephrine and norepinephrine that bind to adrenergic α and β receptors in order to stimulate rapid modulations in the activity of its effector organs (Turner, Keating, & Tilbrook, 2012). One of the main pathways of the SAM to appropriately react to stressors is the stimulation of the cardiovascular system via the autonomic nervous system to increase cardiac output and redistribute blood flow to the pulmonary blood system and necessary organs. As this process necessitates sympathetic activity and the suppression of the parasympathetic nervous system, heart rate (HR) and heart rate variability (HRV) present meaningful indicators of the ANS (Taelman, Vandeput, Spaepen, & Van Huffel, 2009). Considering that these parameters can reliably be assessed in a non-invasive way both in the field and in the laboratory via the use of mobile ECG devices, it stands to reason that they have become some of the most predominant physiological measures of stress (Kim, Cheon, Bai, Lee, & Koo, 2018).

As the aforementioned physiological changes can be understood as indicators of the efforts the body makes in order to re-establish homeostasis after an adverse event, they symbolize imperative measures of the criterion validity of laboratory stress induction paradigms. It is thus not surprising that one of the most commonly used stressors, the cold pressor test (CPT; Hines, 1932), was at first employed to measure vascular constriction to detect disturbances of the cardiovascular system and only later repurposed as a stress induction paradigm when it was discovered that the use of the method stimulates the stress effector systems outlined above (Nabel, Ganz, Gordon, Alexander, & Selwyn, 1988). During the CPT, participants are asked to submerge their hands or feet in 0-4°C cold water, thus activating the sympathetic nervous system via thermal and nociceptor afferents (Bullinger et al., 1984). Following the publication of Dickerson and Kemenys' (2004) influential meta-analysis that identified uncontrollability and social-evaluative threat as the two key components for successful stress induction in a laboratory setting, a refined version of the CPT was developed that incorporates the presence of an experimenter that judges participant’s behavior with the socially evaluated cold-pressor test (SECPT; Schwabe, Haddad, & Schächinger, 2008).

This development toward stress induction via the means of negative social evaluation illustrates a trend that can be traced back to the conception of the Trier Social Stress Test in 1993 (Kirschbaum et al.). the TSST is one of the most momentous stress induction paradigms that has since become the gold standard in psychobiological stress research (Allen et al., 2017). In order to elicit a psychological and physiological stress response, the TSST makes use of a real-life situation by presenting participants with a short preparation time after which they have to introduce themselves in a job interview for a desired position in front of a panel of interviewers. In contrast to a regular job interview, however, these interviewers are trained confederates that are dressed in white lab coats and have practiced to deflect any affiliative verbal or nonverbal behavior that participants employ to alleviate the tension of the situation.

Furthermore, after a set time limit (usually five minutes) the interviewers switch to a mental arithmetic task that involves subtracting from a high number. In addition to those tasks, participants are informed that their behavior will be recorded for later analysis of their performance. Numerous studies have demonstrated that this combination of factors produces a reliable increase in markers of the SAM system as well as the HPA axis. On average, exposure to the TSST prompts a twofold increase of free salivary cortisol in healthy adults and a significant stress-related change in most other physiological markers as well as a substantial amount of psychological distress (Kudielka, Hellhammer, & Kirschbaum, 2007).

The TSST has therefore been adapted for several research contexts and populations, such as group settings (von Dawans et al., 2011) or children (Buske-Kirschbaum et al., 1997) and retains its stress-inducing qualities in these circumstances as well. Moreover, several non-stressful control conditions have been developed that share similar characteristics with the real TSST without socially evaluative threat and uncontrollability, such as the Friendly TSST (Wiemers, Schoofs, & Wolf, 2013) or the Placebo TSST (Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009). Lastly, in order to understand the scope of the present dissertation, reference must be made to the adaptations of the TSST into a computer-generated virtual environment (Kelly et al., 2007). In this version of the paradigm, participants interact solely with virtual avatars that are controlled by the experimenter instead of actual human judges. This method for stress induction and its potential for producing measurable responses of the HPA and SAM systems will be delineated in greater detail in section 2.3. after a brief overview of the application of VR-based paradigms in psychological research in section 2.2.

2.2. Applications of Virtual Reality in Psychological Research

In the clinical domain, the utility of VR-based applications for the treatment of patients has been under investigation since the early 1990s, when Rothbaum and colleagues (1995) first used head-mounted displays to confront acrophobic participants with fear-inducing virtual scenarios. Even though the technical realization of the scenarios they crafted (virtual elevator rides, balconies and suspension bridges) has to be considered seriously limited from a contemporary perspective, the results of repeated exposure give an indication of successful habituation that was in some cases accompanied by voluntary exposure to real-life height situations. This study can be understood as a predecessor to what would later be called Virtual Reality Exposure Therapy (VRET). In the following years, VRET has proven its efficacy in the therapy of numerous pathologies such as specific phobia (Maples-Keller, Yasinski, Manjin, & Rothbaum, 2017), post-traumatic stress disorder (Botella, Serrano, Baños, & García-Palacios, 2015), social anxiety disorder (Bouchard et al., 2017), and panic disorder (Botella et al., 2007). Most noticeably, there is evidence for the assumption that repeated exposure to phobic stimuli *in virtuo* does not only lead to decreased physiological and psychological stress responses when phobic objects are encountered in virtuality. As several studies show, therapeutic progress made in the virtual realm carries over to real scenarios and thus helps participants to assuage their fears in phobic situations *in vivo* (Fodor et al., 2018; Morina et al., 2015).

VR-based approaches in psychotherapy have, however, not exclusively been used to generate scenarios of fear exposure but also to alter dysfunctional cognitions in other pathologies in a constructive manner. Riva and colleagues (Ferrer-Garcia, Gutiérrez-Maldonado, & Riva, 2013; Riva, Bacchetta, & Cesa, 2001) integrated VR sessions into experiential cognitive therapy for the treatment of various eating disorders (among them anorexia nervosa and binge eating disorder) and obesity, reporting a high degree of success in modifying negative body image perceptions. In a similar vein, VR-based applications have been proven effective in reducing self-criticism and in promoting self-compassion in depressive patients (Falconer et al., 2016).

In medical research, there is mounting evidence that VR-based methods do not only alter cognition, but perception of the body and its various signals as well. Working with chronic pain patients, Gromala, Tong, Choo, Karamnejad, and Shaw (2015) demonstrated that a combination of mindfulness-based stress reduction techniques and a virtual environment are helpful in alleviating chronic pain over the long term. Furthermore, paradigms realized in VR have earned their place in rehabilitation science. It has been shown that VR provides an effective exercise setting for regaining upper limb movement after a debilitating brain injury (Levin, Weiss, & Keshner, 2015). In general terms, VR therapy seems to be able to facilitate recovery after brain damage, such as strokes (Lohse, Hilderman, Cheung, Tatla, & Van der Loos, 2014).

In addition to the compensation of abilities that were lost through psychiatric or somatic illness or injury, VR has a lot of potential to be used as an educational tool to practice social skills and prepare for challenging societal situations. In children and adolescents with autism spectrum disorder, for example, VR-based interventions have been employed to practice socially acceptable behavior and emotional skills like emotion recognition and self-regulation (Fernández-Herrero, Lorenzo-Lledó, & Carreres, 2018). Furthermore, Smith and colleagues (2014) demonstrated improvements in performance and self-confidence in adults with autism spectrum disorder who repeatedly completed simulated job interviews. As this study and several others have demonstrated (e.g. Bell & Weinstein, 2011), the interaction with virtual agents in a job interview context can be an adequate practice ground for self-confident appearance and to make corrective experiences in relation to one's self-efficacy under psychological pressure.

2.3. Devising a Paradigm for Standardized Stress Induction in VR

The continually expanding fields of application of VR-based technology highlighted in the previous section hint at the wide array of possibilities yet to be explored in psychological research. Encouraged by a developing body of evidence that suggests that VR is indeed able to provoke strong emotional responses (Felnhofer et al., 2015), researchers of psychobiological stress have started to employ computer-generated environments to confront participants with taxing situations that are devised to elicit distress on a somatic and psychological level (e.g. Kelly et al., 2007). The approach that was predominantly followed in this regard was to attempt and convert well-established stress induction procedures into the virtual realm.

However, one potential pitfall of this endeavor that must be taken into consideration is the fact that the most potent stressors often involve a certain degree of social evaluation in order to elicit the stress response (Dickerson & Kemeny, 2004). It is therefore imperative to critically examine whether an interaction between a human agent and a programmed entity in a virtual space abides by the same rules and can therefore even be considered a “social” exchange. In this regard, Reeves and Nass (1996) were among the first scientists to examine how social rules and expectations are applied to computers and computer-generated characters. The findings that they summarized in what they coined *The Media Equation* predominantly attest to the fact that people tend to behave towards computers in the same manner as towards human agents when confronted with a social context. This holds true for the application of gender stereotypes to computer agents, identification with their ethnicity, adherence to social norms such as politeness and reciprocity, and more (Nass & Moon, 2000). Furthermore Garau, Slater, Pertaub, and Razzaque (2005) demonstrated that people experience presence and exhibit physiological adaptations (e.g. in heart rate and electrodermal activity) in interactions with virtual agents as long as they show a certain, albeit relatively low degree of responsiveness to the human participants. The concept of presence and its potential influence on the psychophysiological reactions of humans to virtual avatars will be further elaborated on in section 3.2.

In light of these findings, it is not surprising that a replication of one of the most audacious psychological experiments of the twentieth century, namely Stanley Milgram's behavioral study of obedience (Milgram, 1963), would have the potential of succeeding in a virtual environment. Indeed, when they had their participants observe the reactions of a virtual avatar to supposedly painful electrical shocks that they themselves had to administer, Cheetham, Pedroni, Antley, Slater, and Jäncke (2009) observed heightened activity in brain areas associated with affective processing, such as the right amygdala, and several parts of the inferior frontal gyrus and superior temporal gyrus as well as posterior cingulate. In sum, empirical evidence supports the assumption that complex social interactions can, in fact, be realized in a simulated virtual environment with computer-generated avatars. The foregoing findings therefore constitute the necessary prerequisites for the adaptation of elaborate socially evaluative stress induction paradigms in the virtual realm.

2.4. Development History of the TSST-VR

In 2007, Kelly and colleagues conducted the first large-scale investigation into the utility of a virtual TSST as a method for standardized psychobiological stress induction. To determine whether their version of the TSST-VR was able to elicit a significant neuroendocrine response, they recruited a large sample of 274 students that consisted in equal parts of male and female subjects and tested different variations of the paradigm in a total of six experimental conditions. Although their version of the TSST-VR appears to have lacked responsiveness to the participants' behavior and has to be considered very limited from a contemporary standpoint ("a helmet with a small viewing screen and headphones through which a prerecorded virtual audience was presented"; p. 659) they found a significant rise in subjective stress levels in the real TSST and three virtual stress conditions (VR task anticipation only, VR speech task only, VR speech and math tasks). There was, however, disparity between reported feelings of distress and the endocrine response as measured by salivary cortisol. While the *in* *vivo* TSST elicited a significant cortisol release that persisted until 30 minutes after stress onset, the response to the virtual conditions was significantly lower and a return to baseline levels was detected at the 30 minute mark. This finding of a statistically significant, albeit comparatively small effect in cortisol reactivity to the TSST-VR was replicated in several studies in the following years (Annerstedt et al., 2013; Fich et al., 2014; Ruiz et al., 2010; Shiban et al., 2016). Although some studies that compare a virtual stressor to its counterpart in the real world do not find significant differences in terms of HPA axis reactivity (e.g. Kothgassner et al., 2016) an overall tendency toward a smaller effect size in virtual paradigms can be detected in the literature. While one might assume that a smaller cortisol response could mainly be attributed to technical constraints of the time, recent studies do not necessarily find larger effects (Linninge et al., 2018; Montero-López et al., 2018). Apparently, while technical capabilities and resources can be considered a crucial factor in the improvement of virtual stressors (as will be further elaborated on in a later part of this section) other aspects that go beyond technical implementation must be considered in order to maximize the neuroendocrine stress response.

Concerning the activation of the sympathetic nervous system, ample evidence for a stimulating effect of the virtual TSST can be found. In 2008, Kotlyar and colleagues reported a significant increase in heart rate and systolic and diastolic blood pressure in response to their version of the TSST-VR. Two years later, Jönsson and colleagues (2010) utilized a TSST-VR in an immersive computer automatic virtual environment (CAVE) to confirm that the increase in heart rate begins in the preparation phase of the TSST, continues throughout the speech task and peaks during the mental arithmetic task. Subsequently, they were able to replicate this result in several studies (Annerstedt et al., 2013; Fich et al., 2014; Jönsson et al., 2015, 2010; Linninge et al., 2018). Moreover, the group also measured cardiac sympathetic activity in the T-wave amplitude in several of the previously mentioned studies (Annerstedt et al., 2013; Fich et al., 2014; Jönsson et al., 2015, 2010; Wallergård, Jönsson, Johansson, & Karlson, 2011) and predominantly found effects that follow the same pattern as the heart rate measurements. Curiously, they found less conclusive results on high frequency heart rate variability. In line with the findings in heart rate, several studies report similar effects of the TSST-VR with regard to tonic skin conductance measures (Montero-López et al., 2016; Ruiz et al., 2010; Shiban et al., 2016). Although this parameter has to be considered somewhat unspecific (Dawson, Schell, & Filion, 2016), it lends further support to the conclusion that a virtual TSST is a suitable stressor for the activation of the SAM system.

In sum, slightly less than twenty studies that employed a virtual TSST as a method for stress induction have been published since 2007. It now seems warranted to assume that the paradigm does indeed represent an effectual method for psychobiological stress induction. Nevertheless, some doubt can be raised as to whether it is reasonable to compare early studies on the TSST-VR with more contemporary adaptations. After all, the second decade of the 21st century has seen the advent of what some scholars termed "the virtual revolution" (Blascovich & Bailenson, 2011) to describe the vastly increased interest of large companies, such as Facebook, Sony, HTC, and Valve in the development of affordable and powerful VR-headsets and software. When the first of a new generation of high-end, cost-effective head-mounted VR displays became commercially available with the Oculus Rift Development Kit One (Oculus VR, Irvine, CA) in 2013, a new pathway for the development of VR-based scientific methods was opened that does no longer require large development teams in order to implement the relatively simple paradigms needed for psychological research and therapy. This trend towards improving accessibility for users that may not have a background in software development is also reflected in a new generation of VR-suited graphic engines, such as current iterations of Unreal (Epic Games, Raleigh, NC) and Unity Engine (Unity Technologies, San Fracisco, CA) that favor self-explanatory user interfaces and helpful toolboxes and thereby substantially facilitate the creation of virtual environments and paradigms. While it has initially taken these developments some years to find their way into psychological research, they are now firmly established and used by research groups worldwide. As a consequence, psychobiological stress research should now command the means to devise a virtual adaptation of the TSST that might potentially lead to larger effects in stress induction by increasing subjective feelings of realism and presence (Steed et al., 2016). The primary focus of the present dissertation is therefore to systematically evaluate a state of the art TSST-VR and its psychobiological correlates and to identify effect mechanisms and possible applications of the paradigm.

3. Aims of the Present Dissertation

In the present dissertation, two empirical journal articles that have already been published and one manuscript that has recently been accepted for publication by the scientific journal *Physiology and Behavior* will be presented. The aim of the dissertation and the studies that are delineated in the publications was threefold: To begin with, an advanced version of the TSST-VR with a high degree of graphical fidelity and responsiveness was tested against the well-established *in vivo* procedure to determine its capabilities in terms of HPA axis and SAM system activation. Secondly, after having established that a sophisticated version of the paradigm in a virtual environment does indeed promote heightened reactivity of the stress effector systems, subjective sense of presence (Riva et al., 2007) was investigated as one potential moderator of the psychobiological stress response. Lastly, the paradigm was employed in a scientific use scenario in an attempt to investigate a novel research question: As some studies have proposed an increase of emotion recognition and processing after social threat (Deckers et al., 2015), we subjected participants to either a virtual TSST or a virtual control condition before testing emotion recognition performance in a novel paradigm based on signal detection theory.

3.1. Virtually Stressed? Investigating the Efficacy of a Psychosocial Stressor in VR

Zimmer, P., Buttlar, B., Halbeisen, G., Walther, E., & Domes, G. (2019). Virtually stressed? A refined virtual reality adaptation of the Trier Social Stress Test (TSST) induces robust endocrine responses. *Psychoneuroendocrinology*, *101*, 186-192 doi:10.1016/j.psyneuen.2018.11.010

As outlined in section 2.4., several research groups have attempted to adapt the TSST for the virtual domain and thereby establish a fully standardized and economic procedure for psychobiological stress research. While successful activation of the sympathetic nervous system is reported in almost all studies using the paradigm (Jönsson et al., 2010; Kothgassner et al., 2016; Linninge et al., 2018), eliciting a reliable response of the HPA axis that is comparable to the magnitude of the regular TSST has been met with various degrees of success (Kothgassner et al., 2016; Shiban et al., 2016). However, since recent findings show a link between better graphical presentation and presence (Gromer et al., 2019), the assumption that a modernized version of the paradigm might elicit stronger physiological responses seems warranted.

The first study that is to be reported in the present dissertation was therefore concerned with the division of a procedure for a virtual TSST that utilizes the technological advantages in the field of VR hard- and software in order to maximize its stress-inducing characteristics. For this purpose, we incorporated the findings reviewed above and used them as a starting point to develop a virtual TSST that can be tailored to each participants' individual behavior. We then tested this new alteration of the TSST-VR in an orthogonal experimental design that comprised of two TSST (*in vivo* vs. VR) and two non-stressful control conditions. Throughout the experimental procedure, participants' subjective feelings of stress as well as salivary cortisol and alpha amylase were measured as indicators of the respective stress effector systems. Furthermore, during the TSST (or control) phase, heart rate and skin conductance levels were recorded. The results of this experimental validation of the TSST-VR are mostly in line with the findings that are described in the published literature. While the TSST-VR did, in fact, cause a substantial raise of free salivary cortisol of about 100 percent, cortisol release in the TSST *in vivo* condition was still more pronounced and persisted for a longer period of time. Although the raise in heart rate was higher in the TSST *in vivo* condition as well, we did not find significant differences in alpha amylase secretion or subjective stress reports between the stress conditions.

In sum, this methodological evaluation of the paradigm demonstrates that the TSST-VR is indeed suitable as a tool for psychobiological stress induction. Consequentially, further investigation into the precise effect mechanisms of psychosocial stress induction in virtual environments was needed.

3.2. The Sense of Presence as a Potential Moderator of Virtual Stress

Zimmer, P., Wu, C., & Domes, G. (in press). Same same but different? Replicating the real surroundings in a virtual Trier Social Stress Test (TSST-VR) does not enhance presence or the psychophysiological stress response. *Physiology and Behavior*.

As described in the previous section, the refined adaptation of the TSST-VR we evaluated differed from previous iterations in its stress-inducing capabilities. Although no changes were made to the experimental protocol by Kirschbaum and colleagues (1993), participants in the TSST-VR we employed showed a comparatively stronger response in stress reactivity parameters than reported in the previous literature. This might potentially be attributed to a higher degree of graphical fidelity, a higher responsiveness of the committee that reacted to the participants based on eye-tracking data and a parallelization of the real and virtual environment that was achieved via modelling the VR environment after the real laboratory. It could be assumed that these modulations have enhanced the experience of presence in the virtual scenario (Sanchez-Vives & Slater, 2005). According to Wirth and colleagues (2007), presence is experienced when people have the sensation of being physically situated in a mediated space and perceive only those action possibilities that are relevant to this space instead of the ones in the real world. Considering that the sense of presence seems to be linked to the onset of strong emotional responses in VR, heightened presence might, in turn, have led to an increased stress response (Diemer, Alpers, Peperkorn, Shiban, & Mühlberger, 2015). In order to examine whether a higher sense of presence leads to a stronger psychophysiological response to a virtual stressor, we conducted a second experiment in which we attempted to modulate presence. For this purpose, we had participants perform the TSST in either an environment that closely resembled their real surroundings (thus facilitating the transition from the real to the virtual world) or in foreign virtual surroundings that did not share any resemblance with the real surroundings. In addition to subjective stress and physiological responses (salivary cortisol, alpha amylase and heart rate), we measured the sense of presence via the use of the well-established IGroup Presence Questionnaire (IPQ; Schubert, Friedmann, & Regenbrecht, 2001). Intriguingly, participants in the parallelized environment did not report stronger subjective presence and did not exhibit a stronger increase in any of the subjective or physiological stress parameters than the participants that performed the TSST-VR in a substantially different environment. While the present study could therefore not entirely elucidate the relationship between presence and physiological responses in virtual environments, it does nonetheless have some practical implications for a potentially more widespread implementation of the paradigm. Namely, the results demonstrate that participants experience presence and react strongly to the virtual scenario regardless of how similar (or different) the real surroundings are, thus making the laborious recreation of environments in virtuality obsolete. With the degree of flexibility gained in this way, the paradigm offers a valid alternative to conventional methods of laboratory stress induction in multi-center research endeavors.

3.3. Socially Evaluative Stress and the Ability of Emotion Recognition

Domes, G., & Zimmer, P. (2019). Acute stress enhances the sensitivity for facial emotions: a signal detection approach. *Stress, 22*(4), 455-460. doi:10.1080/10253890.2019.1593366

The ability to quickly recognize emotions in the facial expression of others is an important skill that is necessary to draw inferences about their mental state and to act accordingly. In the event of a threatening occurrence that challenges one's coping abilities and resources, obtaining social support can often be an expedient strategy (Taylor et al., 2000). Curiously, very few studies have investigated the association between acute stress and emotion recognition performance. Although some findings point to a heightened emotion detection performance after psychosocial stress (Deckers et al., 2015), most studies provide far less conclusive results and indicate complex sex-dependent effects (Duesenberg et al., 2016; Smeets, Dziobek, & Wolf, 2009).

In the third study of the present dissertation, the TSST-VR was used as a procedure for laboratory stress manipulation in order to further investigate whether a promotive effect of psychosocial stress on emotion recognition can be assumed. For this purpose, we recruited two groups of participants that were either subjected to the virtual TSST or a non-stressful virtual control condition (Placebo TSST; Het et al., 2009). Subsequently, all participants undertook a computer-based facial emotion recognition task that required them to decide whether a face that was presented to them displayed a target emotion (anger or happiness) as fast as possible. In a classical signal detection framework (Pessoa, Japee, & Ungerleider, 2005), we calculated discrimination index (average hit rate – average false alarm rate) and response bias and examined response latencies. In line with the findings by Deckers and colleagues (2015), we discovered an overall increase in emotion detection performance for both valences after stress. Furthermore, participants not only showed higher discrimination indices, they also reacted significantly faster to stimuli containing emotional expressions. This enhancement of emotion detection and response time was, however, not predicted by the stress-induced alterations in biological markers such as salivary cortisol or alpha amylase. Taken together, these findings support the hypothesis that psychosocial stress sensitizes for social signals as a precursor to acquiring social support from one's environment. Furthermore, this study again demonstrates the utility of the TSST-VR as a tool for standardized and economic psychobiological stress induction.

4. Original Manuscripts

The following chapter includes two published journal articles and one original manuscript that has recently been accepted for publication. The three original works can be understood as the centerpiece of this dissertation as they summarize the investigations that have been conducted in order to elucidate the research questions delineated in the previous chapter. While the two publications are included in the format in which they have been published in their respective journals, the manuscript that is awaiting production is inserted as it was formatted during submission for assessment by the editor and reviewers.

4.1. Zimmer, P., Buttlar, B., Halbeisen, G., Walther, E., & Domes, G. (2019). Virtually stressed? A refined virtual reality adaptation of the Trier Social Stress Test (TSST) induces robust endocrine responses. *Psychoneuroendocrinology*, *101*, 186-192. doi:10.1016/j.psyneuen.2018.11.010

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Virtually stressed? A refined virtual reality adaptation of the Trier Social Stress Test (TSST) induces robust endocrine responses

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ABSTRACT

In recent years, virtual reality (VR) technology has found its way into nearly all fields of psychology. Previous studies indicated that virtual reality adaptations of the TSST are less potent in stimulating HPA-axis responses, with lower salivary cortisol responses recorded as compared to the in-vivo TSST. (TSST-IV). In the present experiment we tested the stress-induction potential of a refined version of the TSST-VR using a fully orthogonal experimental design in which ninety-three healthy males were either assigned to the TSST condition or a corresponding control condition in a real or virtual environment. We found a significant increase of endocrine, autonomic and self-reported stress markers in both stress conditions. Notably, we found a robust rise in salivary cortisol to the TSST-VR comparable to that observed in the TSST-IV. Despite subtle differences in response between virtual and in vivo settings, we conclude that VR adaptations of in-vivo stressors have the potential to induce real physiological and subjective reactions.

1. Introduction

Over the last decades a number of highly standardized laboratory stressors have been developed to induce psychosocial stress in the laboratory (e.g. the Socially-Evaluated Cold Pressor Test, SECPT, (Schwabe et al., 2013); Maastricht Acute Stress Test, MAST, (Smeets et al., 2012). Among these protocols, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) has become widely used in psychobiological stress research as it has been proven to evoke robust endocrine and cardiovascular responses in the majority of participants.

The TSST mainly consists of a short mock job interview and a mental arithmetic in front of an audience of two or three people. It thus induces the two main factors for robust HPA-axis activation: Social evaluative-threat and uncontrollability (Dickerson and Kemeny, 2004). A recent meta-analysis provided evidence that the TSST is quite robust against protocol variations (Goodman et al., 2017). As long as the protocol comprises both tasks in front of evaluative judges, most participants respond with a significant increase in free salivary cortisol resulting in an overall average two-fold increase over baseline.

Aside from adaptations for specific environments (e.g. MRI, EEG, groups) and populations (e.g. children, elderly), the TSST has been adapted for the use in virtual realities (VR). Using the TSST-VR has three main advantages: Firstly, it significantly reduces the resources needed for research as it makes the presence of extensively trained judges obsolete. Secondly, it offers maximum experimental control, as the agents reliably behave in a highly controlled and standardized way. Lastly, it provides an environment that easily allows for the manipulation of contextual factors (characteristics of the panel, features of the room etc.). It is thus not surprising that a number of preliminary studies have tried to validate their specific adaptation of the TSST-VR (Kelly et al., 2007; Kotlyar et al., 2008; Jönsson et al., 2010; Wallergård et al., 2011) and to provide evidence that the TSST-VR induces a comparable pattern and magnitude of psychobiological reactions as their in-vivo counterpart.

Despite the fact that the published studies on variations of the TSST-VR reported reliable subjective stress responses, most of them demonstrated less robust or lower stress responses of the HPA-axis, concluding that VR adaptations of the TSST are less potent in inducing psychobiological stress reactions. The explicit comparison to a comparable in vivo stressor, however, was not made in most of these studies (e.g. Jönsson et al., 2010; Ruiz et al., 2010; Fich et al., 2014; Montero-López et al., 2016). One recent study by Shiban et al. (2016) implemented a control in vivo condition, and found lower average HPA-axis responding and lower responder rates (using predefined response criteria; Miller et al., 2013) suggesting that the TSST-VR is a milder stressor compared to its in vivo original. One possible explanation is based on

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the assumption that emotional reactions in virtual environments are associated with the individual's feeling of presence (Diemer et al., 2015). Immersion and interactivity have been associated with the subjective presence in VR environments (Baños et al., 2004) and are limited by technical factors such as the graphics engine modeling the virtual environment and the extent to which the agents react to the participants' behavior. In the previous studies, these factors might have limited immersion and interactivity and in consequence may have mitigated the participant's sense of presence which potentially led to lower psychophysiological reactions to the stressful situation.

To overcome these limitations, we designed a virtual reality adaptation of the TSST, in which the virtual surroundings are precisely modeled after the actual laboratory setting. Furthermore, an eye-tracking device was used for real-time feedback of eye-to-eye contact between the participant and the virtual judges. In addition, we modified the VR judges to match their real counterparts as closely as possible, thus maximizing interactivity and sense of presence.

We conducted a standard TSST (Kirschbaum et al., 1993) in VR and in vivo and carefully parallelized both conditions. Similarly, we administered a comparable, but non-stressful placebo version of the TSST (Het et al., 2009) in vivo and in VR. This orthogonal design permitted us to assess the effects of the social stress induction in vivo and in VR independently and therefore detect potentially differential outcomes. We hypothesized that in this rigorous experimental design that uses a refined TSST-VR, similar physiological and psychological stress reactions to a social evaluative stressor will be found in vivo and in VR.

2. Methods

2.1. Participants and design

The experimental design comprised two between-subjects factors: *Strain* (stress or control) and *Reality* (VR or in vivo). An a priori calculation of required sample size for the two-way interaction resulted in a minimum of $N = 84$ for a power of $1\text{-}\beta = .95$ and an effect of $d = 0.8$—an effect size which can be expected in combinations of public speaking and cognitive tasks like the TSST (Dickerson and Kemeny, 2004).

Participants were recruited by on-campus advertisement and were included into the study if they had a BMI between 19 and 26 kg/m^2 and an age between 18 and 50 years. Further exclusion criteria were (a) acute or chronic somatic or psychiatric disease, (b) regular intake of medication, (c) psychotherapeutic treatment during the last year, (d) nicotine intake of more than five cigarettes per day, and (e) regularly working night shifts (Niu et al., 2011). Participants were asked to refrain from physical exercise and alcohol at least 24 h prior to testing and to refrain from consuming anything but water two hours prior. The study was approved by the ethics committee at the University of Trier and conducted in line with the Declaration of Helsinki. All participants gave informed written consent and were paid 30€ for their participation.

Ninety-three male participants ($M = 25.02$; $SD = 4.41$; age range: 19–45) enrolled for the study and were randomly assigned to one of the four conditions: Stress-VR ($n = 29$; age range: 20–45; $M = 24.93$; $SD = 4.63$), stress in vivo ($n = 21$; age range: 21–44; $M = 26.05$; $SD = 4.80$), control-VR ($n = 22$; age range: 18–33; $M = 22.82$; $SD = 3.72$), and control in vivo ($n = 21$; age range: 19–32; $M = 24.30$; $SD = 3.61$). A one-way ANOVA revealed no significant age differences between the groups ($F(3, 88) = 2.17$ $p = .097$, $\eta_p^2 = .07$). Five participants of the stress-VR group and one of the stress in vivo group had to be excluded due to technical errors in the VR procedure, resulting in a total sample of $N = 87$. Furthermore, due to technical errors, one person had to be excluded from the heart rate (HR) data analysis and another four people from the skin conductance level analysis.

2.2. Apparatus

The VR environment was generated using the Steam Source engine (Valve Corporation, Bellevue, Washington, USA) and controlled by the VR simulation software CyberSession 5.6 (VTPlus GmbH, Würzburg, Germany). A Head-Mounted Display (HMD; Oculus Rift DK2, Oculus VR LLC, Menlo Park, CA, USA) and headphones were used. Heart rate and skin conductance were monitored and recorded with Brain Vision Recorder (Version 1.20.0801, Brain Products GmbH, Gilching, Germany). Further technical specifications can be found in the supplementary methods published online with this article.

2.3. Measures

2.3.1. Saliva sampling and analysis

At seven time points throughout the experiment, participants were asked to give saliva samples, using Salivettes (Sarstedt, Nümbrecht, Germany), to determine salivary cortisol and alpha amylase (sAA) levels. After the experiment saliva samples were stored at – 20 °C until biochemical analysis was carried out by the University Laboratory. For details of biochemical analyses, see supplementary methods online.

2.3.2. Heart rate

Heart rate (HR) was recorded using a finger-pulse-plethysmograph (Becker Meditec, Karlsruhe, Germany). The sampling rate was 100 Hz. Brain Vision Analyzer (Version 2.1.1.964, Brain Products GmbH, Gilching, Germany) was used to export RR-intervals. ARTiiFACT (Kaufmann et al., 2011) was used to correct artifacts and export mean HR of the different experimental segments.

2.3.3. Skin conductance level

Skin conductance level (SCL) was recorded using two Ag/AgCl surface electrodes ($\text{Ø} = 8\ \text{mm}^2$) that were covered with isotonic electrode gel and placed on the thenar and hypothenar area of the non-dominant palm (Dawson et al., 2016). The sampling rate was 100 Hz. Again, Brain Vision Analyzer was used to export SCL to Ledalab (Benedek and Kaernbach, 2010) which allowed conduction of artifact correction and exporting of mean SCL of the different experimental segments.

2.3.4. Subjective measures

At five time points, participants rated their subjective feelings of stress on visual analogue scales with a range of 0 (not at all) to 100 (very much) (cf. von Dawans et al., 2012).

2.4. Procedure

Experimental sessions were scheduled to start at 3.30 p.m. or 5.30 p.m. to control for the circadian rhythm of cortisol (Kudielka and Wüst, 2010). After giving informed consent, participants filled out the first subjective stress ratings (VAS1) and gave the first saliva sample (S1) before being lead into the VR laboratory. After application of the equipment for the physiological measurements, participants in the VR conditions put on the HMD and the TSST (or Placebo TSST (Het et al., 2009), began.

After the baseline measurements—that doubled as a period of acclimatization to the new situation—in either the real laboratory or the virtual environment (which was an exact replicate of the real laboratory), participants received instructions on the following task either by the experimenter or via headphones and written on the screen. In the stress conditions, they were told that they would have to do a job interview in front of a panel of judges who would shortly enter. In the control conditions, participants were told that they would have to talk about a self-chosen topic in an empty room. Both conditions were conducted in accordance with their respective original protocols (Kirschbaum et al., 1993), and (Het et al., 2009) although minor

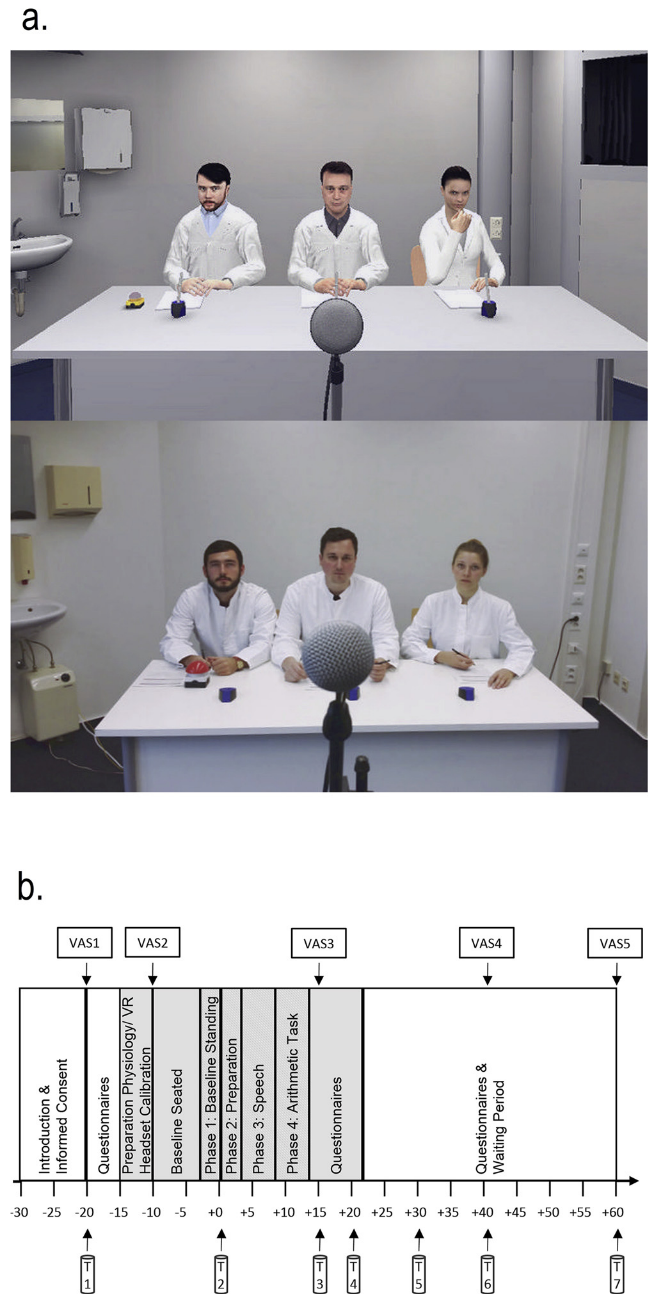

Fig. 1. (a) Picture of the VR (upper panel) and the in vivo (lower panel) judges in the stress conditions and (b) experimental procedure depicting experimental phases and time of assessment of subjective stress ratings (VAS) and saliva samples (S). Procedures in the preparation room are depicted in white; procedures in the VR laboratory have been marked in grey. Hatched patterns represent crucial phases of the (placebo) TSST procedure.

changes were made to facilitate the implementation into a virtual environment. These changes included a shorter preparation time of 3 min in front of the panel after they had been introduced to the task instead of preparing their speech for 10 min in another room alone. In addition, due to the virtual environment they were not able to take notes with paper and pencil. These changes were made to both TSST conditions.

The entire procedure was controlled by the experimenter behind a one-way mirror. Furthermore, prompts to maintain eye contact were automatically triggered in the TSST-VR after five seconds without eye-contact (i.e., not looking into a predefined area surrounding the judges' heads). After the task, the judges left the room (and the screen turned black in VR). The experimenter subsequently reentered the VR laboratory and participants in the VR conditions took off the HMD. All participants remained in the lab until 60 min after TSST started and provided five questionnaires with VAS and seven saliva samples in total. Following the last sample, participants were debriefed and compensated. Further details about the experimental procedure can be found in Fig. 1 or in the supplementary methods.

2.5. *Statistics*

Mixed repeated-measures ANOVAs were conducted to test for effects of stress condition (TSST vs. Control), experimental environment (VR vs. in vivo) and time over the course of the experiment (as a repeated-measures factor) on subjective and physiological measures. In cases where Mauchly's test indicated a violation of the assumption of sphericity, we used Greenhouse-Geisser correction and calculated ε- and corrected *p*-values. All analyses were conducted with SPSS for Windows (Version 24). Significance level was set at $p < .05$. Effect sizes are reported as η_p^2 with 95% Confidence Intervals. All pairwise comparisons were Bonferroni corrected.

3. Results

3.1. *Free salivary cortisol*

The cortisol responder rates to the different experimental conditions give a first indication of the success of the stress manipulation (see Table 1). To further evaluate whether the manipulation of stress was successful, we conducted two separate chi-square tests including the factor Strain and cortisol response—using the conservative criterion of a baseline-to-peak increase of 2.5 nmol/l—for the VR (χ^2 (1) = 3.81, $p = .051$) and in vivo condition (χ^2 (1) = 17.53, $p < .001$). In line with our hypothesis, the odds suggest that it is more likely to show a cortisol reaction to stress than to the control conditions in VR (3.3 times) and in vivo (40 times).

To follow up on these analyses, we conducted a 2 (Strain [stress, control]) x 2 (Reality [VR, in vivo]) x 7 (Time) repeated measures ANOVA (see Fig. 2, upper panels) which revealed significant main effects for Time ($F(6, 498) = 29.04$, $\varepsilon = .51$, $p < .001$, $\eta_p^2 = .26$, 95% CI [.19; .31]), Strain ($F(1, 83 = 13.36$, $\varepsilon = .51$, $p < .001$, $\eta_p^2 = .14$, 95% CI [.03; .28]), and Reality ($F(1, 83 = 4.10$, $\varepsilon = .51$, $p = .046$, $\eta_p^2 = .05$, 95% CI [.00; .16]). In line with our prediction, these main effects were qualified by the significant two-way interaction between the factors Time and Strain ($F(6, 498) = 13.94$, $\varepsilon = .51$, $p < .001$, $\eta_p^2 = .14$, 95% CI [.08; .19]), but also by the three-way interaction between the factors Time, Strain, and Reality ($F(6, 498) = 3.86$, $\varepsilon = .51$, $p = .01$, $\eta_p^2 = .04$, 95% CI [.01; .07]). Pairwise comparisons revealed that stress conditions differed from their corresponding control conditions (VR: +15 to + 60 min. post stress induction, all *ps* < .016; in vivo: +20 to + 60 min post stress induction, all *ps* < .011). Comparing in vivo and VR stress conditions using pairwise comparisons revealed significant effects at + 30 min and + 40 min (all *ps* < .004). All in all, and although cortisol rose and declined earlier in the VR than in the in vivo stress condition, the results indicate that the virtual TSST can activate the HPA-axis in a similar pattern.

Similar results were obtained when computing Area under the

Table 1
Cortisol Responder rates by conditions for a liberal and a conservative response criterion (1.5 vs. 2.5 nmol/l baseline-to-peak increase in free salivary cortisol).

	VR		In vivo	
	Stress	Control	Stress	Control
Response criterion	n (%)	n (%)	n (%)	n (%)
1.5 nmol/l increase	18 (75%)	9 (42.9%)	21 (100%)	10 (47.6%)
2.5 nmol/l increase	15 (62.5%)	7 (33.3%)	20 (95.2%)	7 (33.3%)

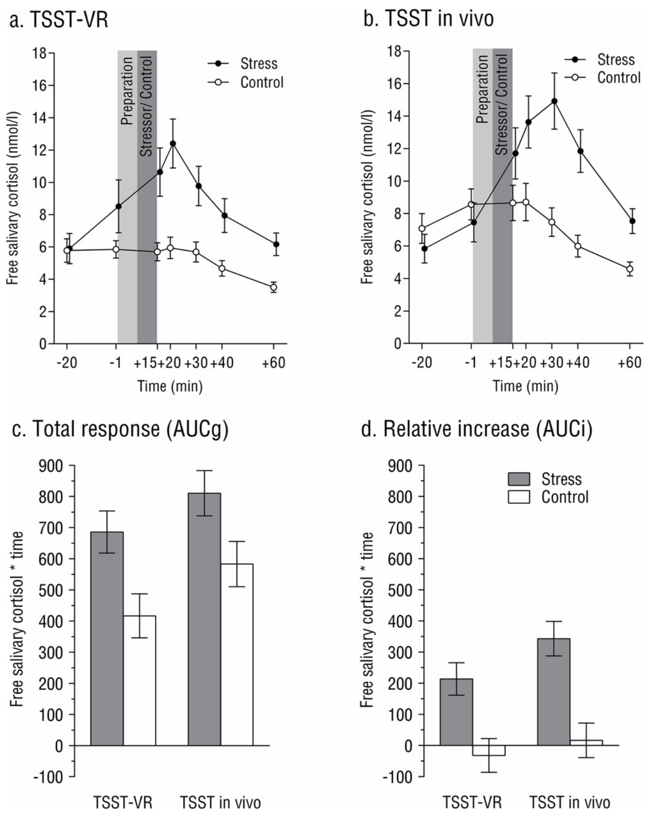

Fig. 2. Concentration of free salivary cortisol in response to the stress and control condition sampled at seven time points over the course of the experiment and as a function of the experimental conditions. (a) VR vs. (b) In vivo condition. (c) Area under the Curve with respect to ground (AUCg). (d) Area under the Curve with respect to increase (AUCi). Error bars denote standard errors.

Curve values using the formulas by (Pruessner et al., 2003) – see Fig. 2 lower panels. Conducting a 2 (Strain [stress, control]) x 2 (Reality [VR, in vivo]) ANOVA with area under the curve with respect to ground (AUCg) as the dependent variable, we found a significant main effect for Strain ($F(1, 83) = 11.70$, $p = .001$, $\eta_p^2 = .12$, 95% CI [.12; .26]) while the factor Reality missed significance ($F(1, 83) = 3.93$, $p = .051$, $\eta_p^2 = .05$). There was no significant Strain*Reality interaction ($F(1,83) = .06$, $p = .809$, $\eta_p^2 > .01$). Similar results were found for area under the curve with respect to increase (AUC_i). Again, a significant main effect for Strain was found ($F(1, 83) = 27.86$, $p < .001$, $\eta^2 = .25$, 95% CI [.10; .39]) while the main effect for Reality did not reach statistical significance ($F(1, 84) = 2.84$, $p = .096$, $\eta_p^2 = .03$). No significant Strain*Reality interaction ($F(1,83) = .46$, $p = .501$, $\eta_p^2 = .01$) was found. In sum, no differences between VR and in vivo stress conditions were found when using area under the curve values as an indicator of cortisol output in response to the experimental manipulation.

3.2. *Salivary alpha amylase*

As with cortisol, we conducted a 2 (Strain [stress, control]) x 2 (Reality [VR, in vivo]) x 7 (Time) repeated measures ANOVA (see Fig. 3) testing the effects on salivary alpha amylase. This ANOVA yielded a significant main effect of the factor Time ($F(6, 498) = 10.57$, $\varepsilon = .71$, $p < .001$, $\eta_p^2 = .11$, 95% CI [.06; .16]). The main effects of the factors Strain and Reality did not reach statistical significance ($F(1, 83) = 1.57$, $\varepsilon = .71$, $p = .21$, $\eta_p^2 = .02$ and $F(1, 83) = .03$, $\varepsilon = .71$, $p = .87$, $\eta_p^2 < .01$, respectively).

Furthermore, the two-way interaction between the factors Time and Strain ($F(6, 498) = 2.89$, $\varepsilon = .71$, $p = .020$, $\eta_p^2 = .03$, 95% CI [.00; .06]) reached statistical significance while the two-way interaction between Time and Reality ($F(6, 498) = .35$, $\varepsilon = .71$, $p = .857$, $\eta_p^2 < .01$) and the three-way interaction between Time, Strain, and Reality ($F(6, 498) = .08$, $\varepsilon = .71$, $p = .992$, $\eta_p^2 < .01$) did not. As predicted, further examination of the significant two-way interaction via pairwise comparisons revealed that the stress conditions differed significantly from the control conditions at +15 ($p = .037$) but not at any other time points (all $ps > .105$). Since the three-way interaction did not reach significance, we did not obtain evidence suggesting differences in the efficacy of in vivo and VR stressors concerning SAM-activation as measured by sAA concentration.

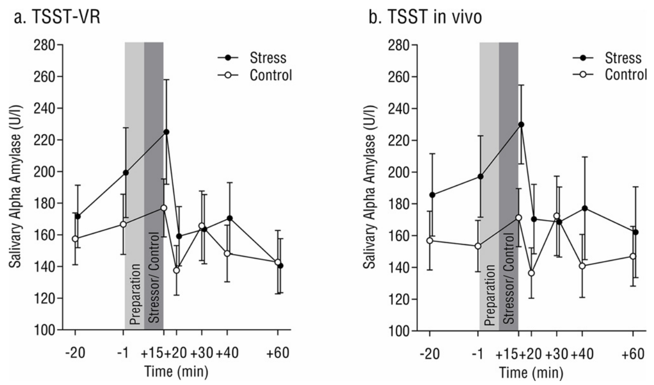

Fig. 3. Concentration of salivary alpha amylase (a) VR vs. (b) in vivo. Error bars denote standard errors.

3.3. *Heart rate*

To analyze another indicator of the SAM, we conducted a 2 (Strain) x 2 (Reality) x 4 (Time) repeated measures ANOVA using HR as a dependent variable (see Fig. 4). The four time points refer to the different phases of the TSST: Baseline measurements while standing, TSST preparation phase, TSST Interview, and TSST arithmetic task. This ANOVA yielded a significant main effect of the factor Time ($F(3, 246) = 112.40$, $\varepsilon = .72$, $p < .001$, $\eta_p^2 = .58$, 95% CI [.50; .63]) and Reality ($F(1, 82) = 4.39$, $\varepsilon = .72$, $p = .039$, $\eta_p^2 = .05$, 95% CI [.00; .17]). The main effect of the Factors Strain did not reach statistical significance ($F(1, 82) = .20$, $\varepsilon = .72$, $p = .656$, $\eta_p^2 > .01$).

Furthermore, the two-way interactions between the factors Time and Strain ($F(3, 246) = 8.47$, $\varepsilon = .72$, $p < .001$, $\eta_p^2 = .09$, 95% CI [.03; .16]) and Time and Reality ($F(3, 246) = 3.03$, $\varepsilon = .72$, $p = .047$, $\eta_p^2 = .04$, 95% CI [.00; .08]) reached statistical significance. These interaction effects were qualified by the significant three-way interaction

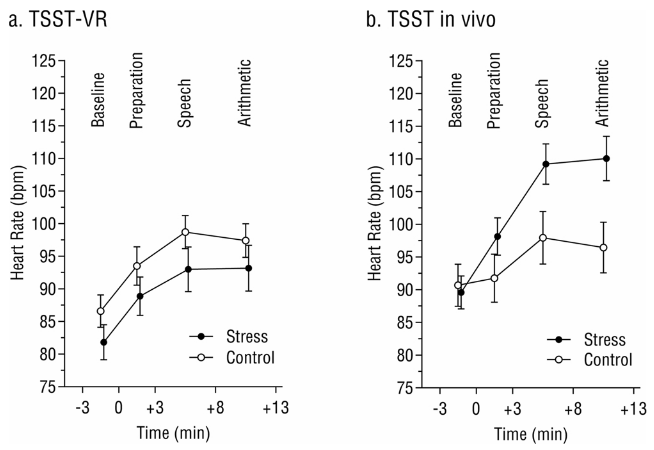

Fig. 4. Average heart rate during the four phases. (a) VR vs. (b) in vivo condition. Error bars denote standard errors.

between Time, Strain, and Reality ($F(3,\ 246) = 8.57$, $\varepsilon = .72$, $p < .001$, $\eta_p^2 = .10$, 95% CI [.03; .16]). Pairwise comparisons revealed that in the in vivo conditions, stress differed significantly from control during the TSST interview and arithmetic task ($p = .021$ and $p = .006$). In the VR conditions, no significant effects emerged at any of the TSST phases (all $ps > .222$). Comparing the respective VR and in vivo conditions, pairwise comparisons revealed significant differences between the stress conditions in all stages of the experimental procedure (all $ps < .046$); these differences in HR indicate that the in vivo stressor was more efficient in activating the SAM.

3.4. Skin conductance level

Analogous to the HR analysis, we conducted a 2 (Strain) x 2 (Reality) x 4 (Time) repeated measures ANOVA with SCL as the dependent variable. This ANOVA yielded only a significant main effect of the factor Time ($F(3,\ 237) = 10.41$, $\varepsilon = .69$, $p < .001$, $\eta_p^2 = .12$, 95% CI [.04; .19]), indicating a significant rise in SCL independent of conditions. None of the other the main effects nor the interactions reached statistical significance (all $Fs < 1.39$ and $ps > .251$).

3.5. Subjective measures

In accordance with previous research, we focused on the question "How stressed are you at the moment?" to analyze participant's subjective stress ratings (Shiban et al., 2016). We conducted a 2 (Strain [stress, control]) x 2 (Reality [VR, in vivo]) x 5 (Time) repeated measures ANOVA (see Fig. 5) that demonstrated a significant main effect of the factor Time ($F(4,\ 332) = 37.36$, $\varepsilon = .81$, $p < .001$, $\eta_p^2 = .31$, 95% CI [.23; .38]). Additionally, the two-way interaction between the factors Time and Strain ($F(4,\ 332) = 6.27$, $\varepsilon = .81$, $p < .001$, $\eta_p^2 = .07$, 95% CI [.02; .12]) reached significance. To examine this interaction, pairwise comparisons were used revealing the predicted data pattern; the stress conditions deviated from the control conditions at VAS3 (directly after the TSST, $p = .002$) but not at any other point in time (all $ps > .155$). No other effects or interactions were significant (all $Fs < 2.09$ and $ps > .152$). We therefore conclude that the VR and in vivo stress conditions are equally efficient in inducing subjective stress.

4. Discussion

The present study assessed whether a refined version of the TSST in VR poses a viable alternative to traditional face-to-face in vivo stress induction methods in the laboratory. Overall, the results suggest that subjective and physiological reactions to the VR and the in vivo version of the TSST were largely comparable. To the best of our knowledge, this is the first study to examine the effects of the TSST-VR and the TSST in vivo in a completely controlled experimental design with control groups in vivo and in VR. This orthogonal design enabled us to compare both versions of the TSST with their respective control group and thus assess the effect of the social stress induction independently. Taken together, we found similar patterns of results on most of our dependent subjective and physiological variables. While participants showed an increase of stress levels on almost all of our psychobiological stress markers, no such rise was observed in our control groups.

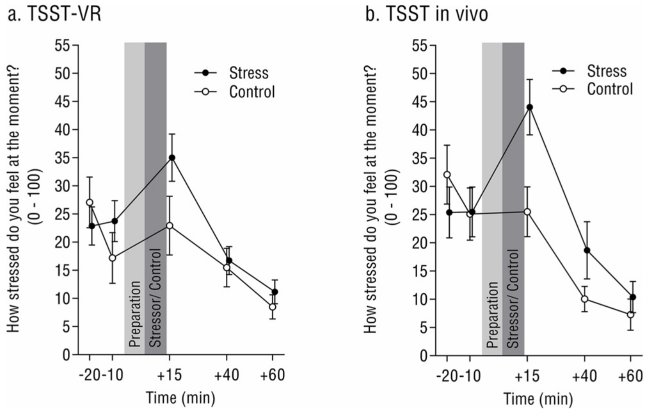

Fig. 5. Subjective ratings of stress (a) VR vs. (b) in vivo. Error bars denote standard errors.

Focusing on salivary cortisol as a major endocrine stress marker, the results are indeed consistent with the assumption that a robust and reliable stimulation of the HPA-axis with a social-evaluative stressor is possible in VR. With a 62.5% responder rate using the 2.5 nmol/l criterion and an on-average twofold increase, our results are comparable with the average cortisol reaction usually obtained with in vivo versions of the TSST (Goodman et al., 2017). The cortisol response to the present, improved version of the TSST-VR was more pronounced and more robust compared to previous studies that used variations of the TSST in VR (e.g., Kelly et al., 2007; Ruiz et al., 2010; Shiban et al., 2016). As stated above, this might be mainly due to rapid technological progress, especially regarding advanced graphics, which can be considered a main prerequisite for an increased immersion and the subjective feeling of presence in the virtual adaptation of the TSST. It is our understanding that the current technological status at the time of experimentation plays an essential role in this field of research. In the past, researchers often had to resort to VR headsets that are described as rather clunky and uncomfortable, (e.g. Kelly et al., 2007 refer to their headset as a helmet with a small viewing screen) and although technical aspects like weight, resolution, or viewing angle are often not reported, it can be assumed that these devices might have hindered the participants from experiencing the degree of presence that modern HMDs achieve. This assumption is largely supported by the fact that studies using a CAVE system to realize their VR conditions (an immersive stereoscopic room in which images are projected onto the walls and participants wear specifically designed glasses instead of HMDs) tend to report large stress effects on physiological markers (Jönsson et al., 2010; Fich et al., 2014). In a direct comparison of both modalities of presentation, Juan and Pérez (2009) found that exposure therapy provoked more anxiety and a higher sense of presence in acrophobic patients when it was conducted in a CAVE system than with an HMD. Since that time, however, technical progression and the introduction of virtual reality headsets to a wider audience via the medium of video games provided researchers with light, relatively comfortable HMDs with high resolution displays and effective motion tracking mechanisms. It would thus be quite informative to experiment with both modes of presentation with state of the art technology in order to elucidate whether there are still significant differences in effectiveness.

In addition, we specifically aimed at maximizing comparability between the different conditions by carefully emulating the in vivo surroundings in the virtual environment and increasing interactivity by introducing automated eye-tracking-based verbal feedback when the participants did not maintain eye-contact with the agents. Both factors—the sophistication of the graphical presentation and the high level of perceived interactivity—might have promoted immersion and presence and thus contributed to the comparability of psychobiological stress responses in the stress conditions (Diemer et al., 2015).

Beyond examining cortisol responses, the comparability of psychological stress reactions in the virtual condition and in vivo can be shown by the rise in sAA—a valid index of sympathetic activation (Nater and Rohleder, 2009) —and the increase in subjective stress ratings. On these measures, the TSST-VR elicited stress responses that were equally high in the VR and the in vivo setting. This supports the conclusion that stress induction paradigms in a virtual environment, such as the TSST-VR, can be potent reflections of a stressful situation in reality.

Moreover, the orthogonal experimental design permits us to infer that the observed stress reactions in the TSST-VR were indeed elicited by the stressful characteristics of the task itself and not by the fact that it was performed in an unfamiliar artificial environment. As assessed via self-report at the end of the experiment, almost all of our participants reported little or no previous experience with immersive virtual reality technology. It is therefore conceivable that the novelty of being immersed in a virtual environment that is entirely under the control of the experimenter might be sufficient to make participants feel uncomfortable and thus induce stress. This alternative explanation for the observed stress effects in previous studies (Jönsson et al., 2010; Fich et al., 2014) cannot be discarded without the implementation of a control group in VR. In the present study, the comparison between the TSST-VR and the control condition in VR showed differential patterns of reactions. The fact that we found stress effects on our dependent measures only in the stress condition suggests that the immersion in a foreign virtual environment is not sufficient to elicit a stress response, at least in terms of HPA-axis activation.

It should be noted, however, that cortisol and HR responses to the in vivo stress condition were still slightly more pronounced than to the VR stress condition, whereas the SCL response was not affected differently in the conditions. Possible explanations for these findings may be that the overall rise in heart rate in the virtual control condition might be attributed to increased activation caused by the speaking task and anticipatory arousal due to the unfamiliar virtual environment, and the low reactivity in SCL to constraints of the measurement (e.g., very sweaty palms). It may, however, also be the case that virtual adaptations of the real world—although potent reflections of many aspects of real situation—are still limited by technological restraints which lead to slightly attenuated psychobiological reactions to these environments. Furthermore and more specifically, the TSST might be especially difficult to replicate in a virtual environment because of its conceptualization as a stressor that uses a performance situation in the presence of unapproachable human judges to generate social evaluative stress. These necessary characteristics—evaluation and negative feedback by human experts and uncontrollability of the situation (Dickerson and Kemeny, 2004)—should make the translation into virtual reality difficult, since participants will still be able to envision that they are not actually performing in front of real human beings but programmed entities. Nevertheless, previous studies and the present findings suggest that a majority of participants still adhere to social conventions (Garau et al., 2005) and experience social evaluative stress in the presence of virtual agents, as indicated by the subjective and endocrine reactions (Jönsson et al., 2010; Kothgassner et al., 2016; Montero-López et al., 2016; Shiban et al., 2016).

As mentioned in the methods section, the implementation of the TSST into virtual reality and the parallelization of the experimental conditions required some alterations to the original study protocol (Kirschbaum et al., 1993) mainly in the preparation phase. A recent meta-analysis on protocol variations of the TSST has, however, shown that the stress induction effect is quite robust against a variety of changes that have been made to the paradigm over the years of its application (Goodman et al., 2017). The substantial stress effects that we report in both TSST conditions in our study seem to further support the idea that the strict adherence to the original protocol might not be a necessary precondition for successful stress induction as long as the main stressful features, social threat and uncontrollability are realized (Dickerson and Kemeny, 2004). It might be an interesting question for future studies, whether the TSST protocol can be generally simplified without reducing its stressfulness.

Some potential limitations of the present experiment should be noted. As in many fundamental studies on endocrine stress reactivity, we started by examining an exclusively male sample of participants. Besides the fact that men and women differ in their endocrine profiles and reactivity to social stress (Kudielka and Kirschbaum, 2005; Kelly et al., 2008), some studies show a differential effect of gender on the perception of virtual environments (Munafo et al., 2017), especially regarding Sense of Presence (Felnhofer et al., 2012). Although the widely assumed concept that men and women differ in their affinity to video gaming and virtual environments in general is slowly being disproved (Rehbein et al., 2016), video games still occupy a larger role in men's free time than in women's (Borgonovi, 2016). Secondly, we assessed the endocrine stress responses by using salivary measures of cortisol and alpha amylase. Although these measures have been proven valid indicators of HPA-axis and catecholaminergic stress reactivity (Hellhammer et al., 2009; Nater and Rohleder, 2009), direct measures of ACTH, cortisol and catecholamines in plasma would have possibly been more sensitive in the assessment of subtle differences between the VR and in vivo version of the TSST. Lastly, it should be noted that although the participant was alone in the room during all VR procedures, the experimenter was in the adjacent room behind a one-way mirror and supervised the experimental sessions and controlled the agents' reactions to the participants' performance. Moreover, the necessity of taking a saliva sample right before the start of the task required the experimenter to re-enter the room and hand the participant the Salivette. In the VR groups, this was done while the participants were wearing the headset so that they consequently saw neither the experimenter nor their own hands while chewing the cotton swab. We therefore cannot rule out that the participants were, to some extent, aware of the experimenter's presence. Thus, the feeling of being socially evaluated might not have been exclusively conveyed by the virtual agents, but to some degree also by the experimenter. Future studies should evaluate the influence of the experimenter's presence on immersion and presence in the virtual reality.

5. Conclusion

Taken together, the present study demonstrates that social evaluative stress can be successfully induced in a virtual environment resulting in stress responses on several physiological measures associated with the HPA axis and the SAM system. By using a refined VR version of the TSST, we could show that situations realized in VR have the potential to realistically simulate complex social interactions and evoke comparable subjective and physiological reactions. Due to its computer-generated nature, the TSST-VR has several key advantages: First, it is entirely standardized with no variation between testing sessions. Secondly, it is very economic insofar as it reduces the necessary amount of personnel from at least three to one and makes training judges obsolete. Lastly, it facilitates the variation of parameters of interest. In sum, the present study demonstrates that a technologically sophisticated version of the TSST-VR that maximizes interactivity and presence might be a valuable alternative to the traditional in vivo stress induction for experiments in psychoneuroendocrinology.

Author contributions

GD, EW and PZ designed the study and drew up the study protocol. PZ, BB and GH performed the experiments. GH supervised the procedures in the VR laboratory and adjusted the VR software to the requirements of the experiment. BB, GD and PZ performed the statistical analyses and wrote the first draft of the manuscript. All authors contributed to writing and have approved the final manuscript.

Declaration of conflicting interests

The authors declare no conflicts of interest with respect to the authorship or the publication of this article.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2018.11.010.

References

Baños, R.M., Botella, C., Alcañiz, M., Liaño, V., Guerrero, B., Rey, B., 2004. Immersion and emotion: their impact on the sense of presence. Cyberpsychol. Behav. 7, 734–741.

Benedek, M., Kaernbach, C., 2010. A continuous measure of phasic electrodermal activity. J. Neurosci. Methods 190, 80–91.

Borgonovi, F., 2016. Video gaming and gender differences in digital and printed reading performance among 15-year-olds students in 26 countries. J. Adolesc. 48, 45–61. https://doi.org/10.1016/j.adolescence.2016.01.004.

Dawson, M.E., Schell, A.M., Filion, D.L., 2016. The electrodermal system. In: Berntson, G.G., Cacioppo, J.T., Tassinary, L.G. (Eds.), Handbook of Psychophysiology, Cambridge Handbooks in Psychology. Cambridge University Press, Cambridge pp. 217–243.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. 130, 355–391.

Diemer, J., Alpers, G.W., Peperkorn, H.M., Shiban, Y., Mühlberger, A., 2015. The impact of perception and presence on emotional reactions: a review of research in virtual reality. Front. Psychol. 6.

Felnhofer, A., Kothgassner, O.D., Beutl, L., Hlavacs, H., Kryspin-Exner, I., 2012. Is virtual reality made for men only? Exploring gender differences in the sense of presence. Proc. Int. Soc. Presence Res. 103–112.

Fich, L.B., Jönsson, P., Kirkegaard, P.H., Wallergård, M., Garde, A.H., Hansen, Å., 2014. Can architectural design alter the physiological reaction to psychosocial stress? A virtual TSST experiment. Physiol. Behav. 135, 91–97.

Garau, M., Slater, M., Pertaub, D.-P., Razzaque, S., 2005. The responses of people to virtual humans in an immersive virtual environment. Presence Teleoper. Virtual Environ. 14, 104–116.

Goodman, W.K., Janson, J., Wolf, J.M., 2017. Meta-analytical assessment of the effects of protocol variations on cortisol responses to the Trier Social Stress Test. Psychoneuroendocrinology 80, 26–35.

Hellhammer, D.H., Wüst, S., Kudielka, B.M., 2009. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 34, 163–171. https://doi.org/10.1016/j.psyneuen.2008.10.026.

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., Wolf, O.T., 2009. Neuroendocrine and psychometric evaluation of a placebo version of the 'Trier Social Stress Test'. Psychoneuroendocrinology 34, 1075–1086.

Jönsson, P., Wallergård, M., Österberg, K., Hansen, Å.M., Johansson, G., Karlson, B., 2010. Cardiovascular and cortisol reactivity and habituation to a virtual reality version of the Trier Social Stress Test: a pilot study. Psychoneuroendocrinology 35, 1397–1403.

Juan, M.C., Pérez, D., 2009. Comparison of the levels of presence and anxiety in an acrophobic environment viewed via HMD or CAVE. Presence Teleoperators Virtual Environ. 18, 232–248. https://doi.org/10.1162/pres.18.3.232.

Kaufmann, T., Sütterlin, S., Schulz, S.M., Vögele, C., 2011. ARTiiFACT: a tool for heart rate artifact processing and heart rate variability analysis. Behav. Res. Methods 43, 1161–1170.

Kelly, M.M., Tyrka, A.R., Anderson, G.M., Price, L.H., Carpenter, L.L., 2008. Sex differences in emotional and physiological responses to the Trier Social Stress Test. J. Behav. Ther. Exp. Psychiatry 39, 87–98. https://doi.org/10.1016/j.jbtep.2007.02.003.

Kelly, O., Matheson, K., Martinez, A., Merali, Z., Anisman, H., 2007. Psychosocial stress evoked by a virtual audience: relation to neuroendocrine activity. Cyberpsychol. Behav. 10, 655–662.

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

Kothgassner, O.D., Felnhofer, A., Hlavacs, H., Beutl, L., Palme, R., Kryspin-Exner, I., Glenk, L.M., 2016. Salivary cortisol and cardiovascular reactivity to a public speaking task in a virtual and real-life environment. Comput. Hum. Behav. 62, 124–135.

Kotlyar, M., Donahue, C., Thuras, P., Kushner, M.G., O'Gorman, N., Smith, E.A., Adson, D.E., 2008. Physiological response to a speech stressor presented in a virtual reality environment. Psychophysiology 45, 1034–1037.

Kudielka, B.M., Kirschbaum, C., 2005. Sex differences in HPA axis responses to stress: a review. Biol. Psychol. 69, 113–132. https://doi.org/10.1016/j.biopsycho.2004.11.009.

Kudielka, B.M., Wüst, S., 2010. Human models in acute and chronic stress: assessing determinants of individual hypothalamus–pituitary–adrenal axis activity and reactivity. Stress 13, 1–14.

Miller, R., Plessow, F., Kirschbaum, C., Stalder, T., 2013. Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: evaluation of salivary cortisol pulse detection in panel designs. Psychosom. Med. 75, 832–840.

Montero-López, E., Santos-Ruiz, A., García-Ríos, M.C., Rodríguez-Blázquez, R., Pérez-García, M., Peralta-Ramírez, M.I., 2016. A virtual reality approach to the Trier Social Stress Test: contrasting two distinct protocols. Behav. Res. Methods 48, 223–232.

Munafo, J., Diedrick, M., Stoffregen, T.A., 2017. The virtual reality head-mounted display Oculus Rift induces motion sickness and is sexist in its effects. Exp. Brain Res. 235, 889–901. https://doi.org/10.1007/s00221-016-4846-7.

Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology 34, 486–496.

Niu, S.-F., Chung, M.-H., Chen, C.-H., Hegney, D., O'Brien, A., Chou, K.-R., 2011. The effect of shift rotation on employee cortisol profile, sleep quality, fatigue, and attention level: a systematic review. J. Nurs. Res. 19, 68–81.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931.

Rehbein, F., Staudt, A., Hanslmaier, M., Kliem, S., 2016. Video game playing in the general adult population of Germany: can higher gaming time of males be explained by gender specific genre preferences? Comput. Hum. Behav. 55, 729–735. https://doi.org/10.1016/j.chb.2015.10.016.

Ruiz, A.S., Peralta-Ramirez, M.I., Garcia-Rios, M.C., Muñoz, M.A., Navarrete-Navarrete, N., Blazquez-Ortiz, A., 2010. Adaptation of the trier social stress test to virtual reality: psycho-physiological and neuroendocrine modulation. J. Cyber Ther. Rehabil. 3 (4).

Schwabe, L., Höffken, O., Tegenthoff, M., Wolf, O.T., 2013. Stress-induced enhancement of response inhibition depends on mineralocorticoid receptor activation. Psychoneuroendocrinology 38, 2319–2326.

Shiban, Y., Diemer, J., Brandl, S., Zack, R., Mühlberger, A., Wüst, S., 2016. Trier Social Stress Test in vivo and in virtual reality: dissociation of response domains. Int. J. Psychophysiol. 110, 47–55.

Smeets, T., Cornelisse, S., Quaedflieg, C.W.E.M., Meyer, T., Jelicic, M., Merckelbach, H., 2012. Introducing the Maastricht Acute Stress Test (MAST): a quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses. Psychoneuroendocrinology 37, 1998–2008.

von Dawans, B., Fischbacher, U., Kirschbaum, C., Fehr, E., Heinrichs, M., 2012. The social dimension of stress reactivity: acute stress increases prosocial behavior in humans. Psychol. Sci. 23, 651–660.

Wallergård, M., Jönsson, P., Johansson, G., Karlson, B., 2011. A virtual reality version of the trier social stress test: a pilot study. Presence Teleoper. Virtual Environ. 20, 325–336.

4.2. Zimmer, P., Wu, C., & Domes, G. (in press). Same same but different? Replicating the real surroundings in a virtual Trier Social Stress Test (TSST-VR) does not enhance presence or the psychophysiological stress response. *Physiology and Behavior.*

Same same but different? Replicating the real surroundings in a virtual Trier Social Stress Test (TSST-VR) does not enhance presence or the psychophysiological stress response

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Abstract

In recent years, adaptations of the Trier Social Stress Test (TSST) have shown that socially evaluative stress can effectively elicit psychobiological responses in a standardized way in Virtual Reality (VR). While these methods hold many advantages, the underlying mechanisms of stress-induction effects via virtual avatars are still largely unclear. The present study tested whether the similarity of the real and virtual world modulates the stress response during a virtual TSST by intensifying the experience of presence. For this purpose, two groups performed the TSST-VR while their virtual surroundings were either a replication of the real laboratory or a foreign environment. Although a significant stress response with regard to salivary cortisol, salivary alpha amylase, heart rate and subjective feelings of stress was found in both groups, the parallelization of the real and virtual environment did not lead to an increase in physiological or subjective stress. Furthermore, both groups did not differ in self-reported presence. Beyond reproducing previous findings of successful psychobiological stress induction in VR, the results indicate that the paradigm is effective regardless of the context it is employed in and therefore could be a promising tool in multi-center research projects or clinical applications.

Keywords: Stress; cortisol; heart rate; Trier Social Stress Test; TSST; virtual reality; TSST-VR

1 Introduction

For several decades, behavioral science has acknowledged the importance of understanding the physiological consequences of stressors induced by our social environment. The need for a paradigm that could reliably emulate the strains of demanding psychosocial situations prompted Kirschbaum, Pirke, and Hellhammer [1] to develop the Trier Social Stress Test (TSST). This procedure, consisting of a mock job interview and a mental arithmetic task, has demonstrated its stimulating capabilities on both stress effector systems in the human body, the sympatho-adrenal-medullary (SAM) system, and the hypothalamic–pituitary–adrenal (HPA) axis, in numerous circumstances [2].

In recent years, Virtual Reality (VR) based paradigms have gained popularity in almost all fields of application in psychology due to their versatility and nearly limitless potential to create scenarios that are difficult to implement in other ways. In 2007, the first adaptation of the TSST in a virtual environment demonstrated the potential of studying the effects of socially evaluative stress induction in VR with respect to subjective and neuroendocrine stress reactivity [3]. Since then, several research groups have furthered the development of this paradigm [4,5] confirming the assumptions collated in the *Media Equation Concept* [6] in 1996. In this framework, Reeves and Nass summarize the findings of a large number of empirical studies that demonstrate that, for the most part, interactions with computers follow the same patterns as real social relationships, if there are sufficient social clues. To explain these findings, they rejected the notion that humans anthropomorphize computers (i.e. believe that computers are essentially human) in favor of what they termed *Ethopoeia*: Responding to a computer and programmed entities as if they were human while being aware that they, in fact, do not warrant the attribution of human characteristics [7].

While several studies point out the effectiveness of the TSST-VR in terms of psychological and autonomous stress responses [8,9], findings concerning the activity of the

HPA axis have been less reassuring with recent studies indicating an attenuated HPA-response in comparison to the traditional in vivo TSST [10].

In a previous study we demonstrated comparable HPA axis reactions in a refined VR-adaptation of the TSST [11]. While retaining the original TSST protocol, the TSST-VR employed in this study differed from previous iterations in its graphical fidelity, eye-tracking based adaptive responses of the committee and parallelization of real and virtual surroundings by modelling the VR room after the real laboratory. All of these modulations presumably enhanced the sense of presence in the computer-generated scenario and might have consequently increased neuroendocrine stress reactivity. The sense of presence is a theoretical concept that describes crucial psychological processes that come into play whenever people interact with a mediated environment, be it simply through a book or via immersive virtual realities. A comprehensive definition of presence that encompasses all relevant aspects has been devised by Wirth and colleagues [12]. According to their two-dimensional model of spatial presence, people experience presence when they have the sensation of physically being in a mediated space and when they perceive only those action possibilities that are relevant to the mediated space and not those that would be possible in the real environment. While the concept of presence can be measured on several dimensions, it is perhaps best described by its transportation component: presence is considered to be experienced when people feel as if they have been transported into a fleshed-out virtual world that allows for the same actions as real environments [13]. In the clinical domain, experiencing presence is often considered a necessary prerequisite for the incurrence of an emotional response in virtual exposure therapy sessions [14].

Based on findings that link presence to a heightened fear response [15] and more pronounced feelings of anxiety [16], it also seems warranted to expect associations between presence and the physiological response to stress-inducing virtual scenarios. Some studies have been able to detect correlations between presence and some markers of the SAM system and even inferred that physiological responses might potentially be suitable indicators of presence [17]. Supporting this conclusion, Slater, Brogni and Steed [18] showed that participants in a CAVE-like VR environment reacted with a skin conductance response and a spike in heart rate when they were interrupted during the exploration of the virtual environment by having to react to a stimulus from the real world (e.g. having to press a button when a colored ball was projected onto the wall of the CAVE). Based on these results, the authors infer that having to switch one's attention from between the physical and the virtual world disrupts the sense of presence and thereby elicits physiological responses. Incorporating additional measures of presence in future studies would enable empirical investigation of the role of presence as a possible moderator of the effectiveness of psychobiological stress induction in virtual settings.

In the present study, we examined whether replicating the real surroundings in VR leads to an increase in presence and the physiological stress responses of both HPA axis and SAM systems. For this purpose, we tested two groups of participants in the TSST-VR in either substantially differing or parallelized environments. We hypothesized that an increased similarity of the real environment and VR would promote an increase of both the physiological stress reaction and heighten the probability of experiencing presence.

2 Methods

2.1. Participants and design

The experimental design comprised of one between-subjects factor with two levels that participants were randomly assigned to: *VR same* and *VR different*. In the first group, participants performed the TSST in a virtual environment that was meticulously modeled after their real surroundings while participants in the second group found themselves in entirely different virtual surroundings.

Participants were recruited via advertisements on the campus of the University of Trier and the Trier University of Applied Sciences. The following inclusion criteria were established: BMI between 19 and 25 kg/m^2 and age between 18 and 50 years, no acute or chronic somatic or psychiatric disease, no regular intake of medication, no psychotherapeutic treatment in the last 12 months, not smoking more than five cigarettes per day and not working night shifts [19]. Furthermore, participants were asked to refrain from physical exercise and consumption of alcoholic or caffeinated beverages at least 24h prior to the start of the testing session and to abstain from consuming anything but water in the two hours before. The study was approved by the University of Trier ethics committee and conducted in accordance with the Declaration of Helsinki and the American Psychological Associations' Ethical Principles of Psychologists and Code of Conduct. All participants gave informed written consent and received 30€.

In total, fifty male participants (age range: 18-36; $M = 24.84$; $SD = 4.00$) were recruited and randomly assigned to the conditions *VR same* ($n = 25$; age range: 19-30; $M = 24.20$; $SD = 3.40$) or *VR different* ($n = 25$; age range: 18-36; $M = 25.48$; $SD = 4.49$). A t-test was calculated to verify that there were no significant age differences between the groups ($t(48)=1.14$, $p = 0.262$). We excluded one participant in the *VR same* group from all analyses due to a post-stress increase in salivary cortisol of over five standard deviations above the mean.

2.2. Apparatus

The virtual environment was generated using the Steam Source engine (Valve Corporation, Bellevue, Washington, USA), interfaced by the VR simulation software CyberSession 5.6 (VTPlus GmbH, Würzburg, Germany), and operated via the CSRemote IOS app running on an Apple IPad Air. The experiment ran on a desktop computer (Intel Core i7

4790K @ 4 Ghz, 16 GB Dual-Channel DDR3 RAM @ 3900 Mhz, NVidia Geforce GTX 980Ti with 6 GB of GDDR5 VRAM). A Head-Mounted Display (HMD; Oculus Rift DK2, Oculus VR LLC, Menlo Park, CA, USA; resolution: 1920 x 1080 [960 x 1080 pixels per eye]; field of view: 100°) with integrated head-tracking was used for VR simulation. Sound was presented via headphones.

The virtual environment in the simulation was designed to closely resemble one specific VR laboratory experimentation room and share all of its distinctive features like the large one-way-mirror (Fig. 1). Furthermore, to make the parallelization of the virtual and real surroundings as salient as possible, certain elements of the VR, such as the white desk and the microphone were also set up in the real laboratory in the *VR same* condition.

In the *VR different* condition, a second laboratory was chosen that shared few properties with the VR laboratory (the second laboratory e.g. lacked its distinctive hexagon-like shape and the blue carpet flooring) and neither the desk nor any other noticeable elements of the VR were present (Fig. 1B). We thereby attempted to maximize the difference between the real and virtual environment so that participants in this group would constantly be reminded that they were put into fabricated virtual surroundings.

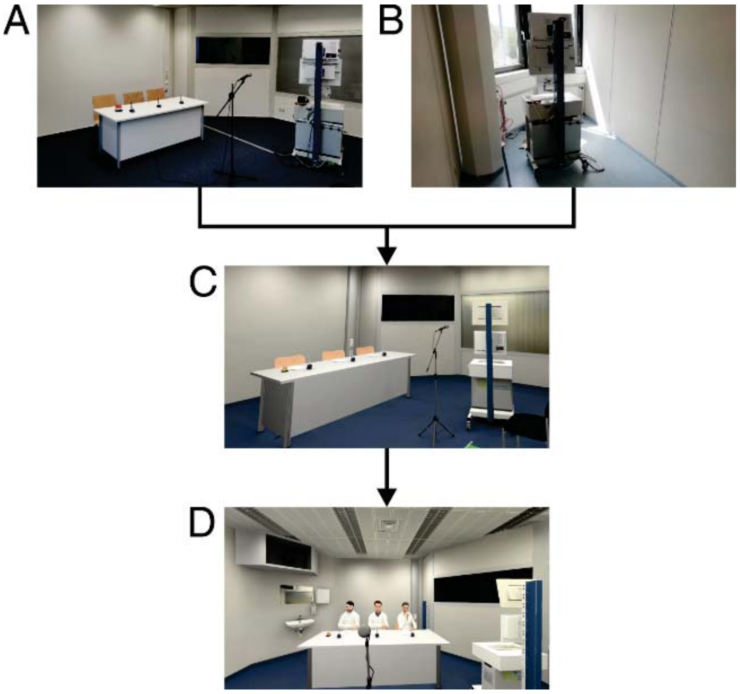

Fig. 1. Depiction of the real laboratories and the virtual environment. (A) Laboratory in the *VR same* condition. (B) Laboratory in the *VR different* condition. (C) Virtual laboratory. (D) Participants’ view during the TSST.

2.3. Measures

At seven time points (Fig. 2), participants were asked to give saliva samples by using Salivettes (Sarstedt, Nümbrecht, Germany). Samples were stored at – 20°C until biochemical analysis by the University Laboratory to determine concentrations of free salivary cortisol and alpha amylase (sAA). For cortisol analysis, a time-resolved fluorescence immunoassay [20] was used. 100µl of saliva were used for duplicate analysis (50µl per well). The Intra-assay coefficient of variation ranged between 4.0% and 6.7% and the corresponding inter-assay coefficients of variation were between 7.1% and 9.0%.

For sAA analysis, the chromogenic molecule 2-Chloro-4-nitrophenyl-a-D-maltotrioside was used [21]. Saliva was diluted 1:200 with assay diluent. 16µl of the diluted saliva were used for duplicate analysis (8µl per well). The intra-assay coefficient of variation was between 2.8% and 6.3%, and the corresponding inter-assay coefficients of variation were between 5.5% and 7.6%.

Heart rate (HR) was recorded using an ANS Recorder flex mobile ECG device (Neurocor Ltd. & Co. KG, Trier, Germany). IBI files were exported using the most artifact-free ECG derivation of the three possible alternatives and entered into ARTiiFACT [22] for automatic artifact detection and correction using cubic spline interpolation [23]. If necessary, automatically corrected files were reintroduced in ARTiiFACT and manually corrected after having undergone visual inspection.

Sense of presence was measured shortly after the stress induction using the Igroup Presence Questionnaire (IPQ; [24]), a self-report scale that measures presence on a one-item global scale (G) and three dimensions: S*patial presence* (SP)*, involvement* (INV)*,* and *realness* (REAL*)*. In total, the questionnaire consists of 14 items that participants have to rate on a Likert scale from -3 (total disagreement) to +3 (total agreement). The subscale SP measures how much participants have the feeling of finding themselves in a real environment with the potential of interacting with their surroundings in a meaningful and plausible way. INV measures how much the virtual environment captivates participants' interest and to what extent they are still aware of their real-world surroundings. The third subscale REAL assesses the feeling of authenticity of the virtual environment in comparison to the real world. Schubert [24] investigated the reliability of the scale and found satisfying internal consistency scores for the three subscales.

At -20, -10, +15, +40 and +60 minutes (in reference to TSST onset) participants rated their perceived levels of distress on eight visual analogue scales (VAS; range from 0—not at all—to 100—very much) with questions such as "how stressed do you feel?" or "how much do you feel physically unwell?" (c.f. Kothgassner et al., [25]). Additionally, participants filled out several visual analogue scales right after the stress induction that referred directly to the stressfulness of the previous task (such as "the situation was challenging for me" or "I felt threatened in the situation").

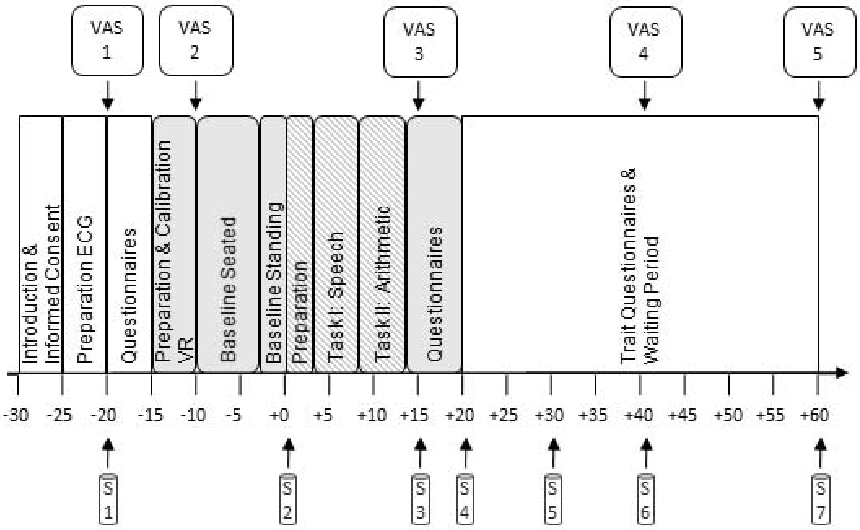

Fig. 2. Experimental procedure depicting experimental phases and time of assessment of subjective stress ratings (VAS) and saliva samples (S). Procedures in the preparation room are depicted in white; procedures in the VR laboratory have been marked in grey. Hatched patterns represent the components of the TSST.

2.4. Procedure

Testing sessions took place in the Virtual Reality laboratory of the University of Trier at either 3 p.m. or 5 p.m. to control for the circadian secretion rhythm of cortisol [26]. Upon arrival, they were greeted by the experimenter and an assistant and informed about the following procedures before declaring their consent. They were then fitted with the ECG device and asked to fill out the first VAS and to give the first saliva sample. Depending on which group they had been assigned to they were either accompanied to the lab that had been replicated in VR (*VR same* condition) or the dissimilar lab (*VR different* condition). After filling out a second set of VAS, participants put on the HMD and headphones. They subsequently had ten minutes to familiarize themselves with their virtual surroundings. After this baseline phase, they received instructions on the following task via head phones and in writing on the screen. Both groups were told that they would have three minutes to prepare for a job interview in front of a panel of judges. Afterwards, three virtual judges entered the room, took their places behind a desk and informed the participants that the preparation period was now beginning. Apart from the shorter preparation time and the need to prepare the job interview without being able to take notes, TSST procedures were directly adapted from the original paradigm [1]. During the tasks, the judges were controlled by the experimenter who triggered pre-recorded follow-up questions and instructions. After the arithmetic task, the judges stood up and left before the screen turned black. Participants were then assisted in taking off the HMD and asked to remain standing while giving the third saliva sample and filling out the IPQ, and several VAS. After completion, participants were led back to the preparation room where they periodically gave additional saliva samples and answered questionnaires. The experimental procedure ended 60 minutes after the beginning of the stress induction when participants gave the last saliva sample and questionnaires before being debriefed and compensated (Fig. 2).

2.5. Statistical analyses

Student's *t*-tests were calculated to compare subjective measures of stress as well as subscales of sense of presence between the two groups (*VR same* vs. *VR different*). For the main analyses, mixed repeated-measures ANOVAs were conducted to test for differential effects of the stress conditions on physiological and subjective stress markers (with time as the repeated-measures factor). When significant Group x Time interactions were found, we carried out Bonferroni-corrected pairwise comparisons. In cases of a violation of the assumption of sphericity (indicated by significant Mauchly's tests), we used Greenhouse-Geisser correction and report ε- and corrected *p*-values. All analyses were performed with SPSS for Windows (Version 25). Significance level was set at $p < .05$. Effect sizes are reported as η_p^2.

3 Results

3.1. Salivary cortisol

In order to test for a stress-related increase in secretion of salivary cortisol we conducted a 2 (*VR same*, *VR different*) x 7 (time) repeated measures ANOVA. The analysis revealed a highly significant main effect of the factor time ($F(6, 282) = 20.20$, $\varepsilon = 0.34$, $p < 0.001$, $\eta_p^2 = 0.30$, 95% CI [0.22; 0.35]). An increase in salivary cortisol over time was found that reached its maximum at 20 minutes after stress onset. Pairwise post-hoc comparisons revealed that the groups did not differ significantly at any of the seven time points (all *p*s > 0.4). Neither the main effect of the factor group ($F(1, 47) = 0.17$, $p = 0.680$) nor the interaction of both factors ($F(6, 282) = 0.76$, $\varepsilon = 0.34$, $p = 0.928$) reached significance, indicating a lack of significant differences between the groups in terms of HPA reactivity (Fig. 3A).

To corroborate these findings, Area under the Curve (AUC) values were calculated using the formulas proposed by Pruessner, Kirschbaum, Meinlschmid, & Hellhammer [25]. Student's *t*-tests showed neither a significant difference between groups for the area under the curve with respect to ground (AUCg; $t(47) = 0.44$, $p = 0.662$) nor for the area under the curve with respect to increase (AUCi; $t(47) = 0.26$, $p = 0.796$; Fig 3B).

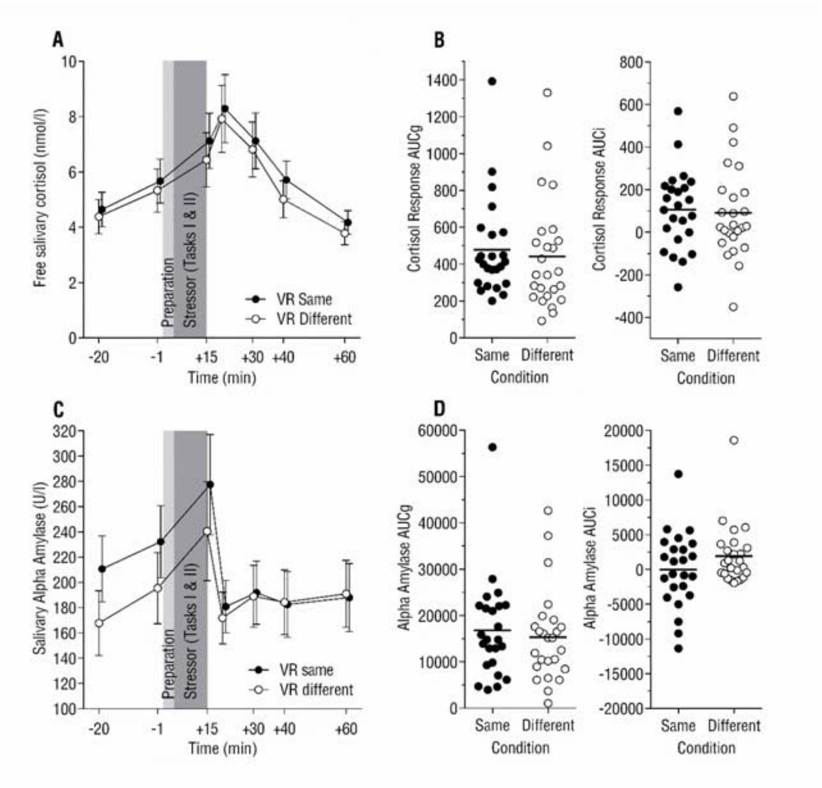

Fig. 3. (A) Free salivary cortisol in response to the stress conditions over the course of the experiment. Preparation and Stressor represent the respective phases of the TSST. Error bars denote standard errors. (B) Area under the Curve (AUC) values with respect to ground (AUCg) and with respect to the increase (AUCi) calculated for free salivary cortisol. (C) Salivary alpha amylase in response to the stress conditions over the course of the experiment. Error bars denote standard errors. (D) Area under the Curve (AUCg and AUCi) values calculated for alpha amylase.

3.2. Salivary alpha amylase

A 2 x 7 ANOVA was carried out with salivary alpha amylase (sAA) as the dependent variable. Again, a significant main effect of the factor time ($F(6, 282) = 13.65$, $\varepsilon = 0.39$, $p < .001$, $\eta_p^2 = 0.23$, 95% CI [0.14; 0.28]) was found. Pairwise post-hoc comparisons revealed an increase in sAA over time that reached its peak shortly after the end of the stress induction before dropping back to pre-stress-exposure levels. Furthermore, the groups did not differ significantly at any of the seven time points (all *p*s > 0.2). There was no differentiated release pattern of salivary amylase between groups as indicated by the nonsignificant main effect of the factor group ($F(1, 47) = 0.23$, $p = 0.634$) and the nonsignificant group x time interaction ($F(6, 282) = 1.74$, $\varepsilon = 0.39$, $p = 0.174$; Fig. 3C).

Area under the curve (with respect to ground and increase) values were calculated for sAA secretion and *t*-tests were performed to test for differences between the groups. There was no significant difference for AUCg ($t(47) = 0.50$, $p = 0.622$) or AUCi ($t(47) = 1.40$, $p = 0.169$; Fig. 3D).

3.3. Heart rate

Six mean HR segments of three to five minutes were calculated from IBI data. Due to missing ECG data in at least one of the experimental phases, one additional participant from each condition had to be excluded, leaving a sample of $N = 47$ for heart rate analysis.

A 2 x 6 (group x time) ANOVA yielded a significant main effect of the factor time ($F(5, 225) = 77.77$, $\varepsilon = 0.61$, $p < 0.001$, $\eta_p^2 = 0.63$, 95% CI [0.57; 0.67]). Mean HR increased over the course of the experiment and reached its peak during the job interview task of the TSST before starting to readjust to normal values (Fig. 4). Pairwise post-hoc comparisons revealed that the groups did not differ significantly at any of the six time points (all *p*s > 0.2).

Both stress groups did not differ in terms of HR activity, as shown by the nonsignificant main effect of the factor group ($F(1, 45) = 0.77$, $p = 0.385$) and the nonsignificant interaction of both factors ($F(5, 225) = 1.65$, $\varepsilon = 0.61$, $p = 0.179$).

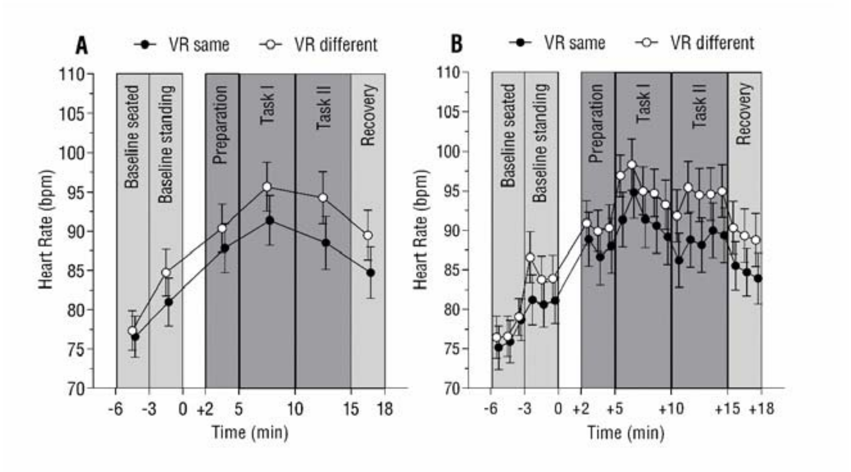

Fig. 4. Heart rate over the course of the experiment separated into six crucial time periods. (A) Averaged over the entire duration of each experimental phase (3-5 minutes). (B) averaged per minute. Error bars denote standard errors.

3.4. Self-reported stress

At five time points throughout the experiment, participants were asked to rate the stressfulness of the experience on several VAS items from 0 to 100. Due to missing data of one participant in the *VR same* group, analyses were carried out with a sample of $N = 48$. A 2 x 5 ANOVA for the item “How stressed do you feel at the moment?” showed a significant main effect of the factor time ($F(4, 184) = 36.69$, $\varepsilon = 0.64$, $p < 0.001$, $\eta_p^2 = 0.44$, 95% CI [0.35; 0.51]). Self-reported stress increased substantially from before stressor onset to immediately afterwards (TSST onset +15) before falling back to baseline levels. Pairwise post-hoc comparisons revealed that the groups did not differ significantly at any of the five time points (all *p*s > 0.2). There was no significant difference between the two stress groups ($F(1, 46) = 2.30$, $p = 0.136$) and no significant group x time interaction ($F(4, 184) = 0.50$, $\varepsilon = 0.64$, $p = 0.653$; Fig. 5A).

Similar results emerged for the item “How much would you like to leave the present situation?” Again, a significant main effect of the factor time was found in the 2 x 5 ANOVA ($F(4, 184) = 7.01$, $\varepsilon = 0.66$, $p < 0.001$, $\eta_p^2 = 0.13$, 95% CI [0.05; 0.19]) indicating a heightened desire to leave the experimental procedure right after stress induction. Pairwise post-hoc comparisons show that except for the second time point before stress onset, at which participants in the VR same group reported a significantly heightened inclination of leaving the situation ($p = 0.035$), the groups did not differ significantly (all *p*s > 0.1). No significant main effect of the factor group ($F(1, 46) = 1.14$, $p = 0.292$) and no significant interaction ($F(4, 184) = 0.89$, $\varepsilon = 0.66$, $p = 0.439$) were found (Fig. 5B).

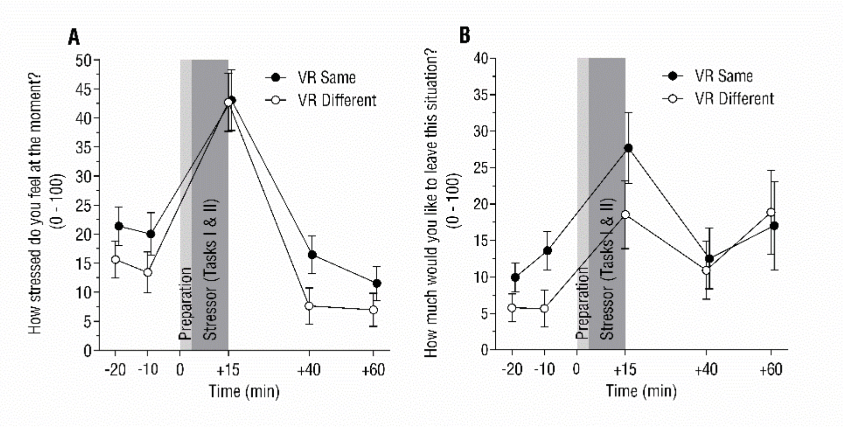

Fig. 5. Subjective stress levels rated on VAS at five time points over the course of the experiment. Preparation and Stressor represent the respective phases of the TSST. (A) How stressed do you feel at the moment? (B) How much would you like to leave the present situation? Error bars denote standard errors.

We analyzed one additional set of VAS collected immediately after the TSST that referred directly to the stressfulness of the VR tasks. Student's *t*-tests were performed to test whether the groups differed in their ratings of the situation in any significant way. None of these questions yielded a significant group difference: for example, "I found the previous situation challenging" ($t(47) = 0.33$, $p = 0.744$), "I felt like I was in control of the situation" ($t(47) = 0.32$, $p = 0.747$), "The situation felt threatening to me" ($t(47) = 0.57$, $p = 0.570$) or "I am content with the outcome of the situation" ($t(47) = 0.17$, $p = 0.865$).

3.5. Sense of presence

Student’s *t*-tests were performed to determine whether the groups differed in the perceived realness of the virtual environment and tasks. There was no significant difference between the groups in the one-item-scale *general presence* ($t(47) = 0.20$, $p = 0.841$), *perceived spatial presence* ($t(47) = 0.92$, $p = 0.362$), *involvement* ($t(47) = 1.86$, $p = 0.069$), or *realness* ($t(47) = 1.97$, $p = 0.054$). This indicates that there was no difference between the groups in the experience of presence. Individual scores for the three main subscales are depicted in Fig. 6.

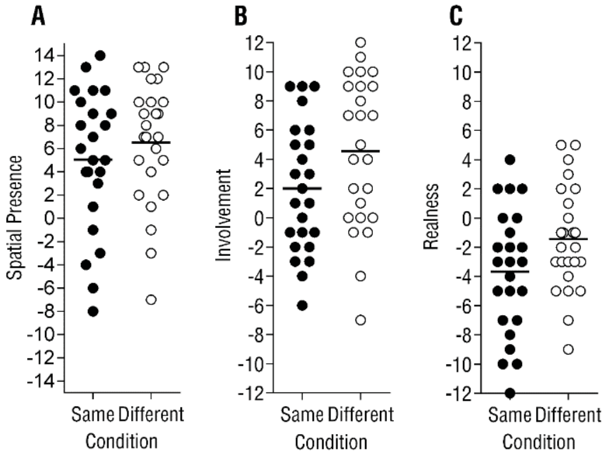

Fig. 6. Individual scores on the three main subscales of the IPQ measured after virtual stress exposure. (A) *Spatial presence*. (B) *Involvement*. (C) *Realness*.

4 Discussion

The results of the present study replicate our previous findings that a refined version of the TSST-VR is an effective paradigm for psychobiological stress induction, including the activation of the HPA-axis [11,28]. Both groups showed a substantial physiological reaction on both stress effector systems in response to the virtual tasks that did not differ depending on the experimental condition. In addition to the on-average twofold increase in salivary cortisol that is comparable to the effects of the invivo TSST [29], participants reported significantly heightened subjective stress levels after the tasks. Our hypothesis that simulating the real environment in VR would lead to an increase in presence and thus to a more pronounced stress response was, however, not confirmed. While both groups reported to have experienced presence in VR no significant differences in presence were detected. Furthermore, participants in the *VR same* condition did not exhibit a larger increase in physiological or subjective stress parameters. The finding that both groups experiences presence equally no matter whether the real environment was replicated in the VR implies that in order for participants to have a sense of presence, other factors must play a more pivotal role.

Moreover, the results can be understood as further evidence for the theoretical framework described in the *Media Equation* [6]. Although participants were confronted with a fabricated environment and an interaction with programmed entities that should not trigger social responses, they nonetheless reacted as if faced with factual psychosocial pressure by other humans. In only very few instances, participants did not comply with the social demands imposed by the virtual judges on account of them not being real human beings. Overall, participants constantly adhered to the tasks the judges asked them to perform and even gave very personal information about their character traits as it would be expected in a real job interview situation. These findings are in line with numerous other studies that have demonstrated how humans employ social norms like politeness, attribution of personality traits, and reciprocity in interactions with computers and computerized agents [7,30,31].

However, a factor that might have influenced subjective presence in the present study was that the management of the VR paradigm required the experimenter to be present at all times. The knowledge that there still was another person in the room while one was performing the job interview and arithmetic tasks might have added an additional layer of social evaluation that was not caused by the TSST-VR itself. While IPQ measures suggested that overall, participants have immersed themselves in the VR, it is possible that the presence of the experimenter led to higher levels of self-awareness during the tasks and thus to a stronger experience of presence [32]. Specifically, if participants were still aware of the experimenter during the virtual stress exposure, they might have viewed him, instead of the virtual committee, as their primary interaction partner. This could have presumably led to an increase in social presence since it might have transformed a participants' perception of the virtual environment from a self-contained situation to a medium through which to communicate with the experimenter [33]. To our knowledge, most VR systems that have been used in psychological research to date have, however, required the experimenter to be present in order to operate the system, and this would also be the case in a clinical setting, where only one room may be available. This makes the systematic evaluation of this factor imperative. Measuring not only spatial presence, but also social presence, might provide some insight concerning this issue.

The present study has some limitations. As described above, the presence of the experimenter might be a factor of influence that warrants further investigation. Additionally, both experimental groups were examined exclusively under laboratory conditions. Further evaluation is warranted in order to improve external validity if the procedure is to be applied in a non-laboratory environment, e.g. for clinical purposes. Furthermore, due to sex-dependent variations in hormonal parameters [34], we decided to test only male participants as a pilot investigation. Future investigations can enhance generalizability by including females as some studies have demonstrated differences in the perception of virtual environments associated with participants' sex [35]. In this sense, the present experiment has to be understood as a pilot investigation that should be supported by future studies that include male and female participants.

Beyond replicating the previous findings of robust stress-induction in VR and elucidating the association of presence and physiological responses in stress-inducing virtual scenarios, the present study might have practical implications. The fact that stress induction and stress-related physiological responses occurred independently of the specific surroundings of the laboratory in which the virtual stressor was applied, shows that the TSST-VR paradigm is a potential candidate to go beyond the exclusive application in science and a promising tool for clinical practitioners. A socially challenging situation like the TSST-VR might be a useful testing ground for patients with anxiety disorders such as social phobia [36] as either a method for therapeutic exposure [37] or to monitor efficacy of conventional therapy. The virtual environment would not have to match each individual clinic environment, making the potential therapy application more flexible and realistic to implement. More minor context variables however, can be changed with relative ease, so that variations of the paradigm with different levels of difficulty could be implemented depending on the progress of the patient. As an example, the TSST-VR could be performed in a calming environment with subsequent relaxing elements [8] when the patient has just recently started therapy in contrast to a more taxing scenario with additional spectators at a later stage [38]. In addition, the robustness of the TSST-VR against variations of the laboratory environment demonstrated in this study is highly relevant for large-scale investigations needing evidence that different laboratory settings can be incorporated while still sustaining high levels of standardization. In particular, multi-center studies using the same virtual environment in different laboratories could thus be more feasible and more easily implemented. This exemplifies one of the strengths of stressors realized in virtual environments. While the original TSST could naturally be used in a multi-center research design, the TSST-VR achieves a maximum degree of standardization while maintaining high cost-efficiency. While further research is still necessary to determine whether the TSST-VR induces a reliable response to psychosocial stress [11], it can nevertheless be considered a promising addition to the canon of experimental paradigms for stress research.

Author Contributions

GD and PZ conceptualized the study and prepared the study protocol. PZ handled project administration and performed the experiments. All authors were involved in the data analysis as well as in writing and editing of the original draft and have approved the final manuscript.

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Declarations of Interest

The authors declare no conflict of interest.

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References

[1] C. Kirschbaum, K.-M. Pirke, D.H. Hellhammer, The ‘Trier Social Stress Test’ – A Tool for Investigating Psychobiological Stress Responses in a Laboratory Setting, Neuropsychobiology. 28 (1993) 76–81. doi:10.1159/000119004.

[2] A.P. Allen, P.J. Kennedy, S. Dockray, J.F. Cryan, T.G. Dinan, G. Clarke, The Trier Social Stress Test: Principles and practice, Neurobiol. Stress. 6 (2017) 113–126. doi:10.1016/j.ynstr.2016.11.001.

[3] O. Kelly, K. Matheson, A. Martinez, Z. Merali, H. Anisman, Psychosocial Stress Evoked by a Virtual Audience: Relation to Neuroendocrine Activity, Cyberpsychol. Behav. 10 (2007) 655–662. doi:10.1089/cpb.2007.9973.

[4] P. Jönsson, M. Wallergård, K. Österberg, Å.M. Hansen, G. Johansson, B. Karlson, Cardiovascular and cortisol reactivity and habituation to a virtual reality version of the Trier Social Stress Test: A pilot study, Psychoneuroendocrinology. 35 (2010) 1397–1403. doi:10.1016/j.psyneuen.2010.04.003.

[5] E. Montero-López, A. Santos-Ruiz, M.C. García-Ríos, R. Rodríguez-Blázquez, M. Pérez-García, M.I. Peralta-Ramírez, A virtual reality approach to the Trier Social Stress Test: Contrasting two distinct protocols, Behav. Res. Methods. 48 (2016) 223–232. doi:10.3758/s13428-015-0565-4.

[6] B. Reeves, C.I. Nass, The media equation: How people treat computers, television, and new media like real people and places, Cambridge University Press, New York, NY, US, 1996.

[7] C. Nass, Y. Moon, Machines and Mindlessness: Social Responses to Computers, J. Soc. Issues. 56 (2000) 81–103. doi:10.1111/0022-4537.00153.

[8] M. Annerstedt, P. Jönsson, M. Wallergård, G. Johansson, B. Karlson, P. Grahn, Å.M. Hansen, P. Währborg, Inducing physiological stress recovery with sounds of nature in a virtual reality forest — Results from a pilot study, Physiol. Behav. 118 (2013) 240–250. doi:10.1016/j.physbeh.2013.05.023.

[9] M. Wallergård, P. Jönsson, G. Johansson, B. Karlson, A Virtual Reality Version of the Trier Social Stress Test: A Pilot Study, Presence Teleoperators Virtual Environ. 20 (2011) 325–336. doi:10.1162/PRES_a_00052.

[10] Y. Shiban, J. Diemer, S. Brandl, R. Zack, A. Mühlberger, S. Wüst, Trier Social Stress Test in vivo and in virtual reality: Dissociation of response domains, Int. J. Psychophysiol. 110 (2016) 47–55. doi:10.1016/j.ijpsycho.2016.10.008.

[11] P. Zimmer, B. Buttlar, G. Halbeisen, E. Walther, G. Domes, Virtually stressed? A refined virtual reality adaptation of the Trier Social Stress Test (TSST) induces robust endocrine responses, Psychoneuroendocrinology. 101 (2019) 186–192. doi:10.1016/j.psyneuen.2018.11.010.

[12] W. Wirth, T. Hartmann, S. Böcking, P. Vorderer, C. Klimmt, H. Schramm, T. Saari, J. Laarni, N. Ravaja, F.R. Gouveia, F. Biocca, A. Sacau, L. Jäncke, T. Baumgartner, P. Jäncke, A Process Model of the Formation of Spatial Presence Experiences, Media Psychol. 9 (2007) 493–525. doi:10.1080/15213260701283079.

[13] M.J. Schuemie, P. van der Straaten, M. Krijn, C.A.P.G. van der Mast, Research on Presence in Virtual Reality: A Survey, Cyberpsychol. Behav. 4 (2001) 183–201. doi:10.1089/109493101300117884.

[14] J. Diemer, G.W. Alpers, H.M. Peperkorn, Y. Shiban, A. Mühlberger, The impact of perception and presence on emotional reactions: a review of research in virtual reality, Front. Psychol. 6 (2015). doi:10.3389/fpsyg.2015.00026.

[15] D. Gromer, M. Reinke, I. Christner, P. Pauli, Causal Interactive Links Between Presence and Fear in Virtual Reality Height Exposure, Front. Psychol. 10 (2019). doi:10.3389/fpsyg.2019.00141.

[16] N. Morina, W.-P. Brinkman, D. Hartanto, P.M.G. Emmelkamp, Sense of presence and anxiety during virtual social interactions between a human and virtual humans, PeerJ. 2 (2014) e337. doi:10.7717/peerj.337.

[17] M. Meehan, S. Razzaque, B. Insko, M. Whitton, F.P. Brooks, Review of Four Studies on the Use of Physiological Reaction as a Measure of Presence in StressfulVirtual Environments, Appl. Psychophysiol. Biofeedback. 30 (2005) 239–258. doi:10.1007/s10484-005-6381-3.

[18] M. Slater, A. Brogni, A. Steed, Physiological responses to breaks in presence: A pilot study, in: Presence 2003 6th Annu. Int. Workshop Presence, Citeseer, 2003.

[19] S.-F. Niu, M.-H. Chung, C.-H. Chen, D. Hegney, A. O'Brien, K.-R. Chou, The Effect of Shift Rotation on Employee Cortisol Profile, Sleep Quality, Fatigue, and Attention Level: A Systematic Review, J. Nurs. Res. 19 (2011) 68–81. doi:10.1097/JNR.0b013e31820c1879.

[20] R.A. Dressendörfer, C. Kirschbaum, W. Rohde, F. Stahl, C.J. Strasburger, Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement, J. Steroid Biochem. Mol. Biol. 43 (1992) 683–692. doi:10.1016/0960-0760(92)90294-S.

[21] K. Lorentz, B. Gütschow, F. Renner, Evaluation of a direct α-amylase assay using 2-chloro-4-nitrophenyl-α-D-maltotrioside, Clin. Chem. Lab. Med. 37 (1999) 1053–1062.

[22] T. Kaufmann, S. Sütterlin, S.M. Schulz, C. Vögele, ARTiiFACT: a tool for heart rate artifact processing and heart rate variability analysis, Behav. Res. Methods. 43 (2011) 1161–1170. doi:10.3758/s13428-011-0107-7.

[23] N. Lippman, K.M. Stein, B.B. Lerman, Comparison of methods for removal of ectopy in measurement of heart rate variability, Am. J. Physiol.-Heart Circ. Physiol. 267 (1994) H411–H418. doi:10.1152/ajpheart.1994.267.1.H411.

[24] T. Schubert, F. Friedmann, H. Regenbrecht, The Experience of Presence: Factor Analytic Insights, Presence Teleoperators Virtual Environ. 10 (2001) 266–281. doi:10.1162/105474601300343603.

[25] O.D. Kothgassner, H. Hlavacs, L. Beutl, L.M. Glenk, R. Palme, A. Felnhofer, Two Experimental Virtual Paradigms for Stress Research: Developing Avatar-Based Approaches for Interpersonal and Evaluative Stressors, in: G. Wallner, S. Kriglstein, H. Hlavacs, R. Malaka, A. Lugmayr, H.-S. Yang (Eds.), Entertain. Comput. - ICEC 2016, Springer International Publishing, Cham, 2016: pp. 51–62. doi:10.1007/978-3-319-46100-7_5.

[26] B.M. Kudielka, S. Wüst, Human models in acute and chronic stress: assessing determinants of individual hypothalamus–pituitary–adrenal axis activity and reactivity, Stress. 13 (2010) 1–14.

[27] J.C. Pruessner, C. Kirschbaum, G. Meinlschmid, D.H. Hellhammer, Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change, Psychoneuroendocrinology. 28 (2003) 916–931. doi:10.1016/S0306-4530(02)00108-7.

[28] G. Domes, P. Zimmer, Acute stress enhances the sensitivity for facial emotions: a signal detection approach, Stress. 22 (2019) 455–460.

[29] W.K. Goodman, J. Janson, J.M. Wolf, Meta-analytical assessment of the effects of protocol variations on cortisol responses to the Trier Social Stress Test, Psychoneuroendocrinology. 80 (2017) 26–35. doi:10.1016/j.psyneuen.2017.02.030.

[30] C. Nass, Y. Moon, J. Morkes, E.-Y. Kim, B.J. Fogg, Computers are social actors: A review of current research, Hum. Values Des. Comput. Technol. 72 (1997) 137–162.

[31] O.D. Kothgassner, A. Felnhofer, H. Hlavacs, L. Beutl, R. Palme, I. Kryspin-Exner, L.M. Glenk, Salivary cortisol and cardiovascular reactivity to a public speaking task in a virtual and real-life environment, Comput. Hum. Behav. 62 (2016) 124–135. doi:10.1016/j.chb.2016.03.081.

[32] A. Felnhofer, H. Hlavacs, L. Beutl, I. Kryspin-Exner, O.D. Kothgassner, Physical Presence, Social Presence, and anxiety in participants with social anxiety disorder during virtual cue exposure, Cyberpsychology Behav. Soc. Netw. 22 (2019) 46–50. doi:10.1089/cyber.2018.0221.

[33] K.L. Nowak, F. Biocca, The effect of the agency and Anthropomorphism on users' Sense of Telepresence, Copresence, and Social Presence in virtual environments, Presence Teleoperators Virtual Environ. 12 (2003) 481–494. doi:10.1162/105474603322761289.

[34] B.M. Kudielka, C. Kirschbaum, Sex differences in HPA axis responses to stress: a review, Biol. Psychol. 69 (2005) 113–132. doi:10.1016/j.biopsycho.2004.11.009.

[35] A. Felnhofer, O.D. Kothgassner, L. Beutl, H. Hlavacs, I. Kryspin-Exner, Is virtual reality made for men only? Exploring gender differences in the sense of presence, Proc. Int. Soc. Presence Res. (2012) 103–112.

[36] E. Klumbies, D. Braeuer, J. Hoyer, C. Kirschbaum, The reaction to social stress in social phobia: Discordance between physiological and subjective parameters, PLoS ONE. 9 (2014) e105670. doi:10.1371/journal.pone.0105670.

[37] S. Bouchard, S. Dumoulin, G. Robillard, T. Guitard, É. Klinger, H. Forget, C. Loranger, F.X. Roucaut, Virtual reality compared with in vivo exposure in the treatment of social anxiety disorder: A three-arm randomised controlled trial, Br. J. Psychiatry. 210 (2017) 276–283. doi:10.1192/bjp.bp.116.184234.

[38] D. Hartanto, I.L. Kampmann, N. Morina, P.G.M. Emmelkamp, M.A. Neerincx, W.-P. Brinkman, Controlling social stress in virtual reality environments, PLoS ONE. 9 (2014) e92804. doi:10.1371/journal.pone.0092804.

4.3. Domes, G., & Zimmer, P. (2019). Acute stress enhances the sensitivity for facial emotions: a signal detection approach. *Stress, 22*(4), 455-460. doi:10.1080/10253890.2019.1593366

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ORIGINAL RESEARCH REPORT

Acute stress enhances the sensitivity for facial emotions: a signal detection approach

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ABSTRACT
Facial emotion recognition is an important prerequisite for social cognition. There is, however, limited evidence on how the ability to detect facial emotions is influenced by acute stress and the associated physiological reactions. In this study, two groups of healthy male participants were either exposed to a psychosocial stressor – an adaptation of the Trier Social Stress Test in virtual reality ($n = 23$) – or a non-stressful control task in the virtual environment ($n = 20$). Afterwards, both groups completed a computerized facial recognition task based on the signal detection theory presenting happy vs. angry faces with three different expression intensities. Saliva samples were taken at seven time points over the course of the experiment and used to analyze concentrations of free salivary cortisol and alpha amylase. Analyses using repeated-measures analyses of variance revealed a significant increase in emotion detection performance and significantly shorter response latencies in the stress group independent of emotional valence or emotion intensity. However, increased task performance in the stress group could not be predicted by stress-induced cortisol or alpha amylase secretion. The results suggest that enhanced detection of emotional cues after stress might be an adaptive response as an increased sensitivity to social cues might help individuals to detect potential threats or sources of social support in their social environment.

LAY SUMMARY
Socially evaluative stress facilitates the subsequent recognition of emotions. After having performed a task in a virtual environment, two groups of participants were asked to detect emotion expressions on pictures of faces that were presented to them on a computer screen. Statistical comparison of groups indicates that the group that had previously been subjected to a stressful job interview showed better results and became faster in detecting displayed emotions than the control group that had previously performed a non-stressful task.

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Facial emotion recognition; psychosocial stress; Trier Social Stress Test (TSST); salivary cortisol; alpha amylase; hypothalamus–pituitary–adrenal axis

1. Introduction

Acute psychosocial stress activates the HPA-axis and the sympathetic-adrenal system which results in the secretion of glucocorticoids (cortisol in humans) and catecholamines (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014). Cortisol is secreted from the adrenal cortex and binds to two different classes of receptors: mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). Catecholamines in turn are mainly secreted from the adrenal medulla. Being unable to cross the blood brain barrier, they mainly act on adrenal receptors in the periphery. Over the last decades, numerous studies have demonstrated that stress affects cognitive function such as episodic memory as a result of complex interactions of glucocorticoids and catecholamines at the level of the amygdala, where a high concentration of MR can be found (Roozendaal, McEwen, & Chattarji, 2009). In addition, other basic cognitive processes such as perception, attention and response selection are prone to the acute effects of psychosocial stress as well (Liston, McEwen, & Casey, 2009).

The cognitive ability to recognize emotions from facial expressions is fundamental to social interaction and largely depends on brain regions also involved in the regulation of the acute stress response (Pessoa & Adolphs, 2010). Surprisingly, research examining the effect of acute psychosocial stress and associated hormonal responses on such an important facet of social cognition is scarce. Deckers et al. (2015) found a general increase in recognition performance following acute psychosocial stress, while Smeets, Dziobek, and Wolf (2009) reported no such effect with a test probing the ability to infer more complex mental states from the eye region. Using blends of mixed emotions, Daudelin-Peltier, Forget, Blais, Deschênes, and Fiset (2017) demonstrated a stress-induced shift toward surprise away from disgust. In contrast, a study in children revealed a shift toward recognizing fear in ambiguous faces (Chen, Schmitz, Domes, Tuschen-Caffier, & Heinrichs, 2014). Following a pharmacological approach, an MR-agonist (fludrocortisone) increased emotional empathy in women (Wingenfeld et al., 2014), but did

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Supplemental data for this article can be accessed here.

not alter emotion recognition performance (Schultebraucks et al., 2016). Furthermore, a complex sex-dependent effect was found for the administration of 10 mg hydrocortisone (Duesenberg et al., 2016). In sum, the few studies so far provide an inconsistent pattern of effects that might have originated in differences in the specific cognitive function studied and the experimental paradigm followed (stress induction vs. pharmacological challenge).

The present study therefore aimed to investigate the effects of acute psychosocial stress on the ability to detect facial emotions within a signal detection framework. We used a virtual-reality adaptation of the Trier Social Stress Test (TSST) that has been shown to serve as an efficient and standardized alternative to the face-to-face TSST to induce robust endocrine stress responses in a previous study (Zimmer, Buttlar, Halbeisen, Walther, & Domes, 2019). In the subsequent emotion recognition task, we chose happy and angry facial expressions because they represent two opposite social signals: a positive signal of social approach and a negative signal of social threat, and thus correspond to the two contrasting reactions to acute stress: “tend-and-befriend” (Taylor, 2006) and “fight-or-flight” (Cannon, 1932). Based on the studies summarized above, we hypothesized that acute stress elicited in a virtual environment would promote the sensitivity for facial emotions, especially for social cues of threat, i.e. angry faces. In addition, we explored the assumed association between HPA axis, sympathico-adrenal activity and emotion detection performance.

2. Methods

2.1. Participants

Healthy male participants were recruited by on-campus advertisement and were included in the study if they fulfilled the following criteria based on self-report given during a telephone interview: age between 18 and 50 years, BMI between 19 and 26 kg/m^2, no acute or chronic somatic or psychiatric disease, smoking less than five cigarettes per day, free of any medication, not working on night shift. In all, $N = 51$ met inclusion criteria and were randomly assigned to one of two experimental conditions (acute stress vs. control condition). An exclusively male sample was chosen to avoid the confounding effects of sex and gonadal steroids related to menstrual-cycle or oral contraceptives on stress-reactivity and facial emotion recognition (Derntl, Kryspin-Exner, Fernbach, Moser, & Habel, 2008; Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Due to technical failure at the beginning of the study, datasets of $n = 6$ participants in the stress condition and $n = 2$ in the control condition had to be excluded, leaving $n = 23$ participants in the stress group and a total of $N = 43$ overall for the emotion recognition analysis. Participants were instructed to refrain from eating and drinking anything other than water two hours before the experimental session. To control for the circadian rhythm of cortisol (Kudielka & Wüst, 2010), experimental sessions started at 3.30 p.m. or 5.30 p.m. The present analysis was part of a larger project that focused on the evaluation of the virtual reality (VR) version of the TSST (Zimmer et al., 2019). The study protocol was approved by the ethics committee of the University of Trier. Participants were reimbursed with 30€ for their participation.

2.2. Procedures

After arriving at the laboratory, participants gave written informed consent and were instructed in how to use the saliva sampling device and how to fill in the visual analog scales (VAS) in a preparation room. They were then brought to the experimental room and familiarized with the VR goggles. After an acclimatization period of 10 minutes in the virtual environment participants were given written and prerecorded spoken instructions about the ensuing task. Subsequently, three virtual judges entered, took their places behind a desk and one informed the participants that they would now have three minutes of preparation time. Thereafter, participants performed a mock job interview and a mental arithmetic task. The reactions of the virtual judges were controlled by the experimenter from an adjacent room behind a one-way mirror. After finishing the TSST-VR, participants were guided back to the preparation room where they performed the emotion detection task on a PC running ePrime (V. 2.0; Psychology Software Tools Inc., Sharpsburg, PA) and completed state questionnaires.

2.2.1. Laboratory stressor – the Trier Social Stress Test in virtual reality

As an acute psychosocial stressor, a VR adaptation of the Trier Social Stress Test (TSST-VR) was used. Several previous studies have demonstrated that other VR adaptations of the TSST reliably elicit a robust physiological and subjective stress responses (Fallon, Careaga, Sbarra, & O’Connor, 2016; Shiban et al., 2016). Detailed procedures and further evaluation of the paradigm have been reported recently (Zimmer et al., 2019). In brief, like the original *in vivo* TSST (Kirschbaum, Pirke, & Hellhammer, 1993), the TSST-VR comprises of five minutes of free talk (mock job interview) and a five minute mental arithmetic task in front of a (virtual) audience of three judges. A non-stressful “placebo version” of the TSST was adapted for the VR environment as the control condition (Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009). This non-stressful version of the TSST mainly differed in terms of the lack of uncertainty and social threat but included comparable cognitive demands as the stressful TSST-VR.

2.2.2. Facial emotion detection task

For the selection of stimuli and leveling of difficulty between positive and negative facial expressions, a pilot study was conducted. For details, refer to the online supplemental methods. The final task comprised of the two different expression categories, anger and happiness, and three different intensities (low, medium, high), resulting in a 2 × 3 design, i.e. six different conditions. In each block, 12 stimuli of a specific condition were shown consecutively in random order (six faces with the specific emotion and six neutral faces). Every block was preceded by a written instruction to decide whether the face presented in the following trials showed an angry (or happy) or a neutral emotion expression. Pictures were presented without a time limit. Participants were asked to decide spontaneously whether the emotion was present or absent in the specific face shown by pressing one of two buttons. The six different blocks (conditions) within one run were presented randomly. Runs were repeated three times, resulting in 18 trials per condition overall, and 216 trials in total, totaling approximately 10 min.

Raw data were analyzed following a classical signal detection theory (SDT) approach previously used in facial emotion recognition research (e.g. Pessoa, Japee, & Ungerleider, 2005). Correct identifications of emotional faces were coded as hits, misidentifications of neutral faces as emotional ones were coded as false alarms. Average hit rate and average false alarms rate of every stimulus category were *z*-transformed and subtracted yielding the discrimination index d' as a measure of signal detection performance ($d' = z[\text{hit rate}] - z[\text{false alarms rate}]$). In addition, bias scores c were calculated ($c = -0.5 \times (z[\text{hit rate}] + z[\text{false alarms rate}])$ – cf. Stanislaw and Todorov (1999).

2.2.3. Saliva sampling and analysis

In reference to the start of the TSST-VR, salivary samples using Salivette sampling devices (Sarstedt, Nümbrecht, Germany) were taken at seven time points over the course of the experiment: −20, −1, +15, +20, +30, +40, and +60 minutes. Saliva samples were frozen and stored at −20 °C until analysis. For details on biochemical analysis, see online supplemental methods.

2.2.4. Self-reported stress – visual analog scales

After the TSST-VR/control condition, participants rated the situation with regard to the perceived stressfulness, challenge, and threat on VASs with a range of 0 (not at all) to 100 (very much).

2.3. Statistical analysis

We calculated two mixed repeated-measures ANOVAs (two groups by seven time points) for salivary cortisol and alpha amylase. *T*-tests were used to test for significant differences in subjective stress ratings of the situation between the two groups.

Emotion detection performance was analyzed by conducting separate mixed repeated-measures ANOVAs (two groups by two emotions by three intensities) for d' and c-scores. In addition, a similar exploratory analysis was conducted for response latencies. To test for possible associations between the physiological stress response and the emotion detection performance, Pearson's correlations for the AUC of free salivary cortisol, alpha amylase and d' and c-values were calculated. In the case of non-sphericity, we used the Greenhouse–Geisser correction and report ε- and corrected p values. All post hoc pairwise comparisons were Bonferroni corrected. Effect sizes for significant tests are reported as η_p^2 and Cohen's d. All statistical analyses were run with SPSS for Windows (Version 25, SPSS Inc., Chicago, IL). Significance threshold was set at $p < .05$.

3. Results

3.1. Manipulation check – acute stress responses

The TSST-VR evoked a marked increase in free salivary cortisol as shown by the significant main effect of the factor time ($F(6,\ 246) = 9.81$, $\varepsilon = 0.37$, $p < .001$, $\eta_p^2 = 0.19$). True to expectations, there was a significantly higher cortisol secretion in the stress condition as indicated by the significant main effect of the factor group ($F(1,\ 41) = 6.54$, $p = .014$, $\eta_p^2 = 0.14$) and the significant group by time interaction ($F(6,\ 246) = 6.03$, $\varepsilon = 0.37$, $p = .003$, $\eta_p^2 = 0.13$) – Figure 1(a). Also in line with our expectations, pairwise comparisons revealed significantly higher cortisol secretion in the stress group at the first time point after the stress induction and all following time points (all *ps* $< .019$).

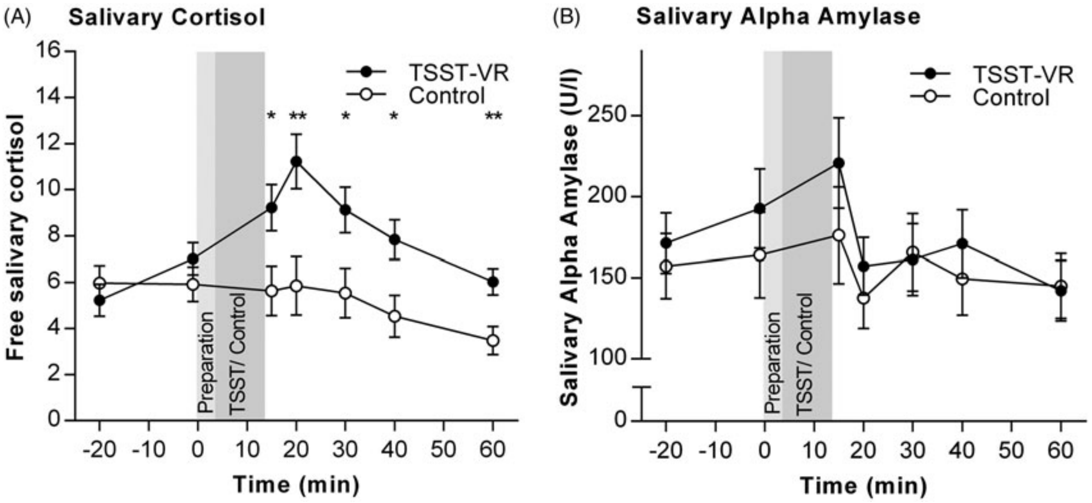

Figure 1. Psychobiological response to the acute stressor. (A) Free salivary cortisol (nmol/L) and (B) alpha amylase (U/L) as a function of time and group. Asterisks denote significant post hoc pairwise comparisons ($^{*}p < .05$; $^{**}p < .01$). Error bars represent S. E.

Although there was a significant increase in alpha amylase over time ($F(6, 246) = 5.13$, $\varepsilon = 0.64$, $p = .001$, $\eta_p^2 = 0.11$), TSST-VR and control condition did not differ substantially as indicated by the nonsignificant main effect of the factor group ($F(1, 41) = 0.38$, $p = .540$) and the nonsignificant group by time interaction ($F(6, 246) = 1.09$, $\varepsilon = 0.64$, $p = .361$) – Figure 1(b). As expected, the TSST-VR was rated more stressful ($t(41) = 3.52$, $p = .001$, $d = 1.10$), more challenging ($t(41) = 4.95$, $p < .001$, $d = 1.55$), and more threatening ($t(41) = 3.35$, $p = .002$, $d = 1.05$) than the control condition.

3.2. Emotion recognition performance

Following the TSST-VR, participants showed an overall increase in emotion detection performance, as indicated by a significant main effect of group on d' values ($F(1, 41) = 4.29$; $p = .045$, $\eta_p^2 = 0.10$) – Figure 2, upper panels. There was also a significant main effect of emotion ($F(1, 41) = 4.44$, $p = .041$, $\eta_p^2 = 0.10$) showing a higher discrimination index for happy faces. The group by emotion interaction did not reach statistical significance ($F(1, 41) = 0.08$, $p = .778$). Moreover, there was a significant main effect of the factor intensity of the presented emotion ($F(2, 82) = 264.26$, $p < .001$, $\eta_p^2 = 0.87$). Post hoc pairwise comparisons confirmed our expectation that the discrimination index significantly differed between each level of intensity (low, medium, high; all $ps < .001$). Still, neither the interaction of the factors group and intensity ($F(2, 82) = 1.04$, $p = .360$) nor the three-way interaction reached statistical significance ($F(2, 82) = 0.85$, $p = .429$). Tables containing descriptive statistics of all measures of emotion recognition performance can be found in the supplemental materials online with this article.

There was no such overall effect of acute stress on response tendency, as indicated by a non-significant main effect of group on c values ($F(1, 41) = 0.49$, $p = .489$). There was, however, a significant main effect of the specific emotion ($F(1, 41) = 44.40$, $p < .001$, $\eta_p^2 = 0.52$), indicating higher bias scores for happy faces. Furthermore, there was no significant interaction between the factors group and specific emotion ($F(1, 41) = 1.77$, $p = .191$). The main effect of intensity of the presented emotion reached significance ($F(2, 82) = 153.08$, $p < .001$, $\eta_p^2 = 0.79$), showing a decrease in bias score from lower to higher emotional intensity (all three $ps < .001$). The interaction of the factors group and intensity ($F(2, 82) = 3.33$, $p = .041$, $\eta_p^2 = 0.08$) also reached significance. The three way interaction of group, emotion and intensity, however, did not ($F(2, 82) = 2.37$, $p = .100$).

Finally, following acute stress participants responded significantly faster to trials showing an emotional expression as indicated by a main effect of group ($F(1, 41) = 8.60$, $p = .005$, $\eta_p^2 = 0.17$). There was also a significant main effect of the specific emotion ($F(1, 41) = 7.16$, $p = .011$, $\eta_p^2 = 0.15$) indicating that it took all participants significantly longer to recognize happiness in facial expressions than anger.

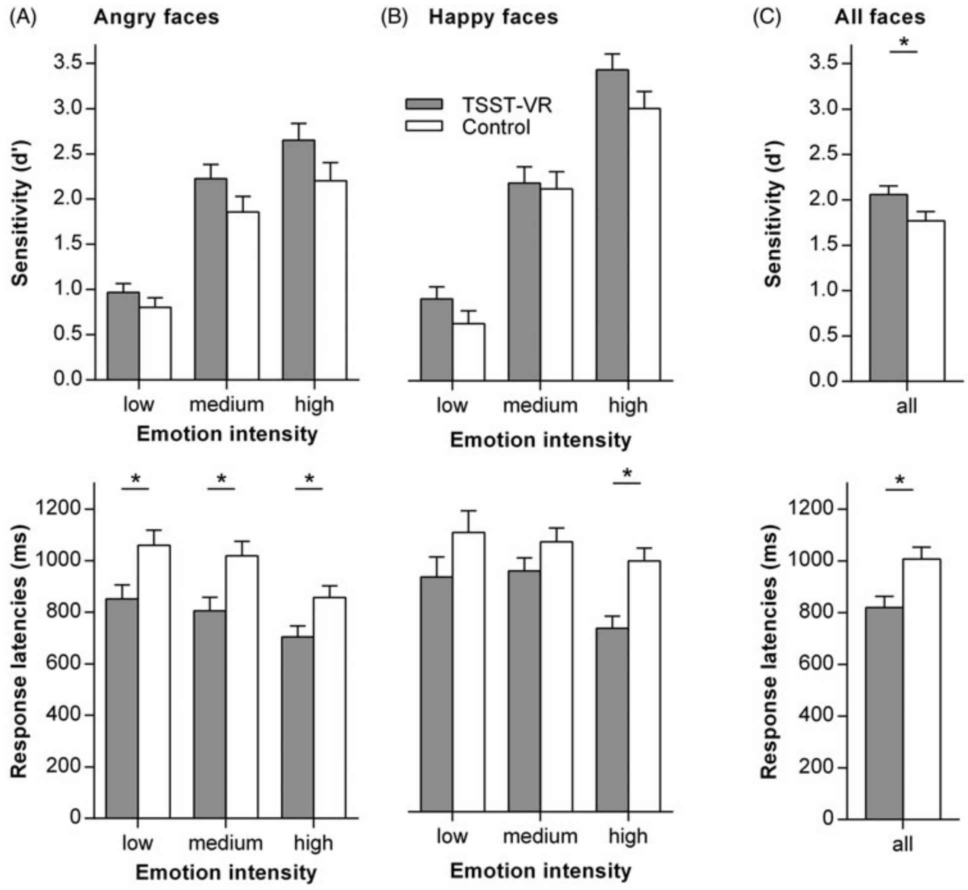

Figure 2. Effects of acute stress on emotion detection performance. Upper panels show mean sensitivity index (d') and lower panels show response latencies in ms for (A) angry faces, (B) happy faces, and (C) averaged over all emotions and intensities. Asterisks denote significant post hoc pairwise comparisons ($*p < .05$). Error bars represent S. E.

Moreover, the main effect of the factor intensity also reached statistical significance ($F(2, 82) = 18.31$, $\varepsilon = 0.79$, $p < .001$, $\eta_p^2 = 0.31$), indicating no significant difference in response latencies for low and medium intensity ($p = 1.00$) but a significantly shortened response time for high emotional intensities (both *ps* $< .001$). There was no significant interaction between the factors group and specific emotion ($F(1, 41) = 0.41$, $p = .841$) or between the factors group and the intensity of the presented emotion ($F(2, 82) = 0.29$, $\varepsilon = 0.79$, $p = .699$). The three-way interaction did also not reach statistical significance ($F(2, 82) = 2.16$, $p = .122$).

3.3. Correlation analyses

Overall, within the stressed group, the individual stress-induced increase in cortisol or alpha amylase did not predict emotion detection performance, response bias or response latencies, i.e. after correction for multiple testing, none of the correlations remained significant (all $p > .05$).

4. Discussion

In sum, the present results support the hypothesis that stress sensitizes for social signals of affective states in general. Within a highly standardized VR environment, acute stress evoked increased detection accuracy for angry and happy facial expressions largely regardless of the specific expression shown or the emotion intensity displayed. In addition, higher detection performance did not come at the cost of increased response latencies; on the contrary, the stress group showed higher detection rates at lower response latencies. Furthermore, increased sensitivity was not associated with altered response tendencies. The present findings are in line with the study by Deckers et al. (2015) who also employed a modified version of the TSST, demonstrating a general increase in emotion recognition performance when participants had to identify the emotions on faces in video sequences that were slowly morphed from low to high intensity. The results of the current study suggest that this performance increase might be based on a heightened sensitivity for subtle facial cues that leads to enhanced detection accuracy.

The present findings of increased detection performance might also relate to more complex social affect and decision making. Studies in this domain demonstrated that stressed participants react with a heightened emotional empathetic response to positive and negative social stimuli (Wolf et al., 2015), and show increased prosocial behavior such as trust (von Dawans, Fischbacher, Kirschbaum, Fehr, & Heinrichs, 2012). Enhanced cognitive performance in detecting and evaluating subtle social cues as found in the present study might in part promote more complex social cognition and decision-making and thus foster positive social interaction as suggested by some previous studies. However, a more general interpretation is that enhanced detection of socio-emotional cues might be adaptive since an increased sensitivity to social cues could help individuals to detect potential threats or, conversely, sources of social support and thus navigate their social environment.

The fact that we did not observe substantial correlations between indicators of the stress-induced endocrine response (cortisol and sAA) and detection performance suggests that there was no close relationship between psychobiological stress markers and cognition in the present study. This finding is at odds with a large body of experimental evidence from animal studies clearly demonstrating that glucocorticoids and catecholamines modulate neural activity involved in cognitive functions such as memory consolidation (as reviewed in Roozendaal et al., 2009). However, some previous studies using experimental stressors and pharmacological challenges in humans also failed to provide congruent evidence for a causal role of these hormones in modulating social cognition (Duesenberg et al., 2016; Wingenfeld et al., 2014; Wolf et al., 2015). Furthermore, the crucial role of timing and the different time courses of action of glucocorticoids and catecholamines as well as the indirect measurement of these hormones in saliva need to be considered as limiting factors that might play a part in the discrepancy of findings between the present and previous studies. Another possible explanation for the inconsistency between the present study and some previous studies which did not report effects of acute stress on emotion recognition relates to the VR setting. For example, it might be speculated that the interaction with the virtual judges in the stress condition (compared to the control condition) might have promoted social priming that subsequently enhanced participants' emotion detection performance from stimuli resembling the artificial agents in the TSST-VR. In line with this notion, Daher et al. (2017) found that the short interaction with a virtual confederate influenced subsequent social interactions with a virtual avatar, resulting in higher scores in several affective and socio-cognitive self-reported parameters. Thus, general conclusions should be drawn with caution until the effects have been replicated with other face-to-face stressors such as the classical TSST.

The present study has some general limitations. First, our sample consisted exclusively of male participants and thus the results might not generalize to women. Furthermore, we employed a merely performance-oriented computer-based measure that operationalizes detection of emotional cues via the SDT approach. Although this task was highly sensitive for the effects of acute stress, it is unclear how this detection rate and response time based measure relates to more complex social cognition and behavior, such as decision making and social interaction in naturalistic settings. Finally, we followed a correlational approach to explore the potential involvement of stress hormones in emotion recognition. Future studies focusing on the causal role of specific stress-induced physiological factors could combine stress induction with pharmacological interventions to isolate the specific effects of the candidate hormones suspected to cause cognitive effects under stress (e.g. Andrews & Pruessner, 2013).

In sum, the present study extends previous findings regarding the cognitive effects of stress on social cognitive functioning by demonstrating that moderate acute stress promotes facial emotion recognition on a basal level. Enhanced detection performance in a signal detection framework provides evidence for heightened sensitivity for social signals after acute stress regardless of the specific valence. This heightened sensitivity might help individuals to detect subtle signals of social threat or support in order to adapt to social stress, and modulate more complex social behavior and decision-making.

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Notes on contributors

GD and PZ designed the study, analyzed the data, interpreted the results and wrote the manuscript. PZ conducted data collection. Both authors have approved the final version of the article.

References

Allen, A.P., Kennedy, P.J., Cryan, J.F., Dinan, T.G., & Clarke, G. (2014). Biological and psychological markers of stress in humans: Focus on the Trier Social Stress Test. *Neuroscience & Biobehavioral Reviews*, *38*, 94–124. doi:10.1016/j.neubiorev.2013.11.005

Andrews, J., & Pruessner, J.C. (2013). The combined propranolol/TSST paradigm-a new method for psychoneuroendocrinology. *PLOS One*, *8*, e57567. doi:10.1371/journal.pone.0057567

Cannon, W.B. (1932). *The wisdom of the body*. New York: Norton.

Chen, F.S., Schmitz, J., Domes, G., Tuschen-Caffier, B., & Heinrichs, M. (2014). Effects of acute social stress on emotion processing in children. *Psychoneuroendocrinology*, *40*, 91–95. doi:10.1016/j.psyneuen.2013.11.003

Daher, S., Kim, K., Lee, M., Schubert, R., Bruder, G., Bailenson, J., & Welch, G. (2017). Effects of social priming on social presence with intelligent virtual agents. In J. Beskow, C. Peters, G. Castellano, C. O'Sullivan, I. Leite, & S. Kopp (Eds.), *Intelligent virtual agents* (Vol. *10498*, pp. 87–100). Cham: Springer International Publishing.

Daudelin-Peltier, C., Forget, H., Blais, C., Deschênes, A., & Fiset, D. (2017). The effect of acute social stress on the recognition of facial expression of emotions. *Scientific Reports*, *7*(1), 1036. doi:10.1038/s41598-017-01053-3

Deckers, J.W.M., Lobbestael, J., Wingen, G.A., van Kessels, R.P.C., Arntz, A., & Egger, J.I.M. (2015). The influence of stress on social cognition in patients with borderline personality disorder. *Psychoneuroendocrinology*, *52*, 119–129. doi:10.1016/j.psyneuen.2014.11.003

Derntl, B., Kryspin-Exner, I., Fernbach, E., Moser, E., & Habel, U. (2008). Emotion recognition accuracy in healthy young females is associated with cycle phase. *Hormones and Behavior*, *53*, 90–95. doi:10.1016/j.yhbeh.2007.09.006

Duesenberg, M., Weber, J., Schulze, L., Schaeuffele, C., Roepke, S., Hellmann-Regen, J., … Wingenfeld, K. (2016). Does cortisol modulate emotion recognition and empathy? *Psychoneuroendocrinology*, *66*, 221–227. doi:10.1016/j.psyneuen.2016.01.011

Fallon, M.A., Careaga, J.S., Sbarra, D.A., & O'Connor, M.-F. (2016). Utility of a virtual trier social stress test: Initial findings and benchmarking comparisons. *Psychosomatic Medicine*, *78*, 835–840. doi:10.1097/PSY.0000000000000338

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., & Wolf, O.T. (2009). Neuroendocrine and psychometric evaluation of a placebo version of the 'trier social stress test'. *Psychoneuroendocrinology*, *34*, 1075–1086. doi:10.1016/j.psyneuen.2009.02.008

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., & Hellhammer, D.H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus–pituitary–adrenal axis. *Psychosomatic Medicine*, *61*, 154–162. doi:10.1097/00006842-199903000-00006

Kirschbaum, C., Pirke, K.-M., & Hellhammer, D.H. (1993). The 'Trier Social Stress Test' – a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*, 76–81. doi:10.1159/000119004

Kudielka, B.M., & Wüst, S. (2010). Human models in acute and chronic stress: Assessing determinants of individual hypothalamus–pituitary–adrenal axis activity and reactivity. *Stress*, *13*, 1–14. doi:10.3109/10253890902874913

Liston, C., McEwen, B.S., & Casey, B.J. (2009). Psychosocial stress reversibly disrupts prefrontal processing and attentional control. *Proceedings of the National Academy of Sciences*, *106*, 912–917. doi:10.1073/pnas.0807041106

Pessoa, L., & Adolphs, R. (2010). Emotion processing and the amygdala: From a "low road" to "many roads" of evaluating biological significance. *Nature Reviews Neuroscience*, *11*, 773–783. doi:10.1038/nrn2920

Pessoa, L., Japee, S., & Ungerleider, L.G. (2005). Visual awareness and the detection of fearful faces. *Emotion (Washington, D.C.)*, *5*, 243–247. doi:10.1037/1528-3542.5.2.243

Roozendaal, B., McEwen, B.S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews. Neuroscience*, *10*, 423–433. doi:10.1038/nrn2651

Schultebraucks, K., Deuter, C.E., Duesenberg, M., Schulze, L., Hellmann-Regen, J., Domke, A., … Wingenfeld, K. (2016). Selective attention to emotional cues and emotion recognition in healthy subjects: The role of mineralocorticoid receptor stimulation. *Psychopharmacology*, *233*, 3405–3415. doi:10.1007/s00213-016-4380-0

Shiban, Y., Diemer, J., Brandl, S., Zack, R., Mühlberger, A., & Wüst, S. (2016). Trier Social Stress Test in vivo and in virtual reality: Dissociation of response domains. *International Journal of Psychophysiology*, *110*, 47–55. doi:10.1016/j.ijpsycho.2016.10.008

Smeets, T., Dziobek, I., & Wolf, O.T. (2009). Social cognition under stress: Differential effects of stress-induced cortisol elevations in healthy young men and women. *Hormones and Behavior*, *55*, 507–513. doi:10.1016/j.yhbeh.2009.01.011

Stanislaw, H., & Todorov, N. (1999). Calculation of signal detection theory measures. *Behavior Research Methods, Instruments, & Computers*, *31*, 137–149. doi:10.3758/BF03207704

Taylor, S.E. (2006). Tend and befriend: Biobehavioral bases of affiliation under stress. *Current Directions in Psychological Science*, *15*, 273–277. doi:10.1111/j.1467-8721.2006.00451.x

von Dawans, B., Fischbacher, U., Kirschbaum, C., Fehr, E., & Heinrichs, M. (2012). The social dimension of stress reactivity: Acute stress increases prosocial behavior in humans. *Psychological Science*, *23*, 651–660. doi:10.1177/0956797611431576

Wingenfeld, K., Kuehl, L.K., Janke, K., Hinkelmann, K., Dziobek, I., Fleischer, J., … Roepke, S. (2014). Enhanced emotional empathy after mineralocorticoid receptor stimulation in women with borderline personality disorder and healthy women. *Neuropsychopharmacology*, *39*, 1799–1804. doi:10.1038/npp.2014.36

Wolf, O.T., Schulte, J.M., Drimalla, H., Hamacher-Dang, T.C., Knoch, D., & Dziobek, I. (2015). Enhanced emotional empathy after psychosocial stress in young healthy men. *Stress*, *18*, 631–637. doi:10.3109/10253890.2015.1078787

Zimmer, P., Buttlar, B., Halbeisen, G., Walther, E., & Domes, G. (2019). Virtually stressed? A refined virtual reality adaptation of the Trier Social Stress Test (TSST) induces robust endocrine responses. *Psychoneuroendocrinology*, *101*, 186–192. doi:10.1016/j.psyneuen.2018.11.010

5. General Discussion

With the present dissertation, I aimed to investigate the potential of the TSST-VR as a standardized and economic procedure for psychobiological stress induction. The three original manuscripts presented in this work indicate that a state of the art version of the TSST-VR with a high level of graphical fidelity and an increased degree of responsiveness does indeed reliably activate the physiological stress effector systems of the HPA axis and the SAM system (Zimmer, Buttlar, Halbeisen, Walther, & Domes, 2019). In this sense, it can be considered a promising addition to the canon of laboratory stress induction paradigms. We further pursued this line of investigation and conducted a second study to examine the influence of the real context and with it the experience of presence on the psychobiological stress response (Zimmer, Wu, & Domes, in press). Lastly, we employed the TSST-VR in a laboratory use scenario by testing the effect of virtual psychosocial stress exposure on emotion recognition performance (Domes & Zimmer, 2019). The results of our investigations will be summarized and their practical implications discussed in the following sections.

5.1. A Psychobiological Investigation of Stress in Virtual Environments

Approximately ten years since the first study that employed the TSST-VR (Kelly et al., 2007), we have conducted a series of investigations using a current iteration of the paradigm. Although our methodological evaluation brought forth evidence that the original TSST still appears to elicit a somewhat stronger neuroendocrine response (Zimmer et al., 2019), we found high cortisol responder rates to the virtual stressor (75% or 62.5% of participants depending on which criterion is used; Miller, Plessow, Kirschbaum, & Stalder, 2013) and an on-average doubling of salivary cortisol. This effect is comparable to the stress-induced changes that are found in most studies that use the original version of the paradigm (Goodman, Janson, & Wolf, 2017). Although HR increased over the course of the preparation phase and peaked during the virtual TSST, the same trajectory was observed in the control conditions with only the TSST *in vivo* displaying a stronger increase. Furthermore, no significant differences between the TSST and control conditions could be discerned in terms of skin conductance levels. In contrast, both salivary alpha amylase and subjective stress levels were significantly elevated in the TSST conditions in comparison to the control conditions.

Taken together, the results of this first study demonstrate the potential of an interactive and immersive version of the TSST-VR for psychobiological research. Although the fact that cortisol secretion is still somewhat less pronounced in comparison to a well-executed TSST *in vivo* might lead researchers to tread cautiously when considering the TSST-VR as a valid alternative, activation of the HPA-axis has consistently been found in all studies on the paradigm that we conducted hereafter (e.g. Domes & Zimmer, 2019). Furthermore, it might be explained by the higher degree to which social evaluation, one of the crucial elements in the origin of stress (Dickerson & Kemeny, 2004), is present in the confrontation with the real human committee. However, as the boundaries of realism and immersiveness are pushed further by rapidly improving technology, the gap between the effects of real and virtual stress induction can be expected to diminish even further.

5.2. Sense of Presence and the Stress Response in VR

The presented findings, which indicate that psychosocial stress can be induced by virtual entities, invite the question of what the underlying effect mechanisms responsible for the onset of the stress response in virtual environments might be. In the second study, we attempted to elucidate the role of the concept of presence that Wirth and colleagues (2007) defined as the experience of physically being in a mediated space with all entailing consequences. Contrary to our initial hypothesis, facilitating the transition from the real to the virtual environment by parallelizing the two settings did not lead to differences in presence in comparison to a control group with differing environments. Moreover, we did not detect any significant differences in endocrine, enzymatic, sympathetic or subjective stress indicators.

This finding is, however, in accord with current theories on spatial presence that propose that when presence is experienced, the impression of being in the respective virtual space is ubiquitous and the real environment is no longer perceived as relevant (Hartmann et al., 2015). Instead, action possibilities in the virtual realm gain in importance, even when committing to them is physically impossible (e.g. escaping through a purely virtual window; Fich et al., 2014).

Beyond the investigation of presence in stressful virtual scenarios, the present study was conducted to answer some pragmatic questions with implications for a more widespread application of the paradigm. Namely, we set out to investigate whether modelling the environment in which the TSST-VR is to be used represents a necessity for successful psychobiological stress induction. The result that the paradigm induces stress even if the user is transported into a virtual environment that in no way bears resemblance to their physical surroundings substantiates its flexibility and makes it an ideal candidate for multi-center research studies in which collaborators wish to test different populations in a standardized environment. Furthermore, it paves the way for clinical application where it will not always be feasible to invest the resources required to program virtual environments. As several studies have shown that the response to the TSST can be used to reflect improvements made over the course of a therapeutic intervention (Britton, Shahar, Szepsenwol, & Jacobs, 2012; Strachowski et al., 2008), employing the virtual adaptation of the TSST in a clinical context is much more feasible due to its cost-efficiency.

5.3. The Influence of Virtual Stress on Social Cognition

In the third study presented in this dissertation, the TSST-VR was employed as a procedure for examining the effects of psychosocial stress on social cognition. Beyond investigating emotion recognition performance, this study was intended to provide a test bed for the scientific application of the TSST-VR in an experimental context. With respect to the research question we sought to examine, the results provide support for the hypothesis that stress sensitizes perception for emotional cues, such as facial expressions of emotions. The participants who were confronted with the virtual stressor had higher values in the discrimination indices for both emotional valences than the control group. Not only did they display higher emotion detection performance, they furthermore showed significantly shortened response latencies over all emotional valences and intensities. This improvement in the processing of displayed emotions can potentially be interpreted as an indication that social threat (in this case represented by the taxing interview situation with the committee) promotes individuals to seek out social support. In this case, detecting emotional cues from others in the vicinity might be a prerequisite for more complex social cognition and decision making. This interpretation is supported by empirical findings that demonstrate not only increases in emotion processing (Deckers et al., 2015), but also in empathy (Wolf et al., 2015) and prosocial behavior (von Dawans, Fischbacher, Kirschbaum, Fehr, & Heinrichs, 2012) after psychosocial stress exposure.

Over and above the findings delineated above, the study provides a proof of concept for the application of the TSST-VR in psychobiological stress induction. In addition to the result that the paradigm appears to elicit a physiological and subjective stress response, it also seems to promote processes that are expected to be heightened specifically after social stress. This assumption is in line with the theoretical framework that Reeves and Nass (1996) proposed in the *Media Equation* concept for the interaction with virtual entities. Specifically, they argue that humans apply the rules of social engagement to mediated spaces as well and that social behavior, such as reciprocity, politeness, and gender stereotyping are displayed even if they know that their interaction partners are programmed entities that do not warrant the attribution of human characteristics or the application of social behavior (*Ethopoeia*; Nass & Moon, 2000).

In conclusion, possible advantages of employing a VR-based stressor when conducting experimental procedures such as the ones described above shall be considered. The most obvious, perhaps, is that using a computer-based stress induction procedure greatly cuts personnel costs by making the use of a committee of judges obsolete and thereby reducing the required number of experimenters to one. Furthermore—and of equal importance—it provides an experimental frame in which all stress-inducing procedures are conducted in a fully standardized manner. While a degree of unsystematic variance in the committees' behavior in the real TSST is arguably unavoidable, interventions in the TSST-VR are fully standardized with a wide range of possibilities to react adequately to the participants' responses. This might be a crucial advantage when investigating research questions such as those elaborated on in the present publication where minor changes in experimenter behavior might influence participants' perception of events and subsequent reactions. In addition, it provides an ideal context in which hypothetical effect mechanisms can be investigated by experimental manipulation. In the publication described, for example, we speculated about how one possible alternative explanation of the effects of psychosocial stress on emotion recognition might be that the interaction with the committee promoted a generalized social priming effect (Daher et al., 2017). In the virtual realm, it would be simple to test this hypothesis by varying the frequency and intensity with which the judges display emotional expressions or even altogether replace them with non-humanoid character models or objects.

5.4. Avenues for Future Research

The present dissertation aimed to investigate the utility and possible fields of application for a virtual adaptation of the well-established Trier Social Stress Test. We have shown over multiple studies that the TSST-VR robustly induces psychosocial stress on a physiological and psychological level and that it holds several key advantages with respect to cost-efficiency, standardization, and versatility. Now that the effectiveness of the paradigm has been demonstrated, a wide array of applications in research and therapy are conceivable. Furthermore, although we attempted to elucidate some potential effect mechanisms, a number of research questions should be investigated in order to further refine the paradigm and to understand its underlying processes in their entirety.

Most importantly, future studies should address the fact that almost all studies concerning stress induction in VR have been conducted exclusively on men. As demonstrated by a recent meta-analysis on the effects of the TSST-VR on cortisol reactivity (Helminen, Morton, Wang, & Felver, 2019), only a small number of studies have investigated the paradigms' efficacy in not only a male, but also a female sample (Fallon, Careaga, Sbarra, & O'Connor, 2016; Jönsson et al., 2015; O. Kelly et al., 2007; Ruiz et al., 2010). Furthermore, all of the studies in existence had very small sample sizes. This is especially problematic since there are several factors that warrant the assumption that females might differ from males in their psychobiological responses to the virtual stressor. Firstly, sex-dependent differences in the somatic responses to psychosocial stressors have been demonstrated in numerous empirical studies (Kelly, Tyrka, Anderson, Price, & Carpenter, 2008; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995; Kudielka & Kirschbaum, 2005; Rohleder, Schommer, Hellhammer, Engel, & Kirschbaum, 2001) and meta-analyses (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014; Liu et al., 2017). Additionally, there seem to be sex differences in how virtual environments are perceived (Munafo, Diedrick, & Stoffregen, 2017) and navigated (Astur, Purton, Zaniewski, Cimadevilla, & Markus, 2016). More specifically, Felnhofer, Kothgassner, Beutl, Hlavacs, & Kryspin-Exner (2012) report that men experience a significantly higher sense of presence in virtual environments. Future studies should examine whether this might at least partially be explained by the fact that men spend a significantly larger amount of their spare time on video game entertainment software, than women (Borgonovi, 2016; Rehbein, Staudt, Hanslmaier, & Kliem, 2016).

Another aspect that warrants further investigation is how the psychophysiological stress response is affected by the experience of presence (Schuemie, van der Straaten, Krijn, & van der Mast, 2001). As reported in section 3.2., the sense of entering a separate reality with entirely different action possibilities is a crucial prerequisite for experiencing a virtual environment in an immersive and involving way (Hartmann et al., 2015). In a stressful virtual environment, the experience of presence seems to be associated with feelings of stress (Meehan, Razzaque, Whitton, & Brooks, 2003). Furthermore, clinical research has shown a link between the sense of presence and anxiety in virtual reality exposure therapy (Gromer et al., 2019; Riva et al., 2007). In patients with social anxiety disorder, for example, the interaction with virtual agents can induce anxiety as long as presence is experienced (Morina, Brinkman, Hartanto, & Emmelkamp, 2014). Similar findings emerged in a non-clinical population when faced with a taxing virtual job interview simulation (Kwon, Powell, & Chalmers, 2013). Consequently, modulating the sense of presence in a TSST-VR scenario might provide additional insight into the role of presence in the onset of a psychobiological stress response after virtual stress exposure.

More specifically, one facet of presence that could be explored is the concept of social presence (Nowak & Biocca, 2003). Social presence describes the degree to which one is aware of the presence and the relationship with another—be it a real person or a computer-controlled entity—while interacting via some technological means of communication. In the studies included in this dissertation, the virtual judges might be interpreted as the vehicle through which the participant interacts with the experimenter. Although it was never explicitly stated, it can be assumed that most participants became aware of the fact that all proceedings in the virtual environment were, in fact, controlled by the experimenter. As a consequence, the socially evaluative threat of the TSST-VR might, to a certain extent, have originated in the fear of negative evaluation by the real person that was present and only to a lesser degree in the actions of the virtual judges. While the first and the third studies presented in this dissertation (Domes & Zimmer, 2019; Zimmer et al., 2019) were conducted with the experimenter directing the VR-based procedures from a separate control room, the experimental design in the second study (Zimmer et al., in press) required the experimenter to be in the same room as the participant at all times. In order to understand the influence of the experimenters' presence during virtual stress induction, future studies should compare both conditions directly. There are reasonable grounds to expect a heightened sense of social presence in instances where the experimenter is present throughout the virtual stress exposure (Biocca & Harms, 2002). While this is in no way meant to deemphasize the psychosocial stress effects of the TSST-VR (that are also consistently found when the paradigm is used in a CAVE environment absent of others; Annerstedt et al., 2013; Fich et al., 2014; Linninge et al., 2018) it might provide an additional pathway to understanding the underlying effect mechanisms of social evaluation by virtual entities.

Another research question that has preoccupied researchers in the field since the early days of laboratory stress induction is the absence of a physiological response to the socially evaluative threat of the TSST in some participants. Concerning the response of the HPA-axis, some thresholds to distinguish cortisol responders from nonresponders have been proposed (Kirschbaum, Wüst, & Strasburger, 1992; Wüst et al., 2000) and are frequently used (Clow, Thorn, Evans, & Hucklebridge, 2004; Petrowski, Herold, Joraschky, Wittchen, & Kirschbaum, 2010) and examined in the literature (Miller et al., 2013). In the discourse of which factors promote the emergence or absence of a physiological stress response in healthy subjects, several influential factors have been investigated, such as gender (Kudielka & Kirschbaum, 2005), age (Kudielka et al., 1998) personality traits (Kirschbaum et al., 1995), genetic factors (Wüst et al., 2004), and dietary habits (Gonzalez-Bono, Rohleder, Hellhammer, Salvador, & Kirschbaum, 2002; Kirschbaum et al., 1997). Interestingly, responder rates to the TSST-VR generally seem to be somewhat lower than to the regular version of the paradigm (e.g. Shiban et al., 2016). It therefore stands to reason that additional factors to the ones mentioned before come into play in virtual adaptations of the TSST. In addition to whether individuals are experiencing presence (Meehan, Insko, Whitton, & Brooks Jr, 2002), the technological means of implementing the simulation—e.g. via a CAVE system or a head-mounted display (Juan & Pérez, 2009)—or graphical fidelity can be presumed to influence the psychobiological stress response (Gromer et al., 2019; Kwon et al., 2013). Future studies should investigate how these factors affect the physiological reactivity to psychosocial stress in virtual environments.

In this last section, several possible fields of application for the TSST-VR beyond foundational research shall be reviewed. Due to its versatility and cost-efficient employment, the paradigm is suitable to a wide range of contexts, be it in therapeutic intervention or training of specific interpersonal skills. One field that might particularly benefit from this economical procedure for stress induction is clinical practice. Providing therapists with the means to expose clients to psychosocial stress in a fully controllable and standardized manner could be beneficial in several ways: For one, the TSST-VR could be used for diagnostic purposes to obtain data on the functionality of physiological responses (Dorn et al., 2003; Gerra et al., 2000). Specifically, several studies have shown that patients with social phobia exhibited hyper-responsiveness of the HPA axis when faced with psychosocial stress (Condren, O'Neill, Ryan, Barrett, & Thakore, 2002; Furlan, DeMartinis, Schweizer, Rickels, & Lucki, 2001). In these instances, the TSST-VR could be used several times over the course of a therapeutic intervention to measure treatment progress on a physiological level. At the same time, it could be an ideal method for exposure therapy since duration and intensity of the stimulus would be under the sole control of the therapist (Parsons & Rizzo, 2008; Powers & Emmelkamp, 2008). Moreover, it could also be used to evaluate whether clients have successfully mastered strategies such as mindfulness-based stress reduction (Britton et al., 2012) or meditation (Pace et al., 2009), to manage stressful occurrences in their daily lives and boost self-confidence.

Lastly, the paradigm could find application for the training of specific skills. As an example, it has been shown that VR-based interventions can support adolescents and adults with high-functioning autism in learning social skills in order to enhance social functioning (Fernández-Herrero et al., 2018; Kandalaft, Didehbani, Krawczyk, Allen, & Chapman, 2013). Furthermore, VR-based job interview training has been shown to be beneficial for patients suffering from schizophrenia (Smith et al., 2015a) and for veterans striving for re-integration into society after post-traumatic stress disorder (Smith et al., 2015b) In individuals with similar deficits, the social interaction incorporated in the TSST-VR might provide an ideal test bed for training to deal with socially challenging situations. Even individuals without any deficits in social cognition could possibly make use of the virtual TSST to improve their public speaking abilities (Chollet, Wörtwein, Morency, Shapiro, & Scherer, 2015) and overall performance in a job interview situation (Baur, Damian, Gebhard, Porayska-Pomsta, & Andre, 2013).

6. Conclusion

In summary, the present dissertation provides a systematic investigation of the efficacy and specific effect mechanisms of a VR-based adaptation of arguably the most frequently employed psychosocial stressor, the TSST. We hereby wish to present the scientific community with a cost-effective, flexible and standardized stress induction paradigm that reliably elicits a psychological and physiological stress response. While this approach is by no means intended to replace the original, face-to-face TSST, it nevertheless has enormous potential to be a valuable alternative for specific research endeavors (Domes & Zimmer, 2019). As technological advances in the wake of the ‘virtual revolution’ (Blascovich & Bailenson, 2011) lead to the development of higher-quality VR equipment and even more immersive virtual worlds, the paradigm might eventually parallel the stress effects of the original TSST in terms of HPA axis reactivity (Zimmer et al., 2019). In the interim, a great deal of knowledge is to be gained from future studies that address the specific mechanisms involved in the origin of the psychobiological stress response in the virtual realm (Zimmer et al., in press). With the empirical studies and the theoretical framework presented in this dissertation, we aim to contribute to a better understanding of the processes involved in socially evaluative stress in virtual environments. Based on the research delineated above, we believe that this technology harbors great potential in a variety of ways. We thus hope to encourage researchers and practitioners alike to consider adopting the TSST-VR into their methodological repertoire and thereby make use of the near limitless range of possibilities in the virtual realm.

7. References

Allen, A. P., Kennedy, P. J., Cryan, J. F., Dinan, T. G., & Clarke, G. (2014). Biological and psychological markers of stress in humans: Focus on the Trier Social Stress Test. *Neuroscience & Biobehavioral Reviews*, *38*, 94–124. doi:10.1016/j.neubiorev.2013.11.005

Allen, A. P., Kennedy, P. J., Dockray, S., Cryan, J. F., Dinan, T. G., & Clarke, G. (2017). The Trier Social Stress Test: Principles and practice. *Neurobiology of Stress*, *6*, 113–126. doi:10.1016/j.ynstr.2016.11.001

Annerstedt, M., Jönsson, P., Wallergård, M., Johansson, G., Karlson, B., Grahn, P., … Währborg, P. (2013). Inducing physiological stress recovery with sounds of nature in a virtual reality forest—Results from a pilot study. *Physiology & Behavior*, *118*, 240–250. doi:10.1016/j.physbeh.2013.05.023

Astur, R. S., Purton, A. J., Zaniewski, M. J., Cimadevilla, J., & Markus, E. J. (2016). Human sex differences in solving a virtual navigation problem. *Behavioural Brain Research*, *308*, 236–243. doi:10.1016/j.bbr.2016.04.037

Bamberger, C. M., Schulte, H. M., & Chrousos, G. P. (1996). Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocrine Reviews*, *17*(3), 245–261. doi:10.1210/edrv-17-3-245

Baur, T., Damian, I., Gebhard, P., Porayska-Pomsta, K., & Andre, E. (2013). A job interview simulation: Social cue-based interaction with a virtual character. *2013 International Conference on Social Computing*, 220–227. doi:10.1109/SocialCom.2013.39

Bell, M. D., & Weinstein, A. (2011). Simulated job interview skill training for people with psychiatric disability: feasibility and tolerability of virtual reality training. *Schizophrenia Bulletin*, *37*(suppl 2), S91–S97. doi:10.1093/schbul/sbr061

Biocca, F., & Harms, C. (2002). Defining and measuring social presence: Contribution to the networked minds theory and measure. *Proceedings of PRESENCE*, *2002*, 1–36.

Blascovich, J., & Bailenson, J. (2011). *Infinite Reality: Avatars, Eternal Life, New Worlds, and the Dawn of the Virtual Revolution*. New York: William Morrow & Co.

Borgonovi, F. (2016). Video gaming and gender differences in digital and printed reading performance among 15-year-olds students in 26 countries. *Journal of Adolescence*, *48*, 45–61. doi:10.1016/j.adolescence.2016.01.004

Botella, C., García-Palacios, A., Villa, H., Baños, R. M., Quero, S., Alcañiz, M., & Riva, G. (2007). Virtual reality exposure in the treatment of panic disorder and agoraphobia: A controlled study. *Clinical Psychology & Psychotherapy*, *14*(3), 164–175. doi:10.1002/cpp.524

Botella, Cristina, Serrano, B., baños, R., & García-Palacios, A. (2015). Virtual reality exposure-based therapy for the treatment of post-traumatic stress disorder: A review of its efficacy, the adequacy of the treatment protocol, and its acceptability. *Neuropsychiatric Disease and Treatment*, 2533. doi:10.2147/NDT.S89542

Bouchard, S., Dumoulin, S., Robillard, G., Guitard, T., Klinger, É., Forget, H., … Roucaut, F. X. (2017). Virtual reality compared with in vivo exposure in the treatment of social anxiety disorder: A three-arm randomised controlled trial. *British Journal of Psychiatry*, *210*(04), 276–283. doi:10.1192/bjp.bp.116.184234

Britton, W. B., Shahar, B., Szepsenwol, O., & Jacobs, W. J. (2012). Mindfulness-based cognitive therapy improves emotional reactivity to social stress: results from a randomized controlled trial. *Behavior Therapy*, *43*(2), 365–380. doi:10.1016/j.beth.2011.08.006

Bullinger, M., Naber, D., Pickar, D., Cohen, R. M., Kalin, N. H., Pert, A., & Bunney, W. E. (1984). Endocrine effects of the cold pressor test: Relationships to subjective pain appraisal and coping. *Psychiatry Research*, *12*(3), 227–233. doi:10.1016/0165-1781(84)90028-3

Buske-Kirschbaum, A., Jobst, S., Wustmans, A., Kirschbaum, C., Rauh, W., & Hellhammer, D. (1997). Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis: *Psychosomatic Medicine*, *59*(4), 419–426. doi:10.1097/00006842-199707000-00012

Cheetham, M., Pedroni, A., Antley, A., Slater, M., & Jäncke, L. (2009). Virtual milgram: Empathic concern or personal distress? Evidence from functional MRI and dispositional measures. *Frontiers in Human Neuroscience, 3*, 29. doi:10.3389/neuro.09.029.2009

Chollet, M., Wörtwein, T., Morency, L.-P., Shapiro, A., & Scherer, S. (2015). Exploring feedback strategies to improve public speaking: An interactive virtual audience framework. *Proceedings of the 2015 ACM International Joint Conference on Pervasive and Ubiquitous Computing - UbiComp '15,* 1143–1154. doi:10.1145/2750858.2806060

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nature Reviews Endocrinology, 5*(7), 374–381. doi:10.1038/nrendo.2009.106

Clow, A., Thorn, L., Evans, P., & Hucklebridge, F. (2004). The awakening cortisol response: methodological issues and significance. *Stress*, *7*(1), 29–37. doi:10.1080/10253890410001667205

Condren, R. M., O'Neill, A., Ryan, M. C. M., Barrett, P., & Thakore, J. H. (2002). HPA axis response to a psychological stressor in generalised social phobia. *Psychoneuroendocrinology*, *27*(6), 693–703. doi: 10.1016/s0306-4530(01)00070-1

Daher, S., Kim, K., Lee, M., Bruder, G., Schubert, R., Bailenson, J., & Welch, G. F. (2017). Can social presence be contagious? Effects of social presence priming on interaction with virtual humans. *2017 IEEE Symposium on 3D User Interfaces (3DUI)*, 201–202. doi:10.1109/3DUI.2017.7893341

Dawson, M. E., Schell, A. M., & Filion, D. L. (2016). The electrodermal system. In G. G. Berntson, J. T. Cacioppo, & L. G. Tassinary (Eds.), *Handbook of Psychophysiology* (4th ed., pp. 217–243). Cambridge: Cambridge University Press. doi:10.1017/9781107415782.010

Deckers, J. W. M., Lobbestael, J., van Wingen, G. A., Kessels, R. P. C., Arntz, A., & Egger, J. I. M. (2015). The influence of stress on social cognition in patients with borderline personality disorder. *Psychoneuroendocrinology*, *52*, 119–129. doi:10.1016/j.psyneuen.2014.11.003

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*(3), 355–391. doi:10.1037/0033-2909.130.3.355

Diemer, J., Alpers, G. W., Peperkorn, H. M., Shiban, Y., & Mühlberger, A. (2015). The impact of perception and presence on emotional reactions: A review of research in virtual reality. *Frontiers in Psychology*, *6*, 26. doi:10.3389/fpsyg.2015.00026

Domes, G., & Zimmer, P. (2019). Acute stress enhances the sensitivity for facial emotions: A signal detection approach. *Stress*, *22*(4), 455–460. doi:10.1080/10253890.2019.1593366

Dorn, L. D., Campo, J. C., Thato, S., Dahl, R. E., Lewin, D., Chandra, R., & Di Lorenzo, C. (2003). Psychological comorbidity and stress reactivity in children and adolescents with recurrent abdominal pain and anxiety disorders. *Journal of the American Academy of Child & Adolescent Psychiatry*, *42*(1), 66–75. doi:10.1097/00004583-200301000-00012

Duesenberg, M., Weber, J., Schulze, L., Schaeuffele, C., Roepke, S., Hellmann-Regen, J., … Wingenfeld, K. (2016). Does cortisol modulate emotion recognition and empathy? *Psychoneuroendocrinology*, *66*, 221–227. doi:10.1016/j.psyneuen.2016.01.011

Falconer, C. J., Rovira, A., King, J. A., Gilbert, P., Antley, A., Fearon, P., … Brewin, C. R. (2016). Embodying self-compassion within virtual reality and its effects on patients with depression. *BJPsych Open*, *2*(1), 74–80. doi:10.1192/bjpo.bp.115.002147

Fallon, M. A., Careaga, J. S., Sbarra, D. A., & O’Connor, M.-F. (2016). Utility of a virtual Trier Social Stress Test: Initial findings and benchmarking comparisons. *Psychosomatic Medicine*, *78*(7), 835–840. doi:10.1097/PSY.0000000000000338

Felnhofer, A., Kothgassner, O. D., Beutl, L., Hlavacs, H., & Kryspin-Exner, I. (2012). Is virtual reality made for men only? Exploring gender differences in the sense of presence. *Proceedings of the International Society on Presence Research*, 103–112.

Felnhofer, A., Kothgassner, O. D., Schmidt, M., Heinzle, A.-K., Beutl, L., Hlavacs, H., & Kryspin-Exner, I. (2015). Is virtual reality emotionally arousing? Investigating five emotion inducing virtual park scenarios. *International Journal of Human-Computer Studies*, *82*, 48–56. doi:10.1016/j.ijhcs.2015.05.004

Fernández-Herrero, J., Lorenzo-Lledó, G., & Carreres, A. L. (2018). A bibliometric study on the use of virtual reality (VR) as an educational tool for high-functioning autism spectrum disorder (ASD) children. In Ş. Çetinkaya (Ed.), *Contemporary Perspective on Child Psychology and Education* (pp. 59-81). Norderstedt: Books on Demand. doi:10.5772/intechopen.71000

Ferrer-Garcia, M., Gutiérrez-Maldonado, J., & Riva, G. (2013). Virtual reality based treatments in eating disorders and obesity: A review. *Journal of Contemporary Psychotherapy*, *43*(4), 207–221. doi:10.1007/s10879-013-9240-1

Fich, L. B., Jönsson, P., Kirkegaard, P. H., Wallergård, M., Garde, A. H., & Hansen, Å. (2014). Can architectural design alter the physiological reaction to psychosocial stress? A virtual TSST experiment. *Physiology & Behavior, 135,* 91–97. doi:10.1016/j.physbeh.2014.05.034

Fodor, L. A., Coteț, C. D., Cuijpers, P., Szamoskozi, Ștefan, David, D., & Cristea, I. A. (2018). The effectiveness of virtual reality based interventions for symptoms of anxiety and depression: A meta-analysis. *Scientific Reports, 8*(1). doi:10.1038/s41598-018-28113-6

Furlan, P. M., DeMartinis, N., Schweizer, E., Rickels, K., & Lucki, I. (2001). Abnormal salivary cortisol levels in social phobic patients in response to acute psychological but not physical stress. *Biological Psychiatry*, *50*(4), 254–259. doi:10.1016/S0006-3223(00)01126-4

Garau, M., Slater, M., Pertaub, D.-P., & Razzaque, S. (2005). The responses of people to virtual humans in an immersive virtual environment. *Presence: Teleoperators and Virtual Environments*, *14*(1), 104–116. doi:10.1162/1054746053890242

Gerra, G., Zaimovic, A., Zambelli, U., Timpano, M., Reali, N., Bernasconi, S., & Brambilla, F. (2000). Neuroendocrine responses to psychological stress in adolescents with anxiety disorder. *Neuropsychobiology, 42*(2), 82–92. doi:10.1159/000026677

Goldstein, D. S., & McEwen, B. (2002). Allostasis, Homeostasis, and the nature of stress. *Stress, 5*(1), 55–58. doi:10.1080/102538902900012345

Gonzalez-Bono, E., Rohleder, N., Hellhammer, D. H., Salvador, A., & Kirschbaum, C. (2002). Glucose but not protein or fat load amplifies the cortisol response to psychosocial stress. *Hormones and Behavior*, *41*(3), 328–333. doi:10.1006/hbeh.2002.1766

Goodman, W. K., Janson, J., & Wolf, J. M. (2017). Meta-analytical assessment of the effects of protocol variations on cortisol responses to the Trier Social Stress Test. *Psychoneuroendocrinology, 80,* 26–35. doi:10.1016/j.psyneuen.2017.02.030

Gromala, D., Tong, X., Choo, A., Karamnejad, M., & Shaw, C. D. (2015). The virtual meditative walk: Virtual reality therapy for chronic pain management. *Proceedings of the 33rd Annual ACM Conference on Human Factors in Computing Systems*, 521–524. doi: 10.1145/2702123.2702344

Gromer, D., Reinke, M., Christner, I., & Pauli, P. (2019). Causal interactive links between presence and fear in virtual reality height exposure. *Frontiers in Psychology, 10,* 141. doi:10.3389/fpsyg.2019.00141

Hartanto, D., Kampmann, I. L., Morina, N., Emmelkamp, P. G. M., Neerincx, M. A., & Brinkman, W.-P. (2014). Controlling social stress in virtual reality environments. *PLoS ONE, 9*(3), e92804. doi:10.1371/journal.pone.0092804

Hartmann, T., Wirth, W., Vorderer, P., Klimmt, C., Schramm, H., & Böcking, S. (2015). Spatial Presence theory: state of the art and challenges ahead. In M. Lombard, F. Biocca, J. Freeman, W. IJsselsteijn, & R. J. Schaevitz (Eds.), *Immersed in Media* (pp. 115–135). Berlin: Springer. doi:10.1007/978-3-319-10190-3_7

Hassard, J., Teoh, K. R. H., Visockaite, G., Dewe, P., & Cox, T. (2018). The cost of work-related stress to society: A systematic review. *Journal of Occupational Health Psychology, 23*(1), 1–17. doi:10.1037/ocp0000069

Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology, 34*(2), 163–171. doi:10.1016/j.psyneuen.2008.10.026

Helminen, E. C., Morton, M. L., Wang, Q., & Felver, J. C. (2019). A meta-analysis of cortisol reactivity to the Trier Social Stress Test in virtual environments. *Psychoneuroendocrinology*, *110*, 104437. doi:10.1016/j.psyneuen.2019.104437

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., & Wolf, O. T. (2009). Neuroendocrine and psychometric evaluation of a placebo version of the 'Trier Social Stress Test.' *Psychoneuroendocrinology*, *34*(7), 1075–1086. doi:10.1016/j.psyneuen.2009.02.008

Hines, E. A. (1932). A standard stimulus for measuring vasomotor reactions: Its application in the study of hypertension. *Mayo Clinic Proceedings*, *7*, 332–335.

Jönsson, P., Österberg, K., Wallergård, M., Hansen, Å. M., Garde, A. H., Johansson, G., & Karlson, B. (2015). Exhaustion-related changes in cardiovascular and cortisol reactivity to acute psychosocial stress. *Physiology & Behavior*, *151*, 327–337. doi:10.1016/j.physbeh.2015.07.020

Jönsson, P., Wallergård, M., Österberg, K., Hansen, Å. M., Johansson, G., & Karlson, B. (2010). Cardiovascular and cortisol reactivity and habituation to a virtual reality version of the Trier Social Stress Test: A pilot study. *Psychoneuroendocrinology*, *35*(9), 1397–1403. doi:10.1016/j.psyneuen.2010.04.003

Juan, M. C., & Pérez, D. (2009). Comparison of the levels of presence and anxiety in an acrophobic environment viewed via HMD or CAVE. *Presence: Teleoperators and Virtual Environments*, *18*(3), 232–248. doi:10.1162/pres.18.3.232

Kandalaft, M. R., Didehbani, N., Krawczyk, D. C., Allen, T. T., & Chapman, S. B. (2013). Virtual reality social cognition training for young adults with high-functioning autism. *Journal of Autism and Developmental Disorders*, *43*(1), 34–44. doi:10.1007/s10803-012-1544-6

Kelly, M. M., Tyrka, A. R., Anderson, G. M., Price, L. H., & Carpenter, L. L. (2008). Sex differences in emotional and physiological responses to the Trier Social Stress Test.

Journal of Behavior Therapy and Experimental Psychiatry, 39(1), 87–98. doi:10.1016/j.jbtep.2007.02.003

Kelly, O., Matheson, K., Martinez, A., Merali, Z., & Anisman, H. (2007). Psychosocial stress evoked by a virtual audience: Relation to neuroendocrine activity. *CyberPsychology & Behavior*, *10*(5), 655–662. doi:10.1089/cpb.2007.9973

Kim, H.-G., Cheon, E.-J., Bai, D.-S., Lee, Y. H., & Koo, B.-H. (2018). Stress and heart rate variability: A meta-analysis and review of the literature. *Psychiatry Investigation*, *15*(3), 235–245. doi:10.30773/pi.2017.08.17

Kirschbaum, C., Wüst, S., & Strasburger, C. J. (1992). ‘Normal’cigarette smoking increases free cortisol in habitual smokers. *Life Sciences, 50*(6), 435–442. doi:10.1016/0024-3205(92)90378-3

Kirschbaum, Clemens, Bono, E. G., Rohleder, N., Gessner, C., Pirke, K. M., Salvador, A., & Hellhammer, D. H. (1997). Effects of fasting and glucose load on free cortisol responses to stress and nicotine. *The Journal of Clinical Endocrinology & Metabolism*, *82*(4), 1101–1105. doi:10.1210/jcem.82.4.3882

Kirschbaum, Clemens, & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology*, *19*(4), 313–333. doi:10.1016/0306-4530(94)90013-2

Kirschbaum, Clemens, Klauer, T., Filipp, S.-H., & Hellhammer, D. H. (1995). Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosomatic Medicine, 57*(1), 23–31. doi: 10.1097/00006842-199501000-00004

Kirschbaum, Clemens, Pirke, K.-M., & Hellhammer, D. H. (1993). The ‘Trier Social Stress Test’ – A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, *28*(1–2), 76–81. doi:10.1159/000119004

Kirschbaum, Clemens, Prussner, J. C., Stone, A. A., Federenko, I., Gaab, J., Lintz, D., … Hellhammer, D. H. (1995). Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. *Psychosomatic Medicine*, *57*(5), 468–474. doi: 10.1097/00006842-199509000-00009

Kothgassner, O. D., Felnhofer, A., Hlavacs, H., Beutl, L., Palme, R., Kryspin-Exner, I., & Glenk, L. M. (2016). Salivary cortisol and cardiovascular reactivity to a public speaking task in a virtual and real-life environment. *Computers in Human Behavior*, *62*, 124–135. doi:10.1016/j.chb.2016.03.081

Kothgassner, O. D., Hlavacs, H., Beutl, L., Glenk, L. M., Palme, R., & Felnhofer, A. (2016). Two experimental virtual paradigms for stress research: Developing avatar-based approaches for interpersonal and evaluative stressors. In G. Wallner, S. Kriglstein, H. Hlavacs, R. Malaka, A. Lugmayr, & H.-S. Yang (Eds.), *Entertainment Computing—ICEC 2016* (pp. 51–62). Berlin: Springer. doi:10.1007/978-3-319-46100-7_5

Kotlyar, M., Donahue, C., Thuras, P., Kushner, M. G., O’Gorman, N., Smith, E. A., & Adson, D. E. (2008). Physiological response to a speech stressor presented in a virtual reality environment. *Psychophysiology*, *45*(6), 1034–1037. doi:10.1111/j.1469-8986.2008.00690.x

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

Kudielka, B. M., Hellhammer, J., Hellhammer, D. H., Wolf, O. T., Pirke, K.-M., Varadi, E., … Kirschbaum, C. (1998). Sex differences in endocrine and psychological responses to psychosocial stress in healthy elderly subjects and the impact of a 2-week dehydroepiandrosterone treatment. *The Journal of Clinical Endocrinology & Metabolism*, *83*(5), 1756–1761. doi:10.1210/jcem.83.5.4758

Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. *Biological Psychology*, *69*(1), 113–132. doi:10.1016/j.biopsycho.2004.11.009

Kwon, J. H., Powell, J., & Chalmers, A. (2013). How level of realism influences anxiety in virtual reality environments for a job interview. *International Journal of Human-Computer Studies*, *71*(10), 978–987. doi:10.1016/j.ijhcs.2013.07.003

Leistner, C., & Menke, A. (2018). How to measure glucocorticoid receptor’s sensitivity in patients with stress-related psychiatric disorders. *Psychoneuroendocrinology*, *91*, 235–260. doi:10.1016/j.psyneuen.2018.01.023

Levin, M. F., Weiss, P. L., & Keshner, E. A. (2015). Emergence of virtual reality as a tool for upper limb rehabilitation: Incorporation of motor control and motor learning principles. *Physical Therapy*, *95*(3), 415–425. doi:10.2522/ptj.20130579

Linninge, C., Jönsson, P., Bolinsson, H., Önning, G., Eriksson, J., Johansson, G., & Ahrné, S. (2018). Effects of acute stress provocation on cortisol levels, zonulin and inflammatory markers in low- and high-stressed men. *Biological Psychology*, *138*, 48–55. doi:10.1016/j.biopsycho.2018.08.013

Liu, J. J. W., Ein, N., Peck, K., Huang, V., Pruessner, J. C., & Vickers, K. (2017). Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A meta-analysis. *Psychoneuroendocrinology*, *82*, 26–37. doi:10.1016/j.psyneuen.2017.04.007

Lohse, K. R., Hilderman, C. G. E., Cheung, K. L., Tatla, S., & Van der Loos, H. F. M. (2014). Virtual reality therapy for adults post-stroke: A systematic review and meta-analysis exploring virtual environments and commercial games in therapy. *PLoS ONE, 9*(3), e93318. doi:10.1371/journal.pone.0093318

Maples-Keller, J. L., Yasinski, C., Manjin, N., & Rothbaum, B. O. (2017). Virtual reality-enhanced extinction of phobias and post-traumatic stress. *Neurotherapeutics, 14*(3), 554–563. doi:10.1007/s13311-017-0534-y

McEwen, B. S., Biron, C. A., Brunson, K. W., Bulloch, K., Chambers, W. H., Dhabhar, F. S., … Weiss, J. M. (1997). The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions. *Brain Research Reviews, 23*(1–2), 79–133. doi:10.1016/S0165-0173(96)00012-4

Meehan, M., Insko, B., Whitton, M., & Brooks Jr, F. P. (2002). Physiological measures of presence in stressful virtual environments. *Acm Transactions on Graphics (Tog), 21*, 645–652. doi:10.1145/566570.566630

Meehan, M., Razzaque, S., Whitton, M. C., & Brooks, F. P. (2003). Effect of latency on presence in stressful virtual environments. *Proceedings of the IEEE Virtual Reality 2003*, 141–148. doi:10.1109/VR.2003.1191132

Milgram, S. (1963). Behavioral study of obedience. *The Journal of Abnormal and Social Psychology, 67*(4), 371–378. doi:10.1037/h0040525

Miller, R., Plessow, F., Kirschbaum, C., & Stalder, T. (2013). Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: Evaluation of salivary cortisol pulse detection in panel designs. *Psychosomatic Medicine, 75*(9), 832–840. doi:10.1097/PSY.0000000000000002

Montero-López, E., Santos-Ruiz, A., García-Ríos, M. C., Rodríguez-Blázquez, M., Rogers, H. L., & Peralta-Ramírez, M. I. (2018). The relationship between the menstrual cycle and cortisol secretion: Daily and stress-invoked cortisol patterns. *International Journal of Psychophysiology, 131*, 67–72. doi:10.1016/j.ijpsycho.2018.03.021

Montero-López, E., Santos-Ruiz, A., García-Ríos, M. C., Rodríguez-Blázquez, R., Pérez-García, M., & Peralta-Ramírez, M. I. (2016). A virtual reality approach to the Trier Social Stress Test: Contrasting two distinct protocols. *Behavior Research Methods*, *48*(1), 223–232. doi:10.3758/s13428-015-0565-4

Morina, N., Brinkman, W.-P., Hartanto, D., & Emmelkamp, P. M. G. (2014). Sense of presence and anxiety during virtual social interactions between a human and virtual humans. *PeerJ*, *2*, e337. doi:10.7717/peerj.337

Morina, N., Ijntema, H., Meyerbröker, K., & Emmelkamp, P. M. G. (2015). Can virtual reality exposure therapy gains be generalized to real-life? A meta-analysis of studies applying behavioral assessments. *Behaviour Research and Therapy*, *74*, 18–24. doi:10.1016/j.brat.2015.08.010

Munafo, J., Diedrick, M., & Stoffregen, T. A. (2017). The virtual reality head-mounted display Oculus Rift induces motion sickness and is sexist in its effects. *Experimental Brain Research*, *235*(3), 889–901. doi:10.1007/s00221-016-4846-7

Nabel, E. G., Ganz, P., Gordon, J. B., Alexander, R. W., & Selwyn, A. P. (1988). Dilation of normal and constriction of atherosclerotic coronary arteries caused by the cold pressor test. *Circulation*, *77*(1), 43–52. doi:10.1161/01.CIR.77.1.43

Nass, C., & Moon, Y. (2000). Machines and mindlessness: Social responses to computers. *Journal of Social Issues*, *56*(1), 81–103. doi:10.1111/0022-4537.00153

Nowak, K. L., & Biocca, F. (2003). The effect of the agency and anthropomorphism on users’ sense of telepresence, copresence, and social presence in virtual environments. *Presence: Teleoperators and Virtual Environments*, *12*(5), 481–494. doi:10.1162/105474603322761289

Ocasio-De Jesús, V., Kennedy, A., & Whittinghill, D. (2013). Impact of graphical fidelity on physiological responses in virtual environments. *Proceedings of the 19th ACM*

Symposium on Virtual Reality Software and Technology - VRST ’13, 73. doi:10.1145/2503713.2503751

Pace, T. W. W., Negi, L. T., Adame, D. D., Cole, S. P., Sivilli, T. I., Brown, T. D., … Raison, C. L. (2009). Effect of compassion meditation on neuroendocrine, innate immune and behavioral responses to psychosocial stress. *Psychoneuroendocrinology, 34*(1), 87-98. doi:10.1016/j.psyneuen.2008.08.011

Parsons, T. D., & Rizzo, A. A. (2008). Affective outcomes of virtual reality exposure therapy for anxiety and specific phobias: A meta-analysis. *Journal of Behavior Therapy and Experimental Psychiatry*, *39*(3), 250–261. doi:10.1016/j.jbtep.2007.07.007

Pessoa, L., Japee, S., & Ungerleider, L. G. (2005). Visual awareness and the detection of fearful faces. *Emotion*, *5*(2), 243–247. doi:10.1037/1528-3542.5.2.243

Petrowski, K., Herold, U., Joraschky, P., Wittchen, H.-U., & Kirschbaum, C. (2010). A striking pattern of cortisol non-responsiveness to psychosocial stress in patients with panic disorder with concurrent normal cortisol awakening responses. *Psychoneuroendocrinology*, *35*(3), 414–421. doi:10.1016/j.psyneuen.2009.08.003

Powers, M. B., & Emmelkamp, P. M. G. (2008). Virtual reality exposure therapy for anxiety disorders: A meta-analysis. *Journal of Anxiety Disorders*, *22*(3), 561–569. doi:10.1016/j.janxdis.2007.04.006

Reeves, B., & Nass, C. I. (1996). *The media equation: How people treat computers, television, and new media like real people and places*. Cambridge: Cambridge University Press.

Rehbein, F., Staudt, A., Hanslmaier, M., & Kliem, S. (2016). Video game playing in the general adult population of Germany: Can higher gaming time of males be explained by gender specific genre preferences? *Computers in Human Behavior*, *55*, 729–735. doi:10.1016/j.chb.2015.10.016

Reiche, E. M. V., Nunes, S. O. V., & Morimoto, H. K. (2004). Stress, depression, the immune system, and cancer. *The Lancet Oncology*, *5*(10), 617–625. doi:10.1016/S1470-2045(04)01597-9

Riva, G., Bacchetta, M., & Cesa, G. (2001). Based experiential cognitive therapy: Rationale and clinical. In G. Riva & C. Galimberti (Eds.), *Towards Cyberpsychology: Mind, Cognition, and Society in the Internet Age* (pp. 273-311). Amsterdam: IOS Press.

Riva, G., Mantovani, F., Capideville, C. S., Preziosa, A., Morganti, F., Villani, D., … Alcañiz, M. (2007). Affective interactions using virtual reality: The link between presence and emotions. *CyberPsychology & Behavior*, *10*(1), 45–56. doi:10.1089/cpb.2006.9993

Rohleder, N., Schommer, N. C., Hellhammer, D. H., Engel, R., & Kirschbaum, C. (2001). Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosomatic Medicine*, *63*(6), 966–972. doi:10.1097/00006842-200111000-00016

Rothbaum, B. O., Hodges, L. F., Kooper, R., Opdyke, D., Williford, J. S., & North, M. (1995). Virtual reality graded exposure in the treatment of acrophobia: A case report. *Behavior Therapy*, *26*(3), 547–554. doi:10.1016/S0005-7894(05)80100-5

Ruiz, A. S., Peralta-Ramirez, M. I., Garcia-Rios, M. C., Muñoz, M. A., Navarrete-Navarrete, N., & Blazquez-Ortiz, A. (2010). Adaptation of the trier social stress test to virtual reality: Psycho-phsyiological and neuroendocrine modulation. *Journal of Cybertherapy & Rehabilitation*, *3*(4), 66–76.

Sanchez-Vives, M. V., & Slater, M. (2005). From presence to consciousness through virtual reality. *Nature Reviews Neuroscience*, *6*(4), 332–339. doi:10.1038/nrn1651

Schubert, T., Friedmann, F., & Regenbrecht, H. (2001). The experience of presence: Factor analytic insights. *Presence: Teleoperators and Virtual Environments*, *10*(3), 266–281. doi:10.1162/105474601300343603

Schuemie, M. J., van der Straaten, P., Krijn, M., & van der Mast, C. A. P. G. (2001). research on presence in virtual reality: A survey. *CyberPsychology & Behavior*, *4*(2), 183–201. doi:10.1089/109493101300117884

Schwabe, L., Haddad, L., & Schachinger, H. (2008). HPA axis activation by a socially evaluated cold-pressor test. *Psychoneuroendocrinology*, *33*(6), 890–895. doi:10.1016/j.psyneuen.2008.03.001

Selye, H. (1956). *The stress of life*. New York: McGraw-Hill.

Selye, H. (1976). Stress without distress. In G. Serban (Ed.), *Psychopathology of Human Adaptation* (pp. 137–146). Berlin: Springer. doi:10.1007/978-1-4684-2238-2_9

Shiban, Y., Diemer, J., Brandl, S., Zack, R., Mühlberger, A., & Wüst, S. (2016). Trier Social Stress Test in vivo and in virtual reality: Dissociation of response domains. *International Journal of Psychophysiology*, *110*, 47–55. doi:10.1016/j.ijpsycho.2016.10.008

Smeets, T., Dziobek, I., & Wolf, O. T. (2009). Social cognition under stress: Differential effects of stress-induced cortisol elevations in healthy young men and women. *Hormones and Behavior*, *55*(4), 507–513. doi:10.1016/j.yhbeh.2009.01.011

Smith, M. J., Boteler Humm, L., Fleming, M. F., Jordan, N., Wright, M. A., Ginger, E. J., … Bell, M. D. (2015). Virtual reality job interview training for veterans with posttraumatic stress disorder. *Journal of Vocational Rehabilitation*, *42*(3), 271–279. doi:10.3233/JVR-150748

Smith, M. J., Fleming, M. F., Wright, M. A., Roberts, A. G., Humm, L. B., Olsen, D., & Bell, M. D. (2015). Virtual reality job interview training and 6-month employment outcomes for individuals with schizophrenia seeking employment. *Schizophrenia Research*, *166*(1–3), 86–91. doi:10.1016/j.schres.2015.05.022.

Smith, M. J., Ginger, E. J., Wright, K., Wright, M. A., Taylor, J. L., Humm, L. B., … Fleming, M. F. (2014). Virtual reality job interview training in adults with autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *44*(10), 2450–2463. doi:10.1007/s10803-014-2113-y

Smoller, J. W. (2016). The genetics of stress-related disorders: PTSD, depression, and anxiety disorders. *Neuropsychopharmacology*, *41*(1), 297–319. doi:10.1038/npp.2015.266

Steed, A., Frlston, S., Lopez, M. M., Drummond, J., Pan, Y., & Swapp, D. (2016). An ‘in the wild’ experiment on presence and embodiment using consumer virtual reality equipment. *IEEE Transactions on Visualization and Computer Graphics*, *22*(4), 1406–1414. doi:10.1109/TVCG.2016.2518135

Strachowski, D., Khaylis, A., Conrad, A., Neri, E., Spiegel, D., & Taylor, C. B. (2008). The effects of cognitive behavior therapy on depression in older patients with cardiovascular risk. *Depression and Anxiety*, *25*(8), E1–E10. doi:10.1002/da.20302

Taelman, J., Vandeput, S., Spaepen, A., & Van Huffel, S. (2009). Influence of mental stress on heart rate and heart rate variability. In J. Vander Sloten, P. Verdonck, M. Nyssen, & J. Haueisen (Eds.), *4th European Conference of the International Federation for Medical and Biological Engineering* (Vol. 22, pp. 1366–1369). doi:10.1007/978-3-540-89208-3_324

Taylor, S. E., Klein, L. C., Lewis, B. P., Gruenewald, T. L., Gurung, R. A. R., & Updegraff, J. A. (2000). Biobehavioral responses to stress in females: Tend-and-befriend, not fight-or-flight. *Psychological Review*, *107*(3), 411–429. doi:10.1037//0033-295X.107.3.411

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, *53*(4), 865–871. doi:10.1016/S0022-3999(02)00429-4

Turner, A., Keating, C., & Tilbrook, A. (2012). Sex differences and the role of sex steroids in sympatho-adrenal medullary system and hypothalamo-pituitary adrenal axis responses to stress. In S. M. Kahn (Ed.), *Sex Steroids* (pp. 115-136). Norderstedt: Books on Demand. doi:10.5772/25611

Vining, R. F., McGinley, R. A., Maksvytis, J. J., & Ho, K. Y. (1983). Salivary cortisol: A better measure of adrenal cortical function than serum cortisol. *Annals of Clinical Biochemistry: An International Journal of Biochemistry and Laboratory Medicine*, *20*(6), 329–335. doi:10.1177/000456328302000601

von Dawans, B., Fischbacher, U., Kirschbaum, C., Fehr, E., & Heinrichs, M. (2012). The social dimension of stress reactivity: Acute stress increases prosocial behavior in humans. *Psychological Science*, *23*(6), 651–660. doi:10.1177/0956797611431576

von Dawans, B., Kirschbaum, C., & Heinrichs, M. (2011). The Trier Social Stress Test for Groups (TSST-G): A new research tool for controlled simultaneous social stress exposure in a group format. *Psychoneuroendocrinology*, *36*(4), 514–522. doi:10.1016/j.psyneuen.2010.08.004

Wallergård, M., Jönsson, P., Johansson, G., & Karlson, B. (2011). A virtual reality version of the Trier Social Stress Test: A pilot study. *Presence: Teleoperators and Virtual Environments*, *20*(4), 325–336. doi:10.1162/PRES_a_00052

Wiemers, U. S., Schoofs, D., & Wolf, O. T. (2013). A friendly version of the Trier Social Stress Test does not activate the HPA axis in healthy men and women. *Stress*, *16*(2), 254–260. doi:10.3109/10253890.2012.714427

Wirth, W., Hartmann, T., Böcking, S., Vorderer, P., Klimmt, C., Schramm, H., … Jäncke, P. (2007). A process model of the formation of spatial presence experiences. *Media Psychology*, *9*(3), 493–525. doi:10.1080/15213260701283079

Wolf, O. T., Schulte, J. M., Drimalla, H., Hamacher-Dang, T. C., Knoch, D., & Dziobek, I. (2015). Enhanced emotional empathy after psychosocial stress in young healthy men. *Stress*, *18*(6), 631–637. doi:10.3109/10253890.2015.1078787

Wüst, S., Federenko, I. S., Rossum, E. F. C., Koper, J. W., Kumsta, R., Entringer, S., & Hellhammer, D. H. (2004). A psychobiological perspective on genetic determinants of hypothalamus-pituitary-adrenal axis activity. *Annals of the New York Academy of Sciences*, *1032*(1), 52–62. doi:10.1196/annals.1314.005

Wüst, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000). The cortisol awakening response-normal values and confounds. *Noise and Health*, *2*(7), 79.

Zimmer, P., Buttlar, B., Halbeisen, G., Walther, E., & Domes, G. (2019). Virtually stressed? A refined virtual reality adaptation of the Trier Social Stress Test (TSST) induces robust endocrine responses. *Psychoneuroendocrinology*, *101*, 186–192. doi:10.1016/j.psyneuen.2018.11.010

Zimmer, P., Wu, C., & Domes, G. (in press). Same same but different? Replicating the real surroundings in a virtual Trier Social Stress Test (TSST-VR) does not enhance presence or the psychophysiological stress response. *Physiology and Behavior*.

8. Author Contributions

Zimmer, P., Buttlar, B., Halbeisen, G., Walther, E., & Domes, G. (2019). Virtually stressed? A refined virtual reality adaptation of the Trier Social Stress Test (TSST) induces robust endocrine responses. *Psychoneuroendocrinology*, *101*, 186–192. doi:10.1016/j.psyneuen.2018.11.010

This article has been published with shared first authorship. P. Zimmer contributed to the conceptualization of the study and conducted the majority of experimental sessions. P. Zimmer furthermore performed data preparation and analysis and wrote the first draft of the manuscript receiving helpful annotations from all authors. Lastly, P. Zimmer was entrusted with the submission and revision of the manuscript during the editorial process of the Journal *Psychoneuroendocrinology*.

Zimmer, P., Wu, C., & Domes, G. (in press). Same same but different? Replicating the real surroundings in a virtual Trier Social Stress Test (TSST-VR) does not enhance presence or the psychophysiological stress response. *Physiology and Behavior.*

This study was conceptualized by G. Domes and P. Zimmer. P. Zimmer conducted the investigation process and handled project administration as well as data curation and preparation. Data analysis and writing of the original draft were handled by P. Zimmer as well as submission and revision of the manuscript during the editorial process at *Physiology and Behavior*. The manuscript has been accepted for publication and is currently awaiting production.

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P. Zimmer was involved in the planning process of the study, conducted the experimental sessions and handled project administration as well as preparation of the data for analysis. P. Zimmer contributed to the original draft and submitted it to *The International Journal on the Biology of Stress*. P. Zimmer revised the manuscript over the course of the editorial process under the supervision of G. Domes.

9. Declaration of Authorship

I hereby certify that this thesis has been authored by me and is based on my own work, unless stated otherwise. No other person's work has been used without acknowledgement in this thesis. All references have been quoted and all sources of information have been specifically mentioned.

Eigenständigkeitserklärung

Hiermit erkläre ich, dass die vorliegende Arbeit von mir und basierend auf meiner Arbeit entstanden ist. Keine andere Person war an der Erstellung beteiligt, die nicht in der Danksagung zu dieser Arbeit erwähnt wird. Alle Referenzen werden entsprechend zitiert und Daten aus anderen Quellen werden unter Angabe der Quelle als solche gekennzeichnet.

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