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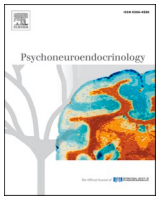

Validation of an online version of the trier social stress test in adult men and women

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ABSTRACT

The Trier Social Stress Test (TSST) is a reliable and efficient protocol to induce acute psychosocial stress in the laboratory. If circumstances do not allow in-person assessments, an online version of the TSST could create more flexible research opportunities. To date, studies have confirmed subjective and autonomic stress responses to online TSST protocols. In this preregistered study (https://osf.io/u57aj), we focused on the effect of a TSST online for adults (TSST-OA) on cortisol and alpha amylase levels, and pleasure and arousal ratings. As cortisol stress reactivity is mediated by sex, we further compared men and women. We hypothesized significant increases in cortisol, alpha amylase and arousal, and a decrease in pleasure in response to the TSST-OA. Also, we expected stronger cortisol responses in males as compared with females, as in the laboratory TSST. $N = 48$ adults (56% female, $mean_{age}$=23.02 years, SD=3.19) participated in the study. Saliva sampling devices were sent to participants' home before testing sessions, during which the experimenter, a mixed-sex panel, and the participant joined a video call. Participants underwent the TSST-OA and overall provided five saliva samples for cortisol and alpha amylase detection. Pleasure and arousal ratings and psychometric questionnaires were also completed online. As hypothesized, the TSST-OA significantly increased cortisol, alpha amylase, and arousal levels, while it decreased pleasure. Moreover, cortisol responses were significantly stronger in males as compared to females. 64% of subjects were classified as responders (cortisol rise>1.5nmol/l). The TSST-OA successfully induced psychophysiological stress in adults. Our protocol offers new possibilities to study stress outside of the laboratory.

1. Introduction

Environmental threats challenge organism's homeostasis and an appropriate response to such stressors is essential for survival. On the physiological level, the autonomic nervous system (ANS) and the hypothalamic pituitary adrenal (HPA) axis orchestrate the restoration and maintenance of homeostasis by mediating adaptive cardiovascular and metabolic processes through their hormonal end products adrenaline and cortisol (Sapolsky, 2000; Ulrich-Lai and Herman, 2009). The regulation of the ANS and HPA axis in response to stress is an important determinant of health and disease (Chrousos, 2009) and has therefore been studied extensively in the last decades.

To study the regulation of the acute stress response in humans, various standardized protocols are in use. Protocols that combine elements of uncontrollability and social-evaluative threat have been shown to elicit a stronger activation of the HPA axis (Dickerson and Kemeny, 2004) as compared to solely physiological stressors (e.g., the Cold Pressor Test; Hines and Brown, 1936). One of the most popular and widely-used protocols that combines these elements is the Trier Social Stress Test (TSST; Allen et al., 2017; Kirschbaum et al., 1993). A core component of the TSST is a video-taped mock job interview, during which the participant presents a free speech and performs a difficult arithmetic task in front of a mixed-sex panel (Kirschbaum et al., 1993). Several modifications of the standard TSST have been developed in the last decades, e.g., a protocol that allows stress induction in children and adolescents (Buske-Kirschbaum et al., 1997), in a group setting (von

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Dawans et al., 2011), or in virtual reality (Zimmer et al., 2019). Yet, to date, these variations rely on the participant visiting the laboratory, which might not always be feasible, as not all circumstances allow in-person assessments (e.g., demonstrated in times of contact restriction measures due to the COVID-19 pandemic). Moreover, subpopulations that have difficulties reaching research sites can only be studied at great expense, or not at all. This in turn limits the generalization of results and the spectrum of research questions that can be studied using the TSST. At the same time, current measurement methods (e.g., determination of hormones via saliva and stability of some metabolites at room temperature for certain time periods) allows performing measurements outside of the laboratory. Consequently, an online version of the TSST could create more flexible research opportunities and offer new possibilities to study psychosocial stress in a standardized manner outside of the laboratory (Kirschbaum, 2021).

Previous reports have shown that online variations of the TSST that take place in online video call settings, can elicit significant subjective stress, and ANS response in adults (Harvie et al., 2021; Huneke et al., 2021; Reed et al., 2021). Further, an online adaptation of the TSST has already been shown to significantly increase cortisol, alpha amylase, and subjective stress levels in children (Gunnar et al., 2021). However, to date, we are not aware of a study that investigated the effects of an online TSST on the endocrine stress system in an adult sample. While a pilot study in our lab showed promising results for the efficacy of an online, adult-version of the TSST (TSST-OA) in activating the HPA axis and triggering a cortisol stress response (Meier, Benz et al., 2021), the small sample size questioned the generalizability of the results. Further, the sample did not allow conclusions about the possible effects of biological sex on the cortisol stress response that is well documented in the literature (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005; Liu et al., 2017).

The aim of this study was therefore to validate the efficacy of the TSST-OA in triggering a cortisol stress response in a sample of adult men and women. We sent study materials (i.e., saliva sampling devices) to eligible participants via mail and scheduled a testing session that took place via video call. During the session, participants underwent the TSST-OA (protocol openly available via: https://osf.io/d3zqk/) and provided five saliva samples for cortisol and alpha amylase detection as markers of the HPA axis and the sympathetic branch of the ANS. Further, participants repeatedly rated their current mood on the dimensions arousal and pleasure. We hypothesized that the TSST-OA triggers a significant increase in salivary cortisol, alpha amylase, and subjective arousal while decreasing subjective pleasure (H1). In line with previous reports (Kirschbaum et al., 1999), we expected that the cortisol stress response was higher in males as compared with females in the follicular phase (H2). Accordingly, we expected that *total cortisol output* during the experiment (as indexed by the area under the curve with respect to ground, AUCg, Pruessner et al., 2003), and *cortisol stress reactivity* (as indexed by the area under the curve with respect to increase, AUCi, Pruessner et al., 2003) was higher in males as compared with females.

2. Methods

2.1. Preregistration

The hypotheses of this study and the statistical analysis plan were preregistered on Open Science Framework prior to any human observation of the data (https://osf.io/u57aj; date of registration: February 9, 2022). This preregistration focused on cortisol as the main outcome of the study.

2.2. Sample size rational

To estimate our sample size, we conducted a power analysis in G*Power (Faul et al., 2007) before data collection. The power analysis was based on the interaction hypothesis (H2), in which we planned to compare the cortisol trajectories (within subject factor, five timepoints) of two groups (between subject factor, men and women). We assumed a small (f=0.1) to medium (f=0.25) effect (mean f=0.175) and wanted to achieve 80% power. In our pilot data (Meier, Benz et al., 2021), the cortisol values correlated with $r = 0.63$ within subjects on average. Using these estimates, a total sample of N = 32 (16 males and 16 females) was needed. Since the effect size is based on rough estimates and to account for potential dropouts or exclusions, we planned to test a minimum of N = 20, and a maximum of N = 25 participants per group. We stopped recruiting participants as soon as a minimum of N = 20 participants were tested in each group (males and females).

2.3. Recruitment and exclusion criteria

We recruited participants via the distribution of flyers at the facilities of the University of Konstanz, the participant database SONA of the University of Konstanz, and different social media platforms (Instagram, Facebook). Before an invitation to a testing session, participants filled in an online screening questionnaire on the platform Qualtrics (duration: approximately 10 min). During the screening, participants reported their assigned sex at birth (male, female, intersex; intersex people were not invited due to our interest in comparing males to females). We applied the following exclusion criteria: 1) age < 18 and > 40 years (to ensure all female participants were pre-menopause), 2) body mass index (BMI) indicating underweight (< 18.5 kg/m^2) or obesity (>30 kg/m^2), 3) use of hormonal contraception (including intrauterine device), lack of (regular) menstrual cycle or current pregnancy in women, 5) smoking more than five cigarettes per day, 6) working nightshifts, 7) physical or mental illness affecting HPA axis regulation, 8) medication intake affecting HPA axis regulation (e.g., antihistaminic medication), and 9) depressive symptoms (Beck's Depression Inventory sum score > 18; Kühner et al., 2007). In addition, participants had to ensure their access to a stable internet connection, a laptop (or similar device) that allowed for video calls, and an undisturbed room.

2.4. Experimental procedure

Eligible participants were invited to participate in the study. The study material (Salivettes to obtain saliva samples for cortisol detection; one piece of Dextro Energy dextrose; paper) was sent to their home via mail. Testing sessions of women were scheduled to take place in the early follicular phase of their menstrual cycle (estimation based on 2–3 last menstrual cycles, invitation at day 1–7 of the next cycle; Schmalenberger et al., 2021). The testing session took place via the videocall platform Zoom™ (https://www.zoom.us) at either 3 or 5 p.m. and lasted for approximately 75 min. Participants were asked to refrain from smoking, eating, and drinking 2 h before the session (except for water and unsweetened tea). They were asked to avoid exercise on the day of the session, and to stick to their usual sleep routine the night before testing. The study procedure is depicted in Fig. 1.

Participants entered the videocall, were welcomed, completed a technical check (video and audio quality), and gave written informed consent. Webcams of the experimenter and participant were activated throughout the experiment. Via the videocall chat, the experimenter sent a link to an online questionnaire, so that participants could fill in the questionnaires online. First, a psychophysiological baseline (salivary sample and mood rating) was assessed. After that, participants consumed one piece of Dextro Energy (5 g dextrose with blackcurrant flavor and minimal amount of citric acid, without caffeine; provided with study material) to control blood glucose levels (Bentele et al., 2021; Meier, Bentele et al., 2021; von Dawans et al., 2020; Zänkert et al., 2020). This was followed by a questionnaire period which allowed the uptake of the dextrose into the bloodstream, and the normalization of salivary pH levels and salivary flow rate (Millward et al., 1997). Then, participants were exposed to the TSST-OA. After a preparation period, participants entered a recorded breakout session and performed a free

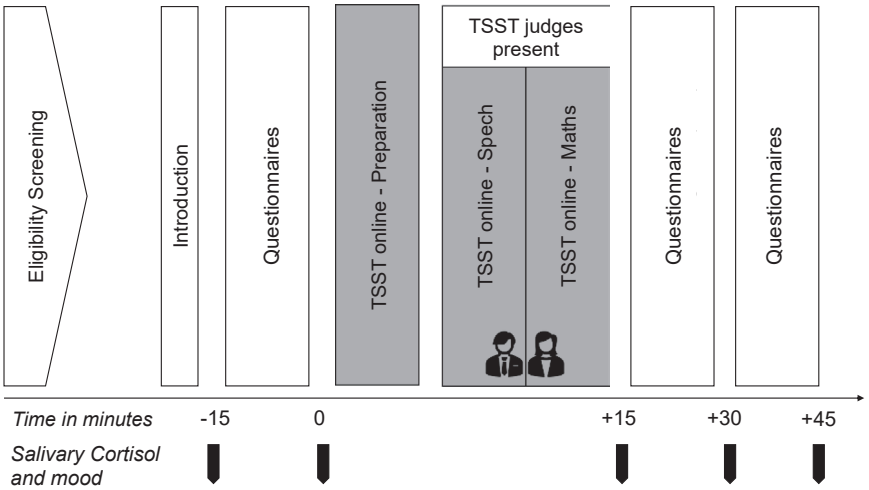

Fig. 1. Study procedure. Sessions took place via videocall. Participants received a dextrose load after baseline (−14 min). TSST = Trier Social Stress Test.

speech task and an arithmetic task in front of a two member, mixed-sex panel. In the subsequent recovery period, participants returned to the main videocall session, in which the experimenter was present, and filled in questionnaires. Overall, five saliva samples for later cortisol detection, and five concurrent mood ratings (Affect Grid and single item visual analog scales) were assessed throughout the session. In the end, participants were debriefed. They received a 15€ Amazon voucher or 1.5 h course credits as soon as the Salivettes were returned using the provided stamped envelopes.

2.5. Tasks and measures

2.5.1. Online, adult-version of the Trier Social Stress Test (TSST-OA)

Acute stress was induced by exposing participants to the Trier Social Stress Test (TSST), with the main modification being that the task was performed online via videocall (e.g., on the platform Zoom™). Inspired by a TSST study in children (Gunnar et al., 2021), we translated the standard protocol for adults to an online setting while maintaining the core components and tasks.

The procedure was introduced by the experimenter just before the start of the TSST-OA. The participant was told that they will undergo a fictious, videotaped job interview for a job of choice including a free speech and an arithmetic task which they perform in front of a two-member, mixed-sex committee. The participant was given 5 min to prepare their free speech in the main session of the videocall. During this preparation, the participant was allowed to take notes on a white paper provided with the study material. At the end of the preparation period, they had to fold the paper and put it aside. Then, the participant was asked to stand up and back away from the camera so that they were visible from the waist upwards. As soon as audio and video quality from distance were confirmed, the participant joined a recorded breakout session, in which the mixed-sex panel awaited them (panel members joined the call separately). The panel members wore neutral, professional clothes, and sat in front of a neutral background. As in the standard TSST, they were trained to only show neutral facial expressions. After ensuring that the screen of the participant was showing the "gallery view" (so that both panel members were visible to the participant), the panel asked the participant to present their speech (5 min) and perform a mental arithmetic task (5 min). At the end of the task, the recording was stopped, and the participant was redirected to the experimenter in the main videocall session. A more detailed protocol of the TSST-OA is available online (https://osf.io/q6tr9/).

2.5.2. Salivary Cortisol and Alpha Amylase

Five saliva samples for detection of free cortisol (nmol/l) and alpha amylase (U/ml) were collected using Salivettes (Sarstedt, Nümbrecht, Germany; Gröschl et al., 2008). The biochemical analysis took place in the biochemical laboratory of the Department of Neuropsychology of the University of Konstanz. Participants were asked to store the samples in the fridge until they shipped them back to the laboratory, where they were stored at − 20 °C until analysis. For cortisol analysis, samples were analyzed in duplicates using a commercially available competitive enzyme immunosorbent assay (Cortisol Saliva ELISA, RE-52611, IBL International GmbH, Hamburg, Germany). Thawed samples were centrifuged at 2500 g for 10 min. For alpha amylase analysis, samples were thawed a second time and analyzed in duplicates using a commercially available liquid phase enzymatic assay (alpha-Amylase Saliva Assay, RE-80111, IBL International GmbH, Hamburg, Germany). In five samples, the amount of saliva was too small for analysis, and they were thereby excluded. Furthermore, 24 samples exceeded the upper detection limit, so they were re-analyzed after dilution. All inter- and intra-assay coefficients of variation were in the acceptable range.

2.5.3. Subjective arousal and pleasure

Concurrently to taking saliva samples, participants rated their current mood using the Affect Grid (Russell et al., 1989). The Affect Grid is a single item scale that is rated on a 9 × 9 grid. The grid spans the two dimensions displeasure/pleasure, and sleepiness/arousal. Values on each dimension range from 1 to 9, with higher values indicating higher arousal, or higher pleasure respectively.

2.5.4. Questionnaires

Participants filled in several questionnaires during the experimental session. The questionnaire data are not part of the current preregistered hypotheses but are used to describe the sample. We used the sum score of the Beck's Depression Inventory II (Kühner et al., 2007) to index self-reported depressive symptoms. Self-esteem was assessed using the sum score of the Rosenberg Self-Esteem Scale (Rosenberg, 2002). Self-reported perceived stress was measured using the sum score of the 10 item Perceived Stress Scale (Klein et al., 2016). Childhood trauma was measured using the sum score of the Childhood Trauma Questionnaire (Bernstein et al., 2003). A complete list of the questionnaires assessed can be found on the OSF website related to this project (https://osf.io/d3zqk/).

2.6. Participants

Overall, $N = 48$ adults (56.00% females, $mean_{age}$=23.02 years, SD=3.19) participated in the study. Since prior exposure to the TSST can lead to habituation of the stress response, we excluded participants that

reported that they had been exposed to any variation of the TSST within the last 4 months (Kexel et al., 2021). This applied to one person who took part in another TSST study 4 days before the testing session. The sample analyzed in the following thus comprised $n = 47$ adults (55.00% females, $mean_{age}$=23.11 years, SD=3.16).

2.7. Data processing

The cortisol, alpha amylase, and subjective arousal and pleasure data were screened for missing values. Missing data were imputed using the mean of the respective *group* (males, females) at the respective timepoint. We defined outliers in the cortisol data as values that exceed the mean of the *group* (male, female) by more than 3 standard deviations (SD). To decrease the impact of such values on our results, cortisol and alpha amylase values were winsorized across groups, so that outliers were replaced with values that were equivalent to 3 SD above the respective group mean (applied to 3 cortisol and 3 alpha amylase values). Using the winsorized cortisol data, we calculated the area under the curve with respect to ground (AUCg) across the complete time course of the study as an index of *total cortisol output* during the experiment, and the area under the curve with respect to increase (AUCi) across the complete time course of the study as an index of *cortisol stress reactivity* (Pruessner et al., 2003). Analogously, AUCg and AUCi of alpha amylase, subjective arousal and pleasure levels were computed. Since the cortisol data lacked normality (Shapiro-Wilk test: W=0.787, $p < .001$), we transformed the values using Box Cox transformation as recommended for longitudinal endocrine data (Miller and Plessow, 2013).

To compare cortisol responder rates of the TSST-OA to other published studies in the field, we calculated the percentage change in cortisol values from baseline (−15 min) to expected peak (+30 min) concentrations (baseline-to-peak-increase in %) and defined cortisol non-responders as participants with a baseline-to-peak-increase of < 15.5%, or 1.5nmol/l (Miller et al., 2013), or *cortisol stress reactivity* < 0 respectively.

2.8. Statistical analysis

Analyses were conducted using R version 4.0.3 (R Core Team, 2019), RStudio version 1.4.1106 (RStudio Team, 2016), and *nlme* (Pinheiro et al., 2018). Graphs were created using *ggplot2* (Wickham, 2016) and *patchwork* (Pedersen, 2019). The level of significance was set to alpha= 0.05.

For descriptive purposes, we compared demographic and personality characteristics of males and females using t-tests and Chi squared tests.

To test whether the TSST-OA triggered a significant cortisol stress response, we modeled cortisol changes over time using a growth curve approach within a multilevel modeling framework. By doing so we could consider individual differences in cortisol baseline (random intercepts) and cortisol trajectories over time (random slopes) in our model (Curran et al., 2010). We modeled a linear, quadratic, and cubic fixed effect of time. Further, since repeated measures of cortisol are usually correlated (r~.63) we added a first-order autoregressive covariance structure (AR1). We used a stepwise approach to build the models and compared the overall model fit of the nested models using the log-likelihood ratio and evaluated the final model including all random and fixed effects. In case of model convergence problems, we simplified the complexity of the random effect structure (e.g., by excluding higher order random slopes).

In addition to the growth model, we used Bonferroni corrected post-hoc t-test to conduct pairwise comparisons of the five timepoints (e.g., to contrast the baseline at −15 min and the expected post-stress peak at +30 min). To allow for comparison between the magnitude of the cortisol stress response to the TSST-OA and the standard TSST, we calculated the effect size of cortisol change from baseline to peak (Goodman et al., 2017).

To test whether the cortisol stress response to the TSST-OA was significantly higher in males as compared to females, we added the independent variable *group* (male, female) to our growth curve and evaluated the main effect of group, and the interaction effect of group by time in the final model. We followed up with the calculation of Bonferroni corrected post-hoc t-tests to test whether the cortisol values after stress onset are higher in males as compared to females. Further, we compared *total cortisol output* (AUGg) and *cortisol stress reactivity* (AUCi) of males and females by using t-tests.

Complementing the preregistration, the same analyses were conducted using alpha amylase, subjective arousal, and pleasure as outcome variables. Further, we exploratively compared whether cortisol stress responders were distributed differently among men and women using Chi squared tests.

3. Results

To maintain the clarity and brevity of this report, we summarize the core results in the following. The detailed results (including results of nested model comparisons etc.) can be found in the supplemental material and recalculated using the available analysis script and data (see https://osf.io/d3zqk/). Descriptive statistics of the groups (males, females) are summarized in Table 1.

3.1. Cortisol stress response

The inclusion of random intercepts, random slopes, a quadratic, and cubic trend of time as well as both, a main effect of sex, and the sex by time interactions led to significant increases in model fit of the growth curve. Coefficients of the final model can be retrieved from Table 2. We found that cortisol levels changed significantly throughout the experiment, with the time trend being best described by a cubic effect (see Fig. 2A). Bonferroni corrected post-hoc t-test confirmed that the TSST-OA led to a significant increase in cortisol levels from levels before stress to post stress levels, with the peak being reached + 30 min after stressor onset, which is in line with previous standard TSST studies (Kudielka and Kirschbaum, 2005). The effect size of cortisol change from baseline to peak was d= 1.08, which is comparable to effect sizes reported for the standard TSST (cf. d'=0.925 in Goodman et al., 2017).

The cortisol response to the TSST-OA was significantly higher in males as compared with females, which was reflected in a significant *group* by quadratic *time* interaction effect (see Table 2), and significantly higher peak levels in males (mean=14.39, SD=9.61) as compared with females (mean=5.83, SD=4.65) at timepoint + 30 min. The results of all Bonferroni corrected post-hoc t-test and the mean cortisol values per

Table 1
Descriptive characteristics of the sample.

	Females ($n = 26$)	Males ($n = 21$)	*p-value*
age	21.65 ± 2.06	24□90 ± 3.40	$p < .001$
BMI[a]	21.86 ± 2.09	24.11 ± 3.51	$p = .015$
depressiveness[b]	5.08 ± 3.38	4.76 ± 4.53	$p = .793$
childhood trauma[c]	1.44 ± 1.16	1.33 ± 1.06	$p = .747$
chronic stress level[d]	31.32 ± 3.35	31.71 ± 4.27	$p = .733$
self-esteem[e]	31.77 ± 4.97	34.29 ± 4.22	$p = .070$
cortisol baseline (nmol/l)[f]	3.26 ± 2.28	4.49 ± 2.73	$p = .105$
alpha amylase baseline (U/ml)[f]	176.4 ± 111.96	201.86 ± 120.17	$p = .471$

Note. If not otherwise specified, an independent t-test comparing groups was calculated to test whether groups differed in respect to the listed variables. In these cases, data is expressed as *mean* ± *standard deviation*.
[a] BMI=body mass index,
[b] indexed by Beck's Depression Inventory II sum score,
[c] indexed by Childhood Trauma Questionnaire sum score,
[d] indexed by Perceived Stress Scale sum score,
[e] indexed by Rosebnerg Self-Esteem Scale sum score,
[f] based on raw values.

Table 2
Coefficients of the growth curve model (number of observations: 235, number of participants: 47) predicting cortisol changes over time by group (females, males).

	Unconditional		Conditional	
Fixed effects	Estimate	SE	Estimate	SE
Baseline level, β_0	1.99 * **	0.17	1.46 * **	0.20
Time linear, $\beta_{1.1}$	4.32 * **	0.95	2.55 *	1.22
Time quadratic, $\beta_{1.2}$	-3.54 * **	0.71	-1.95 *	0.89
Time cubic, $\beta_{1.3}$	-2.23 * **	0.60	-2.48 * *	0.82
Group, β_2	–	–	1.20 * **	0.30
Time linear by Group, $\beta_{3.1}$	–	–	3.97 *	1.83
Time quadratic by Group, $\beta_{3.2}$	–	–	-3.56 * *	1.33
Time cubic by Group, $\beta_{3.3}$	–	–	0.56	1.22
Random effects	SD	covariance baseline-slope	SD	covariance baseline-slope
Variance baseline level, b_{0i}	1.14	–	.98	–
Variance slope linear, $b_{1.1i}$	5.92	.34	5.58	.21
Variance slope quadratic, $b_{1.2i}$	4.07	-0.90	3.69	-0.87
Variance slope cubic, $b_{1.3i}$	3.23	.18	3.23	.15
Residual, ε_{ti}	0.37	–	0.37	–

Note. The unconditional growth model does not include the main effect of *group* and *group*time* interaction terms, while the conditional model does. Please note that the model was calculated using Box Cox transformed values, which is why coefficients do not represent nmol/l. SE=standard error. SD=standard deviation * $p < .05$, * * $p < .01$, * ** $p < .001$.

group at each timepoint are reported in the supplemental material.

We found significantly higher *total cortisol output* (AUGg) in males (mean=615.63, SD=376.05) as compared with females (mean=266.89, SD=186.91), $t(27.91) = -3.88$, $p < .001$, $d = -1.22$, and higher *cortisol stress reactivity* (AUCi) in males (mean=346.06, SD=317.85) as compared with females (mean=71.42, SD=149.09), $t(27.06) = -3.65$, $p = .001$, $d = -1.15$.

Counting cortisol responder rate based on the baseline-to-peak-increase in % (Miller et al., 2013), all participants were rated as responders. In contrast, when counted based on the 1.5nmol/l criterium (Miller et al., 2013), 64% of participants were responders, with no significant difference between men and women, $Chi^2(1) = 3.57$, $p = .059$. A comparable picture emerged when using positive *cortisol stress reactivity* (AUCi>0) as responder criterium (79% responders, no significant difference between men and women, $Chi^2(1) = 1.99$, $p = .158$.

3.2. Alpha amylase stress response

The inclusion of random intercepts, and a quadratic, and cubic trend of time led to significant increases in model fit of the growth curve. Neither the main effect of *group* (males, females) nor the *group* by *time* interactions led to significant improvements of the model. Coefficients of the final model can be retrieved from Table 3. Alpha amylase levels changed significantly over the course of the experiment (see Fig. 2B), with Bonferroni corrected post-hoc t-test confirming that alpha amylase levels increased significantly in response to the TSST-OA. Peak levels could be observed + 15 min after stressor onset, which is in line with previous standard TSST studies (Nater and Rohleder, 2009). The effect size of alpha amylase change from baseline to peak was $d = 0.46$.

The response did not significantly differ between males and females (no significant *group*time* interaction effect in the growth curve approach, no difference in *total alpha amylase output* or *alpha amylase stress reactivity*). The results of all Bonferroni corrected post-hoc t-test and the mean alpha amylase values per *group* at each timepoint are reported in the supplemental material.

3.3. Subjective arousal and pleasure ratings

Modeling changes in subjective arousal over time, only the inclusion of a quadratic time trend led to significant increases in model fit. Arousal levels increased in response to the TSST-OA and decreased thereafter, with the highest ratings being observed + 15 min after stressor onset (see Fig. 2C). The response did not significantly differ between males

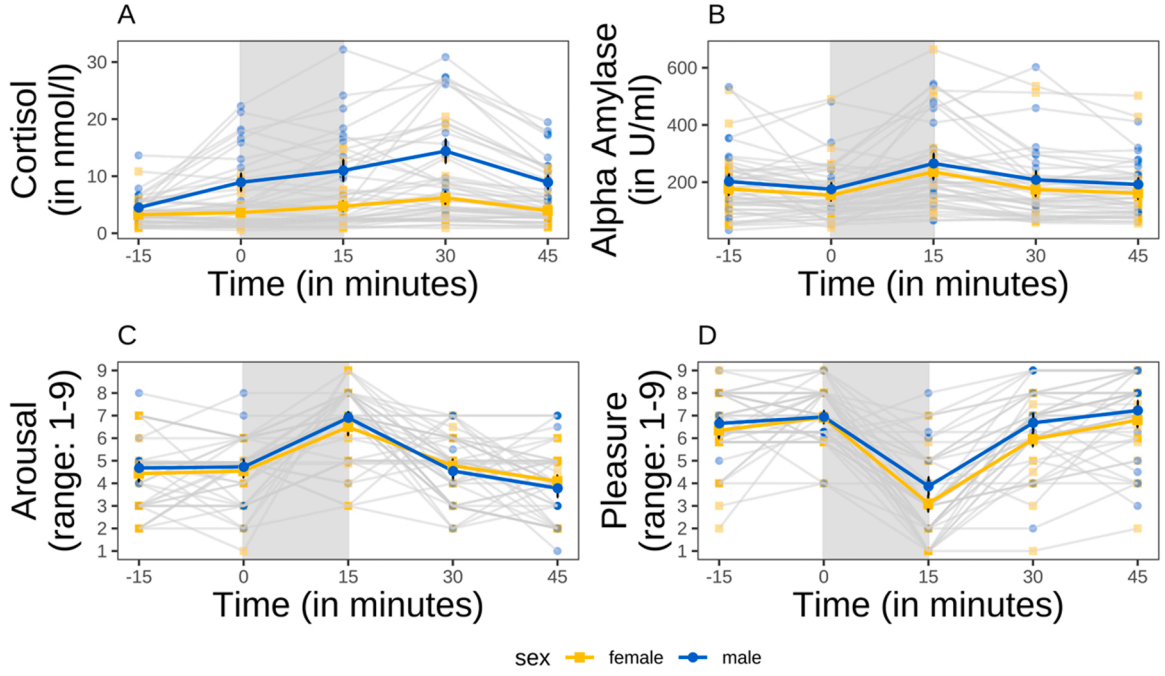

Fig. 2. Changes in (A) cortisol, (B) alpha amylase, (C) subjective arousal, and (D) subjective pleasure in males (blue circles) and females (yellow squares) over the course of the experiment. Shaded area=TSST-OA speech and mental arithmetic task.

Table 3
Coefficients of the growth curve model (number of observations: 235, number of participants: 47) predicting alpha amylase changes over time by *group* (females, males).

	Unconditional		Conditional	
Fixed effects	Estimate	SE	Estimate	SE
Baseline level, β_0	192.48 * **	14.77	179.56 * **	19.82
Time linear, $\beta_{1.1}$	-1.15	78.61	-23.28	106.54
Time quadratic, $\beta_{1.2}$	-218.48 * *	69.49	-209.94 *	94.16
Time cubic, $\beta_{1.3}$	-137.00 *	62.61	-116.65	84.85
Group, β_2	–	–	28.93	29.65
Time linear by Group, $\beta_{3.1}$	–	–	49.76	159.38
Time quadratic by Group, $\beta_{3.2}$	–	–	-19.11	140.87
Time cubic by Group, $\beta_{3.3}$	–	–	-45.55	126.94
Random effects	SD	covariance baseline-slope	SD	covariance baseline-slope
Variance baseline level, b_{0i}	96.44	–	95.36	–
Variance slope linear, $b_{1.1i}$	323.11	.26	322.85	.25
Variance slope quadratic, $b_{1.2i}$	204.92	-0.73	204.56	-0.73
Residual, ε_{ti}	62.07	–	62.03	–

Note. The unconditional growth model does not include the main effect of *group* and *group*time* interaction terms, while the conditional model does. SE=standard error. SD=standard deviation * $p < .05$, * * $p < .01$, * ** $p < .001$.

and females (no significant *group*time* interaction effect in the growth curve approach, no difference in AUCg or AUCi). Results of the model comparisons and coefficients of the final model can be retrieved from the supplemental information.

Correspondingly, when looking at subjective pleasure ratings, only the inclusion of the correlation structure led to a significant increase in model fit. Results of the model comparisons and coefficients of the final model can be retrieved from the supplemental information. Pleasure ratings decreased in response to the TSST-OA and increased in the recovery phase, with the lowest ratings being observed directly after stressor cessation (see Fig. 2D). Males and females did not significantly differ in their ratings (no significant *group*time* interaction effect in the growth curve approach, no difference in AUCg or AUCi).

4. Discussion

The results showed that our TSST-OA protocol successfully triggered a cortisol, alpha amylase, and subjective stress response in adults. The cortisol stress response was higher in males as compared with females in the follicular phase (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005; Liu et al., 2017), but alpha amylase stress responsivity (van Stegeren et al., 2008), and arousal and pleasure ratings did not differ between sexes. Based on effect size measures, the cortisol response elicited by the TSST-OA (d=1.08) was comparable to the standard TSST in adults (cf. d'=0.925 in Goodman et al., 2017). Based on the 1.5nmol/l criterium (Miller et al., 2013), 64% of subjects were classified as responders, with no significant difference between sexes. Compared to the standard TSST, the TSST-OA might thus be slightly less effective (e.g., responder rate of > 70% reported in Kudielka and Kirschbaum, 2005; yet we must note that responder rate criteria are inconsistently used and reported, which complicates comparability between studies). Overall, our results are in line with and expand previous studies that showed significant subjective and autonomic stress responses to online TSST protocols (Harvie et al., 2021; Huneke et al., 2021; Reed et al., 2021). By confirming a significant increase in cortisol, we could moreover ensure an activation of the main endocrine stress system, the HPA axis.

The sex-dimorphic pattern in cortisol stress responses is consistent with previous studies investigating stress-induced changes in free, biologically active cortisol. It might be related to basal gonadal hormone differences, i.e. estradiol and testosterone levels, that have been linked to HPA axis responsivity in animals and humans (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005). Yet, besides hormonal differences, other factors could have contributed to the sex dimorphic pattern. For example, women are particularly sensitive to stress paradigms involving social rejection, as compared with achievement challenges (Kudielka and Kirschbaum, 2005; Stroud et al., 2002). If judges in the TSST-OA are not as salient as compared with the standard TSST, because of limited exposure through a computer screen for example, this could particularly affect women's cortisol stress responses. Indeed, a panel out-version of the TSST, in which the judges are sitting behind a one-way mirror (Andrews et al., 2007; Juster et al., 2012; Lupien et al., 1997; Marin et al., 2012; Raymond et al., 2019; Wadiwalla et al., 2010), seems to maximize sex-specific effects by decreasing heterosexual women's cortisol response as compared to the standard TSST (Juster et al., 2015). As such, the decreased responsivity of women as compared with men in our study could either be due to gonadal hormone differences (Kudielka and Kirschbaum, 2005), or effects of sex and gender attribution on the perception of the stressor (Juster et al., 2015; Stroud et al., 2002). While our data do not serve to answer which factor weighs particularly strong, one strength of our study is that we controlled for the match of sex and gender identification (all participants reported to be cis) and tested all female participants in the early follicular phase of their menstrual cycle. Future studies should elaborate on the question of whether the TSST-OA is equally effective in eliciting a cortisol stress response in different population subgroups as compared to the standard TSST or variants of it.

Overall, we can conclude that the TSST-OA successfully triggered an acute psychophysiological stress response in adults. While shipping the study material (e.g., Salivettes) to participants before the testing session involves an extra but manageable amount of planning and financial effort, the TSST-OA protocol offers new opportunities to study acute psychosocial stress without the need of bringing participants to the laboratory.

A limitation of the TSST-OA procedure is that participants need to have access to a stable internet connection, an undisturbed room, and a laptop with a webcam to conduct the testing session. Along that line, we cannot assume that all populations are equally familiar with the use of laptops and video conferencing platforms, or equally willing to (at least partly) share their living conditions by turning on the webcam during the testing session. These factors can on the one hand lead to a sampling bias that impacts the generalizability of results. On the other hand, they can complicate running the testing session with a direct impact on data quality or usability. If it is unclear whether participants can join without any obstacles due to unfamiliarity with the remote setup, it might be useful to conduct an introductory session before the actual experiment, during which the handling of the video conferencing platform is explained (Gunnar et al., 2021). Overall, however, we believe that the possibility of conducting acute stress research remotely can enrich the spectrum of research questions being investigated in the future at large.

While this validation of the TSST-OA protocol showed that it can induce acute psychosocial stress, we need to consider some limitations of our study when interpreting the results. First, the recruited participants were young adults, which limits generalizability regarding the findings and regarding ease of conducting the remote protocol, as this age group is probably very familiar with video calls. Second, we did not ask participants for the size of the laptop screen they used to conduct the study (e.g., seen in Gunnar et al., 2021). Related to this, although we asked participants to run the video conference in gallery mode, we did not check this by, for example, letting participants show a captured cell

phone image (Gunnar et al., 2021). These are two differences to the TSST-OL protocol introduced by Gunnar and colleagues (2021), and these variables could impact the visibility and therefore salience of the panel during the stressor, and consequently, as discussed above, the cortisol stress response of women in particular (Kudielka and Kirschbaum, 2005; Stroud et al., 2002). Future studies should thus test the applicability of the TSST-OA in various populations and age groups, and, if possible, control for variables that could impact the presence of the judges during the stressor.

Taken together, we demonstrated that delivering the TSST in an online environment via videocall can induce robust cortisol and alpha amylase stress responses in adult men and women. This remote version offers several opportunities that, in our opinion, remedy the cost of organising the shipping of study material. For example, while we previously had problems recruiting eligible male participants in on-site stress studies (Bentele et al., 2021; Meier, Bentele et al., 2021), we were able to draw on a larger reach using the TSST-OA (indeed, almost 50% of the sample were not present locally at the time of the study), and thus achieve a balanced male-female ratio. Further, as already discussed by colleagues (Gunnar et al., 2021), we could work independently of room availability at the faculty, all people involved in testing could join the session from home, and the panel could even work on parallel sessions as they only joined the experiment for 20 min. This overall highly increased flexibility and efficiency of testing sessions. While possible sampling bias might need to be considered, this is an issue that also plays a role in on-site studies. As such we conclude that the TSST-OA offers exciting new and more flexible opportunities to study stress in the digital age, where online meetings are becoming the norm rather than the exception in remote work environments, and the TSST-OA may open new avenues to more fully examine stress in the digital work context.

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Preregistration, open material, data, and scripts

Preregistration, materials (the protocol of the TSST-OA), and analysis script, as well as data of this study and of a pilot study (Meier, Benz et al., 2021) are available online: https://osf.io/d3zqk/. A preprint of this manuscript has published on psyarxiv: https://psyarxiv.com/d2rph/.

CRediT authorship contribution statement

Maria Meier: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – original draft, Visualization. **Kristina Haub**: Methodology, Investigation, Data curation, Writing – review & editing. **Marie-Luise Schramm**: Methodology, Investigation, Data curation, Writing – review & editing. **Marc Hamma**: Methodology, Investigation, Data curation, Writing – review & editing. **Ulrike U. Bentele**: Writing – review & editing. **Stephanie J. Dimitroff**: Writing – review & editing. **Raphaela Gärtner**: Writing – review & editing. **Bernadette F. Denk**: Writing – review & editing. **Annika B. E. Benz**: Conceptualization, Writing – review & editing. **Eva Unternaehrer**: Writing – review & editing. **Jens C. Pruessner**: Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare to have no conflict of interest.

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