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Title: A virtual reality approach to the Trier Social Stress Test: Contrast of two distinct protocols

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Abstract

Virtual reality adaptations of the Trier Social Stress Test (TSST-VR) constitute useful tools in studying the physiological axes involved in the stress response. Here, we aimed to determine the most appropriate experimental approach to the TSST-VR when investigating the modulation of the axes involved in the stress response. Hence, we compared the use of goggles versus a screen projection in the TSST-VR paradigm. Forty-five healthy participants were divided into 2 groups: the first one (goggles condition; 13 females, 11 males) wore goggles in performing the TSST-VR; the second (screen condition; 15 females, 6 males) was exposed to the TSST-VR projected on a screen. Sympathetic reactivity to stress was measured by continuously recording skin conductance (SC), while the hypothalamic-pituitary-adrenal axis (HPA) was evaluated by sampling salivary cortisol throughout the experiment. At the end of the task, there was an increase in SC and cortisol level for either means of delivering the TSST-VR, although the SC increase was greater in the goggles condition, while salivary cortisol was comparable in both groups. Immersion levels were reportedly higher in the screen presentation than in the goggles group. In terms of sex differences, females experienced greater involvement and spatial presence, though comparatively less experienced realism, than their male counterparts. These findings help us determine which protocol of the TSST-VR is most suitable to the stress response under study. They also emphasize the need to consider the sex of the participants, as males and females show distinct responses in each protocol.

Keywords: TSST; virtual reality; hypothalamic-pituitary-adrenal axis; sympathetic activation; salivary cortisol

A Virtual Reality Approach to the Trier Social Stress Test: Contrast of two distinct protocols

The Trier Social Stress Test (TSST) is an experimental paradigm used as a tool in investigating the psychobiological stress response in the laboratory, as it activates the hypothalamic-pituitary-adrenal axis (HPA) (Kirschbaum, Pirke, & Hellhammer, 1993). The response is reflected in an increase in heart rate, blood pressure, and adrenaline and cortisol levels, as well as in a negative effect on mood (Al'Absi et al., 1997; Nicolson, Storms, Ponds, & Sulon, 1997). In the traditional TSST, as proposed by Kirschbaum et al. (1993), participants have to prepare a speech, claiming they are suitable to obtain a given job appointment, pronounce the speech in front of an audience, and subsequently complete an arithmetic task in public. The audience is generally composed of people who had been previously trained on neutral verbal and non-verbal behaviors (Foley & Kirschbaum, 2010).

Vinkers et al. (2013) recorded psychophysiological variables to investigate the effects of stress on core and peripheral body temperature in humans. They used the TSST and obtained distinct response patterns in men (i.e., increased cheek temperature) and women (i.e., decreased nasal skin temperature).

The original TSST has been utilized, since its inception, to measure stress reactivity in diverse populations such as children (TSST-C; Buske-Kirschbaum et al., 1997), middle-age adults (Fiocco, Joober, & Lupien, 2007), or retired people (Kudielka et al., 1998); as well as in various pathologies, such as psychiatric patients (Brenner et al., 2009), metabolic syndrome (Chrousos, 2000), systemic hypertension (Esler et al., 2008), systemic lupus erythematosus (Pawlak et al., 1999; Santos-Ruiz et al., 2010), and myalgias (Sjörs et al., 2010). Likewise, the TSST has been used to test the relationship between stress and a variety of psychological variables, such as depression (Parker, Schatzberg, & Lyons, 2003), social anxiety (Shirotsuki et al., 2009), and personality traits (Kirschbaum, Bartussek, & Strasburger, 1992; Pruessner et al., 1997).

The adaptation of the TSST into a virtual reality environment elicits a significant physiological response, thus solving some of the common TSST limitations that come from constraints both in the design of different environments or set-ups and from the assembling of acceptably large audiences for each experimental

session, given that the magnitude of the stress response depends in part on the size of the audience. An additional limitation results from the cost of TSST implementation, as it is necessary to have separate rooms for experimental subjects and audiences, a certain time is spent in training audience participants to show neutral behaviors in response to subjects' performance, and audiences may be unreliable and show distinct behaviors from one participant to the next. Therefore, it would seem that the TSST in the virtual environment constitutes a viable alternative to the usual forms of presentation of stress tasks (Kotlyar et al., 2008).

Virtual reality tasks are increasingly used in the laboratory setting, given their convenience and efficacy in providing 3D computerized environments and set-ups with which to study various pathologies and their associated responses (e.g., stress). In reviewing recent research on the application of VR to the treatment of mental disorders, Malbos, Boyer, and Lançon (2013) concluded that there was sufficient evidence to attest to the efficacy of this methodology in the assessment and treatment of distinct mental disorders.

In a related investigation, Kelly, Matheson, Martinez, Merali, and Anisma (2007) utilized the TSST-VR, as delivered by the *Virtually Better Inc., Atlanta, GA*, in which a previously recorded virtual audience was presented in 3D. Results indicated that giving a speech and carrying out an arithmetic task in front of a virtual audience produced a significant increase in salivary cortisol (30%), even though the increase was greater when both tasks were conducted in front of a real audience (90%).

In their study, Kotlyar et al. (2008) demonstrated that the TSST-VR resulted in a physiological response with significant increases observed in response to each stress task in systolic and diastolic blood pressure and heart rate, a finding that suggests the TSST-VR constitutes a viable alternative to previous TSST methodologies, with the additional advantages of reducing variability in the mode of presentation, and decreasing human and financial costs.

Several studies have used the TSST-VR in recent years. For instance, Jönsson et al. (2010) used a CAVE™ system developed at the *Electronic Visualization Laboratory* of the University of Illinois in Chicago, which facilitates total immersion by means of a VR room, a head tracking system, and passive stereoscopy.

This study's main results center on the habituation of the various measures of sympathetic activity obtained in response to the second implementation of the TSST-VR. On the first performance of the TSST-VR there was an 88% increase of salivary cortisol with respect to baseline levels, which habituated in the second session.

Another version of the TSST-VR was implemented by Santos Ruiz et al. (2010) by projecting a 3D virtual audience onto a screen. Results showed that this version of the TSST-VR resulted in significant sympathetic activation, as well as activation of the HPA axis in the majority of participants. It was, therefore, concluded that this form of the TSST-VR stimulated the main axes involved in the stress response.

Given the results obtained heretofore, it seems that the TSST-VR constitutes a useful technique in the study of the activation of the basic axes involved in the stress response. Considering the advantages of this way of presenting the TSST paradigm, it would be beneficial to further investigate which methodological variations produce greater or lesser immersion in the virtual environment, as we hypothesize that the level of immersion will determine the level of activation of the physiological axes involved in the stress response. Additionally, it would be worthwhile to explore whether there are sex differences in the stress response brought about by the TSST-VR, as traditional TSST studies have shown that this task results in larger increases of salivary cortisol in men than in women (Foley & Kirschbaum, 2010; Kirschbaum et al., 1993; Williams, Hagerly, & Brooks, 2004).

Annerstedt et al. (2013) carried out a pilot study in which a VR environment was used with two distinct objectives; first, producing physiological stress by means of the TSST-VR, and, secondly, comparing physiological recovery in different virtual environments after inducing the stress response. Their results showed that the TSST-VR activates the HPA axis and that recovery from the induced stress response varied as a function of the type of virtual environment to which participants were exposed.

Previous studies of the TSST in the virtual environment have differed in the way they projected 3D images, with some projecting images onto a screen and others using 3D goggles.

Morina, Brinkman, Hartanto, and Emmelkamp (2014) examined levels of anxiety and sense of presence during exposure in two types of virtual environments: a head-mounted display (HMD) with motion tracker and stereoscopic view, and a one-screen projection-based virtual reality display. Their results suggest that both VR displays were equally effective in evoking moderate levels of anxiety, although the HDM display appeared to produce a stronger sense of presence.

We have explored the potential differences between studies of the TSST-VR paradigm that have used goggles and those that have used a projection screen, and it appears that none of them show a conclusive advantage of using one method versus the other in terms of activating the axes of the stress response. Thus, our objectives were twofold. First, we aimed to investigate which of two TSST-VR protocols induced greater sympathetic activation, as measured by skin conductance, and HPA axis activation, as measured by secretion of salivary cortisol. Both protocols included a 3D virtual audience but, while one was presented on a large screen, the other was presented by virtual goggles. A second objective in this study was to examine whether there were sex differences in the activation of either axis, as is the case in the traditional TSST, and whether they differed according to the two TSST-VR protocols.

Method

Participants

Forty-five students attending the University of Granada volunteered their participation in this study. They were divided into two groups, according to which protocol of the TSST-VR was used: 3D goggles or a large screen projection. The goggles group included 13 females and 11 males, with a mean age of 21.9 years (SD=5) and 14 years of schooling on average (SD=0). The screen group contained 15 females and 6 males, with a mean age of 20.4 years (SD=1.25) and 14 years of schooling on average (SD=0).

The following were the exclusion criteria, adopted because of their potentially negative effect on cortisol levels (Williams et al., 2004): hypertension, heart disease, obesity, clinical diagnosis of depression or

anxiety, personality disorders, and substance use (i.e., amphetamines, methadone, barbiturates, or muscle relaxants). We also recorded sleep duration and excluded any participant that had not slept a minimum of 4 hours on the night prior to the experiment. Participants provided data on sleep duration as part of the semi-structured interview.

On arrival at the laboratory, participants were given information about the experiment and asked to sign an informed consent approved by the Ethics Committee of the University of Granada and performed according to the recommendations of the Declaration of Helsinki. Immediately afterwards, they were administered the semi-structured interview.

Instruments

The questionnaires below provided meaningful covariates that could be used in statistical analyses of the psychophysiological measures and cortisol.

Semi-structured Interview. Including socio-demographic data, daily life and sleep habits, medication, menstrual cycle, and history of psychiatric or psychological treatment.

Stress Vulnerability Inventory (SVI) (Beech, Burns, & Scheffield, 1986; Spanish adaptation, validated by Robles-Ortega, Peralta-Ramirez, & Navarrete-Navarrete, 2006). This instrument consists of 22 items and evaluates the individual's predisposition to be affected by perceived stress. The Spanish adaptation by Robles-Ortega et al. (2006) shows a Cronbach's alpha of 0.87. As for convergent validity, the results show a significant positive correlation (p<0.01) with the following assessment scales: STAI-R, Beck Depression Inventory, Somatic Symptom Scale, and Survey of Recent Life Experiences (SRLE).

Perceived Stress Scale (PSS) (Cohen, Kamarak, & Mermeistein, 1983; Spanish adaptation by Remor & Carrobles, 2001). The PSS is a self-report scale used to evaluate perceived stress level and the degree to which people find their lives unpredictable, uncontrollable or overwhelming (aspects that contribute to stress). It consists of 14 items with five response alternatives. The highest score corresponds to the highest perceived

stress level. The Spanish version of the PSS (14 items) has adequate reliability (internal consistency=0.81 and test-retest=0.73), concurrent validity, and sensitivity (Remor, 2006). Here, we have considered those scores over 22 (i.e., the mean score for the Spanish population; Remor & Carrobles, 2001) as reflecting high levels of perceived stress.

SCL-90-R Symptoms Inventory (Derogatis, 1994; Spanish adaptation by Gonzalez de Rivera & De las Cuevas, 1988). We used this instrument to rule out potential psychopathology in the participants. This self-report questionnaire was developed to assess symptoms of psychopathology and it includes 90 items with five response alternatives (0-4) on a Likert scale. Subjects respond according to how they have felt within the past seven days, including the day the inventory is administered. The inventory is scored and interpreted according to nine main dimensions (somatization, obsessive-compulsive symptoms, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism) and three global indices of psychological distress (Global Severity Index [GSI], Positive Symptom Total [PS], and Positive Symptom Distress Index [PSDI]). In this study, we have analyzed these last three global indices. This instrument is thought to have satisfactory reliability and validity (De las Cuevas et al., 1991).

Igroup Presence Questionnaire (IPQ; Schubert, Friedmann, & Regenbrecht, 2001). The IPQ is used to measure the sense of presence experienced in a virtual environment. The questionnaire was used to rule out the possibility that potential differences between groups could be attributable to variations in the sense of presence experienced by participants within the virtual environment. IPQ responses range from -3 (=totally disagree) to +3 (=totally agree). The IPQ includes a total of 14 items, consisting of a *global* scale (with scores between -3 and 3) and three subscales: *spatial presence* (i.e., the sense of being physically present in the virtual environment), with scores between -15 and 15; *involvement* (i.e., the attention devoted to the virtual environment and the degree of involvement experienced), with scores between -12 and 12; and *experienced realis*m (i.e., the subjective experience of realism in the virtual environment), with scores between -12 and 12. Reliability data are not yet available for the Spanish population.

Virtual reality version of the Trier Social Stress Test (TSST-VR). This is a virtual-reality adaptation of the traditional TSST. It includes a computer program that enables the design of a 3D audience, which can be projected into a set of VR goggles (eMagin Z800 3DVisor) with viewing equivalent of 105 inch diagonal movie screen viewed at 12 feet, with 40º field of view and stereoscopic image with a 800 x 600 triad pixels per display (http://www.3dvisor.com/) connected to a computer or, alternatively, can be projected onto a screen.

Pertaub, Slater, and Barker (2002) showed that participants that are exposed to 3 virtual audiences whose behavior is different toward the speaker (i.e., neutral, positive, or negative) generate distinct responses in the speaker. In the present study, all participants were exposed to the same type of audience, in identical VR and protocol conditions. Presentations were all stereoscopic, since Ling, Brinkman, Nefs, Qu, and Heynderickx (2012) had shown that stereoscopic VR presentations improved the sense of spatial presence in the case of public speaking. Sound volume and audience behavior were the same for all presentations.

World Viz (http://www.worldviz.com/) was adopted as the software of choice, as it provides VR environments that can be integrated with fMRI signals, biological responses, motion-tracking input, and 3D rendering. The Vizard program, with its high-level scripting language, affords the creation of interactive 3D content and the integration of biological signals.

The screen was 2.21 m wide and 2.30 m high, with 44º field of view and stereoscopic image of 1600 x 1200 triad pixels per display and a distance of 1.75 m from the participant.

Both VR delivery protocols require that participants wear earphones to listen to the sounds coming from the virtual environment, as well as a microphone to mislead them into thinking that their speech will be recorded and thus enhance the threatening effect of the social evaluation component and the participants' level of psychosocial stress. Once the participants were ready to start the experiment, they were informed that the task included four different phases. During the 3-minute initial phase, participants were instructed to relax and remain still. This phase was designated as the *period of psychophysiological adaptation to the virtual environment (baseline of skin conductance)*. Under both experimental conditions, participants were seated. In

the Screen Projection condition, they faced a screen showing a 3D image of a stage curtain, while in the

Goggles condition, the same image was projected by means of virtual goggles. The second phase, labeled the

anticipatory stress period, lasted 5 minutes during which participants were required to prepare a speech,

describing their positive and negative traits, to be delivered in front of the virtual audience. The third phase,

also of 5 minutes in duration, was called the *speech delivery period*. In it, the curtain came up, the virtual

audience appeared (Figure 1), and participants had to begin delivering their speeches. They were instructed to

speak for the whole 5 minutes without interruption and attend to the form and content of the speech, as they

would greatly determine the audience's response to their speech. The end of the speech constituted the

beginning of the last phase of the stressful task, the *arithmetic task*, based on the Paced Auditory Serial

Addition Test (PASAT) in which participants were asked to add two consecutive numbers (i.e., the number

they just heard and the one that just preceded it), delivered by means of an auditory recording.

Figure 1. 3D virtual audience as projected onto a large screen

Procedure

   The study was scheduled according to the diurnal cortisol curve, between 3:00 pm and 6:00 pm, when

levels of salivary cortisol are more stable. Although cortisol levels are reportedly stable between 2:00 pm and

4:00 pm (Kudielka & Kirschbaum, 2005), Spanish metabolic and circadian rhythms could differ from those of other European countries, due to the greater number of light hours and the distinctive schedule of meals. Therefore, following a pilot study, Santos-Ruiz et al. (2010) determined in a Spanish sample that cortisol levels were stable between 3:00 pm and 6:00 pm, so all participants were tested in the afternoon, within this temporal window.
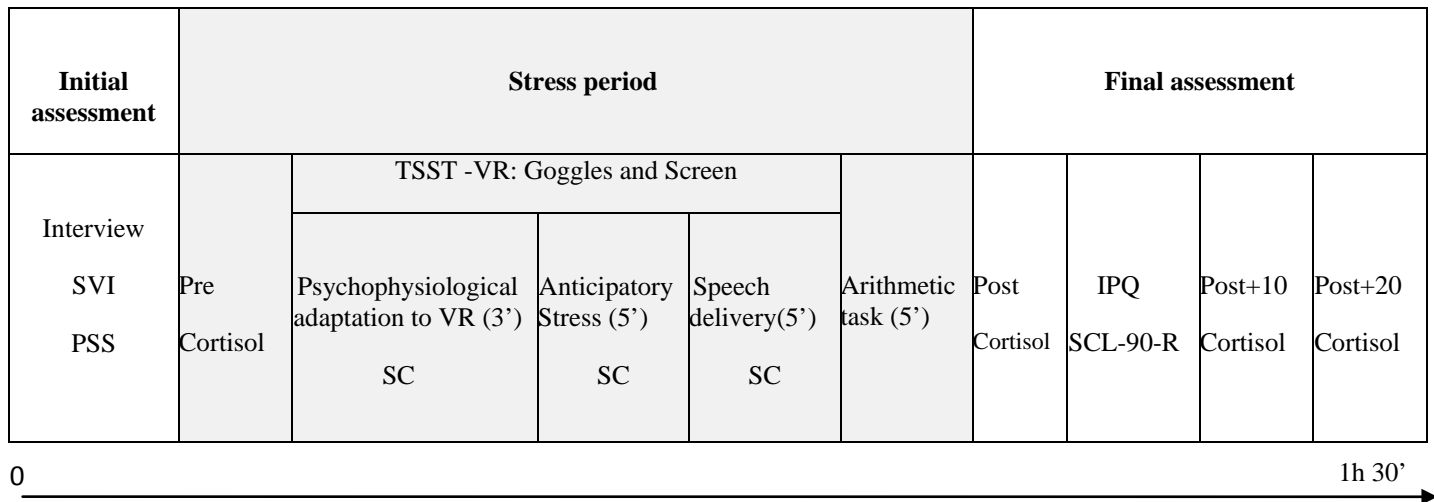
Once the participants arrived at the laboratory, they were given information about the experiment and a consent form to be read and signed. Afterwards, they were interviewed and completed the following questionnaires: SV, PSS, and SCL-90-R.

Next, participants were fitted with electro-dermal electrodes to record their skin conductance responses at various time intervals (see assessment of the sympathetic reactivity section below).

Right afterwards, the TSST –VR was explained to the participant, the first salivary cortisol sample *(pre-exposure cortisol)* was collected and the stressful task started (anticipatory stress, a situation of speech delivery and arithmetic task). Right after the end of arithmetic task, the second cortisol sample *(post-exposure cortisol)* was collected and the degree of immersion into the VR environment was assessed by administering the IPQ. Ten minutes and 20 minutes later, the third *(post-exposure cortisol +10')* and fourth *(post-exposure cortisol +20')* cortisol samples were withdrawn. Participants were given interior design magazines to leaf through in the interval between the third and fourth drawing of salivary cortisol. A diagram of the protocol of the TSST-VR is shown in Figure 2.

At the end of the experiment, participants are told that their speech has not been recorded and that the objective of the procedure was to elicit a stress response and not to have their performance be evaluated in the speech and arithmetic tasks.

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Figure 2. Diagram of the TSST-VR protocol.

Initial assessment	Stress period					Final assessment			
Interview SVI PSS	Pre Cortisol	TSST -VR: Goggles and Screen			Arithmetic task (5')	Post Cortisol	IPQ SCL-90-R	Post+10 Cortisol	Post+20 Cortisol
		Psychophysiological adaptation to VR (3') SC	Anticipatory Stress (5') SC	Speech delivery(5') SC					

0   1h 30'

Note: SVI: Stress Vulnerability Inventory; PSS: Perceived Stress Scale; TSST -VR: Trier Social Stress Test adapted to Virtual Reality; Pre Cortisol: pre-exposure cortisol; Post Cortisol: post-exposure cortisol; Post +10 Cortisol: cortisol at 10 minutes after exposure; Post +20 Cortisol: cortisol at 20 minutes after exposure; SC: skin conductance; IPQ: Igroup Presence Questionnaire; SCL-90-R: Symptom Checklist SCL-90-R

Assessment of the physiological axes involved in the stress response

Assessment of the sympathetic reactivity. Skin conductance (SC) was recorded by means of two standard-size Ag/AgCl electrodes, with isotonic electrolyte gel, placed on the palm of the non-dominant hand. Subsequently, data were recorded during visualization of the virtual scenarios, and consisted of a three-minute baseline prior to the TSST-VR (*period of psychophysiological adaptation to the virtual environment*) and continued recording throughout the *anticipatory stress* (5 minutes) and *speech delivery* (5 minutes) periods. Recording of skin conductance ended right after the conclusion of the speech delivery task (see Figure 2). Since one of the aims of the study was to test the sympathetic reactivity evoked during the speech delivery task under the two experimental conditions, we chose to record measures of skin conductance because they provided a measure of sympathetic reactivity during the speech delivery task that was faster than that of the HPA axis in the presence of threatening stimuli.

Assessment of the hypothalamic-pituitary adrenal axis (HPA). Collection of salivary cortisol samples was performed using Salivette® Cortisol (*Sarstedt*, Numbrecht, Germany, Ref.51.1534), which consists of two small tubes, one of them containing a small piece of cotton. Participants chewed the cotton for about 60 seconds, after which it was introduced into the *salivette* for analysis. Samples were analyzed at the San Cecilio University Hospital, using the electrochemiluminescence immunoassay "ECLIA" method. This method is designed for use in Roche Elecsys 1010/2010 automated analyzers and in the Elecsys MODULAR ANALYTICS E170 module. Salivary cortisol samples were obtained at four collection times in the study: *pre-exposure cortisol*, *post-exposure cortisol*, *post-exposure cortisol +10' and post-exposure cortisol +20'*.

Statistical analyses

Initially, Student's *t-tests* were used to check for differences between groups (goggles vs. screen) in terms of socio-demographic (age and education level), and psychological (SV, PSS, and SCL-90-R) variables. Chi-square analyses were performed to check for differences between groups in sex, use of tobacco, and contraception.

Next, we conducted a 2x2x2 ANCOVA in order to test for differences in conductance between the 2 types of VR presentation protocols (goggles vs. screen), the sexes (males vs. females), and the time of data acquisition (anticipatory stress vs. speech delivery). Baseline levels of conductance for each participant were used as a covariate.

Additionally, we carried out a 2x2x3 ANCOVA to analyze if there were significant differences in cortisol levels associated with the 2 types of VR protocols (goggles vs. screen), the sexes (males vs. females), and the time of cortisol sampling (post-exposure, post-exposure +10' and post-exposure +20'). Pre-exposure levels of cortisol for each participant were used as a covariate.

The Greenhouse-Geisser correction was applied to all analyses involving repeated measures and sphericity violations. Also, in the case of cortisol responses, Bonferroni post-hoc analyses were conducted when analyzing within-group differences across time during the experimental session.

When significant Group x Sampling Time interactions were found, follow-up Student's *t*-tests were carried out to determine whether there were differences in either skin conductance or cortisol level between groups at each sampling time.

Finally, we conducted a 2x2 ANOVA that included the type of VR protocol (goggles vs. screen) and sex (males vs. females) to analyze the 4 subscales of the IPQ: global, spatial presence, involvement, and experienced realism. Significant interactions were further examined by means of Student's *t*-tests. Further, correlation analyses were used to test the relationship between the stress variables (PSS and SVI) and the IPQ.

Results

Sample description Sample description. Socio-demographic and psychological data for the participants can be found in Table 1. Results showed significant statistical differences between type of protocols in the PSS, with the screen group scoring higher than the goggles group. Thus, PSS scores were added as covariate in the following analyses.

Table 1. Means (M) and standard deviations (SD) of socio-demographic and psychological variables for the participants in both groups.

Variables	Groups		Analyses	
	Goggles	**Screen**	t / χ^2	p
	M ± SD	M ± SD		
Age (years)	21.9 ± 5	20.4 ± 1.25	1.36	0.181
Education Level (years)	14 ± 0.00	14 ± 0.00	-1.07	0.290
Sex (%, χ^2)				
Males	45.83%	25%	1.4	0.230
Females	54.17 %	75%		
Smoking (%, χ^2)				
Males	27.27 %	0 %	2.7	0.100
Females	30.77 %	6.5 %		
Contraceptives (%, χ^2)	4.33 %	1.3 %	2.6	0.140
Perceived Stress Scale	20.08 ± 10.03	26.90 ± 5.15	-2.81	0.007*
Stress Vulnerability Inventory	4.91 ± 3.92	6.62 ± 4.07	-1.43	0.161
Symptom Checklist SCL-90-R				
Somatization	54.20 ± 11.39	55.52± 6.83	-0.46	0.647
Obsessions and compulsions	58.96 ± 9.32	61.71± 6.02	-1.16	0.253
Interpersonal sensitivity	57.96 ± 12.99	59.81± 7.51	-0.57	0.569
Depression	48.56 ± 14.30	54.09 ± 6.03	-1.65	0.107
Anxiety	52.87 ± 10.21	54.38 ± 7.89	-0.55	0.587
Hostility	50.37 ± 11.40	52.05 ± 8.13	-0.56	0.579
Phobic Anxiety	43.71 ± 13.12	42.19 ± 9.23	0.44	0.660

|				
Paranoia	57.50 ± 10.09	55.38 ± 8.76	0.75	0.459
Psychoticism	52.62 ± 14.11	53.95 ± 8.50	-0.37	0.709

Note: χ^2: Chi-square analysis; t : t-student value; *p≤0.05

Sympathetic reactivity: Skin conductance

Results showed a main effect of type of VR protocol [$F(1,42)=4.440$; $p=0.041$], with the goggles condition evoking higher sympathetic activity overall.

A significant interaction between type of VR protocol and assessment time [$F(1,42)=3.959$; $p=0.050$] revealed a higher activation between anticipatory stress (M=2.85; SD=1.95) and speech delivery (M=3.81; SD=2.72) in the goggles group than in the screen group (anticipatory stress: M=2.98; SD=1.20; speech delivery: M=3.34; SD=1.10).

Figure 3. Skin conductance for the two protocols of TSST-VR delivery (Goggles vs. Screen), recorded at 3 points in the experiment
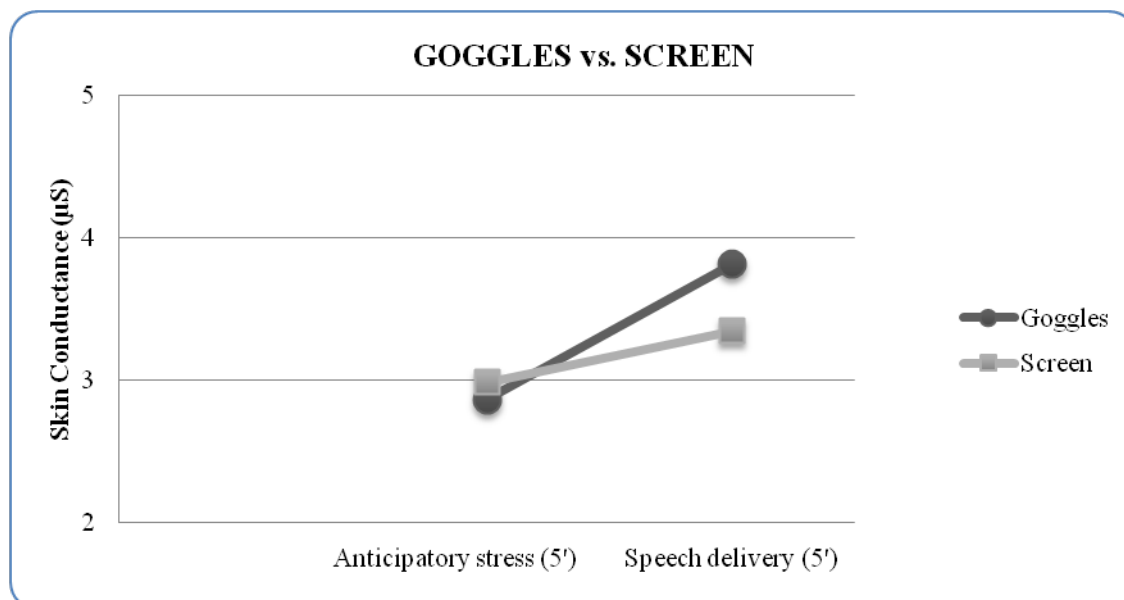
Activation of the hypothalamic-pituitary-adrenal axis (HPA): Salivary cortisol

In terms of HPA axis activation, there was no evidence of any main effect or an interaction between the level of activation of the HPA axis and the type of TSST-VR protocols, goggles or screen projection, at any of the cortisol extraction times.

Finally, results showed that there was no interaction between sex and cortisol secretion levels, regardless of whether the TSST-VR was presented by means of goggles or projected on a screen.

Immersion in the virtual environment (IPQ)

Statistical analyses demonstrated statistically significant differences in the *involvement* subscale of the IPQ as a function of the sex of the participant (independent of VR protocol used), with males (M= -1.06; SD=5.43) endorsing lower levels of involvement than females (M=2.92; SD=3.50) [F(1,42)=7.29; p=0.01].

On the *experienced realism* subscale, there was a significant interaction between sex and type of TSST-VR protocol [F(1,42)=6.63; *p*=0.014], such that males obtained higher scores than females only on the screen projection condition (males: M=-1.66; SD= 2.16 vs. females: M=-4.26; SD=2.05). No significant differences were found on the *global* and *spatial presence* subscales.

Significant correlations were found between scores on the PSS and on the IPQ subscales *involvement* (*r*=0.348; *p*=0.02) and *spatial presence* (*r*=0.519; *p*<0.001). Similarly, SVI scores correlated with those obtained on the IPQ subscales *involvement* (*r*=0.298; *p*=0.049) and *spatial presence* (*r*=0.428; *p*=0.004).

Discussion

The objective of this study was twofold: firstly, we aimed to determine which of two protocols of the TSST-VR, whether a 3D virtual audience projected onto goggles or a large screen, produced greater sympathetic reactivity and activation of the HPA axis. Secondly, we sought to find out whether males and females showed distinct levels of activation of the sympathetic nervous system and HPA axis in these two TSST-VR protocols.

In terms of activation of the HPA axis, we found no statistical differences between the two TSST-VR protocols: goggles versus screen. However, sympathetic reactivity, as measured by skin conductance, did vary

across presentation protocols, being higher in the goggles projection condition than in the screen condition. This effect could not be attributed to the level of immersion associated with each of the experimental conditions.

Here, it is worth emphasizing that, although an adequate level of immersion in the virtual environment seems indispensable to activate the stress response axes, some studies (e.g., Santos-Ruiz et al., 2010) have failed to find a significant relationship between the HPA axis and the level of immersion in the virtual environment. In the present study, we did not find a significant relationship of the HPA axis and sympathetic reactivity with the level of immersion in the virtual environment. In fact, although females obtained higher scores in the *involvement* subscale of the IPQ, regardless of VR protocol, there were no significant sex differences associated with HPA axis activation and sympathetic reactivity. Similar results were found with the screen protocol in male participants that, despite obtaining higher scores on the *experienced realism* subscale of the IPQ, did not show higher sympathetic activation either. It seems that the goggles elicited significantly higher conductance values overall, but this effect was not associated with any subscale of the IPQ and did not vary across sexes.

On the other hand, these results are in agreement with the recent study by Ling, Brinkman, Nefs, Qu, and Heyndericky (2013) in which real-size screen presentations of a virtual classroom were associated with a greater sense of presence when compared to real- or reduced-size TV projections.

A positive correlation was found between stress levels (as measured by the PSS and VSI) and the IPQ *involvement* and *spatial presence* subscales, independent of VR protocol used (goggles or screen) or the participant's sex. In other words, those participants reporting higher stress levels were also more likely to feel involved and spatially present during the task, regardless of the manner in which the virtual audience was presented. These results concur with those of Ling, Nefs, Morina, Heynderickx, and Brinkman (2014), who found a correlation between sense of presence and anxiety, but not for sex or type of projection of the virtual environment (e.g., field of view, degree of freedom of the tracker, etc.). Since we did not measure anxiety

levels in the present study, we cannot say whether a greater spatial presence leads to a higher level of anxiety, but we did find that those participants with higher levels of stress prior to the TSST-VR protocol showed a higher level of involvement and a greater sense of spatial presence, as measured by the IPQ.

The results showed sex differences in that females manifested a greater degree of *involvement* than males, regardless of the VR protocol used. In contrast, females exposed to the 3D audience of the TSST-VR by means of the screen projection manifested less *experienced realism* than males. Such sex differences need to be considered in designing research that uses VR methodologies, given that differences between males and females could be erroneously attributed to distinct HPA axis responses when, in fact, they are due to differences in the degree of VR immersion of each gender. In fact, the two aims of this study stemmed from results such as those reported by Kotlyar et al. (2008), showing that the TSST-VR produces sympathetic activation and engages the HPA axis. Similarly, Kelly et al. (2007) concluded that the TSST-VR activates the stress response and constitutes a useful tool in that it solves some of the limitations associated with the traditional TSST.

In recent years, there have been reports of various investigations demonstrating the utility of the TSST-VR. Nevertheless, despite common agreement on the suitability of the VR version of this task in engaging the axes mediating the stress response, certain controversial issues still remain. One of these issues pertains to the presentation of the virtual environments that are common to most. Some investigators have projected 3D environments onto large screens (Santos-Ruiz et al., 2010), while others have used goggles (Jönsson et al., 2009; Kelly et al., 2007). Despite these differences, no study has contrasted the various versions of the TSST-VR to detect which form of presentation results in a greater response to the stress generated by the task. Additionally, although it is well known that the traditional TSST evokes more activation in males than in females (Foley & Kirschbaum, 2010; Hemmeter et al., 2005; Williams et al., 2004), sex differences in response to the TSST-VR have not been systematically studied. The results obtained in this study provide some answers to these questions.

It should be noted, however, that use of the VR goggles could have caused *cybersickness*, according to theories like the Sensory Conflict Theory, the Poison Theory, and the Postural Instability Theory (La Viola Jr., 2000). Still, no member of the goggle presentation group reported symptoms of cybersickness, nor were there any indications of cybersickness in the skin conductance records.

The present study has limitations that need to be taken into account when considering the study and its findings. Firstly, given that one of our objectives was to characterize sex differences in the two versions of the TSST-VR used here, it would have been desirable to have an equal number of males and females. Likewise, it would have been preferable to count on matched levels of perceived stress across groups at the baseline level, since the initial differences could be selectively influencing stress responses at both the sympathetic and HPA axis levels.

In summary, our results appear to support the use of the TSST-VR, whether it is delivered via goggles or via a large screen projection, as a useful paradigm to investigate the modulation of the principal axes that intervene in the stress response. More concretely, if the aim of the study is to specifically investigate one of the two axes, it seems that it would be preferable to use a goggles protocol to activate the sympathetic nervous system and projection of the 3D image onto a screen to activate the HPA axis, particularly if participants are mainly females. In investigating the stress response in both axes and mostly with males, our results seem to favor the use of the goggles protocol. Finally, if the study were to include both stress response axes and both sexes, our findings would indicate that the use of a 3D image projected onto a large screen would be the recommended TSST-VR protocol.

<div align="center">**Acknowledgment**</div>

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