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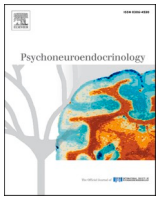

A Semi-Virtual Trier Social Stress Test (SV-TSST)

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ABSTRACT

The Trier Social Stress Test (TSST) is a strategy for inducing acute psychological stress and increases in glucocorticoid levels. Here we describe the methodology and implementation of a Semi-Virtual Trier Social Stress Test (SV-TSST) which combines the control of a laboratory environment with reduced need for in-person logistical support and enhanced social distancing without the need for specialized equipment. During the SV-TSST, the participant is guided through the baseline, anticipatory, challenge, and recovery phases of the test by an in-person experimenter. Confederate judges involved in the challenge phase of the protocol connect with the participant via live video teleconference. Fifty-five healthy male and female participants aged 18–25 completed measures of self-report stress and provided saliva samples for cortisol assay throughout the SV-TSST session. The SV-TSST protocol was found to induce a significant acute increase in subjective psychological stress and salivary cortisol, with elevated psychological stress in SV-TSST female compared to male participants. Results indicate that the SV-TSST can be implemented as protocol for acute stress induction in a within-subject design that can serve as an alternative to classic, virtual, and virtual reality adaptations of this methodology.

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) response to stress plays a critical role in physiological and behavioral adaptations to environmental challenges (McEwen, 2007). Experimental studies of the response of the HPA axis in humans have relied on the Trier Social Stress Test (TSST), an acute stress induction protocol which increases glucocorticoid levels and self-report psychological stress (Kirschbaum et al., 1993), though other acute stress protocols are similarly effective (Bali and Jaggi, 2015). The TSST is designed to combine social-evaluative threat and uncontrollability in a context in which participants are tasked with engaging in public speaking and impromptu mental arithmetic performance before a panel of emotionally non-responsive confederate judges (Birkett, 2011). Decades of research indicates that measures of psychological and HPA response to the TSST provide critical insights into the interplay between the neurobiology of stress and mental health (Allen et al., 2016; McEwen and Akil, 2020).

The traditional TSST methodology can pose logistical challenges due to the need to coordinate the presence of five individuals for the in-person session (*i.e.,* the experimenter, the subject, and two to three confederate judges). This protocol is not feasible to conduct when social distancing is necessary (e.g. during the Covid-19 pandemic) and potentially introduces sources of environmental noise (e.g. exposure to human volatilome signals; Tang et al., 2016). There have been a number of studies investigating virtual-reality (VR) or online formats of the TSST, which require only the participant or a participant and investigator to be present. These protocols have been shown to effectively induce acute stress (Allen et al., 2016; Gunnar et al., 2021; Halbeisen et al., 2023; Harvie et al., 2021; Helminen et al., 2019; Kothgassner et al., 2021; Santl et al., 2019; Shiban et al., 2016), however, they may require specialized equipment and/or lack the controlled conditions of a laboratory environment (Fallon et al., 2021).

In the current study, we describe the methods and implementation of a semi-virtual TSST (SV-TSST) protocol, which integrates the control of an in-person laboratory experience while reducing logistical burdens and promoting social distancing by having judges participate virtually (without use of VR). Within the SV-TSST, participants deliver their speech and math performance via live video teleconference (*i.e.,* Zoom) on an iPad to a panel of confederate judges projected on a large television screen. The SV-TSST requires only two in-person individuals in attendance (*i.e.,* the experimenter and the participant). In the current study, we demonstrate the implementation of the SV-TSST using measures of self-report stress and salivary cortisol and illustrate the importance of considering sex differences in TSST responses.

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2. Material and methods

2.1. Participants

This study was approved by the Institutional Review Board at the University of Texas at Austin. Participants were recruited from a large undergraduate class at the University of Texas at Austin and required to be 18–25 years of age and provided informed consent via Qualtrics. The study was conducted during the Covid-19 pandemic, when Zoom-based video teleconferencing and social distancing were normative experiences for participants. Respondents were excluded if taking endocrine-related medications other than hormonal contraception, were taking a high dose or mixture of medications, reported current substance abuse or current nicotine use, reported having a mental health disorder, were diagnosed with Cushing's or Addisons's disease, were pregnant, breastfeeding, or had an irregular menstrual cycle. Female participants were scheduled for the SV-TSST during the luteal phase of their menstrual cycle which was calculated based on self-report of the start date of their last menstrual period. BMI was not used as an exclusion criterion but self-report height and weight data suggest that participants were in the normal weight range (average BMI = 22.9 ± 0.55).

2.2. Salivary cortisol measures

Participants provided saliva samples at four sampling times (see Fig. 1**A**). Saliva was collected after the 30-minute baseline period and at 15, 30, and 60 minutes after the cessation of the SV-TSST challenge. Immediately after collection, saliva samples were stored at −80°C. Cortisol concentration was measured using a high throughput liquid chromatography–tandem mass spectrometry assay by Dresden Lab-Service GmbH. The within-subject change in cortisol concentration between baseline to post-challenge (Fig. 1**A**) was calculated in order to categorize participants as cortisol responders or non-responders based on a threshold of 1.5 nmol/l (Miller et al., 2013).

2.3. Subjective stress assessment

Participants self-reported their subjective stress using a digital visual analog scale (VAS) slider on an iPad (Qualtrics) with end points at 0 and 100, with lower numbers indicating less stress and higher numbers indicating more stress (Hellhammer and Schubert, 2012). The VAS was administered after the 30-minute baseline period, 7 minutes into the anticipatory period, immediately after the acute stress induction challenge, and at three sampling times during the recovery period (15, 30, and 60 minutes after the cessation of the stress challenge).

2.4. Study protocol

A summary of the study timeline is shown in Fig. 1**A**. Participants were not made fully aware of the study details prior to their appointment. Participants were asked to abstain from caffeine, intense exercise, and dairy before their appointment and to avoid eating or drinking for at least 30 minutes prior to their appointment. Appointments were scheduled between 11:30 am and 6:00 pm. At the time of scheduling, 55 participants were assigned to the SV-TSST protocol. An additional 7 participants were assigned to a no-stress condition. While the within-

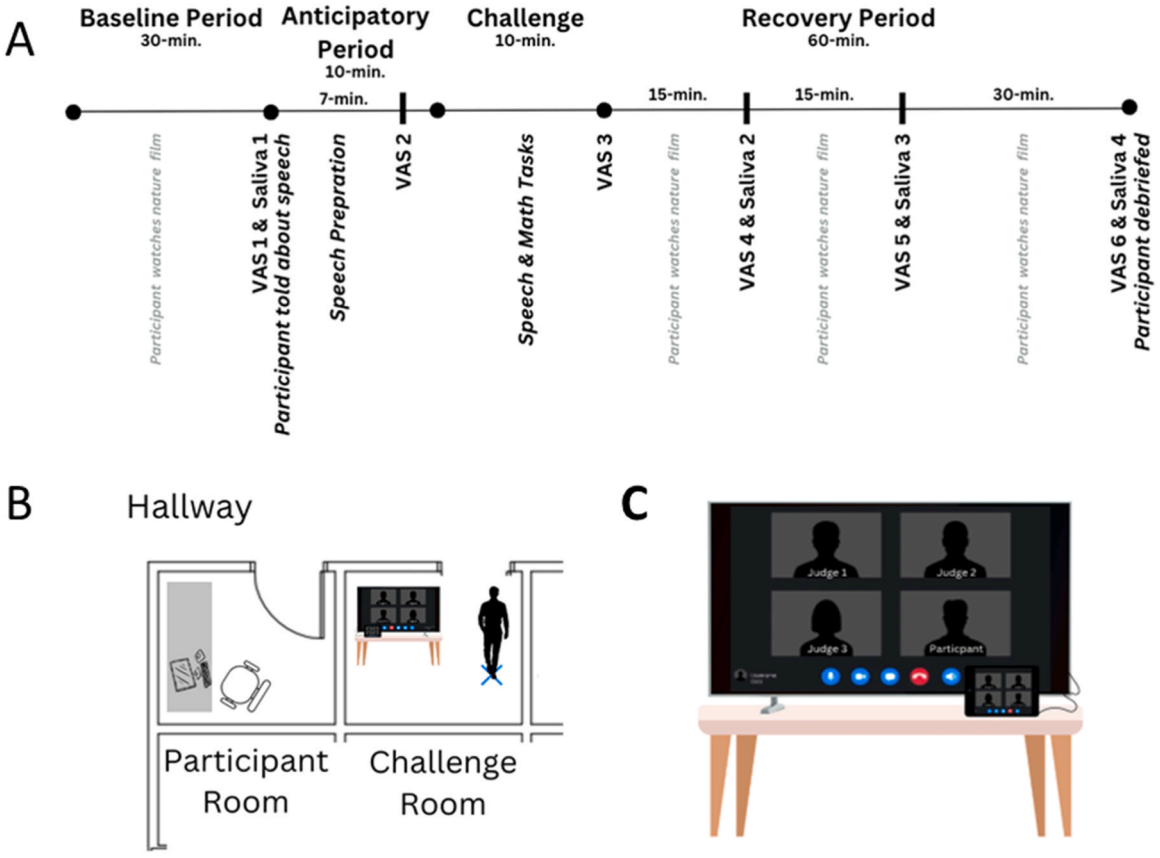

Fig. 1. SV-TSST study session timeline and set-up. A. The protocol consisted of a baseline, anticipatory, challenge and recovery period with VAS psychological stress assessed at 6 timepoints and saliva samples for cortisol analyses collected at 4 timepoints. B. Participants completed VAS and saliva collection in the Participant Room which was connected via a hallway to the Challenge Room. C. Participants delivered their speech and math performance via live video teleconference (*i.e.,* Zoom) on an iPad to a panel of confederate judges projected on a large television screen. The in-person experimenter waited outside the Challenge Room during the SV-TSST.

subject analyses of the SV-TSST participants are the primary focus of this study protocol and its applications, a small no-stress condition group of participants was also included to observe any trends in stress measures while participants where in the lab environment (see Fig. 2 for no-stress condition psychological stress and salivary cortisol values).

Upon arrival at the lab, a researcher of the opposite sex greeted participants. Participants were asked to enter the participant room for the 30-minute baseline period (see Fig. 1**A**), wherein they watched a nature documentary (episode "One Planet" from the series "Our Planet" on an iPad. During this baseline, all communication between the participant and researcher was carried out via FaceTime (Apple Inc.) on the iPad. After the 30-minute baseline period, participants rated their subjective stress via Qualtrics survey on the iPad using the VAS (t1 VAS), provided their first saliva sample, and then SV-TSST participants were sent to the challenge room (see Fig. 1**B**). No-stress condition participants remained in the participant room and continued to watch the nature documentary.

In the challenge room, the SV-TSST participant was instructed to stand on a marked location facing a video camera on a tripod and a microphone. A live Zoom call was displayed on a large TV screen and set to gallery view so that all three confederate judges (head and shoulders) were visible (see Fig. 1**C**). The judges and the researcher were trained to not emotionally respond to the participant. The participant was directed to listen to a brief audio instruction for the speech task read in a voice of someone of the opposite sex. The participant was then directed to return to the participant room to prepare their speech. Seven minutes into the anticipatory period, participants were asked to complete the second VAS (t2) before returning to the challenge room.

Once in the challenge room, the first confederate judge (opposite sex of participant), directed the participant to begin their speech. If the participant paused for 10 seconds, they were prompted to continue and if they ended their speech early, they were probed with questions. After five minutes, an alarm sounded, and the first judge abruptly stopped the participant. That judge then directed the participant to complete an impromptu mental math challenge out loud as quickly and accurately as possible: subtracting from 1022 in increments of thirteen. Participants

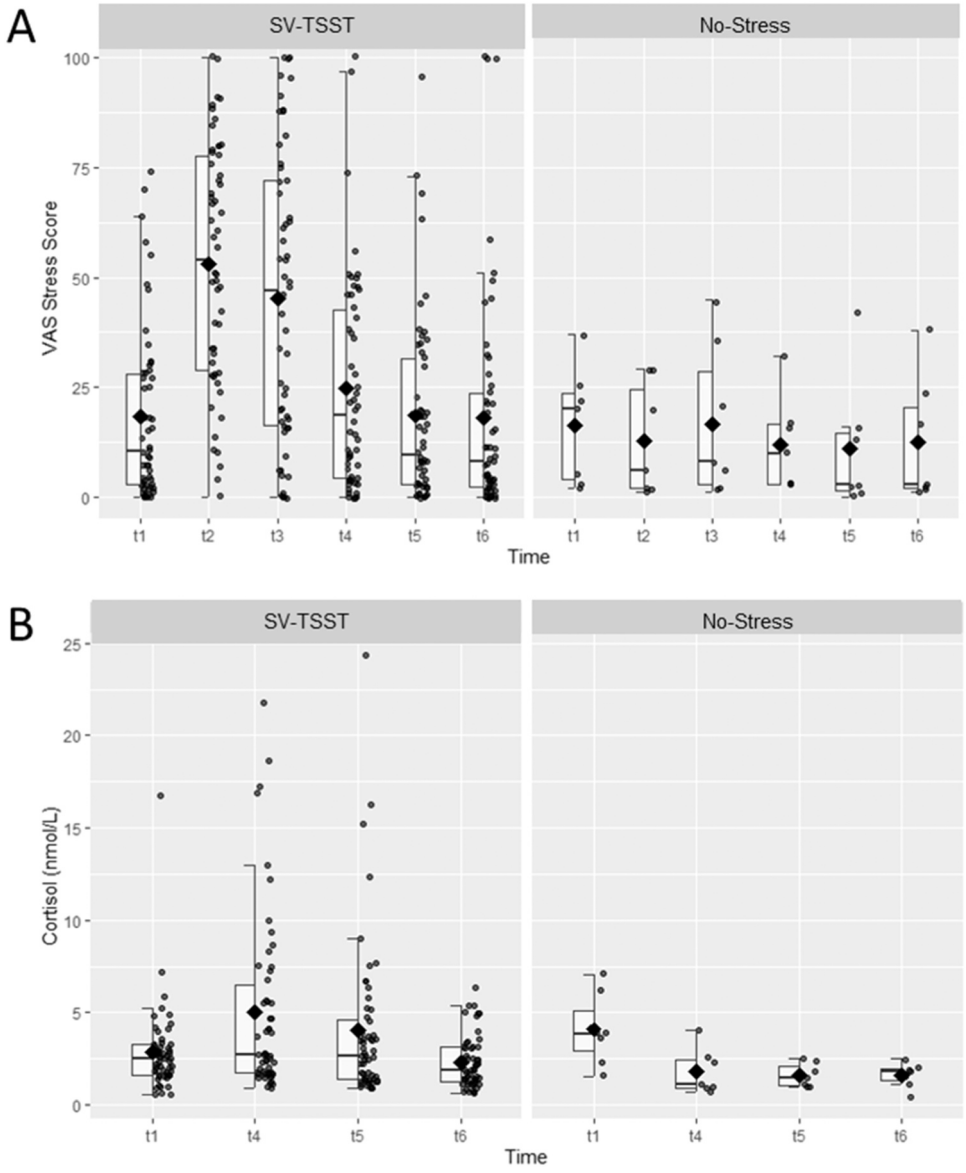

Fig. 2. Psychological and biological stress effects during the SV-TSST. A. Subjective stress in SV-TSST and no-stress conditions. A significant ($p < 0.05$) change in self-reported VAS score was found in the SV-TSST group when comparing baseline (t1) to immediately before the challenge (t2) and immediately after the TSST challenge (t3). B. Salivary cortisol concentration stratified by condition. The SV-TSST group showed a significant ($p < 0.05$) increase in cortisol, between baseline (t1) and after the challenge (t2).

were reminded to speak loudly, enunciate, and were told to begin again if an error was made. After five minutes, the participant was told to stop and return to the participant room.

Immediately after completing the SV-TSST challenge, the participant completed a third VAS (t3) before resuming the nature documentary on the iPad. Fifteen minutes later (t4), participants completed a fourth VAS and provided a second saliva sample. A fifth VAS and third saliva sample were collected 30 minutes (t5) after the challenge. Sixty minutes (t6) after the challenge, the sixth VAS and final saliva sample were collected before the participant was debriefed.

2.5. Data analysis

All statistical analyses were performed in RStudio. Given that linear mixed models allow more flexibility in the analysis of longitudinal data than ANOVA models and have been previously utilized in TSST studies (Fallon et al., 2016), linear mixed-effects models were implemented with the lme4 package version 1.1–31 (Bates et al., 2015) and lmerTest version 3.1–3 (Kuznetsova et al., 2017) to test differences between sampling times of VAS scores and salivary cortisol. For subjective stress, this model was fitted to VAS score regressed on sex and time, with participant ID included as a random intercept term, which models clusters of observations (e.g., repeat measures). For salivary cortisol, the linear mixed-effects model was fitted to cortisol concentration regressed on sex and time, with participant ID included as a random intercept term. Sex as a predictor of subjective stress and cortisol concentration was also tested. Area under the curve with respect to increase (AUCi) was calculated for VAS and cortisol responses (Pruessner et al., 2003). G*Power estimates indicated a sample size of 24 participants per sex would be sufficient to achieve a power 0.80 with an alpha of 0.05 with a small effect size (0.20). This study was not pre-registered.

3. Results

3.1. Summary of participants

Participants [n = 55 SV-TSST (28 female, 27 male); n = 7 no-stress (4 females, 3 males)] were comprised of the following race/ethnicity groups: Black/African American (1 %), Hispanic White (24 %), Non-Hispanic White (30 %), Asian/Pacific Islander (37 %) and Other/Mixed (8 %). The average age was 19.63 ± 1.46 years. There were no sex differences in age or education level (all participants were undergraduate students). Consistent with prior recruitment efforts from this type of participant pool (McAfee et al., 2025), approximately one third of female participants (9/28) reported using hormonal contraceptives.

3.2. Subjective stress (VAS) during the SV-TSST

During the SV-TSST, subjective stress increased from baseline (t1) to the anticipatory phase (t2) and then subsequently decreased during the recovery phase (t4-t6; see Fig. 2**A**). A linear mixed model revealed a significant difference between t1 and t2 (b = 34.83, SE = 3.74, df = 265, t = 9.30 p < 2e-16) and t1 and t3 (b = 27.04, SE = 3.74, df = 265, t = 7.22, p = 5.44e-12), and that sex was a significant (b = −12.51, SE = 4.66, df = 52, t = -2.68, p = 0.00978) predictor of VAS score, with females reporting higher levels of subjective stress during the SV-TSST. Mean AUCi for VAS was 894.54 ± 235.42.

3.3. Salivary cortisol levels during the SV-TSST

When salivary cortisol concentration assessed during the SV-TSST was compared between sampling times (Fig. 2**B**), a significant increase in cortisol concentration was identified between t1 (baseline) and t4 (15 minutes post-challenge). A linear mixed model revealed a significant difference in cortisol between t1 and t4 (b = 2.79, SE = 0.60, df = 162, t = 4.67 p = 6.33e-06) and t1 and t5 (b = 1.22, SE = 0.60, df = 162, t = 2.04, p = 0.04) in the SV-TSST group. However, sex was not a significant predictor of cortisol concentration. Cortisol responder rates were found to be 33 %; a level consistent with previous applications of virtual versions of the TSST (Shiban et al., 2016). Mean AUCi for cortisol was 91.70 ± 39.72, indicating consistency with previous studies of young healthy adults undergoing a TSST challenge (Morris et al., 2014).

4. Discussion

The objective of this study was to implement a novel semi-virtual TSST protocol for inducing acute stress, including subjective stress and physiological stress as indexed by salivary cortisol (Fallon et al., 2021). During the SV-TSST, there was a significant increase in self-reported subjective stress emerging during the anticipatory phase of the protocol which then trended back towards baseline levels for the remainder of the recovery period. The SV-TSST protocol also induced an increase in salivary cortisol concentration measured 15 minutes after the challenge phase of the protocol. Similar to self-report stress, levels of cortisol decreased during the recovery phase, returning to baseline levels 60 minutes after the challenge. Overall, this protocol was found to induce acute stress in both male and female participants, with responder rates and AUCi values consistent with prior studies utilizing classic and virtual TSST protocols (Morris et al., 2014; Shiban et al., 2016).

Though sex differences in response to the TSST have been previously reported, there is inconsistency in these effects, likely attributable to methodological variations, including whether participants were taking hormonal contraceptives or how menstrual phase was determined. However, our findings of increased self-reported psychological stress in female luteal phase participants (based on self-report) compared to male participants, is consistent with prior research (Childs et al., 2010; Kelly et al., 2008). Individual differences in response to the SV-TSST are also evident (see Fig. 2), a finding consistent with prior work using the TSST or virtual reality-TSST and suggests that the SV-TSST can be effectively used in studies comparing stress responders and non-responders as well as generating a broad range of area under the curve (AUC) values for both psychological and biological stress.

The SV-TSST protocol provides a novel alternative to both the classic TSST and virtual reality adaptations of the TSST, combining the control of a lab-based environment with reduced in person contact needed when social distancing is necessary. Similar to other online versions of the TSST (Gunnar et al., 2021; Harvie et al., 2021), the virtual participation of the panel of confederate judges in the SV-TSST reduces the logistical burden of this test and can be facilitated with non-specialized equipment and videoconferencing software. Standardization of the physical environment experienced during the challenge phase is critical to experimental endpoints, thus, this protocol may be ideal when biosampling of participants requires a laboratory environment (Tang et al., 2016). Integration into this protocol of additional physiological measures associated with stress would provide further insight into the utility of this approach (Harvie et al., 2021).

Though our goal was to describe the SV-TSST, there are limitations to the interpretation of the psychological and biological stress results from this implementation. First, since there is no control group, it is not possible to examine group differences in outcome variables associated with specific features of the protocol (Harvie et al., 2021) and the small no-stress group (see Fig. 2) was only exposed to the lab environment. Second, approximately one third of the luteal phase female participants were using hormonal contraceptives and we do not have sufficient sample sizes to explore the contributions of contraceptive use to the stress measures in the SV-TSST or the sex-differences in psychological stress observed in these participants. Third, the responder rates generated within the current study suggest that variables contributing to individual differences in stress reactivity need careful consideration when using this protocol. Future studies using the SV-TSST protocol with diverse participants and exploring the experiential and hormonal contributions to psychological and biological stress would contribute

significantly to our understanding of the dynamics of this protocol and to individual differences in stress reactivity.

CRediT authorship contribution statement

Shelby Sears: Writing – review & editing, Investigation, Data curation. **Robin Brown:** Writing – review & editing, Investigation, Data curation. **Ciara McAfee:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Madeline Divine:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Melissa Miller:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Frances A Champagne:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Robert A. Josephs:** Writing – review & editing, Supervision, Conceptualization. **Rhea Gogia:** Writing – review & editing, Investigation. **Cole Krautkramer:** Writing – review & editing, Investigation.

Declaration of Competing Interest

None

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References

Allen, A.P., Kennedy, P.J., Dockray, S., Cryan, J.F., Dinan, T.G., Clarke, G., 2016. The Trier Social Stress Test: Principles and practice. Neurobiol. Stress 6, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001.

Bali, A., Jaggi, A.S., 2015. Clinical experimental stress studies: Methods and assessment. Rev. Neurosci. 26, 555–579. https://doi.org/10.1515/revneuro-2015-0004.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Birkett, M.A., 2011. The Trier Social Stress Test protocol for inducing psychological stress. J. Vis. Exp. JoVE. https://doi.org/10.3791/3238.

Childs, E., Dlugos, A., De Wit, H., 2010. Cardiovascular, hormonal, and emotional responses to the TSST in relation to sex and menstrual cycle phase. Psychophysiology 47, 550–559. https://doi.org/10.1111/j.1469-8986.2009.00961.x.

Fallon, M.A., Careaga, J.S., Sbarra, D.A., O'Connor, M.-F., 2016. Utility of a Virtual Trier Social Stress Test: Initial findings and benchmarking comparisons. Psychosom. Med. 78, 835. https://doi.org/10.1097/PSY.0000000000000338.

Fallon, M.A., Riem, M.M.E., Kunst, L.E., Kop, W.J., Kupper, N., 2021. Multi-modal responses to the Virtual Reality Trier Social Stress Test: A comparison with standard interpersonal and control conditions. Int. J. Psychophysiol. 161, 27–34. https://doi.org/10.1016/j.ijpsycho.2021.01.010.

Gunnar, M.R., Reid, B.M., Donzella, B., Miller, Z.R., Gardow, S., Tsakonas, N.C., Thomas, K.M., DeJoseph, M., Bendezú, J.J., 2021. Validation of an online version of the Trier Social Stress Test in a study of adolescents. Psychoneuroendocrinology 125, 105111. https://doi.org/10.1016/j.psyneuen.2020.105111.

Halbeisen, G., Domes, G., Walther, E., 2023. Is stress colorblind? Exploring endocrine stress responses in intergroup contexts using a virtual reality-based Trier Social Stress Test (TSST-VR). Psychoneuroendocrinology 147, 105970. https://doi.org/10.1016/j.psyneuen.2022.105970.

Harvie, H.M.K., Jain, B., Nelson, B.W., Knight, E.L., Roos, L.E., Giuliano, R.J., 2021. Induction of acute stress through an internet-delivered Trier Social Stress Test as assessed by photoplethysmography on a smartphone. Stress 24, 1023–1032. https://doi.org/10.1080/10253890.2021.1995714.

Hellhammer, J., Schubert, M., 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, 119–124. https://doi.org/10.1016/j.psyneuen.2011.05.012.

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

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

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

Kothgassner, O.D., Goreis, A., Glenk, L.M., Kafka, J.X., Pfeffer, B., Beutl, L., Kryspin-Exner, I., Hlavacs, H., Palme, R., Felnhofer, A., 2021. Habituation of salivary cortisol and cardiovascular reactivity to a repeated real-life and virtual reality Trier Social Stress Test. Physiol. Behav. 242, 113618. https://doi.org/10.1016/j.physbeh.2021.113618.

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82, 1–26. https://doi.org/10.18637/jss.v082.i13.

McAfee, C.A., Josephs, R.A., DiVita, A., Telch, M.J., Champagne, F.A., 2025. CO2 challenge-evoked hormonal changes predicting TSST changes in cortisol and subjective distress. Psychoneuroendocrinology 171, 107187. https://doi.org/10.1016/j.psyneuen.2024.107187.

McEwen, B.S., 2007. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol. Rev. 87, 873–904. https://doi.org/10.1152/physrev.00041.2006.

McEwen, B.S., Akil, H., 2020. Revisiting the stress concept: Implications for affective disorders. J. Neurosci. 40, 12–21. https://doi.org/10.1523/JNEUROSCI.0733-19.2019.

Miller, R., Plessow, F., Kirschbaum, C., Stalder, T., 2013. Classification criteria for distinguishing cortisol responders from nonresponders to psychosocial stress: Evaluation of salivary cortisol pulse detection in panel designs. Psychosom. Med. 75, 832. https://doi.org/10.1097/PSY.0000000000000002.

Morris, M.C., Rao, U., Wang, L., Garber, J., 2014. Cortisol reactivity to experimentally-manipulated psychosocial stress in young adults at varied risk for depression, 10.1002/da.22125 Depress Anxiety 31. https://doi.org/10.1002/da.22125.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931. https://doi.org/10.1016/s0306-4530(02)00108-7.

Santl, J., Shiban, Y., Plab, A., Wüst, S., Kudielka, B.M., Mühlberger, A., 2019. Gender differences in stress responses during a virtual reality Trier Social Stress Test. Int. J. Virtual Real. 19, 2–15. https://doi.org/10.20870/IJVR.2019.19.2.2912.

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

Tang, X., Misztal, P.K., Nazaroff, W.W., Goldstein, A.H., 2016. Volatile Organic compound emissions from humans indoors. Environ. Sci. Technol. 50, 12686–12694. https://doi.org/10.1021/acs.est.6b04415.