ADAPTATION OF THE TRIER SOCIAL STRESS TEST TO VIRTUAL REALITY: PSYCHO-PHYSIOLOGICAL AND NEUROENDOCRINE MODULATION

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The Trier Social Stress Test (TSST; Kirschbaum et al., 1993) is currently the most commonly used psychosocial stressor to generate a response of the axes involved in stress. The TSST has proven effective in the activation of the hypothalamic-pituitary-adrenal axis. In addition, new technologies, such as virtual reality (VR), are being integrated into stress research protocols (Kelly et al., 2007).

To determine whether TSST as applied to VR leads to the sympathetic and neuroendocrine activation in a group of healthy individuals. Also, this study aims to connect this response with different psychological variables regarding stress vulnerability, psychopathology, and personality. Twenty-one university students (6 male and 15 female) were exposed to a modified version of the TSST adapted to a virtual environment (VE), in which they have to deliver a speech. Electrodermal activity and salivary cortisol secretion were simultaneously registered at different instances.

After the task, sympathetic activation was observed in all participants, as well as increase in the cortisol secretion in 14 of the students. This increase was statistically significant in the moment prior to the speech and the moment after in the responder group. In the same fashion, statistically significant differences were found in the responder group only regarding obsession and compulsion scales and extroversion, which were higher in the responder group. Our findings support the use of the TSST paradigm in VR as an experimental situation appropriate to research designs in laboratory aiming to study the modulation of the axes implied in response to stress.

Keywords: TSST, Virtual Reality, Hypothalamic-Pituitary-Adrenal Axis, Psychophysiological Activation, Salivary Cortisol

INTRODUCTION

In recent decades, neuroscientific research on stress has notably increased. In order to induce psychophysiological responses to stress similar to those in real life a great number of stressor agents has been used, such as acute psychological stressors that activate the sympathetic-adrenomedullary system and affect the immune system (Cacioppo, 1994). The tasks that have been used most often are public speaking, arithmetic, Stroop test, video-games, problem solving, and reaction time (Moya-Albiol & Salvador, 2001). In the same fashion, response to stress originated in the hypothalamic-pituitary-adrenal (HPA) axis has been widely used in the increase of blood or salivary cortisol levels when an in-
individual faces acute stress-inducing situations. In their revision of acute stressors used to activate cortisol secretion, Dickerson & Kemeny (2004) highlight the following stressors used in the laboratory – cognitive tests (e.g. mental arithmetic tasks), Stroop test, time-vigilance tasks, analytic perception tasks, public speaking, combined cognitive and public speaking task, noise exposure, and emotion induction by means of films or images. These authors found that the most effective type of stressor task to generate significant cortisol responses was a combined cognitive and public speaking task, as its size effect was twice as much as for cognitive and public speaking tasks performed separately. Therefore, the Trier Social Stress Test (TSST; Kirschbaum, Pirke & Hellhammer, 1993) has become the psychosocial stressor most used in the laboratory to generate the response of the involved axes. The TSST integrates a public speaking task with an arithmetic task and has proven effective in activating the HPA axis (Kelly et al., 2008; Kudielka, Shommer, Hellhammer & Kirschbaum, 2004; Williams, Hagerty & Brooks, 2004).

The traditional TSST proposed by Kirschbaum et al. (1993) consists of a brief anticipatory stress period, where the individual must prepare a speech addressing their eligibility for a job. Then, a second phase follows, in which the individual delivers the speech and completes an arithmetic task in front of an audience who has previously been trained to be neutral in both their verbal and nonverbal behavior (Foley & Kirschbaum, 2010). The main components of this task are the social-evaluative and uncontrollable threats, which are necessary for a stressor stimulus or situation to generate cortisol responses (Dickerson & Kemeny, 2004).

Ever since its design, this psychosocial stressor has been used to measure the response to stress in different population groups, such as children (TSST-C, Buske-Kirschbaum et al., 1997), middle-aged adults (Fiocco, Joobber & Lupien, 2007) and seniors (Kudielka et al., 1998), and in different pathologies, such as psychiatric patients (Brenner et al., 2009), metabolic syndrome (Chrousos, 2000), systemic hypertension (Esler et al., 2008), systemic erythematosus lupus (Pawlak, 1999), and myalgia (Sjörs et al., 2010). In addition, it has been used to study the relationship between stress and several psychological variables, such as depression (Parker et al., 2003), social anxiety (Shirotzuki et al., 2009) or personality features (Kirschbaum, Bartussek & Strasburger, 1992; Pruessner et al., 1997). Despite several studies that have tried to establish a relationship between personality and reactivity to stress, very few have succeeded, which means that only repeated exposure to a stressor (Kirschbaum et al., 1995) and data aggregation (Pruessner et al., 1997) enable the relationship between personality and increased cortisol levels in a stressful situation. However, studies measuring blood or salivary cortisol levels with no manipulation have shown that personality features most closely related to cortisol levels were psychoticism and disinhibition (Ballanger et al., 1983) or anxiety, depression, and extroversion (Dabbs & Hopper, 1990).

Despite the fact that TSST has facilitated a breakthrough in laboratory research on stress, its application needs a wide array of resources including a real audience available for several sessions that must receive some type of honorarium, and the availability of several rooms including a room for the audience and a room for the speakers (experimental subjects). On the other hand, it is essential to bear in mind the variability of the audience, as their attitude may not be neutral or equal for all participants, despite the previous briefing. For this reason, one of the proposals in order to reduce these disadvantages would be introducing other technologies, such as VR, which can act as a neutral tool and saving human and material costs associated with the traditional TSST. In addition, the use of VR provides a measure of human interaction within a dynamic and realist 3-D environment, which facilitates the use of instruments for psychophysiological evaluation (e.g. continuous psychophysiological registration).

To date, few studies have introduced a VR component in order to study psychophysiological and neuroendocrine changes in a stressor task. Regarding psychophysiological activation, Kotlyar et al. (2008) verified the effectiveness in a speech delivered in front of a virtual audience, together with an arithmetic task, in order to generate physiological activation (blood pressure, heart rate, catecholamine in blood and blood cortisol levels) in 12 healthy individuals. The researchers found that the speech increased blood pressure, heart rate, and catecholamine of participants, while no significant changes were recorded in cortisol levels. Regarding neuroendocrine changes, a study by Hemmeter et al. (2005) showed differences in cortisol secretion depending on whether the individuals were exposed to one mental task in a static or dynamic virtual environment (VE). In this
study, four conditions were included: 1) exposure control in a static VE, without cognitive stress; 2) static VE, where participants had to perform a cognitive stressor task: speed and concentration; 3) exposure to a dynamic VE, with objects of different colors and shapes in motion; and 4) exposure to a dynamic VE, where the participants had to perform the same task as in condition 2). The authors found that the cognitive stress condition in a VE significantly increased cortisol secretion compared with the sole exposition to the virtual (static or dynamic) environment or the performance of the mental task in a static VE. However, it was Kelly et al. (2007) who were the first to use the TSST as a stressor task integrated in VR to measure neuroendocrine changes. In the same study, the TSST was adapted to a VE and its effects were compared with those of the traditional TSST, i.e. an imaginary audience vs. a control group that did not perform the task. The authors found that the traditional TSST produced a higher increase in salivary cortisol levels, even though the imaginary audience and the VR TSST also produced significant neuroendocrine changes compared to the control group. Therefore, they were another instrument for the evaluation of the implied axes in response to stress. Nonetheless, this study did not consider other related variables, such as the immersion of the subject in the VR situation or other closely related psychological factors, such as personality features, stress vulnerability, etc.

Therefore, the main goal of our study is to verify the modulation of the axes involved in the response to stress, i.e. sympathetic activation (by means of a skin conductance test) and the HPA axis (by means of the salivary cortisol level) when exposed to a psychosocial stressor, specifically the virtual reality version of the TSST [TSST (VR)].

Secondly, we aim to establish whether or not that modulation may be connected with the different psychological variables of stress vulnerability and personality, as well as immersion in the said virtual situation.

**METHODOLOGY**

**PARTICIPANTS**

For the purpose of this study, we recruited 21 Physiotherapy students at the University of Granada; six were men and 15 were women. The mean age of the participants was 24 (SD=1.2) and mean education was 14.1 years (SD=0.43). All participants were informed about the study objectives, after which they signed an informed consent form. Exclusion criteria were low cultural level (illiteracy), high blood pressure, heart disease, clinical depression, clinical anxiety or other personality disorders, use of drugs or other substances (amphetamines, alcohol, barbiturates, methadone, muscle relaxants or lithium), obesity, use of contraceptives or menopause. Additionally, menstrual course was controlled in women, considering that salivary cortisol levels are higher in the luteal phase than in the follicular phase (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005).

The participants completed a series of psychological questionnaires, after which they were informed about the TSST (VR), which was delivered as described in the procedure section below.

All of the patients gave their signed informed consent to participate in this study, which was approved by the ethics committee in our University and carried out according to the recommendations of the Helsinki Declaration.

**INSTRUMENTS**

- Semi-structured interview, covering sociodemographics, life and sleep habits, medication, menstrual course, and psychiatric or psychological treatment.

- Stress Vulnerability Inventory (Beech, Burns, Sheffield, 1986) in Spanish, validated by Robles-Ortega, Peralta-Ramírez & Navarrete-Navarrete (2006): consists of 22 items and evaluates the individual’s predisposition to be influenced by perceived stress. Regarding reliability, Cronbach’s alpha was 0.87. Regarding convergent validity, the results show a statistically positive correlation (p<0.01) with other evaluation instruments, such as STAI-R, Beck Depression Inventory, Somatic Symptom Scale and SRLE.

- Eysenck Personality Questionnaire for Adults, EPQ-A (Eysenck and Eysenck, 1997) composed of 94 items in Yes/No modality. It gives information about three personality variables: neuroticism, extraversion, and psychoticism, as well as a fourth scale on sincerity. It has satisfactory reliability and validity.

- Iggroup Presence Questionnaire (Schubert, Friedmann, & Regenbrecht, 2001, IPQ): is a scale to measure the sense of experimental presence in a VE. It consists of a global scale and three subscales: spatial presence, involvement, and experimental realism.
Perceived Stress Scale, PSS, by Cohen, Kamarak, & Mermeistein (1983), Spanish, by Remor & Carrobles (2001) is a self-report instrument evaluating the perceived stress level and the degree in which people find their lives unpredictable, uncontrollable or overcharged, aspects that have repeatedly been confirmed as major components of stress. It consists of 14 items with four response alternatives. The highest score corresponds to the highest perceived amount of stress. The Spanish version of the PSS (14 items) showed an adequate reliability (internal consistency=0.81 and test-retest=0.73), concurrent validity, and sensitivity (Remor, 2006).

Symptom Check-List 90-R, SCL-90-R (Derogatis, 1994) was developed to evaluate symptom patterns of individuals. It is a self-report scale, made up of 90 items with five response alternatives (0-4). The participants must respond according to their feelings over the last seven days, including the administration day. This is evaluated and interpreted according to nine primary dimensions (somatizations, obsessions and compulsions, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism) and three global indices of psychological distress (i.e. Global Severity Index, GSI; Positive Symptom Total PST; and Positive Symptom Distress Index, PSDI). This test is administered in order to discard any psychopathologies in the participants, and as a measure of anxiety and depression. It has satisfactory reliability and validity.

EVALUATION OF THE AXES IMPLIED IN RESPONSE TO STRESS SYMPATHETIC ACTIVATION
Physiological data of the dermal conductance were registered by the Biopac data acquisition system MP150WSW, specifically by acquisition unit GSR100C. It was registered during visualization of the virtual scenarios, taken at baseline three minutes prior to the TSST. Registering continued during the entire performance of the task, divided into three periods – anticipatory stress (5 minutes), public speaking (5 minutes) and the arithmetic task (5 minutes).

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS
We collected four salivary cortisol samples and allocated them in four time periods of the study (see Protocol). For the salivary sampling we used Salivettes, consisting of two small tubes and a small piece of cotton. Participants chewed the cotton for 60 seconds and then put it into the Salivette for analysis. The samples were analyzed at San Cecilio University Hospital by the electrochemiluminescence immunoassay (ECLIA) method, to be used in automatic analyzers Roche Elecsys 1010/2010 and module Elecsys MODULAR ANALYTICS E170.

PROTOCOL

TIMETABLE
The study was performed between 3:30-6 p.m. for all participants. Despite European studies showing that the period where cortisol levels are considered stable is between 2-4 p.m. (Kudielka & Kirschbaum, 2005), we consider that circadian and metabolic cycles in Spain differ from those in other countries. In fact, we believe that the number of sunlight hours and the differing diets would make cortisol levels in humans different than those in other European countries. In order to define the timetable for our study, we performed a pilot study aiming to collect salivary cortisol samples in ten non-smoking participants – five men and five women – with a mean age of 34.90 (Sd=11.41), every two hours throughout one day, from 8 a.m. to 10 p.m. The description of the daytime cortisol secretion together with the graph (Graph 1) is included in the Results section below.

VIRTUAL REALITY-ADAPTED TRIER SOCIAL STRESS TEST–TSST (VR)
This program consists of a 3-D screen where a virtual audience is presented; a pair of headphones through which the participant will hear the sounds of the VE; and a microphone, which will be used to make the participant believe that their speech is going to be recorded. Even though it is not recorded, they will only learn this upon completion of the task.

The task consists of several stages. Once it has been explained to the participants, the VE starts being projected. The participant is behind a curtain and can hear noise from the virtual room, then a three-minute psychophysiological adaptation period starts in the VE during which the subject remains immobile.

Over the next five minutes, i.e. anticipatory stress period, the participant must prepare a speech about their own qualities and defects. They will have to elicit them and then explain why they identify with them.

The exposure period starts when the curtain lifts and the virtual audience appears. The participant must then begin their speech. They have previously been informed...
that their speech must last five minutes and they must be mindful of both the content and the manner in which they convey the information, as the audience could react according to the quality of the delivery itself. The participant is reminded that they must keep talking during the five minutes. From minute 2 onwards, a change of attitude occurs in the audience, and the original "interested audience" turns into a "restless audience," irrespective of the performance of the participant. This new attitude will continue until the end of the speech.

Once the speech is over, the arithmetic task starts, where the participant must serially subtract the number 13 from 1022 as quickly as possible over the last five minutes. In case of error, they will have to start again.

**Cortisol Sampling Moments**

After the explanation of the TSST (VR), an initial cortisol sample is taken (pre-exposure cortisol). Then, after the arithmetic task has been completed, another sample is taken (post-exposure cortisol). The third sample is taken 10 minutes after completing the task (cortisol post+10), and the last sample is taken 20 minutes after completing the task (cortisol post+20).

**Procedure**

At the beginning of the study, the participants received general information and then gave their signed consent. During the laboratory adaption period (one hour), they completed the aforementioned psychological questionnaires, i.e.: Positive Affect and Negative Affect Scale (PANAS-State1), Stress Vulnerability Inventory, Eysenck Personality Questionnaire for Adults (EPQ-A), Perceived Stress Scale, and the Symptom Check-List 90-R. After this stage, they learned what the TSST (VR) was and the stressor task began. Upon completion of the task, and after collection of post-exposition cortisol, the participants completed two questionnaires: PANAS-State2, and the Igroup.

**Presence Questionnaire**

Once the last salivary cortisol sample was collected, the participants were told that their speech had not been recorded and that the goal of the study was not to analyze their performance in public speaking and the arithmetic task, but to generate a response to stress for its further analysis. The protocol diagram of the TSST (VR) is in Figure 1 below.

**Statistical Analyses**

In order to establish the daytime cortisol curve in the Spanish population, we performed a pilot study in ten people. For that purpose, mean scores of each sampling moment were graphically represented.

Secondly, in order to establish sympathetic and adrenomedullary activation of the participants in the different moments we performed several T analyses in related samples.

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**Figure 1.** Diagram explaining the (VR) TSST application protocol throughout the whole stay of the participants in the laboratory, indicating duration of each phase, cortisol sampling moments, psychophysiological response, and questionnaires used.
Also, in order to determine the relationship between the different psychological variables (stress, psychopathology, and immersion) and the activation of the HPA axis, we performed a student t for independent samples. Finally, in order to verify the relationship between personality features and the activation of the HPA axis, we performed a contingency table based on a Chi-Square analysis.

RESULTS

CORTISOL CURVE
Before presenting the VR paradigm aimed to the activation of stress actuation axes, we performed a research to establish the cortisol curve in the Spanish population. The objective was to determine the moment of the day where cortisol is more stable and set the study timetable accordingly. This allows us to connect cortisol variations with the experimental design and not with the evolution of the daytime cortisol cycle itself. For that purpose, we performed eight cortisol measurements (one every two hours) in a sample of ten people, among which five were men and five were women with a mean age of 34.9 (SD=11.4). This evaluation was performed in a daytime timetable from 8 am to 10 pm (see Graph 1).

As can be observed, cortisol secretion is represented in a descending curve – the most stable levels correspond to the period from 3:30 p.m. to 6 p.m., together with a steep decrease. Based on these results, we decided to set the timetable for the experimental protocol in this time slot.

SYMPATHETIC ACTIVATION
As for the activation of the adrenomedullary axis, we established three time periods, taking mean conductance in each of these moments (psychophysiological adaptation in baseline, anticipatory stress, and speech). The results showed activation of the adrenomedullary axis through the paradigm TSST (VR) in all participants. Highest mean scores in conductance were observed in the preparatory and the speech phase versus baseline. In order to determine whether or not there were statistically significant differences between these scores, we performed two t analyses for related samples, comparing mean conductance in baseline with that in the speech. The results showed statistically significant differences between mean conductance in baseline and mean conductance in anticipatory stress \([t=-2.771; p<0.012]\), the latter being higher (3.13 vs 3.5). Similarly, statistically significant differences were also found between mean conductance in baseline and mean conductance in speech phase \([t=-2.89; p<0.009]\), the latter being higher (3.13 vs 4.05) (see Graph 2).

ACTIVATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS
Regarding the evaluation of modulation of the HPA axis, we took four cortisol measures – before beginning the TSST (VR), immediately after completion of the task, at 10 minutes after completion, and at 20 minutes after completion. The results show that seven of the partici-

Graph 1. Representation of cortisol secretion in healthy people in the period ranging from 8 am to 10 pm.
pants did not present any increase in cortisol secretion after completing the TSST (VR). However, cortisol secretion of the remaining fourteen did increase as a response to stress due to the TSST (VR) paradigm. Representation of both groups is shown in Graph 3.

Regarding the responder group, we found significant differences between pre-and post-exposure cortisol \( [t=2.32; p<0.007] \). However, no differences in cortisol levels were found between the first moment and the moments post +10’ and post +20’.

**TSST (VR) and Psychological Variables**

In order to detect any statistically significant differences between the psychological variables evaluated and the response to stress, we performed several Student t analyses, the independent variable being the response to the TSST (VR) with two levels (responders vs. non-responders), and the dependent variables being the scores of stress vulnerability, perceived stress, and the scores in the psychopathological symptoms in SCL-90. The results showed statistically significant differences between the responders and non-responders in the obsession-compulsion scale in SCL-90 \( (t=-2.43; p<0.025) \), the scores of responders being higher than those of non-responders \( (64.07 \text{ vs. } 57.85) \) and marginally significant in the scores for hostility in the same scale \( (t=-1.90; p<0.073) \), where the responder group also scored higher \( (54.38 \text{ vs. } 47.42) \). No differences in stress variables were found between the groups.

Regarding the personality features in EPQ-A (neuroticism, extraversion, and psychoticism), we performed a contingency analysis with Chi-Square and found statistically significant differences between both groups in the variable extraversion, where subjects with increased cortisol levels recorded after the TSST (VR) obtained higher scores \( (1.6 \text{ versus } 1.1) \).

**Virtual Environment Immersion**

In order to determine whether non activation of the HPA axis could be due to an inadequate immersion in the VR situation, we performed four T analyses for independent samples, the independent variable being the response to the TSST (VR) with two levels (responders and non-responders) and the dependent variables the scores obtained in each of the four IPQ scales (presence perception, spatial presence, implication, and experimental realism). The results showed that there were not statistically significant differences between both groups in either of the variables studied.
DISCUSSION
The results of our study show that the TSST integrated in a VE acts as a stressor to activate electrodermal responses and increase salivary cortisol levels in the participants. Therefore, this task is presented as a valid instrument for the activation of both responses in a stressful situation.

According to the results obtained in the representation of the Spanish daytime cortisol curve, we find that the most appropriate time to perform a study including measurement of variability of salivary cortisol levels depending on a stressful situation ranges from 3:30-6 p.m., so that variations will be attributed to the stressor and not to the daytime cortisol cycle itself.

As for the activation of the two axes of response implied in response to stress, the data obtained in the sympathetic axis show how the TSST (VR) is effective in the activation of skin conductance, which is a variable that was not previously measured in the study by Kotlyar et al. (2008). These authors had used a stressor in VR to generate physiological changes, obtaining an increase in systolic and diastolic blood pressure, as well as in heart rate. The results obtained place our study in line with the research by Kelly et al. (2007), who found that the TSST (VR) was appropriate to produce the activation of the HPA axis, although said activation was inferior to the one generated by a real audience. In contrast, we have found that the TSST (VR) is an appropriate task that can solve some of the limitations of the traditional TSST and becomes a useful tool in stress research.

Nonetheless, our study takes an important step in studying the different psychopathological, stress and personality variables related to the activation of the HPA axis. On the one hand, psychopathological symptoms, such as obsessive-compulsive and hostilities were connected with the activation of the axis. Thus, a higher score in these scales was linked to a higher activation of the HPA axis in a psychosocial stressor. On the other hand, the responder group was found to have a higher score in extroversion than non-responders, which was contrary to the expected. In a study by Dabbs et al. (1990), a salivary cortisol sample was taken in 102 students in order to connect cortisol concentrations with personality features; participants with high cortisol levels scored lower in sociability. However, according to our results, sub-

![Graph 3. Representation of the activation of the HPA axis based on salivary cortisol samples taken in four moments: pre-exposure, post-exposure, post+10 minutes, and post+20 minutes in both groups (responders and non-responders).](image-url)
jects with higher scores in extroversion would more likely seek the approval of others more than those with low scores – activation being even more common in situations involving evaluative threats.

Finally, it is important to highlight that no factor related to VR immersion was connected with the activation of the HPA axis, as no differences were found between the responder and the non-responder group in any of the variables implied in perception of VE presence. Therefore, non activation of this axis observed in some subjects cannot be explained by the artificial nature that is often attributed to VE’s.

In the same line, a limitation of this study would be the characteristics of the selected sample composed of physiotherapy students, possessing a University degree related to students with a high academic performance who are used to public speaking situations due to oral expositions in class performed in front of their teachers and classmates. They may have had a chance to develop resources and strategies to counteract the physiological activation in a stressful situation. We hypothesize that activation of response to stress would be higher in a population with an academic level closer to the mean.

In conclusion, this is the first study to evaluate simultaneous activation of the two axes involved in response to stress, the sympathetic and the HPA axis, by means of the TSST (VR), together with other related variables. The findings of this study support the use of TSST (VR) paradigm as an appropriate experimental situation in laboratory research designs aiming to check the modulation of both stress axes.

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