Sex Differences in Stress Reactivity to the Trier Social Stress Test in Virtual Reality

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Abstract

Background: The aim of the present study is to investigate the sex differences in stress reactivity to the Trier Social Stress Test (TSST) in a virtual reality(TSST-VR).

Methods: Healthy young male (n = 30) and female (n = 30) undergraduates were randomly assigned to a psychosocial stress protocol (TSST) condition or to a non-stressful control condition (Placebo-TSST) under VR. Electrodermal activity (EDA), heart rate (HR) and heart rate variability (HRV) were measured throughout the study. The subjective scales of stress and emotion were also conducted.

Results: The results showed that after VR, the stress group reported higher stress perceptions than the non-stress group. Compared with females, the males stronger EDA and higher HRV before the VR, and lower HR during VR as well as higher HRV after VR. The correlation between subjective and objective reactivity demonstrated that HRV during VR was negatively correlated to depression and negative affect. The HRV after VR was negatively correlated to the positive coping but was positively correlated to the depression.

Conclusions: These findings suggest that the TSST-VR could be used as an available tool for testing gender differences to social stress induction in experimental settings. Compared with females, males were more sensitive to stress.

1. Background

Stress has been defined as "the adaptive responses of the body that maintain homeostasis in response to stressors that enable an organism to make adaptations to environmental demands" (McEwen and Akil, 2020). The effective induction of a stressor and measured stress reactivity were important to stress research, which has both theoretical and practical indications (Allen et al., 2014; Schoofs and Wolf, 2011). Researchers have explored many means of inducing acute stress reactivity in experimental settings, and the Trier Social Stress Test (TSST) has been one of the most popular methods because its validity has been shown in both ordinary participants and clinical samples (Chopra et al., 2009; Dickerson and Kemeny, 2004; Vergaelen et al., 2015; Villada et al., 2014). In brief, the TSST requests the participants to give a speech and perform a math task in front of panels that are formed by professionally trained actors who do not express any emotional variation during the experiment. The participants are thus nervous because of social evaluation and uncontrollable factors (Kirschbaum et al., 1993).

One of the disadvantages of the TSST is the difficulty of maintaining the constancy of the test conditions, especially requesting the experimenter to maintain the identical demeanor for all the participants (Fallon et al., 2016; Kotlyar et al., 2008). The TSST in a virtual reality environment (TSST-VR) uses the virtual persons as experimenters who give unbiased responses to all the participants and become one of the modified versions of the TSST (Diemer et al., 2014; Jonsson et al., 2010). The use of TSST to induce participants' stress reactivity in the laboratory needs a control (non-stress) condition (Birkett and Melissa, 2011). The purpose of the control design was to show that the stress reactivity that

we observed in the experiment was caused by the social evaluation and uncontrollable factors of the TSST rather than by other factors such as the physical or cognitive demands of the task itself (e.g., give a speech or perform a calculation). Het and his colleagues (2009) believed that the control condition that comprised a simple talk and a simple calculation was the most appropriate manner in which to test the effects of psychosocial stressors on the individual. They named this the 'Placebo Version' of the TSST. The present study combines the virtual reality environment and the 'Placebo Version' setting; this is the first time the placebo has been used as the control condition to investigate the stress reactivity induced by the TSST in the virtual reality environment.

As a psychosocial stressor, the TSST induces the arousal of the HPA (hypothalamic-pituitary-adrenal) axis and SNS (sympathetic nervous system). The common physical indices are cortisol and heart rate (Hellhammer and Schubert, 2012). In addition to physiological reactivity, the TSST will also induce psychological reactivity such as stress perception (Childs et al., 2006), anxiety and the insecurity affect (Fiksdal et al., 2019; Firk and Markus, 2009; Yim et al., 2010). According to neuroendocrine theory, when exposed to stressors, at first the prefrontal cortex integrates the sensory input and evaluates the significance of information and then produces available coping strategies; second, the limbic system forms emotional responses; and in the end, physical systems such as the HPA system is aroused (Campbell and Ehlert, 2012). Therefore, there is a certain correlation between physical and subjective reactivity in stress situations. Studies regarding the correlation between subjective and objective reactivity to stress have been controversial although the neuroendocrine theory has been widely accepted by stress researchers (Het et al., 2012; Wager et al., 2009).

Engert and his colleagues (2010) observed that during public speech, the increase in the negative mood correlated to the stress reactivity of the HPA and endocrine system. Similar results resulted from the studies of Dedovic et al. (2014), Oldehinkel et al. (2011) and Schlotz et al. (2008), and whose results showed that the psychological and physiological reactivity to the TSST were correlated. Conversely, other studies observed no relation between the subjective and objective reactivity to stress (Cohen et al., 2000; Hjortskov et al., 2004; Shiban et al., 2016). In fact, the HPA reactivity caused by the TSST was influenced by many factors such as age (Almela et al., 2011), sex (Foley and Kirschbaum, 2010), personality (Ryan et al., 2010), and coping style (Diehl and Hay, 2010). In other words, the physiological reactivity to acute stressors was mediated by additional variables, particularly sex. Understanding sex differences during stress reactivity would provide insights into the physiological differences between males' and females' brains as well as differences in vulnerability and coping mechanisms for men and women who suffer from chronic mental disorders that are related to stress.

The review of sex differences in stress reactivity revealed that previous studies that considered the relation between subjective and objective reactivity to the TSST were mainly conducted as a combination of behavior and neuroimaging to investigate the neurobiological effects of stress; the connections between the SNS system, behavior, cognition and emotion were rarely mentioned. To eliminate the influence of hormonal variations, the majority of the previous studies used males as participants (Allen et al., 2017). The studies of young and middle-aged participants observed that compared to females, the

males displayed greater elevations in cortisol, which indicated differences between women and men in terms of HPA reactivity to stress (Aguilera, 2012; Eisenberger et al., 2007; Uhart et al., 2006). However, sex was not able to mediate the correlation between cortisol and subjective feelings of stress (Dedovic et al., 2009; Hodes and Epperson, 2019; Kumsta et al., 2007). Schoof and Wolf (2011) even proposed that the TSST would not induce quick and intense changes in cortisol whereas the sex differences in HPA arousal that the above researchers observed were simply because the cortisol measures were more appropriate for testing male' reactivity to the TSST. Our former study also observed that when the stressor was canceled, the SNS recovered to the baseline; although the glucocorticoids continued to secrete, the effect of stress on females had been eliminated (Liu et al., 2013).

For this reason, we believe that the reason why the study outcomes regarding the correlation between subjective and objective reactivity to stress were inconsistent may be because of the imperfect coupling of different stress reactivity systems (physiology and psychology). For example, when exposed to stressors, the subjective psychological reactivity immediately occurred whereas the stable physiological reactivity occurred far more slowly (Reinelt et al., 2019). Considering the biological stress principle, in stress situations, the glucocorticoids first influence the participants' SNS and then the HPA system (Schoofs and Wolf, 2011). The changes in SNS indices occurred in seconds and returned to baseline within a minute whereas the cortisol hormones took 20 to 40 minutes to reach peak values and an hour to return to baseline (Bale and Epperson, 2015; Verhasska et al., 2004). This may indicate that the changes in the HPA measures were not appropriate dependent variables for the investigation of the correlation between physiological and psychological reactivity to stress whereas the SNS indices were more applicable. Based on the above reviews, we hypothesized that the stress reactivity produced by the TSST-VR was different in men and women on both the subjective report and physiological arousal. Compared to women, men were more vulnerable and less resilient to stress. In addition, the subjective and objective reactivity to stress were correlated.

2. Methods

2.1. Participants

The participants were recruited via flyers in the university and campus network. We randomly selected 60 young and healthy adults (30 males and 30 females). The participants were between 18 and 30 years old (M = 23.28, SD = 2.13). The education level of participants was bachelor's degree and above. They were all right-handed and non-athletes. The participants who were eligible for participation in the present study met the following criteria: physically and psychologically healthy, having no cardiovascular and endocrine disorders, no psychiatric or family medical history; normal or corrected-to-normal vision and hearing; because salivary cortisol levels are higher in the luteal phase than in the follicular phase, menstrual cycles were controlled in females (all the females were not under menstruation, including 14 females in the luteal phase and 15 females in the follicular phase). The females included in the present study reported not using any oral contraceptive medication and having a normal menstrual cycle for the previous four months.

We randomly divided the participants into two relatively equal groups, the stress group and the non-stress group. Three participants were eliminated from the experiment because of missing data regarding subjective or physiological reactivity to stress. The final effective data came from fifty-seven participants. There were 29 participants in the stress group (15 males and 14 females) and 28 participants in the non-stress group (13 males and 15 females). The participants were informed that they would fill in a series of psychological scales before the stress test and participate in the stress test in a virtual reality environment.

2.2. Materials

2.2.1. Subjective Scales

Visual Analog Scale for stressful situations (VAS)

A VAS is a 100 mm bipolar line that measures a characteristic across a continuum (Hellhamer and Schubert, 2012). One end of the line is zero, indicating no stress; and the other end of the line is 10 cm, indicating unbearable stress. The middle portion of the line indicates varying degrees of stress. The participants marked a spot on the line resembling their subjective appraisal of stress perception. Scores were determined by measuring from the left end to the mark using a ruler.

Eysenck Personality Questionnaire Short Scale for Chinese (EPQ-RSC)

The EPQ-RSC includes four subscales: extroversion (E), neuroticism (N), psychoticism (P) and lying (L). There were a total of 48 items, and each subscale comprised 12 items. The questionnaire has good reliability and validity, which were evaluated by the Committee of Psychometrics, Chinese Psychology Society (Mingyi et al., 2000).

Emotional Scales

The emotional scales included the Beck Anxiety Inventory (BAI), Beck Depression Inventory (BDI) and positive affect and negative affect scale (PANAS). The BAI comprises 21 items to assess degree of anxiety. It utilizes a four-level scoring rubric in which 1 indicates no discomfort. The BDI also comprises 21 items, each of which presents a category. The description of each category divides it into four levels, and scores range from 0 to 3 for different levels. We used standardized scores for further analyses of the BAI; we calculated the total raw scores for the 21 items and then used the equation Y = int (1.19X) to transform them into a standardized score. We used the total raw scores of the BDI's 21 items for further analysis. Both the validity and reliability of the Chinese version of the BAI and BDI are well established (Wang et al., 1999).

The PANAS includes 20 items and contains two emotion dimensions (positive, 10 items; negative, 10 items). The participants are requested to make decisions according to their current emotional state (Laurent et al., 1999). The PANAS is a Likert-style questionnaire (from 1, indicating"very slightly or not at all," to 5, indicating "extremely"). The sum of the positive affect and negative affect scores are utilized

separately for data analysis. The Chinese version of the PANAS has well-established validity and reliability (Huang et al., 2003).

Simplified Coping Stress Questionnaire (SCSQ)

The SCSQ comprises 20 items, 12 positive items and eight negative items, yielding two coping dimensions. The SCSQ is also a Likert-style questionnaire (from 1, indicating 'no use,' to 4, indicating 'always use'). The results of the SCSQ are the mean score of positive coping and the mean score of negative coping. The reliability and validity of the SCSQ have been demonstrated (Wang et al., 1999).

2.2.2. The Trier Social Stress Test in Virtual Reality Environment (TSST-VR)

The TSST-VR attempted to maintain similarity to the traditional version. The WorldViz Vizard4.0 software, the PPT E8 optical inertial hybrid wide-area tracking system and the nVisor SX60 head-mounted display were used to present the virtual audience. The virtual room had a table, four chairs (one real chair for the participant), a surveillance camera and a microphone (height-adjustable). The committee panel comprised three virtual persons with neutral facial expressions (a middle-aged man in the middle, a young woman to the left and a young man to the right, all sitting down behind a table, facing the participant). The virtual persons nod, look at the participant or elsewhere and move their feet during the test to improve the realism of the VR simulation (Fig. 1).

2.3. Procedure

2.3.1. The first phase (Room A): baseline physiology data collection and subjective reports

After the participants reached the experiment room (Room A), we attached the physiological equipment to the participants to collect the five-minutes-resting data for the baseline. The indices included electrodermal activity (EDA), heart rate (HR) and heart rate variability (HRV). Next, the participants filled in a series of scales (BAI, BDI, PANAS, VAS, EPQ and SCSQ). When they finished the scales, the participants were led to Room B to participate in the virtual reality test (stress or control conditions).

2.3.2. The second phase (Room B): the induction of stress

The TSST-VR with the placebo as the control condition was administered to the participants to induce their stress or as the control group. The specific procedures were as follows:

(1) The experimenter took the participants to the virtual reality room, gave the helmet to the participants and explained the introductions.

(2) Preparation for the speech included voices coming from the helmet by virtual persons requesting the participants to prepare a speech about running for a position. The voices were controlled by the

experimenter with a remote keyboard that the participants could not see. A surround-sound system played the recorded voices in advance.

(3) The public speech was given by the stressed group for the monitor of the class, the speech time was five minutes. The non-stress group spoke loudly while standing for five minutes, the contents of their talk could be a film, a book, a trip, etc.

(4) Mental arithmetic was performed by the stressed group. They had to count backwards from 2011 in steps of 13 for five minutes; the non-stress group added the number 15 starting at 0 for five minutes.

(5) After the arithmetic task, the experimenter approached the participants, took off the helmet, and guided the participants back to Room A.

2.3.3. The third phase (Room A): The evaluation of stress reactivity

The participants were led back to Room A after the virtual reality test. They filled in the VAS again for their subjective stress perception; they then were again recorded for five minutes of physiological activity for the recovery reactivity.

2.4. Data Recording

Participants wore an nVisor SX60 head-mounted display (HMD, NVIS, Reston, VA) that featured dual 1,280 horizontal by 1,024 vertical pixel resolution panels that refreshed at 60 Hz. Stereoscopic images were rendered by a 2.8G CPU Intel computer with a 4G graphics card. An optical tracking system (Worldviz PPT E8) with an orientation sensor (InterSence InertiaCube2-US/JP, Billerica, MA, USA) provided tracking of six degrees of freedom (x, y, z positions and pitch, yaw, and roll) for the head. The system latency, or delay between the participant's movement and the resulting update in the HMD was no greater than 45 ms. Vizard 4.0 software was used to assimilate tracking and computer image generation (WorldViz, Santa Barbara, CA, USA).

Physiological activity data were collected via the BioNomadix remote tracking system (BIOPAC MP150, Biopac Systems, Inc., Goleta, CA), which included specific modules for acquisition, conversion, amplification and storage of signals. A BioNomadix device consists of two components, a wireless transmitter that is worn by the subject to amplify and send the physiological data and a receiver module. The range of motion for participants was 10 meters. The bipolar electrodes that were used to collect the wireless electrodermal activity (EDA100C) were attached to the ring and middle fingers of the subjects' left hands (VIN + and VIN−, respectively). The amplifier gain of the EDA100C was 5μmho/V, the high-pass filter was DC and the low-pass filter was 0.1 Hz. The sample rate was 250 Hz, and the units were in μmho/V.

The Dual Wireless electrocardiogram (ECG) BioNomadix Pair consists of a matched transmitter and receiver module specifically designed to measure ECG data on one or both channels. ECG signal data are

transmitted at a rate of 2,000 Hz. Raw data from the pair are band-limited from 0.05 Hz to 150 Hz. The heart rate (HR) of each participant was obtained on the basis of the R-R interval, which was immediately extracted from the ECG signal. The unit of the HR was beats/minute (bpm). The ground (GND) was connected to the right abdomen, the VIN + was connected to the left fourth and fifth intercostal spaces, and the VIN−, which showed the electrode connections to the ECG for the lead measurements, was connected to the left collarbone underneath (Precordial Lead).

2.5. Statistical Analysis

The SPSS16.0 software (SPSS Inc., Chicago, IL) was used to process and analyze data in the present study. We conducted a mixed-factor ANOVA on the sex differences in stress reactivity to the TSST-VR for which the test time (baseline, VR and recovery phases) was the within-subjects variable. All of the significant analyses used the two-way test ($p < 0.05$), and the partial eta squared (η^2_p) was the effect size. We adopted the logarithmic transformation to normalize the HRV data because of the skewness of data distribution (Buchanan et al., 2010). Paired-sample *t* tests were used for significant tests for the main effects, and the simple effect analysis was used to test significant interactions. For within-subject analysis, the Greenhouse-Giesser correction was used where appropriate. The data were all presented as the mean ± S.D.

The resting-state EDA and ECG data were recorded while the subjects were in comfortable and relaxed states and were recorded serially for five minutes after all of the components of the apparatuses had been attached. We used the Acknowledge 4.2 software to extract and analyze the EDA, HR and heart rate variability (HRV). High signal-to-noise ratio and high time-based sampling resolution permit the pair to be used for exacting HRV studies. The Fast Fourier Transformation (FFT) was used to transform the R-R interval, which converted from the raw R wave to the HRV frequency domain information. The frequency indices included the high frequency of the HRV (HF, 0.15Hz—0.40 Hz), the low frequency of the HRV (LF, 0.04 Hz—0.15 Hz), the ratio of the LF and HF (LF/HF), the very low frequency of the HRV (VLF, 0.01 Hz—0.04 Hz) and the total power (TP).

3. Results

3.1. Subjective reactivity to stress

3.1.1. The scores of VAS

To test the effect of the TSST-VR on participants' subjective perception, a mixed-factor ANOVA was performed on the scores of the VAS. The within-subjects variable was the TIME (before and after the VR) whereas the between-subject variables were GROUP (stress, non-stress) and SEX (male, female). The results showed that the main effects of group ($F_{(1,53)} = 14.531$, $p < 0.001$, $\eta 2 = 0.215$) and sex ($F_{(1,53)} = 4.110$, $p = 0.048$, $\eta 2 = 0.072$) were significant whereas the time main effect on the VAS was not significant, $F_{(1,53)} = 3.248$, $p = 0.077$, $\eta 2 = 0.058$. The only significant interaction that we obtained was the

TIME × GROUP on the VAS, $F_{(1,53)} = 6.569$, $p = 0.013$, $\eta 2 = 0.110$. Further simple effect analysis revealed that compared to the non-stress group ($M = 37$ mm.25, $SD = 14.43$), the stress group ($M = 56.75$ mm, $SD = 17.55$) reported higher scores on the VAS after the VR, $F_{(1,55)} = 10.16$, $p = 0.002$. The specific trends are presented in Fig. 2.

3.1.2. The scores of the EPQ, SCSQ, BAI, BDI and PANAS

The independent sample *t* tests were conducted on the scores of the EPQ, SCSQ, BAI, BDI and PANAS for men and women. The results showed that men and women performed significantly differently on the neuroticism subscale of the EPQ, $t_{(55)} = 2.495$, $p = 0.016$, $d = 0.662$. Specifically, compared to women ($M = 5.00$, $SD = 3.40$), men ($M = 7.11$, $SD = 2.55$) reported higher scores on the neuroticism subscale. In addition, men and women showed no significant differences on the scores of the BAI, BDI, PANAS, SCSQ and the extroversion, psychoticism and lying subscales of the EPQ, which are presented in Table 1.

Table 1
The scores of males ($N = 28$) and females ($N = 29$) on emotion scales (BAI, BDI, PANAS), coping scale (SCSQ) and personality scale (EPQ-RSC).

Scales	Male ($M \pm SD$)	Female($M \pm SD$)
BAI	32.61 ± 7.41	30.59 ± 5.32
BDI	8.79 ± 7.50	6.48 ± 5.60
Positive Affect	29.18 ± 9.62	28.17 ± 7.98
Negative Affect	17.82 ± 5.38	16.83 ± 4.53
Positive Coping	21.21 ± 6.02	20.34 ± 5.86
Negative Coping	9.00 ± 3.91	9.10 ± 3.63
Psychoticism	2.89 ± 2.03	2.45 ± 1.66
Extraversion	7.32 ± 3.24	8.21 ± 3.78
Lying	3.39 ± 2.32	4.24 ± 2.21
Neuroticism	7.11 ± 2.55*	5.00 ± 3.40

3.2. Objective reactivity to stress

We conducted mixed-factor ANOVAs on the participants' EDA, HR and HRV with the TIME (baseline, virtual reality and recovery) as the within-subjects variable. The between-subject variables were GROUP (stress, non-stress) and SEX (male, female). The results showed that there were significant time main effects on EDA ($F_{(2,106)} = 31.304$, $p < 0.001$, $\eta 2 = 0.371$), HR ($F_{(2,106)} = 42.983$, $p < 0.001$, $\eta 2 = 0.448$) and HRV (VLF: $F_{(2,206)} = 7.483$, $p = 0.001$, $\eta 2 = 0.124$; LF: $F_{(2,106)} = 10.458$, $p < 0.001$, $\eta 2 = 0.165$; HF: $F_{(2,106)} = 9.433$, $p < 0.001$, $\eta 2 = 0.151$; TP: $F_{(2,106)} = 9.808$, $p < 0.001$, $\eta 2 = 0.156$). In addition, the main effects of sex

on EDA ($F_{(1,53)}$ = 4.445, p = 0.040, $\eta 2$ = 0.077), HR ($F_{(1,53)}$ = 4.771, p = 0.033, $\eta 2$ = 0.083), and the balance of HRV ($F_{(1,53)}$ = 10.362, p = 0.002, $\eta 2$ = 0.164) were significant.

We did not identify the significant interactions of time, group and sex on the EDA, HR and HRV. However, the interactions of TIME × SEX on the EDA ($F_{(1,53)}$ = 5.759, p = 0.020, $\eta 2$ = 0.098) and HRV (VLF, $F_{(1,53)}$ = 4.375, p = 0.037, $\eta 2$ = 0.079; LF, $F_{(1,53)}$ = 4.294, p = 0.043, $\eta 2$ = 0.075; TP, $F_{(1,53)}$ = 4.228, p = 0.045, $\eta 2$ = 0.074) showed quadratic increases. Moreover, we also obtained a significant quadratic increase interaction of TIME × SEX × GROUP on HRV (HF, $F_{(1,53)}$ = 4.020, p = 0.050, $\eta 2$ = 0.070; the balance, $F_{(1,53)}$ = 4.588, p = 0.030, $\eta 2$ = 0.080).

Further analysis showed that in the baseline phase, the EDA of men was significantly higher than that of women (Fig. 3A). During the virtual reality, the HR of men was significantly lower than that of women (Fig. 3B). In the recovery phase, the balance of HRV for men was significantly greater than for women (Fig. 3C). The specific variations of HRV for men and women in the stress and non-stress groups during different test times are shown in Table 2.

Table 2
The very low frequency (VLF), low frequency (LF), high frequency (HF) and total power (TP) of heart rate variability of males and females in the stress (N = 28) or non-stress (N = 29) groups during virtual reality.

Index	Time	Stress		Non-Stress	
		Male	Female	Male	Female
VLF	R1	-3.76 ± 0.38	-3.84 ± 0.60	-3.86 ± 0.49	-3.79 ± 0.86
(nu)	R2	-3.87 ± 0.50	-4.06 ± 0.55	-3.67 ± 0.46	-4.13 ± 0.56
LF	VR	-3.68 ± 0.41	-3.30 ± 0.62	-3.55 ± 0.67	-3.57 ± 0.63
(nu)	R1	-3.58 ± 0.50	-3.66 ± 0.72	-3.65 ± 0.47	-3.65 ± 0.98
HF	R2	-3.72 ± 0.57	-3.81 ± 0.67	-3.36 ± 0.57	-4.09 ± 0.57
(nu)	VR	-3.31 ± 0.52	-3.06 ± 0.69	-3.23 ± 0.75	-3.27 ± 0.75
TP	R1	-3.69 ± 0.78	-3.65 ± 0.64	-3.85 ± 0.83	-3.63 ± 0.94
(nu)	R2	-3.96 ± 0.78	-3.73 ± 0.64	-3.53 ± 0.74	-4.08 ± 0.62
	VR	-3.40 ± 0.69	-3.08 ± 0.69	-3.47 ± 0.91	-3.24 ± 0.68
	R1	-11.02 ± 1.60	-11.14 ± 1.92	-11.35 ± 1.73	-11.07 ± 2.72
	R2	-11.55 ± 1.67	-11.59 ± 1.77	-10.35 ± 1.71	-12.30 ± 1.61
	VR	-10.38 ± 1.58	-9.44 ± 1.93	-10.25 ± 2.27	-10.08 ± 2.02

3.3. The correlations of subjective and objective reactivity to stress

The Pearson's correlations were conducted on the data of subjective scales and the physiological activity at different time points (R1, VR and R2). The specific trends are presented in Table 3. The results of correlations showed that first, in R1, the EDA and anxiety had a positive correlation, which suggested that the participants who had higher EDA in R1 would experience more anxiety. Second, during the VR, EDA and anxiety was positively correlated, which indicated that the participants who showed higher EDA during HR would have more feelings of anxiety. Moreover, during the VR, the HRV (VLF, LF, HF and TP) were negatively correlated to depression and negative affect. Finally, in R2, HR and depression exhibited a negative correlation. The HRVs (VLF, LF and TP) were positively correlated to depression and negatively correlated to positive coping in R2.

Table 3
The Pearson's correlations between subjective scales (BAI, BDI, EPQ, PANAS and SCSQ) and physiological activities (EDA, HR, VLF, LF, HF, TP) during the first resting state (R1), the second resting state (R2) and virtual reality (N = 57).

Time	Index	BAI	BDI	NA	PC
R1	EDA	0.357(0.006)**	-0.256(0.054)	-0.324(0.014)*	-0.340(0.010)*
R2	HR	0.267(0.045)*	0.284(0.032)*	-0.305(0.021)*	-0.349(0.008)**
VR	VLF		0.277(0.037)*	-0.288(0.030)*	-0.352(0.007)**
	LF		0.283(0.033)*		-0.373(0.004)**
	HF		-0.389(0.003)**		
	TP		-0.384(0.003)**		
	EDA		-0.326(0.013)*		
	VLF		-0.375(0.004)		
	LF				
	HF				
	TP				

4. Discussion

In the present study, we investigated the sex differences in stress reactivity to the TSST-VR and the correlations between subjective and objective reactivity to stress. Consistent with our hypotheses, compared to the non-stress group, the stress group reported more stress perception; and compared to the resting baseline, the participants' HR went up in VR. Compared with women, men experienced more stress before and after VR. Men's EDA was stronger before VR, their HR was lower during VR and their HRV was

greater after VR. The EDA before and during the VR were negatively correlated with anxiety. The HRV during VR had negative correlations with depression and negative affect. After VR, the HRV was positively correlated with depression and negatively correlated with the positive coping style.

The present study steadily induced participants' stress reactivity to the TSST consistent with the studies of Kelly et al. (2007), Josson et al. (2010) and Montero-López et al. (2016), which used the virtual reality environment to conduct the TSST. Similarly, because we used the placebo as a control condition consistent with Het et al. (2009), we observed the correlation between subjective and objective reactivity to the TSST. This suggested that on the one hand, the TSST-VR could be an available tool to induce acute stress reactivity in an experimental setting; on the other hand, the placebo version of the TSST would more easily and clearly detect the covariance of participants' psychological and physiological reactivity to psychosocial stress.

We used the wireless polygraph system to record participants' physiological changes throughout the experiment, consistent with Hellhamer and Schubert (2012), who posited that the dynamic measurement of stress reactivity (before, during and after the TSST) would at best provide details. Here, compared with women, men felt more stress and stronger EDA reactivity before the TSST-VR, and men's neuroticism levels were higher as well. We considered this an indication that vulnerability to stress is higher for men. Hellhamer explained it as the influence of anticipation on psychological measurement: the anticipation of stress induces negative feelings that in turn affect the participants' physiological and psychological reactivity to stress. In this study, we further limited the effects of anticipation on negative affect in the male participants.

The low HR of men during VR compared with women's indicated men's weaker ability to cope with stress. The HR levels of women during VR were higher than they were before and after VR, which indicates that women can easily mobilize their physiological resources to cope with stressful situations whereas men cannot. This expands the results of Schoof and Wolf (2011) that the HPA arousal caused by the TSST performed differently in men and women. Our results fully demonstrate that sex differences in physiological reactivity to the TSST would be differentiated by the arousal of HPA and the SNS on the basis of the findings of Schoof and Wolf (2011). Schoof and Wolf believed that the sex differences they observed in HPA reactivity to the TSST may be why the HPA indices were more appropriate for detecting men's reactivity to stress than women's. Based on this, we observed that the effective arousal of the SNS to the TSST-VR in our study only occurred with women, suggesting that HR indices were more appropriate for examining women's stress reactivity than men's. Moreover, this result was consistent with the study that used the Stroop task to induce women's stress reactivity (Liu et al., 2013).

The greater stress perception and HRV of men after the TSST-VR indicates that the resistance to stress was worse for men than for women. Men cannot quickly return their physiological arousal to baseline when the stressor has been withdrawn and the larger fluctuation in physiological reactivity in turn increased men's subjective stress feelings. These results coincide with the fMRI results of Wang et al (2007), who observed that men simultaneously showed increased activation in the right prefrontal cortex

(rPFC) and decreased activation in the left orbitofrontal cortex (lOFC) under stress. Moreover, this dissymmetrical activation pattern remained even after the stress test. Here, at the level of ANS activity, we observed the continuous reactivity (larger HRV) of men after the TSST-VR, which indicated that we once again showed that men are less resilient to stress.

The correlation between subjective and objective reactivity to the TSST-VR in our study connected ANS activity with anxiety, depression, negative affect and coping styles. This confirms the neuroendocrine theory that subjective and objective reactivity to stress are correlated, also corroborating the perspective of Campbell and Ehlert (2012), who considered that the choices of measurement and methodology features are critical to detecting stress reactivity. In other words, the combination of a dynamic tracking measurement and the multidimensional evaluation method were necessary to establish the relation between physiological and psychological reactivity to psychosocial stress. Specifically, we observed that EDA before and during the VR were positively correlated with anxiety, suggesting that participants who demonstrated hyperactivity of the SNS before and during VR were more likely to experience anxiety. This correlation was more significant than the correlation reported by Cohen et al. (2000). Cohen et al. observed that the increased HR during the TSST had a moderate positive correlation with anxiety, which indicated that the participants who had intense SNS activity would have greater anxiety. Based on this, in the present study, we further show that in the baseline phase, the intense hyperactivity of the SNS predicts more anxiety. The difference between Cohen's study and ours is the disparate SNS indices that may indicate that compared to HR, EDA was more sensitive to physiological reactivity variation to stress (Bale and Epperson, 2015; Verhasska et al., 2004).

In addition, the negative correlation of HRV during VR and the negative affect in our study duplicated the results of Child et al. (2006) and extended the results of Olderhinel et al. (2011). The study of Child et al. (2006) observed that the TSST induced participants' cardiovascular reactivity and increased their negative affect. In this study, we discovered that the HRV during the VR was negatively correlated to negative affect before VR. However, the results of Olderhinel et al. (2011) demonstrated that there was no relation between physiological activity during the TSST and emotional states before the TSST although the HR during the TSST could predict the negative affect after the TSST. Compared with the study of Olderhinel et al., our study lacks the effective predication data of physiological reactivity during the TSST and participants' emotional variations after the TSST. This may explain why our study is inconsistent with the study of Schlotz et al. (2008). Schlotz et al discovered that the psychological and physiological (HPA) reactivity to the TSST contains a time lag: the former was faster than the latter. According to this lag effect, we hypothesized that the time-coupling design of SNS reactivity and psychological reports was more appropriate for examining the covariance of subjective and objective reactivity to stress. In the follow-up studies, we must retest the participants' emotional reactivity after the TSST to detect whether or to what extent the variation of physiology during VR predicts emotional variations after stress.

Overall, our study demonstrates that the TSST-VR could be an available tool to induce acute stress reactivity in experimental settings. Moreover, the multidimensional data collection was essential to obtaining maximum information regarding stress reactivity. This method will improve our understanding

of stress psychophysiology. Sex differences in stress reactivity to the TSST may be expressed separately on HPA and SNS activity. We revealed that compared to women, men had greater vulnerability and less resilience to stress. Finally, because of the combination of multidimensional psychological evaluations and the synchronous tracking of physiological indices, we showed that SNS arousal and psychological reactivity to stress were time-coupled to some extent. Specifically, anxiety, depression, negative affect and positive coping may predict SNS arousal during and after the TSST.

List Of Abbreviations

Trier Social Stress Test in a Virtual Reality	TSST–VR
Non–Stressful Control Condition under VR	Placebo–TSST
Electrodermal Activity	EDA
Heart Rate	HR
Heart Rate Variability	HRV
Hypothalamic–Pituitary–Adrenal Axis	HPA
Sympathetic Nervous System	SNS
Visual Analog Scale for Stressful Situations	VAS
Eysenck Personality Questionnaire Short Scale for Chinese	EPQ–RSC
Beck Anxiety Inventory	BAI
Beck Depression Inventory	BDI
Positive Affect and Negative Affect Scale	PANAS
Simplified Coping Stress Questionnaire	SCSQ
Electrocardiogram	ECG
High Frequency of Heart Rate Variability	HF
Low Frequency of Heart Rate Variability	LF
Ratio of the LF and HF	LF/HF
Very Low Frequency of Heart Rate Variability	VLF
Total Power of Heart Rate Variability	TP
Fast Fourier Transformation	FFT
Right Prefrontal Cortex	rPFC
Left Orbitofrontal Cortex	lOFC

Declarations

Ethics approval and consent to participate

All participants provided written informed conset to participate the present experiment. The stuy was also followed by the Declaration of Helsinki. Experimental procedures were approved by the Institutional Review Board of the State Key Laboratory of Cognitive Neuroscience and Learning of Beijing Normal University.

Consent for publication

Not applicable.

Availability of data and materials

All data, models, and code generated or used during the study appear in the submitted article.

Competing interests

The author has declared that no competing interests exist.

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Authors’ contributions

Conceived and designed the experiments: QL WZ.

Performed the experiments: QL.

Analyzed the data: QL.

Contributed reagents/materials/analysis tools: QL.

Wrote the paper: QL WZ.

All authors have read and approved the manuscript.

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Figures

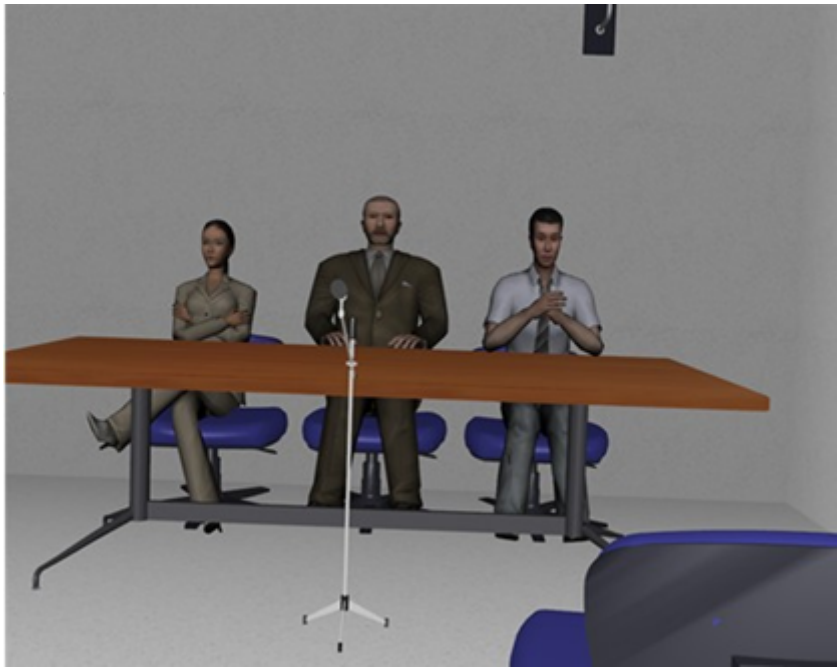


Figure 1

The figure shows the committee in front of a participant.

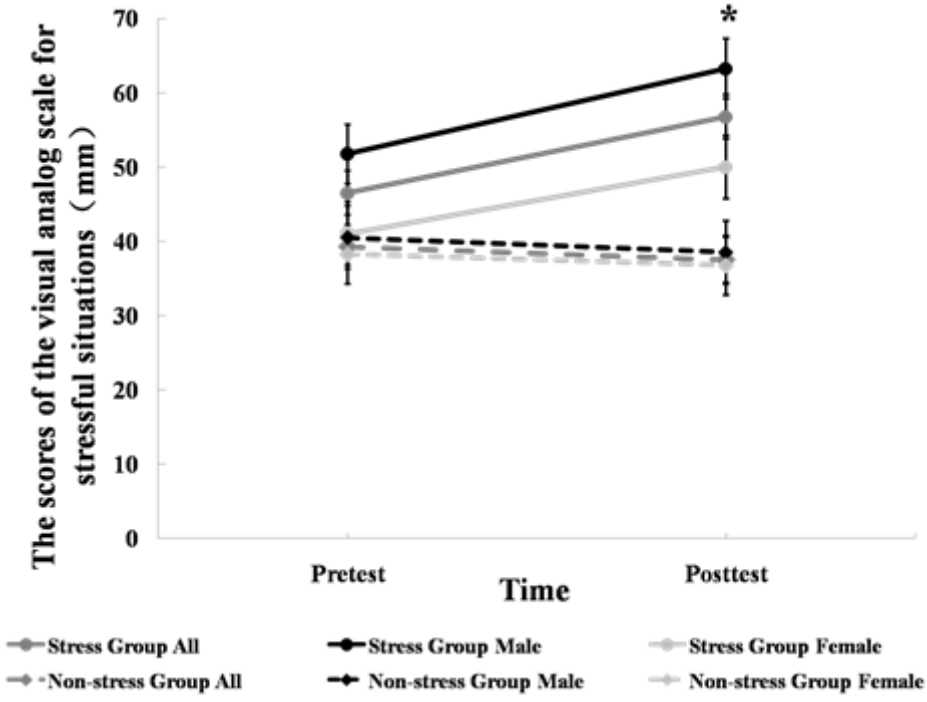
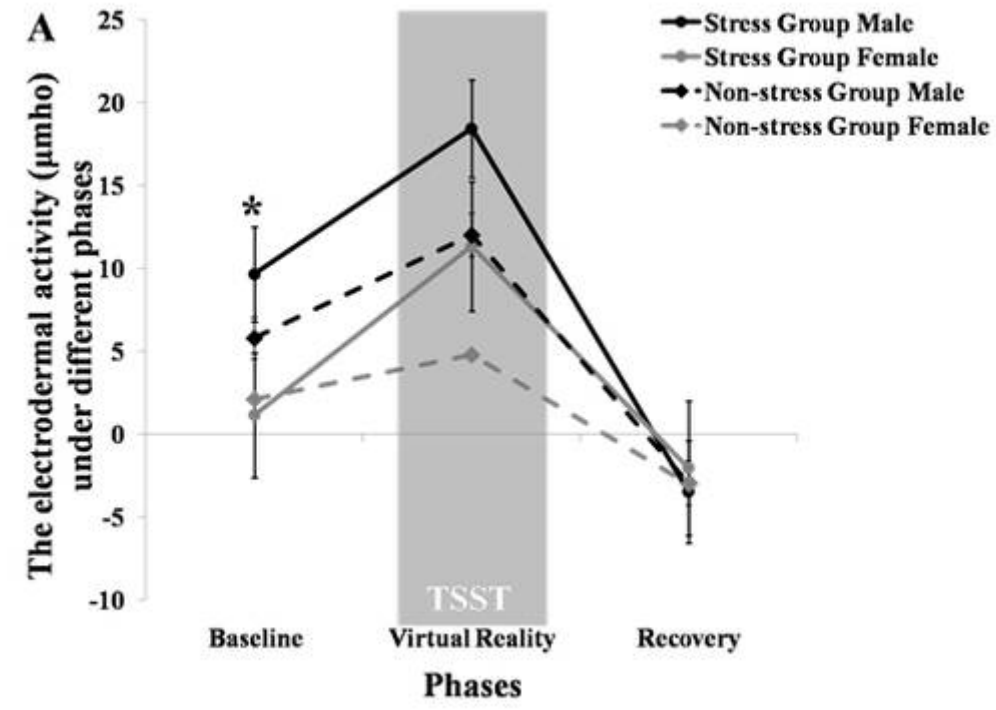
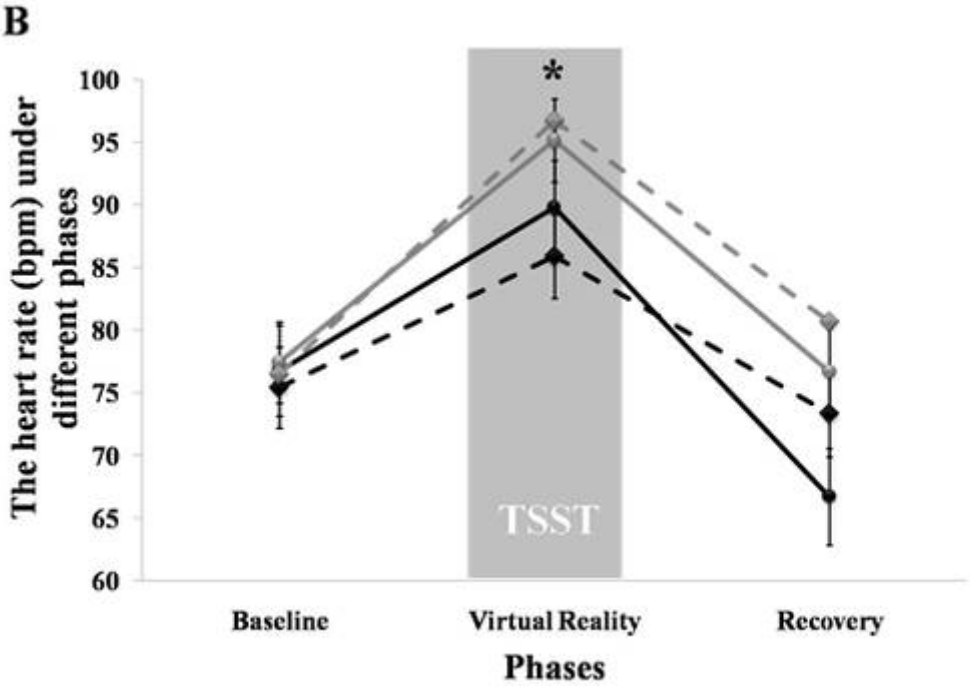
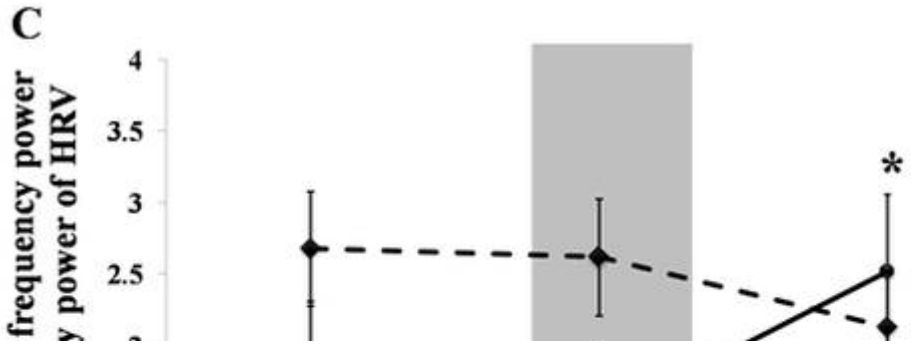


Figure 2

The scores on the VAS (Visual Analog Scale for Stressful Situations) of men and women from the stress (N=28) or non-stress (N=29) groups before and after the virtual reality environment (VR).


A
Stress Group Male
Stress Group Female
Non-stress Group Male
Non-stress Group Female
The electrodermal activity (μmho) under different phases
25
20
15
10
5
0
-5
-10
*
TSST
Baseline
Virtual Reality
Recovery
Phases


B
The heart rate (bpm) under different phases
100
95
90
85
80
75
70
65
60
*
TSST
Baseline
Virtual Reality
Recovery
Phases


C
frequency power
y power of HRV
4
3.5
3
2.5
*

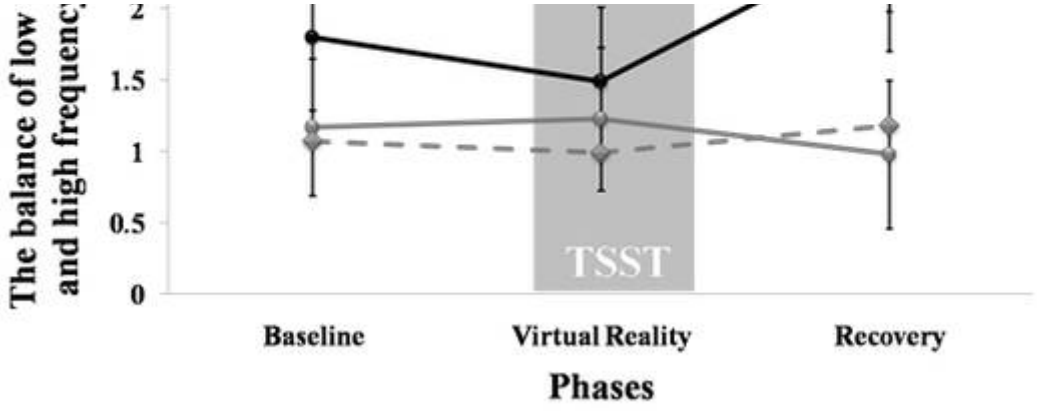


Figure 3

The electrical activity (EDA, μhmo), heart rate (HR, bpm) and the ratio of low power and high power of heart rate variability (balance of HRV) for men and women in the stress (N=28) or non-stress (N=29) groups during different test time phases (baseline, virtual reality and recovery). A: The EDA (μhmo) of men and women from the stress and non-stress groups in different test times. B: The HR (bpm) of men and women in the stress and non-stress groups during different test times. C: The balance of HRV for men and women in the stress and non-stress groups during different test times.