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Assessing the effect of the Trier social stress test (TSST) on psychological and physiological measurements of Vietnamese students

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Abstract:

The Trier social stress test (TSST) is considered as the most appropriate standardized protocol for studying stress hormone reactivity. The TSST is distinct from other stress induction protocols in laboratory settings due to its unique ability to create a natural context that includes elements of social and psychological acute stress. However, there has been no investigation of the efficacy of the TSST among the Vietnamese population. Therefore, the purpose of this study is to examine the impact of the TSST on Vietnamese university students. A group of 10 healthy male and female volunteers underwent the TSST protocol, and self-reported values (the positive and negative affect schedule and the visual analogue scale) as well as physiological stress response (heart rate) were measured. The results suggest that the TSST mildly induces acute psychological stress among Vietnamese participants. To enhance the potency of the TSST in future studies, a larger sample size and more consistent adherence to the TSST protocol are crucial.

Keywords: **electrocardiogram, questionnaires, stress, Trier social stress test, university students.**

Classification number: **3.6**

1. Introduction

Stress, as defined by the World Health Organization, is the body's response to stimuli that require attention or action, resulting in physical, emotional, or psychological pressure. Stressors are increasingly prevalent in modern society, encompassing factors from family to work stress [1, 2]. As stress affects individuals of all ages, stress-related disorders such as depression, anxiety, physiological changes (cardiovascular, hormonal), and cognitive impairments have become more common [3-7]. Consequently, there is a pressing need for a deeper understanding of stress mechanisms to develop effective treatments. Laboratory-based protocols that can induce the human stress response are essential [8].

To establish a stress-inducing protocol in a laboratory setting, it is crucial to differentiate between different types of stress. Chronic stress arises from long-term stressors that individuals frequently encounter over an extended period, such as unemployment, financial strain, or caregiving responsibilities [9-11]. Acute stress, on the other hand, results from sudden, traumatic, or surprising events, like having an argument, presenting to a large audience, or facing imminent work deadlines. While chronic stress is a more severe and pervasive form of stress associated with disease and mortality [12], acute stress is particularly relevant for understanding how individuals respond to threatening situations. Exploring acute stress responses is vital for informing adaptive stress management strategies that promote long-term well-being. Achieving this requires a laboratory protocol capable of inducing significant changes in endocrine and cardiovascular activities.

Regarding acute psychological stressors, a meta-analysis of 208 studies conducted by S.S. Dickerson, et al. (2004) [13] identified the TSST as one of the few stress protocols consistently eliciting hypothalamic-pituitary-adrenocortical (HPA) axis responses. Social-evaluative threat, where individuals face potential negative judgment from others, and uncontrollability are key psychological components that generate the largest HPA axis response. These components are prominently present in the TSST's procedural stages. Social-evaluative threat is incorporated by requiring participants to prepare a presentation in front of expert judges while being video recorded. Elements of uncontrollability are integrated throughout the procedure: participants are abruptly informed of a short preparation time and are not informed about the mental arithmetic task [14].

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Up until 2007, the TSST had been employed in over 4000 studies, consistently producing results that established its position as the gold standard for inducing laboratory-based acute psychological stress [15]. The TSST's applicability in non-western populations, such as Chinese [16-22], Japanese [23], and African [24], further enhances its desirability. However, due to its wide usage across cultures, variations in how researchers implement the TSST protocol have been observed. A recent systematic review by N.N. Linares, et al. (2020) [25] identified several discrepancies in testing procedures, particularly in exclusion criteria, selected time intervals for physiological data collection, and the number of judges. These variations may complicate research comparison and replication. To address these issues, the authors provided comprehensive guidance for the systematic implementation and detailed reporting of the TSST methodology, which this study aims to follow.

The effects of the TSST are typically analyzed using multiple methods at different levels of analysis: physiological (electrocardiogram), self-reports, and cortisol. Electrocardiogram (ECG) is a valuable tool for assessing stress levels during specific stages [26]. During the stress experience, the release of adrenaline triggers the body's fight-or-flight response, resulting in physiological changes, including an increased heart rate compared to a non-stress state. Therefore, heart rate (beats per minute) is a reliable metric for evaluating current stress levels [27]. Recent studies have also utilized heart rate variability (HRV) as an indicator of stress [28]. Additionally, common data used to assess the effectiveness of the TSST in inducing stress include heart rate, standard deviation of HRV (RMSSD), root mean square of successive differences (RMSSD), and the percentage of N-N intervals larger than 50 ms (pNN50) [25, 29, 30]. Previous studies have shown that RMSSD and pNN50 decrease in the presence of stressors [28, 31]. Skewness and kurtosis are also employed to understand the distribution of HRV values [32, 33]. Alongside physiological measures, a minimum of two self-report stress questionnaires are typically recommended to assess participants' subjective stress levels before and after the TSST. The positive and negative affect schedule (PANAS), the visual analogue scale (VAS), and the state-trait anxiety inventory (STAI) are commonly selected scales due to their sensitivity to change and validity across languages [25].

Despite the TSST's essential role in studying the neurobiological response to acute stress, no previous studies have investigated its effects on Vietnamese or Southeast Asian samples. K. Lewin's field theory, proposed in 1997, asserts that behavior is a product of the individual and the environment [34]. This notion is supported by numerous studies demonstrating significant differences in mental health outcomes across ethnicities, highlighting the role of culture in perceiving stress and anxiety [35]. Therefore, this preliminary study aims to lay the foundation for future investigations by evaluating the effects of the translated TSST protocol on a small sample of Vietnamese students. Ten healthy Vietnamese university students were exposed to the TSST protocol, during which self-reported measures (PANAS and VAS) and electrocardiogram activity were recorded. This study hypothesizes that post-TSST, participants would exhibit lower positive affectivity, higher negative affectivity, and higher perceived psychological stress. Additionally, a significant increase in heart rate was predicted, while the remaining electrocardiogram values (standard deviation of HRV, root mean square of successive differences between normal heartbeats (RMSSD), and pNN50) during the resting phase with speech and arithmetic tasks were expected to significantly decrease. Furthermore, the study employed kurtosis and skewness of HRV to understand the distribution of HRV values.

2. Materials and methods

2.1. Participants

Ten participants (six men and four women), aged 19 to 22, all of whom were current university students, took part in this study. Exclusion criteria for the participants included regular consumption of energy drinks or beverages containing stimulant compounds (less than two cups per week), diagnosis of mental illness by professionals, use of psychotherapeutic therapies, and exhibiting signs of anxiety or obsession with their surroundings.

This study received approval from the Institutional Review Board (IRB) of the School of Biomedical Engineering, International University, Vietnam National University, Ho Chi Minh City. Prior to the study, all participants were provided with the informed consent form to read and sign if they agreed to participate.

2.2. The Trier social stress test protocol

The TSST was utilised to induce psychosocial stress [14]. This study followed the recommendations of a systematic review conducted by N.N. Linares, et al. (2020) [25], which synthesized data on the procedures from 35 articles.

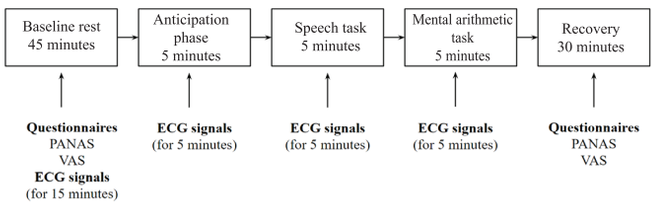

Fig. 1. The Trier social stress test laboratory protocol (total of 90 minutes).

In our study, all participants underwent a 45-minute resting stage, which involved completing questionnaires (PANAS and VAS) and a 15-minute acquisition of EEG signals. Subsequently, the participants had 5 minutes to prepare for a 5-minute speech task. This was followed by a 5-minute mental arithmetic task, concluding the TSST. Finally, there was another 30-minute resting stage during which the participants completed the two questionnaires once again (Fig. 1).

2.2.1. Baseline rest (resting stage) (45 minutes)

The participants were instructed to wait comfortably for 30 minutes. After the 30-minute waiting period, the participants completed questionnaires (Supplementary Tables 1 and 2) to assess their baseline stress level and subjective well-being, including stress symptoms, positive and negative affect (using the PANAS), and current stress level (using the VAS). Physiological measures (subjects' ECG signals) were collected for 15 minutes following the initial 30-minute waiting period.

2.2.2. The Trier social stress test protocol

Anticipation phase (5 minutes): The researcher informed the participant that they were applying for their ideal job and would need to give a 5-minute speech describing why they would be the ideal candidate for the position in front of a judge. The researcher explained that the presentation would be audio and video-recorded for later analysis. The participants were provided with a sheet of paper and a pen and given five minutes to prepare for the presentation. The researcher then started a digital timer to count down from five minutes and left the room. Subjects' ECG signals were collected for five minutes during this stage.

Speech task (5 minutes): Immediately after the preparation stage, the researcher returned to the testing room and instructed the participant to deliver their speech. The researcher set a timer for five minutes. If the participant paused for more than 20 seconds during the speech or finished before five minutes, the judge would use a standardized prompt to ask the participant to continue. If necessary, the judge would ask prepared questions to ensure the participant spoke for the entire allotted time.

Mental arithmetic task (5 minutes): At the conclusion of the speech task, the judge introduced a new task to the participant. The participant was asked to subtract the number 13 from 1022 sequentially, aloud, as quickly and accurately as possible. The judge informed the participants that their progress would be closely monitored, and any mistakes would result in starting over from the beginning. Pauses lasting longer than 15 seconds were also counted as miscalculations. The task began immediately after the researcher set a timer to count down from five minutes. Subjects' ECG signals were collected for five minutes during this stage.

2.2.3. Recovery (30 minutes)

After the mental arithmetic task, the researcher returned the participants to the original waiting room and instructed them to wait comfortably for 30 minutes. After the 30-minute period, the participants completed the questionnaires again, and post-stress physiological measures were taken.

During the debrief, the researcher informed the participants that their performance was not recorded and that no analysis of their speech or math performance would be conducted. Additionally, the tasks presented to them were intentionally challenging and did not reflect their true abilities.

2.3. Psychological measures

Affect was measured using the PANAS, which is a self-reported questionnaire consisting of 20 multiple-choice questions, with 10 questions measuring positive affect and 10 measuring negative affect [36]. Each question presented a word describing a feeling or emotion experienced at the present moment, and participants rated their response on a scale from one (very slightly or not at all) to five (extremely). The positive and negative affect subscales each comprised 10 words, with a possible score range of 10 to 50. Higher scores indicated a higher level of positive or negative affectivity.

Perceived stress was assessed using the VAS, which has been reliably used to measure psychological distress in Vietnam [37-39]. Participants rated their current stress level on a scale from 1 (not at all) to 10 (extremely).

2.4. Physiological measures: ECG

To measure participants' ECG activities, ECG sensors developed by the ITR company (Fig. 2), and approved by the FDA, were used. The device was placed in the middle of the participants' chest, and the sensors were in direct contact with their skin to record their heart activities. Participants were instructed to remain seated and avoid sudden movements during the TSST procedure to minimize noise caused by muscular movement.

The collected data was then processed using a bandpass filter (range from 0.5 to 30 Hz), and a fifth-order Butterworth filter was applied to remove any remaining noise, particularly

electromyography noise [40, 41] (Fig. 3). The data processing was conducted in Python, utilizing the WFDB processing tools (https://wfdb.readthedocs.io/en/latest/index.html) for signal analysis (Fig. 4). Five values were selected for further analysis, calculated using Python: average heart rate, standard deviation, kurtosis and skewness of HRV, root mean square of successive differences (RMSSD) between normal heartbeats, and PNN50 (the ratio of the number of standard heartbeat intervals larger than 50 ms to the total of the N-N intervals). These values indicate the participants' physiological responses to each task condition [42, 43]. Additionally, the average heart rate was calculated by summing all heart rate values between 30 and 200 bpm and dividing them by the total number of suitable heart rate values.

Fig. 2. The ITR electroencephalogram sensor.

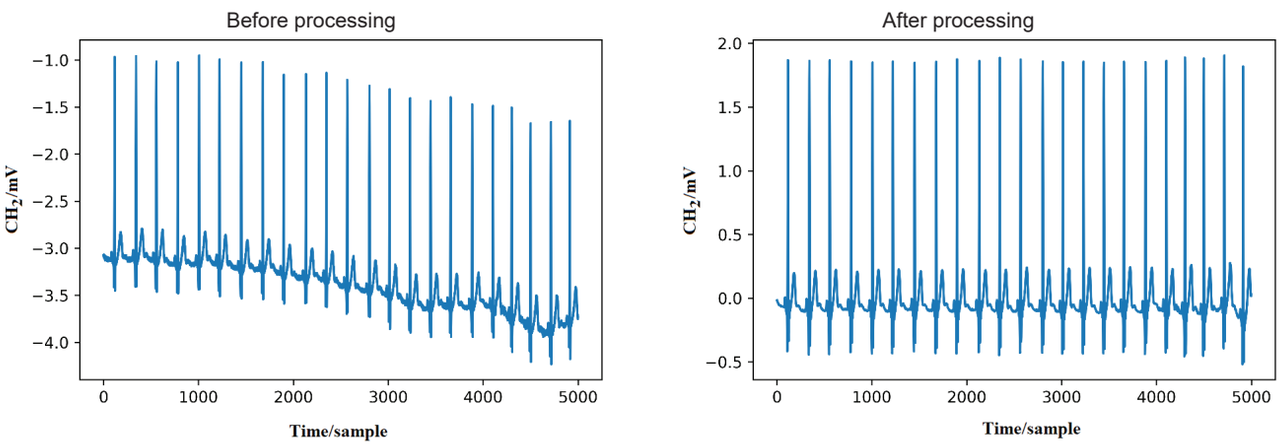

Fig. 3. Acquired data before and after signal processing.

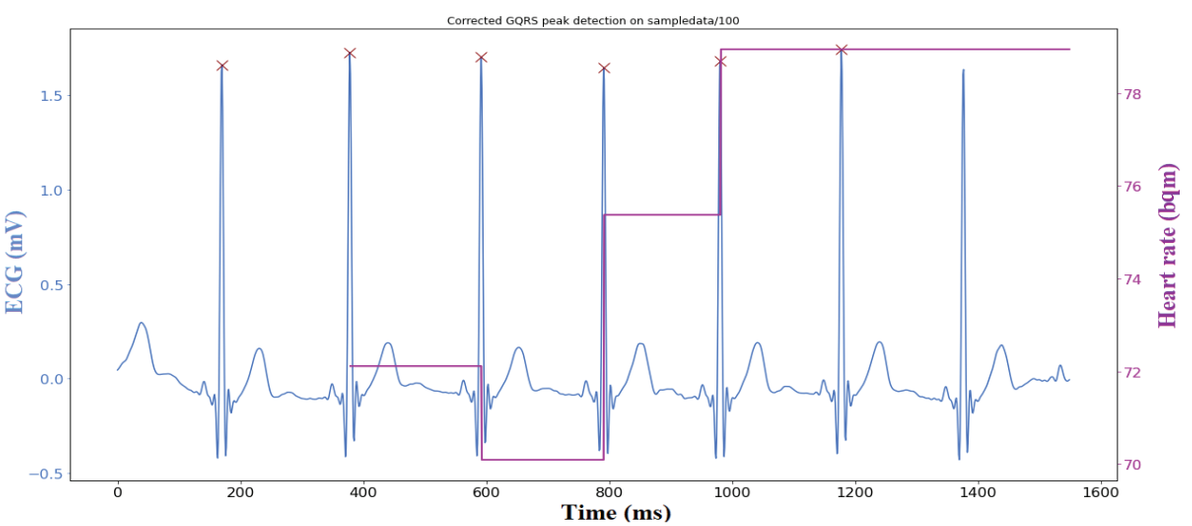

Fig. 4. Peak finding using WFDB functions.

2.5. Statistical analysis

Paired samples t-tests were conducted to compare affective states and perceived psychological stress before and after the administration of the TSST. Additionally, repeated-measures one-way ANOVA was used to compare the ECG signal results across the four stages of the TSST. All statistical analyses were performed using GraphPad Prism. Unless otherwise stated, all values and graphs presented in this article are reported as data ± standard error (SE). In addition, the asterisk symbol (*) in the graph indicates that there are significant differences between the two comparison groups. The statistical power and effect size of the testing population were calculated using G*Power 3.1.9.7 [44].

3. Results

3.1. Psychological measures

3.1.1. Positive affective state

We hypothesised that the positive affective score, as measured by the PANAS, would significantly decrease after the induction of stress. The average positive affect score prior to the TSST was

28.9±8.621. This score slightly decreased to 26.9±8.212 after the administration of the TSST. However, there was no significant difference in the positive affect scores before and after stress induction (t(9)=0.956, p=0.364) (Fig. 5). The statistical power of the conducted t-test was 0.668 with an effect size of 0.849.

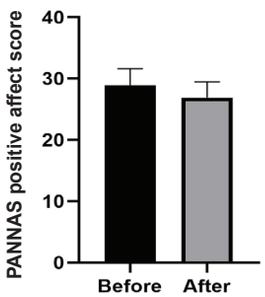

Fig. 5. Positive affect score before and after Trier social stress test.

3.1.2. Negative affective state

We also tested the hypothesis that participants would experience higher negative affectivity after undergoing the TSST. The average negative affective score during the baseline rest was 18.8±8.230. After the participants underwent the TSST, the average score increased to 25.8±11.094, indicating an increase in negative emotions. Our analysis revealed a significant effect of the TSST on negative affect (t(9)=-2.881, p=0.020) (Fig. 6). The statistical power of the conducted t-test was 0.266 with an effect size of 0.472.

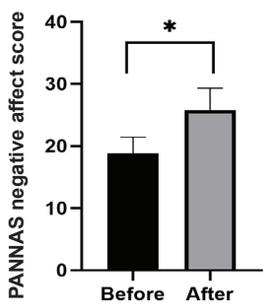

Fig. 6. Negative affect score before and after Trier social stress test.

3.1.3. Perceived stress

Our prediction was that participants would experience increased perceived stress after completing the TSST procedure. Comparing the VAS scores before and after the TSST, we observed a 42% increase in the self-reported stress score (mean of 4.8±1.751 and 6.8±1.687, respectively). The paired sample t-test indicated a significant effect of the TSST on participants' perceived stress (t(9)=-3.078, p=0.013) (Fig. 7). The statistical power of the conducted t-test was 0.29 with an effect size of 0.497.

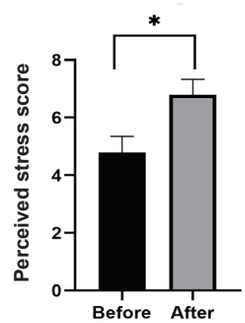

Fig. 7. Perceived stress score before and after Trier social stress test.

3.2. Physiological measures

3.2.1. Heart rate

To assess the influence of different phases of the TSST on participants' heart rate, a one-way repeated measures ANOVA was conducted. We expected significant differences between the baseline rest and the speech and mental arithmetic task phases (Fig. 8). Comparing the four phases of the TSST, significant differences were observed in the participants' average heart rates after applying the repeated measures ANOVA one-way statistical analysis between the rest and anticipation phase (means of 80.4±2.95 and 86.8±3.04, p=0.043), the rest and speech phase (means of 80.4±2.95 and 90.8±2.77, p=0.012), and the rest and arithmetic phase (means of 80.4±2.95 and 88.0±3.02, p=0.010). No significant differences were observed among the remaining phases (anticipation and speech task, anticipation and arithmetic task, speech and arithmetic task). In summary, the TSST significantly affected participants' heart rate, with an increase observed during the speech task and arithmetic task stages.

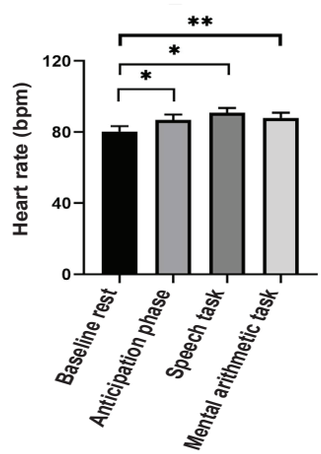

Fig. 8. Subject's heart rate during the four sessions of Trier social stress test.

3.2.2. Heart rate variability (HRV) measurement

Standard deviation of R-R intervals: Assessing the standard deviation of the R-R Interval, a significant difference was only found between the Anticipation and Speech phases (means of 0.0507±0.0052 and 0.062±0.0051, respectively, p=0.016). No significant differences were observed in the remaining comparisons between any two phases (Fig. 9).

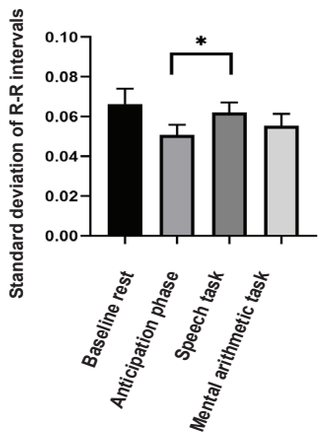

Fig. 9. Subjects' standard deviation of R-R intervals between four sessions of Trier social stress test.

Kurtosis and skewness of R-R intervals: Analysing the participants' kurtosis of R-R intervals, no significant differences were observed. For the skewness of R-R intervals, significant differences were found between the rest and anticipation phases (means of -0.181±0.153 and 0.267±0.13, respectively, p=0.015), the rest and speech task phase (means of -0.181±0.153 and 0.337±0.165, respectively, p=0.005), and the rest and arithmetic phase (means of -0.181±0.153 and 0.536±0.156, respectively, p<0.0001). No differences were observed in the remaining comparison groups. In summary, no conclusive findings can be drawn from the results of kurtosis. However, the significant increase in skewness between each phase of the TSST indicates that the R-R intervals tend to lean towards smaller values, indicating lower variance among R-R intervals and suggesting increased stress in later stages (Fig. 10).

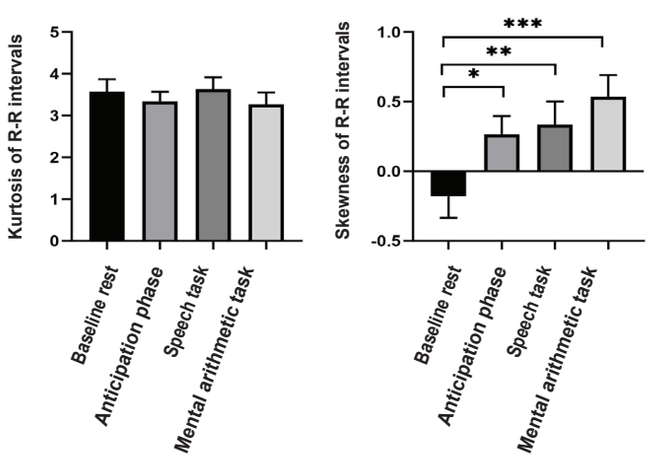

Fig. 10. Subjects' kurtosis and skewness of R-R intervals among the four sessions of Trier social stress test.

Root mean square of successive difference (RMSSD) and pNN50: The results of the root mean square of successive differences and pNN50 indicate a decrease in their mean values between the rest session and the anticipation and speech sessions (Fig. 11 and Supplementary Table 3). However, no significant differences were found among the four stages of TSST in these two results. Therefore, no conclusive indications can be interpreted from the RMSSD and pNN50 values (Fig. 11).

The statistical power of all heart rate measures ranged from 0.139 to 0.479, with effect sizes ranging from 0.3 to 0.676.

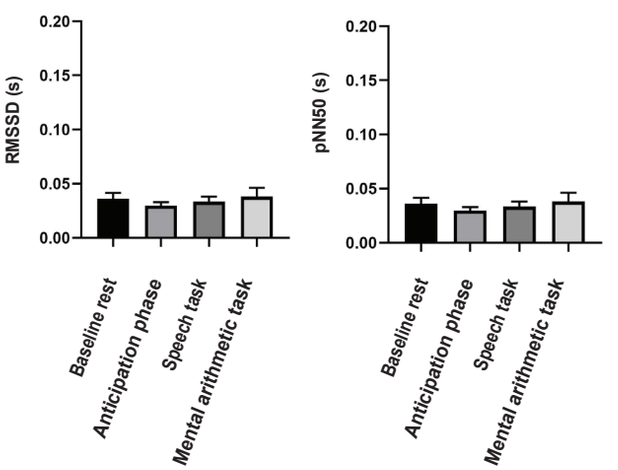

Fig. 11. Subjects' root mean square of successive difference (RMSSD) and pNN50 among the four sessions of Trier social stress test.

4. Discussion

In our experiment investigating the effect of the TSST on stress responses in the Vietnamese population using questionnaires and electrocardiogram (ECG) measurements, we observed several notable results indicating the stress-inducing effects of the TSST. The analysis of the questionnaires revealed a significant increase in participants' negative affective state after the TSST, supporting our hypothesis that negative emotions would intensify during the speech and arithmetic task stages, leading to a more stressful state. However, no significant differences were found in the positive affective state, indicating that positive emotions remained unaffected by the TSST. Additionally, the VAS questionnaire confirmed a significant increase in participants' perceived stress levels after the TSST. Analysing the ECG

measurements, we observed a significant increase in heart rate between the resting stage and the speech and arithmetic stages. The increase in skewness of HRV further indicated that participants' heart rates beat at a faster rate than usual after these two final stages, suggesting a heightened state of stress.

However, some of the remaining ECG results contradicted our hypotheses. There were no differences in the standard deviation and kurtosis of HRV, root mean square of successive differences, and pNN50 values between the resting stage and the speech and arithmetic tasks. These mixed results deviated from previous studies that demonstrated the effectiveness of the TSST in inducing stress in other Asian populations [16-23]. We will discuss potential explanations and recommendations for these contradictions in the following section.

5. Conclusions

The results of our project indicate that the TSST had a stimulating effect on the stress levels of participants, as evidenced by the increased heart rate and alternations in psychological measurements (increased negative affective state and VAS scores). However, non-significant findings in measures such as HRV (kurtosis, RMSSD, and pNN50) may be attributed to confounding variables, including familiarity with the test procedure and ethnic characteristics. Future experiments using the TSST to induce stress should consider several modifications. Firstly, an increased number of interviewers, preferably more than two as in previous TSST studies, should be employed. Secondly, interviewers' rigor during the speech and arithmetic tasks should be consistent to minimise participant bias. Furthermore, future extensions of the study should incorporate a control group and a larger number of participants to enhance the study's validity.

6. Limitations and recommendations

Some of the results that contradict our hypotheses can be attributed to several reasons, which also serve as limitations of our preliminary study. *Firstly,* in the speech task and the arithmetic task, the presence of only one interviewer may have been insufficient to induce a significant level of stress or intensity in the participants. Additionally, the current level of arithmetic difficulty might not have been challenging enough to elicit stress in Vietnamese students. This could be attributed to the exceptional mathematical skills of the Vietnamese population, as evidenced by their performance in the PISA examination compared to other nations (India, Ethiopia, Peru) [45]. *Secondly,* variations in the interviewers' strictness could have led to different emotional responses from participants, resulting in varying stress levels. Furthermore, the lack of uniformity in the questions asked during the speech task may have led to some participants perceiving the interview as easier than others. *Thirdly,* the small number of participants in our study may have yielded undesirable results and affected the statistical analysis. Particularly for this preliminary study, with only ten participants, the statistical analysis may not provide conclusive findings. The statistical power of the conducted analyses was all below 0.8, which is the ideal threshold for statistical tests [46]. However, the effect size was notable, ranging from 0.472 to 0.849 for psychological measures, indicating strong effects of the TSST intervention on these measures. Conversely, the effect size ranged from 0.3 to 0.67 for physiological measures, indicating a moderate independence between the TSST intervention and these measures [47]. *Fourthly,* some participants may have previously been in similar situations, particularly in the speech task, as reported after the study. This familiarity with job interviews could have reduced the perceived stress of the interview task. Consequently, participants may have experienced a milder response to stress during the TSST. *Fifthly,* the absence of a control group (groups that do not undergo the TSST stage) limits our ability to confirm further details about the effects of the TSST. *Lastly,* the small sample size (N=10) may have reduced the study's ability to replicate the TSST, as the original study by C. Kirschbaum, et al. (1993) [14] found that a sample size of at least 20 participants was necessary to detect significant cortisol responses to the TSST.

SUPPLEMENTARY

Table 1. Positive and negative affective schedule questionnaire's English version.

Positive and negative affective schedule (PANAS-SF)

Indicate the extent you feel this way right now:

	Very slightly or not at all	A little	Moderately	Quite a bit	Extremely
Interested	○	○	○	○	○
Distressed	○	○	○	○	○
Excited	○	○	○	○	○
Upset	○	○	○	○	○
Strong	○	○	○	○	○
Guilty	○	○	○	○	○
Scared	○	○	○	○	○
Hostile	○	○	○	○	○
Enthusiastic	○	○	○	○	○
Proud	○	○	○	○	○
Irritable	○	○	○	○	○
Alert	○	○	○	○	○
Ashamed	○	○	○	○	○
Inspired	○	○	○	○	○
Nervous	○	○	○	○	○
Determined	○	○	○	○	○
Attentive	○	○	○	○	○
Jittery	○	○	○	○	○
Active	○	○	○	○	○
Afraid	○	○	○	○	○

Table 2. Visual analogue scale questionnaire (English version).

0-1 Numeric visual analogue scale - VAS

Choose a number from 0 to 10 that best describes your stress level:

No stress	1	2	3	4	5	6	7	8	9	10	Extremely stressed
	○	○	○	○	○	○	○	○	○	○	

Table 3. Mean values of different heart signal information in four sessions of the Trier social stress test.

	Baseline rest (mean ± SE)	Anticipation phase (mean ± SE)	Speech task (mean ± SE)	Mental arithmetic task (mean ± SE)
Average heart rate	80.4±2.95	86.8±3.04	90.8±2.77	88.0±3.02
R-R standard deviation (s)	0.066±0.0078	0.05±0.0052	0.062±0.0051	0.055±0.006
R-R kurtosis	3.58±0.296	3.34±0.229	3.63±0.285	3.27±0.291
R-R skewness	-0.18±0.153	0.26±0.130	0.33±0.165	0.536±0.156
RMSSD (s)	0.036±0.0053	0.029±0.0032	0.033±0.0046	0.038±0.008
pNN50 (%)	16.1±4.55	9.85±3.01	10.05±2.36	13.0±3.26

CRediT author statement

Lam Quang Nguyen: Conceptualisation, Software, Validation, Formal analysis, Writing - Original draft preparation; Huu Phuc Ngan Nguyen: Conceptualisation, Methodology, Formal analysis, Writing - Original draft preparation; Phuc Hoang Anh Le: Conceptualisation, Methodology, Investigation, Writing - Reviewing and Editing; Hoai Bao Pham, Kim Vy Huynh, Hoang Minh Thien Cao: Investigation, Writing - Reviewing and Editing; An Dong Bui: Software, Resources; Khon Huynh, Thi Thanh Huong Ha: Conceptualisation, Project administration, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] N.P. Podsakoff, J.A. LePine, M.A. LePine (2007), "Differential challenge stressor-hindrance stressor relationships with job attitudes, turnover intentions, turnover, and withdrawal behavior: A meta-analysis", *Journal of Applied Psychology*, **92(2)**, pp.438-454, DOI: 10.1037/0021-9010.92.2.438.

[2] D.S. Carlson, P.L. Perrewé (1999), "The role of social support in the stressor-strain relationship: An examination of work-family conflict", *Journal of Management*, **25(4)**, pp.513-540, DOI: 10.1177/014920639902500403.

[3] R. Beiter, R. Nash, M. McCrady, et al. (2015), "The prevalence and correlates of depression, anxiety, and stress in a sample of college students", *Journal of Affective Disorders*, **173**, pp.90-96, DOI: 10.1016/j.jad.2014.10.054.

[4] P.F. Lovibond (1998), "Long-term stability of depression, anxiety, and stress syndromes", *Journal of Abnormal Psychology*, **107(3)**, pp.520-526, DOI: 10.1037//0021-843x.107.3.520.

[5] J.R. Turner (1994), "Cardiovascular reactivity and stress: Patterns of physiological response", *The Springer Series in Behavioral Psychophysiology and Medicine (SSBP)*, DOI: 10.1007/978-1-4757-9579-0.

[6] A.J. Crum, M. Akinola, A. Martin, et al. (2017), "The role of stress mindset in shaping cognitive, emotional, and physiological responses to challenging and threatening stress", *Anxiety, Stress & Coping*, **30(4)**, pp.379-395, DOI: 10.1080/10615806.2016.1275585.

[7] A. Saleh, G.G. Potter, D.R. McQuoid, et al. (2017) "Effects of early life stress on depression, cognitive performance and brain morphology", *Psychological Medicine*, **47(1)**, pp.171-181, DOI: 10.1017/S0033291716002403.

[8] D.B. O'Connor, J.F. Thayer, K. Vedhara (2021), "Stress and health: A review of psychobiological processes", *Annual Review of Psychology*, **72**, pp.663-688, DOI: 10.1146/annurev-psych-062520-122331.

[9] L. Dettenborn, A. Tietze, F. Bruckner, et al. (2010), "Higher cortisol content in hair among long-term unemployed individuals compared to controls", *Psychoneuroendocrinology*, **35(9)**, pp.1404-1409, DOI: 10.1016/j.psyneuen.2010.04.006.

[10] S. Gallagher, R.C. Sumner, O.T. Muldoon, et al. (2016), "Unemployment is associated with lower cortisol awakening and blunted dehydroepiandrosterone responses", *Psychoneuroendocrinology*, **69**, pp.41-49, DOI: 10.1016/j.psyneuen.2016.03.011.

[11] Y. Kim, R. Schulz (2008), "Family caregivers' strains: Comparative analysis of cancer caregiving with dementia, diabetes, and frail elderly caregiving", *Journal of Aging and Health*, **20(5)**, pp.483-503, DOI: 10.1177/0898264308317533.

[12] E.S. Epel, A.D. Crosswell, S.E. Mayer, et al. (2018), "More than a feeling: A unified view of stress measurement for population science", *Frontiers in Neuroendocrinology*, **49**, pp.146-169, DOI: 10.1016/j.yfrne.2018.03.001.

[13] S.S. Dickerson, M.E. Kemeny (2004), "Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research", *Psychological Bulletin*, **130(3)**, pp.355-391, DOI: 10.1037/0033-2909.130.3.355.

[14] C. Kirschbaum, K.M. Pirke, D.H. Hellhammer (1993), "The 'Trier social stress test' - A tool for investigating psychobiological stress responses in a laboratory setting", *Neuropsychobiology*, **28(1-2)**, pp.76-81, DOI: 10.1159/000119004.

[15] B.M. Kudielka, D.H. Hellhammer, C. Kirschbaum (2007), "Ten years of research with the Trier social stress test - revisited", *Social Neuroscience: Integrating Biological and Psychological Explanations of Social Behavior*, The Guilford Press, pp.56-83.

[16] J. Fu, T. Weng, F. Tao (2012), "Cortisol response to the Trier social stress test among Chinese adolescents", *Journal of Pediatric Endocrinology & Metabolism*, **25(11-12)**, pp.1213-1216, DOI: 10.1515/jpem-2012-0234.

[17] Y. Juan, H.O.U. Yan, Y. Yu, et al. (2011), "Impact of Trier social stress test (TSST) on salivary cortisol secretion", *Acta Psychologica Sinica*, **43(04)**, pp.403-409.

[18] Y. Sun, F. Li, T. He, et al. (2023), "Physiological and affective responses to green space virtual reality among pregnant women", *Environmental Research*, **216**, Part 1, DOI: 10.1016/j.envres.2022.114499.

[19] Y. Wei, W. Tianfang, H. Wu, et al. (2018), "Biological mechanisms underlying the liver's regulation of emotions in women: A study using the Trier social stress test", *Journal of Traditional Chinese Medical Sciences*, **5(2)**, pp.110-118, DOI: 10.1016/j.jtcms.2018.03.003.

[20] L. Ying, Q. Yan, X. Shen, et al. (2022), "Social value orientation moderated the effect of acute stress on individuals' prosocial behaviors", *Frontiers in Psychology*, **13**, DOI: 10.3389/fpsyg.2022.803184.

[21] C.L.J. Lai, D.Y.H. Lee, M.O.Y. Leung (2021), "Childhood adversities and salivary cortisol responses to the Trier social stress test: A systematic review of studies using the children trauma questionnaire (CTQ)", *International Journal of Environmental Research and Public Health*, **18(1)**, DOI: 10.3390/ijerph18010029.

[22] Q. Liu, W. Zhang (2020), "Sex differences in stress reactivity to the Trier social stress test in virtual reality", *Psychology Research and Behavior Management*, **2020(13)**, pp.859-869, DOI: 10.2147/PRBM.S268039.

[23] Y. Yamanaka, H. Motoshima, K. Uchida (2018), "Hypothalamic-pituitary-adrenal axis differentially responses to morning and evening psychological stress in healthy subjects", *Neuropsychopharmacology Reports*, **39(1)**, pp.41-47, DOI: 10.1002/npr2.12042.

[24] C. Plooy, K. Thomas, M. Henry, et al. (2014), "The fear-factor stress test: An ethical, non-invasive laboratory method that produces consistent and sustained cortisol responding in men and women", *Metabolic Brain Disease*, **29**, pp.385-394, DOI: 10.1007/s11011-014-9484-9.

[25] N.N. Linares, V. Charron, A.J. Ouimet, et al. (2020), "A systematic review of the Trier social stress test methodology: Issues in promoting study comparison and replicable research", *Neurobiology of Stress*, **13**, DOI: 10.1016/j.ynstr.2020.100235.

[26] S. Pourmohammadi, A. Maleki (2020), "Stress detection using ECG and EMG signals: A comprehensive study", *Computer Methods and Programs in Biomedicine*, **193**, DOI: 10.1016/j.cmpb.2020.105482.

[27] S. Sriramprakash, V.D. Prasanna, O.R. Murthy (2017), "Stress detection in working people", *Procedia Computer Science*, **115**, pp.359-366, DOI: 10.1016/j.procs.2017.09.090.

[28] H.G. Kim, E.J. Cheon, D.S. Bai, et al. (2018), "Stress and HRV: A meta-analysis and review of the literature", *Psychiatry Investigation*, **15(3)**, pp.235-245, DOI: 10.30773/pi.2017.08.17.

[29] J.A. Seddon, V.J. Rodriguez, Y. Provencher, et al. (2020), "Meta-analysis of the effectiveness of the Trier social stress test in eliciting physiological stress responses in children and adolescents", *Psychoneuroendocrinology*, **116**, DOI: 10.1016/j.psyneuen.2020.104582.

[30] J. Hellhammer, M. Schubert (2012), "The physiological response to Trier social stress test relates to subjective measures of stress during but not before or after the test", *Psychoneuroendocrinology*, **37(1)**, pp.119-124, DOI: 10.1016/j.psyneuen.2011.05.012.

[31] E. Filaire, H. Portier, A. Massart, et al. (2010), "Effect of lecturing to 200 students on HRV and alpha-amylase activity", *European Journal of Applied Physiology*, **108(5)**, pp.1035-1043, DOI: 10.1007/s00421-009-1310-4.

[32] A.L. Rivera, B. Estanol, H.S. Madrid, et al. (2016), "Heart rate and systolic blood pressure variability in the time domain in patients with recent and long-standing diabetes mellitus", *PLOS ONE*, **11(2)**, DOI: 10.1371/journal.pone.0148378.

[33] A.V. Machado, M.G. Pereira, G.G. Souza, et al. (2021), "Association between distinct coping styles and HRV changes to an acute psychosocial stress task", *Scientific Reports*, **11(1)**, DOI: 10.1038/s41598-021-03386-6.

[34] K. Lewin (1997), *Resolving Social Conflicts and Field Theory in Social Science*, American Psychological Association, 422pp, DOI: 10.1037/10269-000.

[35] L. Jobson, S. Haque, S.Z. Abdullah, et al. (2022), "Examining cultural differences in the associations between appraisals and emotion regulation and posttraumatic stress disorder in Malaysian and Australian trauma survivors", *International Journal of Environmental Research and Public Health*, **19(3)**, DOI: 10.3390/ijerph19031163.

[36] D. Watson, L.A. Clark, A. Tellegen (1988), "Development and validation of brief measures of positive and negative affect: The PANAS scales", *Journal of Personality and Social Psychology*, **54(6)**, pp.1063-1070, DOI: 10.1037//0022-3514.54.6.1063.

[37] J.C. Beckham, A.L. Crawford, M.E. Feldman, et al. (1997), "Chronic posttraumatic stress disorder and chronic pain in Vietnam combat veterans", *Journal of Psychosomatic Research*, **43(4)**, pp.379-389, DOI: 10.1016/s0022-3999(97)00129-3.

[38] G.B. Langley, H. Sheppeard (1985), "The visual analogue scale: Its use in pain measurement", *Rheumatology International*, **5(4)**, pp.145-148, DOI: 10.1007/BF00541514.

[39] F.X. Lesage, S. Berjot, F. Deschamps (2012), "Clinical stress assessment using a visual analogue scale", *Occupational Medicine*, **62(8)**, pp.600-605, DOI: 10.1093/occmed/kqs140.

[40] P. Zhou, B. Lock, T.A. Kuiken (2007), "Real time ECG artifact removal for myoelectric prosthesis control", *Physiol. Meas.*, **28(4)**, pp.397-413, DOI: 10.1088/0967-3334/28/4/006.

[41] H.L. Butler, R. Newell, C.L.H. Kozey, et al. (2009), "The interpretation of abdominal wall muscle recruitment strategies change when the electrocardiogram (ECG) is removed from the electromyogram (EMG)", *Journal of Electromyography and Kinesiology*, **19(2)**, pp.e102-e113, DOI: 10.1016/j.jelekin.2007.10.004.

[42] B.M. Kudielka, A. Buske-Kirschbaum, D.H. Hellhammer, et al. (2004), "Differential heart rate reactivity and recovery after psychosocial stress (TSST) in healthy children, younger adults, and elderly adults: The impact of age and gender", *International Journal of Behavioral Medicine*, **11(2)**, pp.116-121, DOI: 10.1207/s15327558ijbm1102_8.

[43] A. Arza, J.M. Garzón, A. Hemando, et al. (2015), "Towards an objective measurement of emotional stress: Preliminary analysis based on HRV", *37th Annual International Conference of The IEEE Engineering in Medicine and Biology Society (EMBC)*, **2015**, pp.3331-3334, DOI: 10.1109/EMBC.2015.7319105.

[44] F. Faul, E. Erdfelder, A.G. Lang, et al. (2007), "G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences", *Behavior Research Methods*, **39(2)**, pp.175-191, DOI: 10.3758/bf03193146.

[45] P. Glewwe, Z. James, J. Lee, et al. (2021), *What Explains Vietnam's Exceptional Performance in Education Relative to Other Countries? Analysis of The Young Lives Data from Ethiopia, Peru, India and Vietnam*, RISE Working paper 21/078, 75pp.

[46] C.C. Serdar, M. Cihan, D. Yücel, et al. (2021), "Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies", *Biochemia Medica*, **31(1)**, pp.27-53, DOI: 10.11613/BM.2021.010502.

[47] T. Schäfer, M.A. Schwarz (2019), "The meaningfulness of effect sizes in psychological research: Differences between sub-disciplines and the impact of potential biases", *Frontiers in Psychology*, **10**, DOI: 10.3389/fpsyg.2019.00813.