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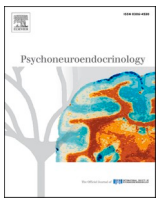

TSST-OL: Comparison between online and laboratory application and effects on empathy

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ABSTRACT

Online test protocols are increasingly popular in psychological and neuroscientific research. Despite its relevance to the social functioning, the influence of acute stress on cognitive and affective state empathy is not clearly understood. Recently, a remote online version (TSST-OL) of the Trier Social Stress Test (TSST) was established for use in research with both children and adults. In general, the TSST-OL offers the opportunity for context-independent application (e.g., at the participants' home or in field contexts). However, in order to exploit this opportunity, it seems crucial to validate the TSST-OL across different settings and contextual variables. We compared stress reactivity in response to the TSST-OL at home and in the laboratory. In a 2 ×2 factorial design, N=120 participants (n=60 women) underwent the TSST-OL and an online adaption of the friendly TSST (fTSST-OL) either at home (n=60) or at the laboratory (n=60). Stress induction was evaluated in terms of physiological (cortisol and salivary alpha-amylase, sAA) and subjective stress and affect measures. Participants also completed an empathy performance task after stress and control exposure. Results confirmed that the TSST-OL successfully induced stress both when conducted at participants' homes and in the laboratory. Still, cortisol levels were higher during laboratory participation compared to application at home, likely due to anticipatory stress. Consequently, the TSST-OL in a home-based application seems to buffer anticipatory stress thus making it an attractive tool to study experimentally induced stress reactivity. Concerning empathy, positive emotions were generally better identified (cognitive empathy) and empathized (affective empathy) than negative emotions. For the latter, this difference was absent after stress, indicated by decreased affective empathy for positive emotions. Overall, this study indicates that the TSST-OL induces stress and validates the tool using a rigorous study design with sufficient participants and relevant stress parameters. Thus, future studies may apply the TSST-OL in different contexts and diverse samples. The findings on empathy under stress align with mixed results in existing research, highlighting the necessity for further investigations into empathy, considering various measurements, stimulus valence, and sex of the participant.

1. Introduction

The current study aimed at further validating an online version of an established stress induction paradigm across different contexts. Such approaches offer opportunities for context-independent testing of more diverse samples, for instance. Moreover, we investigated whether stress affected state empathy in this online setting.

Situations in which external demands surpass internal resources trigger an acute stress response that results in adaptive physiological changes (Dickerson and Kemeny, 2004; Joëls and Baram, 2009; Lazarus and Folkman, 1984). Importantly, the sympathetic nervous system (SNS) is activated, initiating the secretion of catecholamines such as (nor)adrenaline which mediate an increase of physiological activation parameters (e.g., heart rate). Simultaneously, the

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hypothalamus-pituitary-adrenal (HPA) axis is activated with a rapid release of corticotropin-releasing hormone (CRH) in the hypothalamus (Joëls and Baram, 2009), which ultimately results in the secretion of cortisol from the adrenal cortex with a certain temporal delay (Ulrich-Lai and Herman, 2009).

Experimental stress induction paradigms are used to investigate the acute stress response in standardized laboratory contexts. The Trier Social Stress Test (TSST; Kirschbaum et al., 1993) constitutes the gold standard for experimental stress induction (Allen et al., 2017). However, driven by the COVID-19 pandemic, researchers adapted the TSST for online use (Kirschbaum, 2021; Pfeifer et al., 2021). During the so-called TSST-OL, the original TSST protocol is implemented in online video communication software. Gunnar et al. (2021) validated the procedure for an adolescent sample in terms of subjective measures, cortisol and salivary alpha-amylase (sAA) without a control condition. Of note, a preprint by DeJoseph et al. (2019) took a similar approach in exposing adolescents to a screen-based TSST including a prerecorded sham panel in their own homes. However, we refer to the TSST-OL as an online procedure involving a real panel joining live in online communication software. Since then, other studies (e.g., Harvie et al., 2021; DuPont et al., 2022; Eagle et al., 2021) applied different versions of the TSST-OL in adult samples but did not measure cortisol. The absence of cortisol assessment in remote stress procedures is likely due to challenges with implementing saliva sampling, including logistical issues and external factors affecting measurement validity (Heyers et al., 2024; Pfeifer et al., 2021). Recently, Meier et al. (2022) confirmed that the TSST-OL triggers physiological stress reactivity (cortisol and sAA) in adult men and women. Interestingly, men reacted stronger in terms of cortisol – a well-replicated finding for the in-person TSST (e.g., Liu et al., 2017). This similarity in sex-specific responses underscores the qualitative comparability of the two procedures. Recently, Shields et al. (2024) confirmed cortisol reactivity towards the TSST-OL in an adult sample in comparison to a control condition (i.e., an online version of the placebo TSST, pTSST, Het et al., 2009). Moreover, Shields et al. (2024) showed that exposure towards the TSST-OL affected executive processes as assessed online subsequently, suggesting that the TSST-OL serves for studying stress-induced effects on cognition.

Previous studies (Gunnar et al., 2021; Meier et al., 2022; Shields et al., 2024) confirmed cortisol reactivity towards the TSST-OL, but responder rates were slightly lower than for the in-person TSST: According to the 1,5nmol/l criterion (Miller et al., 2013), 43 % (Shields et al., 2024) to 63 % (Gunnar et al., 2021) or 64 % (Meier et al., 2022) were classified as responders towards the TSST-OL while the in-person TSST produces responder rates of 70–80 % (Kudielka et al., 2007), indicating slightly lesser HPA axis activation in the TSST-OL. Through meta-analysis, Goodman et al. (2017) revealed effect sizes of cortisol reactivity in the in-person TSST to be reduced by an extended familiarization phase. In the TSST-OL, participants are in their own homes – a context that is extremely familiar. Thus, one may hypothesize attenuated cortisol reactivity at home. Empirical evidence might be found in the above-mentioned preprint by DeJoseph et al. (2019) who reported decreases in cortisol during their home-based TSST in adolescents. Hence, it is worth investigating whether the context affects cortisol reactivity towards the TSST-OL in adult samples.

Exploring the impact of online stress exposure on emotional functions such as empathy is relevant, as social interactions shift to digital platforms. Acute stress has been documented to influence empathy even though mechanisms are not yet clearly understood (Nitschke and Bartz, 2023; Shields et al., 2016; von Dawans, Strojny, and Domes, 2021). Empathy can be considered a trait or a state. State empathy is the situated expression of trait empathy and is influenced by external factors (Cuff et al., 2016; Håkansson Eklund and Summer Meranius, 2021; Zhao et al., 2022). Importantly, empathy can be divided into cognitive empathy (understanding what another person feels) and affective empathy (feeling the same emotion as the observed person) (Cuff et al., 2016). Both facets have been reported to be influenced by acute stress (Nitschke and Bartz, 2023). Emotion recognition (a key component of cognitive empathy) benefits from prior stress exposure (Deckers et al., 2015; Domes and Zimmer, 2019), even though this effect is restricted to positive emotions (von Dawans et al., 2020). Importantly, several studies also reported no stress-induced effect on cognitive state empathy (Graumann et al., 2021; Smeets et al., 2009; Wingenfeld et al., 2018). Nitschke and Bartz (2023) highlighted that these differences could arise from the choice of the empathy task, the sex of the participant (Baez et al., 2017; Dorris et al., 2022; Nitschke et al., 2022) or the protagonist (Gamsakhurdashvili et al., 2021), and the valence of stimuli (Mogg et al., 1990; Wolf, 2009). Interestingly, when it comes to affective empathy several studies conclusively suggested stress exerts beneficial effects (Gonzalez-Liencres et al., 2016; Wingenfeld et al., 2014; Wolf, 2009).

With the current preregistered study, we aimed to further validate the TSST-OL in an adult sample by measuring subjective and physiological stress measures and in comparison to a control condition, the friendly TSST (Wiemers et al., 2013) in an online variant (fTSST-OL; preregistered hypothesis H1). Moreover, we extended the validation of the TSST-OL by a context dimension in comparing stress reactivity at home and in the laboratory (preregistered hypotheses H2.a-H2.b). Finally, the current study examined whether cognitive and affective state empathy might be affected by exposure to the TSST-OL, context, sex of participant, and valence of stimuli (preregistered hypotheses H3.a-H3.f).

2. Methods

2.1. Participants

An a-priori power analysis using G*Power3.1 (Faul et al., 2007) suggested a required sample size of N=116 participants for an analyses of variance (ANOVA) with repeated measures and within-between interactions (α=.05, 1-ß>.95, f=.20, r=.30). The final sample consisted of N=120 healthy participants (n=60 women). Table 1 provides an overview over demographic sample characteristics divided for the two contexts (home vs. laboratory) while a summary across the two contexts as well as a detailed description of inclusion criteria can be found in the Appendix A.1.

Participants were recruited via online advertisements and local announcements. The study was approved by the local ethics committee of the faculty of Psychology at Ruhr University Bochum and conducted in accordance with the Declaration of Helsinki. Participants were reimbursed with 35€ or course credit. The study design as well as statistical analyses were preregistered at Open Science Framework (OSF) https://osf.io/q6tn7/.

2.2. Design and procedure

The current study used a 2 ×2 mixed factorial design with the within-subjects factor *session* (TSST-OL vs. fTSST-OL) and the between-subjects factor *context* (home vs. laboratory). Half of the participants (n=60, n=30 women) were randomly assigned to either participate in the laboratory or at their own homes. In both groups, participants alternated

Table 1
Overview over demographic sample characteristics.

	Home-based participants		Laboratory-based participants		*p*
n (*women*)	60 (*30*)		60 (*30*)		
	M	*SD*	*M*	*SD*	
BMI (kg/m²)	22.49	2.09	22.48	1.84	>.05
Age	24.78	4.53	23.28	3.72	>.05

Note. M=Mean; SD=Standard Deviation; BMI=Body Mass Index.

starting with the stress or control session. Both sessions were completed within seven days.

The experimental procedure is illustrated in Fig. 1. Participants tested from the laboratory were welcomed by an experimenter and seated in a laboratory room. A computer was started, and they were left alone since the rest of the experimental session took place via Zoom™ (https://www.zoom.us). Participants tested from home were welcomed in the Zoom meeting directly. After having given written consent, participants completed the first assessment (T1) of stress and affect measures (see Section 2.4.). Subsequently, we inserted a familiarization phase which was used to explain behavioral tests that were executed after stress or control induction (day 1) or to perform an audiometer test (day 2) (results on the audiometer test are not reported in the current manuscript). After having given stress and affect measures for the second time (T2), participants were transferred to a breakout room in which they completed the TSST-OL or the fTSST-OL (see Section 2.3.). Here, they also gave a third assessment of stress and affect measures (T3). After completion of the TSST-OL and the fTSST-OL, participants returned to the main session where they completed stress and affect measures for a fourth time (T4). Finally, participants completed behavioral tasks assessing empathy (see Section 2.5.) and lateralization outcomes (for results concerning these lateralization outcomes, see Pfeifer et al., 2024). Afterwards, stress and affect measures were assessed for the fifth time (T5). At the end of the session, participants were reminded of the second session (day 1) or debriefed and compensated (day 2).

2.3. Stress and control induction

To induce stress, participants were exposed to the TSST-OL (Gunnar et al., 2021) in the breakout room of the Zoom meeting. That is, after a 5-min. preparation phase including a third assessment of stress and affect markers, participants gave a 5-min. speech in front of a social-evaluative panel (one man, one woman in white lab coats). After the speech, participants performed a 5-min. math part which required serial subtraction. Of note, participants were not aware of the duration of the different task parts. Moreover, during the whole procedure, the panel was neutral and did not provide any feedback. Participants were asked to take an upward position and to fixate the screen to keep eye contact with the panel. As a control condition, participants underwent the fTSST-OL which was realized in line with the procedure of the validated fTSST (Wiemers et al., 2013) in an online setting. That is, participants were requested to talk about a preferred topic of their choice with a friendly, interactive panel for 10 min (of note, participants were also informed about the duration of the talk in this condition). Moreover, participants were not video- or audiotaped during the fTSST-OL. Likewise, one panel member turned off the camera during the preparation phase (in the fTSST participants are also allowed to prepare for the casual talk). Hence, the fTSST-OL can be considered an appropriate control condition for the TSST-OL, since it omits or reduces the critical stress-inductive components (i.e., social evaluation and task difficulty). Still, it seems noteworthy that there are also other control conditions of the TSST. Most importantly, the above-mentioned pTSST (Het et al., 2009) does not include a panel at all. Instead, participants give their speech in front of an empty room. In the current design, we opted for the fTSST, however, since it might have appeared bizarre (and perhaps stressful therefore) to the participants to give a speech in an empty breakout room of a Zoom conference. Of note, for each participant, we ensured that the experimenter was kept the same for the two sessions while the panel was a different one for the TSST-OL and the fTSST-OL.

2.4. Stress and affect measures

To evaluate the stress-inductive potential of the TSST-OL, we assessed subjective and physiological stress parameters at: −26 min (T1), −6 min (T2), +7 min (T3), + 26 min (T4), +56 min (T5) relative to stressor (or control) onset.

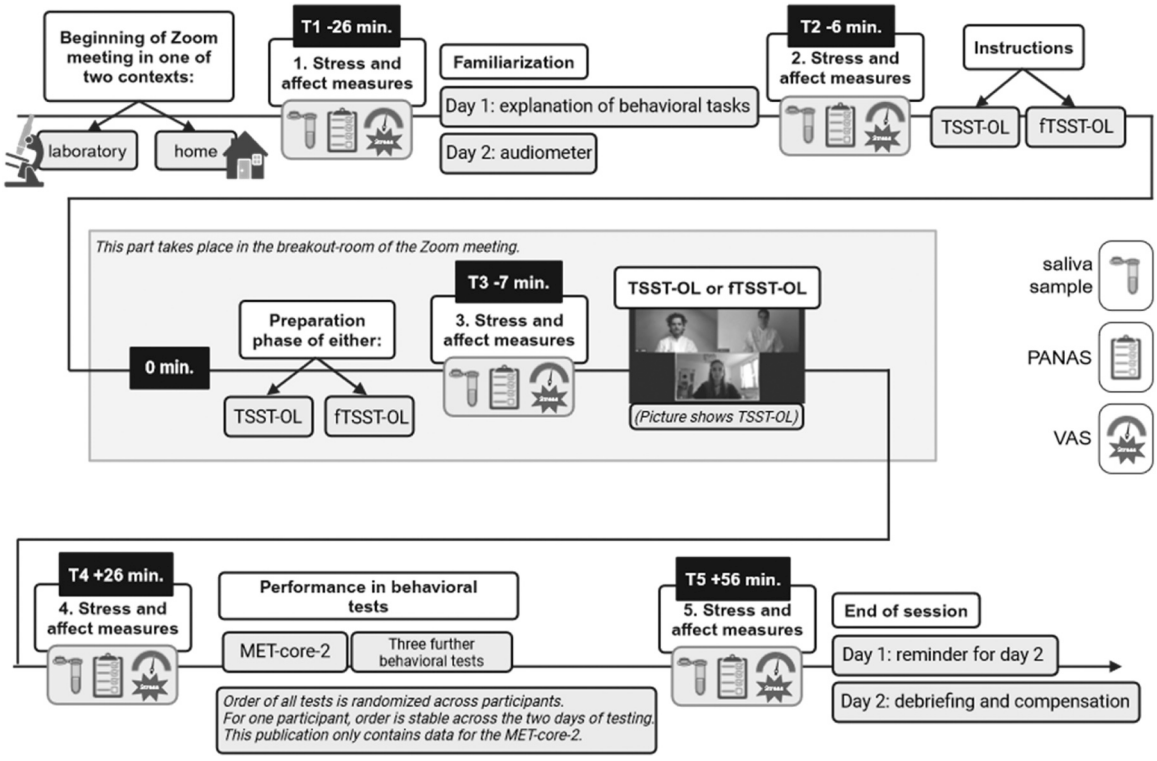

Fig. 1. Schematic display of the procedure of the session. Note. Created with biorender.com. TSST-OL=Trier Social Stress Test (online); fTSST-OL=friendly Trier Social Stress Test (online); PANAS=Positive and Negative Affect Schedule; VAS=Visual Analogue Scale; MET-core-2=Multifaceted Empathy Test Condensed and Revised.

Participants' affect was measured by means of the Positive and Negative Affect Scale (PANAS; German version: Krohne et al. 1996). Moreover, in order to assess perceived stressfulness, participants rated their agreement with the statement "*The situation was stressful for me.*" (adapted from Kudielka et al., 2004) on a visual analogue scale (VAS) from 0 (*absolute disagreement*) to 100 (*absolute agreement*).

To assess physiological stress, we collected saliva samples using Salivettes® (Sarstedt, Nümbrecht, Germany) for the later analysis of sAA and cortisol. For participants tested at their own homes, Salivettes were sent and returned via postal delivery after each test session. Participants were not instructed to store Salivettes in a freezer or refrigerator at their own homes. As soon as Salivettes arrived at the university (typically after seven days), they were frozen at −20°C until final analysis (alike Salivettes collected at the laboratory). Analyses were performed at the joint laboratory of the Department of Genetic Psychology and Cognitive Psychology at Ruhr University Bochum. sAA activity was measured via a colorimetric test using e 2-Chloro-4-nitrophenyl-a-D-maltotrioside (CNP-G3) as substrate reagent and dilution of samples as described elsewhere (Lorentz et al., 1999). Free cortisol concentrations were determined with a commercially available enzyme-linked immunosorbent assay (ELISA; Demeditec, Kiel, Germany). Intra-assay coefficients of variation (CV) were below 6.5 % for cortisol and below 8.5 % for sAA. Inter-assay CV were below 5 % for cortisol and below 10.5 % for sAA.

2.5. Cognitive and affective empathy measures

To assess cognitive and affective state empathy, we used the Multifaceted Empathy Test Condensed and Revised (MET-core-2), which is an adapted version of the Multifaceted Empathy Test (Drimalla et al., 2019; Dziobek et al., 2008, 2011). Participants were presented with 40 photographs for an undefined time that display people in emotionally charged situations. Twenty photographs displayed a person expressing a positive emotion (e.g., *satisfied*), while the other 20 photographs showed a person expressing a negative emotion (e.g., *frustrated*). Likewise, 20 of the photographs showed male, and 20 showed female protagonists.

The MET-core-2 included two tasks. First, to assess cognitive state empathy, participants were asked to respond to the question '*What does the person feel?*' by selecting the correct answer out of four possible options (forced choice). The four options displayed similar yet distinct emotions. Second, to assess affective state empathy, participants were asked to rate how much they feel with the person on a Likert scale from 1 (*not at all*) to 9 (*very much*) by answering the question '*How much do you feel with the person?*'. Participants were told that there is no right answer. Rather, they were encouraged to express their current, subjective perception of how much they felt about the person. For both tasks, participants used numbers on their keyboard to give responses. Response time was not fixed. Before the start of the actual experiment, participants engaged in two practice trials. See the Appendix A.2 for details on the specific presentation of the MET-core-2.

In total, the MET-core-2 took about 9 min to complete and was executed on average at +37 min after the TSST-OL or the fTSST-OL. The paradigm was programmed using PsychoPy® (Peirce et al., 2019) and hosted on Pavlovia (https://pavlovia.org/).

2.6. Statistical analyses

Statistical analyses were conducted using R (version 4.3.2) implemented in RStudio (RStudio Team.. 2021). First, we performed an outlier exclusion. Outliers were defined as individuals who deviated more than three standard deviations from the mean of the respective variable. Details of the outlier exclusion can be found in the Appendix (Table A.2). Second, we checked statistical assumptions of preregistered ANOVA (normality: Shapiro-Wilk tests and visual inspection of QQ plots, homogeneity of variance: Levene tests). In line with our preregistration, we performed non-parametric statistical models when assumptions of ANOVA were violated. In detail, for hypotheses concerning the TSST-OL (H1, H2.a, H2.b) or the MET-core-2 (H3.a-H3.f), we applied the nparLD-package (Noguchi et al., 2012). In cases where we applied the nparLD-package, we report ANOVA-type statistics (ATS). For all analyses, we applied a significance level of $\alpha<.05$. For significant main effects of repeated-measures variables as well as for significant interaction effects, pairwise post-hoc test were realized by Wilcoxon-signed-rank tests with Holm-correction. In contrast, we applied unpaired Mann-Whitney U tests for the follow-up of significant main effects of between-subjects variables (i.e., context or sex of participant). Effects sizes for pairwise post-hoc tests are given as rank biserial correlations (r_b).

Hypotheses concerning the validation of the TSST-OL including the effect of context (H1, H2.a, H2.b) were analyzed using 2 (session: TSST-OL vs. fTSST-OL) x 5 (timepoints: T1 at −26 min vs. T2 at −6 min vs. T3 at +7 min vs. T4 at + 26 min vs. T5 at +56 min) x 2 (context: home vs. laboratory) models separately for all of our stress-related dependent variables (PANAS positive affect, PANAS negative affect VAS, sAA, cortisol). Subjective and physiological stress measures that were assessed repeatedly, were further analyzed in terms of the area-under-the-curve with respect to increase (AUCi, Pruessner et al., 2003) in a 2 (session) x 2 (context) model. Importantly, AUCi measures were calculated using timepoints T2-T5. We opted to omit timepoint T1 (at −26 min) since this measurement was considered to capture anticipatory stress. Therefore, T2 was considered the true baseline since it was the most recent assessment before stress or control exposure. With AUCi measures, we performed 2 (session) x 2 (context) models.

Hypotheses concerning the MET-core-2 (H3.a-H3.f) were checked for cognitive and affective state empathy separately. Cognitive state empathy was approached using a sum score of correct responses. Affective state empathy was integrated in terms of mean intensity ratings. Empathy data were analyzed using two different models. (1) We analyzed cognitive and affective empathy as a function of a 2 (session) x 2 (valence: positive vs. negative) x 2 (context) model. (2) We analyzed cognitive and affective empathy as a function of a 2 (session) x 2 (valence) x 2 (sex of participant: men vs. women) model.

Moreover, as preregistered for explorative purposes, we analyzed responder rates concerning cortisol as produced by the TSST-OL for the two test contexts (home vs. laboratory). To do so, we used two different criteria as reviewed by Miller et al. (2013): (1) we defined responders as individuals showing an increase of 1,5nmol/l from timepoint T2 (-6 min) to timepoint T4 (+26 min), and (2) we defined responders as individuals showing an increase of 15,5 % from timepoint T2 (-6 min) to timepoint T4 (+26 min). The choice of these timepoints was carefully reasoned. Timepoint T2 (-6 min) was considered the true baseline of our experiment. In contrast, timepoint T1 (-26 min) was considered to reflect cortisol levels of anticipatory stress. The time between timepoint T1 (-26 min) and timepoint T2 (-6 min) was considered a familiarization phase. Likewise, timepoint T4 (+26 min.) was expected to show cortisol peak levels since it falls right into the window of heightened cortisol levels between +21 min and +40 min after stressor onset as identified by Dickerson and Kemeny (2004). Last but not least, we ran several covariance analyses which can be found in the Appendix A.5.

In the following "Results" section, if not specified otherwise, we refer to our preregistered hypotheses.

3. Results

3.1. Subjective stress and affect measures

Data on our subjective stress and affect measures (positive affect scale of the PANAS; negative affect scale of the PANAS, self-reported stressfulness as assessed with the VAS) are shown in Fig. 2.

As presented in terms of statistical evidence in Table 2, we found significant main effects of session and time for all of our subjective stress and affect measures. The main effect of session showed that across all timepoints, positive affect was higher during the fTSST-OL (M=3.11,

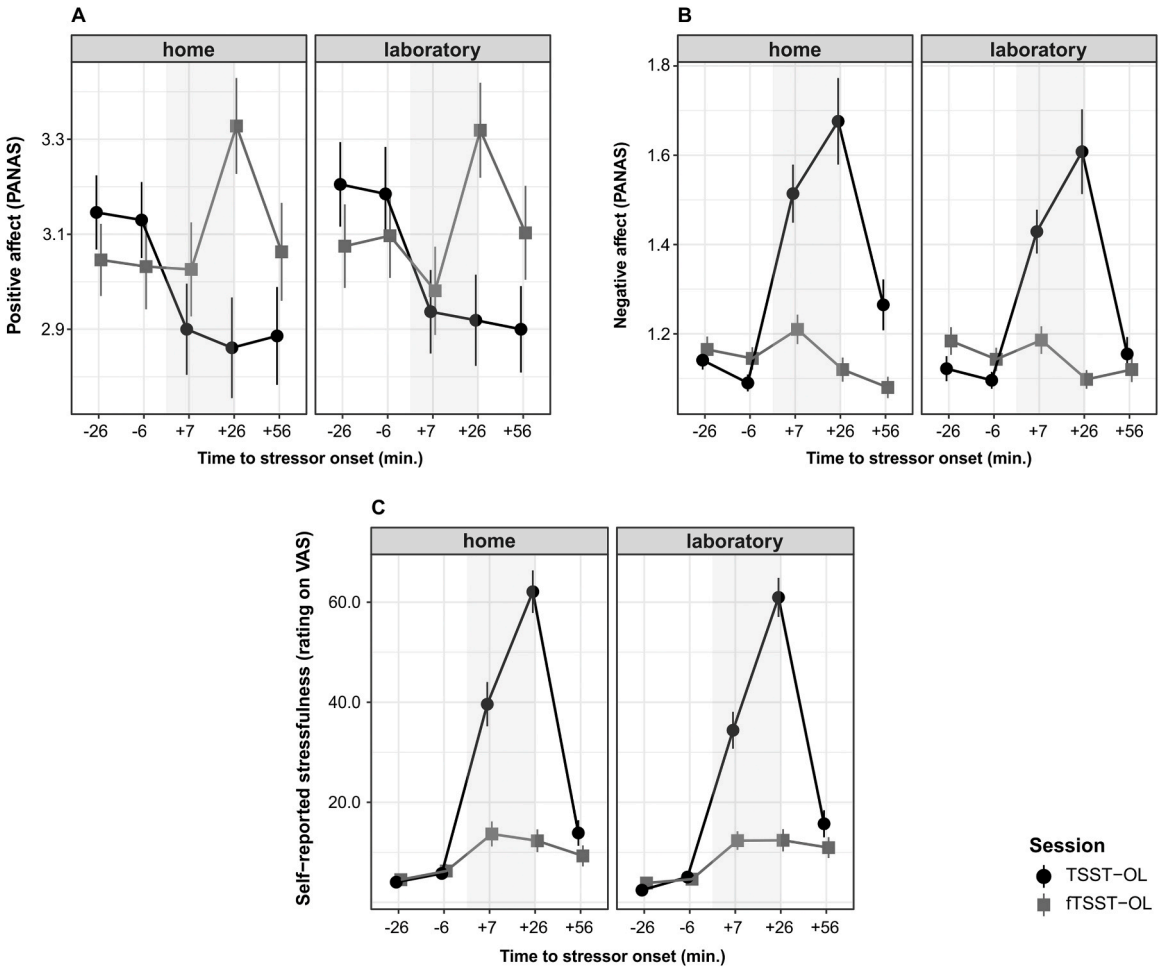

Fig. 2. Subjective stress and affect measures. Note. This figure illustrates mean values (and standard errors) of subjective stress and affects measures separately for home- and laboratory-based participation with different curves for the two sessions (TSST-OL and fTSST-OL). The duration of TSST-OL or fTSST-OL is illustrated by the grey shadow in the background of the plot. Stress and affect measures were assessed at five different time points with varying temporal distance to stressor or control onset, respectively (as marked on the x-axis) during both sessions: T1 at −26 min, T2 at −6 min, T3 at +7 min., T4 at +26 min., T5 at +56 min. Importantly, data are averaged across participants that were available for analyses of the different dependent variables so that *N* varies between the panels. Participants were not included in the analysis (and the figure) either due to missing data points or because they were categorized as outliers. Panel A: PANAS - positive affect scale (home: *n*=57, laboratory: *n*=59). Panel B: PANAS - negative affect scale (home: *n*=49, laboratory: *n*=51). Panel C: VAS (Participants had to rate the stressfulness of the previous situation on a scale ranging from 0 (not stressful at all) to 100 (maximally stressful), home: *n*=49, laboratory: *n*=52). TSST-OL=Trier Social Stress Test (online); fTSST-OL=friendly Trier Social Stress Test (online); PANAS=Positive and Negative Affect Schedule; VAS=Visual Analogue Scale.

Table 2
Overview over main and interaction effects for the subjective stress and affect measures.

	Positive affect (PANAS)			**Negative affect (PANAS)**			**Self-reported stressfulness (VAS)**		
	Statistic	*df*	*p*	*Statistic*	*df*	*p*	*Statistic*	*df*	*p*
Context	0.25	1.00	.616	0.30	1.00	.586	0.03	1.00	.865
Session	6.67	1.00	.001*	33.62	1.00	<.001*	95.53	1.00	<.001*
Timepoint	9.69	3.17	<.001*	60.92	3.34	<.001*	154.83	3.42	<.001*
Context x session	0.01	1.00	.935	1.61	1.00	.205	0.24	1.00	.624
Timepoint x session	19.29	2.68	<.001*	55.80	3.62	<.001*	54.31	3.45	<.001*
Context x timepoint	0.25	3.17	.873	0.44	3.34	.743	0.66	3.42	.599
Context x session x timepoint	0.14	2.68	.921	1.52	3.62	.199	0.12	3.45	.965
AUCi - context	1.19	1.00	.275	0.25	1.00	.614	0.05	1.00	.827
AUCi - session	29.19	1.00	<.001*	148.72	1.00	<.001*	252.77	1.00	<.001*
AUCi - context x session	0.05	1.00	.818	1.08	1.00	.280	0.34	1.00	.558

Note. Overview over main and interaction effects of our omnibus models as well as of AUCi analyses for our subjective stress and affect measures. We provide ATS-statistics, degrees of freedom (df) and *p*-values. Significant effects are marked with an asterisk. PANAS=Positive and Negative Affect Schedule; VAS=Visual Analogue Scale; ATS=ANOVA-type statistics; AUCi=Area under the Curve with Respect to Increase.

SD=0.72) compared to the TSST-OL (*M*=3.01, *SD*=0.72), while negative affect was lower during the fTSST-OL (*M*=1.15, *SD*=0.20) than during the TSST-OL (*M*=1.31, *SD*=0.45) and participants reported higher stressfulness during the TSST-OL (*M*=24.38, *SD*=30.08) compared to the fTSST-OL (*M*=9.02, *SD*=13.40). Results of pairwise post-hoc comparisons for the main effect of time can be found in the Appendix A.3. Moreover, the interaction between session and time reached statistical significance for all our subjective stress and affect measures (see Table 2). Pairwise post-hoc comparisions revealed differences between the two sessions at different (see Appendix A.4) and at the same points in time (see Table 3). Finally, for all our subjective stress and affect measures, AUCi analyses suggested a main effect of session (see Table 2). That is, participants showed a higher AUCi of positive affect during the fTSST-OL (*M*=5.64, *SD*=21.16) compared to the TSST-OL (*M*=-14.34, *SD*=33.89) but higher AUCi of negative affect during the TSST-OL (*M*=21.21, *SD*=24.28) compared to the fTSST-OL (*M*=-0.65, *SD*=7.35). Likewise, AUCi of self-reported stress was higher during the TSST-OL (*M*=2020.30, *SD*=1142.56) compared to the fTSST-OL (*M*=361.53, *SD*=597.83).

3.2. Physiological stress measures

Data on our physiological stress and measures (sAA and cortisol) are shown in Fig. 3.

As suggested by statistical evidence presented in Table 4, we found a significant main effect of session for cortisol, but not for sAA. For cortisol, pairwise post-hoc comparisons calirified that across all timepoints, cortisol levels were higher during the TSST-OL (*M*=2.86, *SD*=1.94) as compared to the fTSST-OL (*M*=2.36, *SD*=1.32). A main effect of time reached statistical significance for both physiological measures and is resolved in terms of pairwise post-hoc comparisons in the Appendix A.3. Of note, for cortisol (but not for sAA), we found a significant main effect of context indicating that across all sessions and timepoints, participants had lower levels of cortisol when joining from home (*M*=2.38, *SD*=1.65) as compared to participants who were tested at the laboratory (*M*=2.85, *SD*=1.67). Furthermore, the interaction between session and time reached statistical significance for sAA and cortisol. Again, results of pairwise post-hoc comparisions concerning different points in time between the two sessions can be found in the Appendix A.4 while effects concering the same points in time are given in Table 3. The above-mentioned main effect of session for cortisol was further confirmed by AUCi analyses indicating that significantly higher AUCi of cortisol was observed during the TSST-OL (*M*=37.95, *SD*=81.04) compared to the fTSST-OL (*M*=-20.31, *SD*=46.13) while there was no effect for sAA.

Finally, for cortisol, we identified responders towards the TSST-OL using criteria as proposed by Miller et al. (2013). In total, *n*=31 (25.83 %) (home: *n*=20 (*n*=10 women), laboratory: *n*=11 (*n*=4 women)) showed an increase in cortisol of at least 1,5nmol/l between timepoints T2 (-6 min) and T4 (+26 min.) whereas *n*=57 (47.50 %) (home: *n*=32 (*n*=16 women), laboratory: *n*=25 (*n*=12 women)) were classified as responders for an increase of 15,5 %.

Table 3
Pairwise post-hoc comparisons of the same timepoint between TSST-OL and fTSST-OL.

		fTSST-OL		TSST-OL			
		M	*SD*	*M*	*SD*	p_{Holm}	r_b
PANAS - Positive Affect							
	T1	3.06	0.63	3.18	0.64	.577	/
	T2	3.07	0.68	3.16	0.69	>.999	/
	T3	3.00	0.73	2.92	0.70	>.999	/
	T4	3.32	0.76	2.89	0.77	<.001	4.53
	T5	3.08	0.77	2.89	0.74	.015	2.43
PANAS - Negative Affect							
	T1	1.18	0.21	1.13	0.18	.781	/
	T2	1.14	0.18	1.10	0.13	.042	2.04
	T3	1.20	0.23	1.47	0.40	<.001	6.33
	T4	1.11	0.17	1.64	0.68	<.001	7.13
	T5	1.10	0.19	1.21	0.34	.048	1.98
VAS - Self-reported Stress							
	T1	4.19	6.93	3.22	5.38	>.999	/
	T2	5.43	8.71	5.41	8.02	>.999	/
	T3	12.99	15.49	36.93	28.77	<.001	6.86
	T4	12.36	15.96	61.52	28.80	<.001	8.21
	T5	10.14	14.89	14.82	18.70	.537	/
sAA							
	T1	131.66	87.54	134.77	79.59	>.999	/
	T2	130.99	87.89	126.78	72.53	>.999	/
	T3	137.03	94.39	153.41	98.12	.041	2.04
	T4	188.83	130.05	180.21	113.57	>.999	/
	T5	134.00	83.28	138.26	82.78	>.999	/
Cortisol							
	T1	2.79	1.58	2.63	1.74	>.999	/
	T2	2.55	1.30	2.47	1.43	>.999	/
	T3	2.26	1.04	2.36	1.43	>.999	/
	T4	2.36	1.47	3.59	2.42	<.001	4.81
	T5	1.83	0.90	3.28	2.21	<.001	5.87

Note. M=Mean, SD=Standard Deviation, r_b =Rank Biserial Correlation. Rank Biserial Correlations are only given for significant comparisons. TSST-OL=Trier Social Stress Test (online); fTSST-OL=friendly Trier Social Stress Test (online); PANAS=Positive and Negative Affect Schedule; VAS=Visual Analogue Scale; sAA=salivary alpha-amylase.

3.3. Cognitive and affective empathy measures

Data on state empathy are illustrated in Fig. 4.

Statistical analysis (see Table 5) of empathy data revealed a main effect of valence for cognitive and for affective empathy state empathy for both models (model 1 accounting for context and model 2 accounting for sex), respectively. Pairwise post-hoc comparisons clarified that across sessions, participants were better at identifying positive (*M*=14.76, *SD*=2.48) compared to negative emotions (*M*=14.18, *SD*=2.61) and expressed higher levels of affective state empathy for positive emotions (*M*=5.01, *SD*=1.62) compared to negative emotions (*M*=4.45, *SD*=1.60). For affective state empathy, model 1 further revealed a statistically significant interaction between valence and session. Pairwise post-hoc comparisons revealed that after the fTSST-OL, participants expressed higher levels of affective state empathy for positive emotions (*M*=5.15, *SD*=1.59) compared to negative emotions (*M*=4.38, *SD*=1.59, p_{Holm}<. 001, r_b=4.47). This effect also concerned affective state empathy for positive emotions after the fTSST-OL compared to positive emotions (*M*=4.88, *SD*=1.64, p_{Holm}=.012, r_b=2.50) and negative emotions (*M*=4.52, *SD*=1.60) after the TSST-OL (p_{Holm}<. 001, r_b=4.01). Furthermore, affective state empathy for negative emotions after the fTSST-OL was significantly lower compared to affective state empathy as indicated for positive emotions after the TSST-OL (p_{Holm}=.009, r_b=2.60). Finally, applying model 1, we observed a significant threeway interaction between context, session, and valence for affective state empathy which is described in more detail in the Appendix A.5. Model 2 further revealed a significant interaction between session and sex for cognitive empathy as after the fTSST-OL, women (*M*=14.93, *SD*=2.54) were better at identifying correct emotions compared to men (*M*=14.05, *SD*=2.51, p_{Holm}=.034, r_b=2.12). No such effect was observed between men and women after the TSST-OL (*p*>.05). For affective state empathy model 2 further identified an interaction between session and valence in that participants expressed higher levels of affective state empathy for positive emotions (*M*=5.15, *SD*=1.59) after the fTSST-OL compared to negative emotions after the fTSST-OL (*M*=4.38, *SD*=1.59, p_{Holm}<. 002, r_b=3.11) and after the TSST-OL (*M*=4.52, *SD*=1.60, p_{Holm}=.014, r_b=2.46). Moreover, for affective state empathy, model 2 revealed a significant interaction between valence and participant sex. Men indicated significantly higher levels of affective state empathy for positive emotions (*M*=5.20, *SD*=1.48)

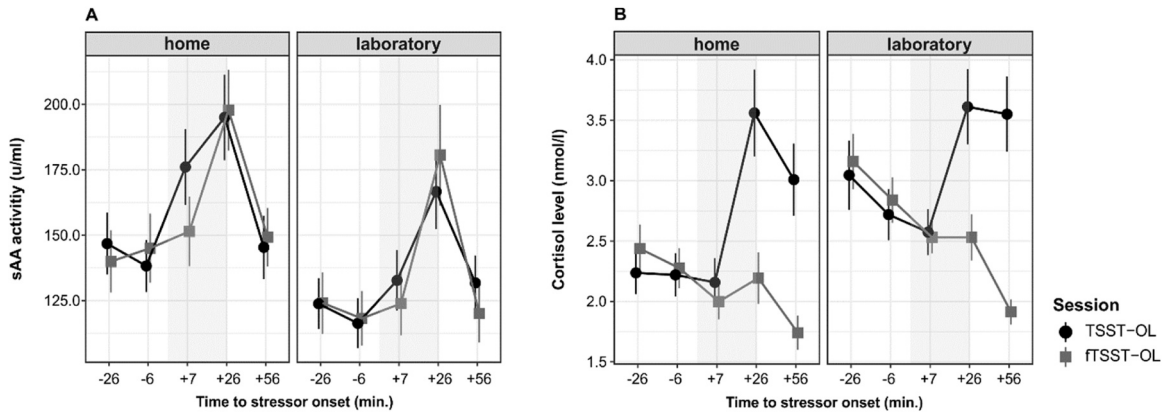

Fig. 3. Physiological stress measures. Note. This figure illustrates mean values (and standard errors) of physiological stress measures. That is, salivary alpha amylase (sAA) activity and cortisol are plotted separately for home- and laboratory-based participation with different curves for the two sessions (TSST-OL and fTSST-OL). Duration of TSST-OL or fTSST-OL is illustrated by the grey shadow in the background of the plot. Physiological measures were assessed at five different timepoints with varying temporal distance to stressor or control onset (as marked on the x-axis) during both sessions: T1 at −26 min, T2 at −6 min, T3 at +7 min., T4 at +26 min., T5 at +56 min. Data are averaged across participants that were available for analyses of the different dependent variables so that *N* varies between the panels. Participants were not included in the analysis (and the figure) either due to missing data points or because they were categorized as outliers. Panel A: sAA activity (home: n=52, laboratory: n=57). Panel B: Cortisol (home: n=53, laboratory: n=51). TSST-OL=Trier Social Stress Test (online); fTSST-OL=friendly Trier Social Stress Test (online); sAA=salivary alpha-amylase.

Tabe 4
Overview over main and interaction effects for the physiological stress measures.

	sAA			Cortisol		
	Statistic	*df*	*p*	*Statistic*	*df*	*p*
Context	3.07	1.00	.080	8.66	1.00	.003*
Session	2.32	1.00	.128	5.19	1.00	.023*
Timepoint	37.38	3.34	<.001*	7.22	1.61	.002*
Context x session	0.22	1.00	.638	0.25	1.00	.614
Timepoint x session	2.52	3.39	<.050*	40.34	1.90	<.001*
Context x timepoint	0.95	3.34	.423	0.66	1.61	.485
Context x session x timepoint	1.85	3.39	.128	0.64	1.90	.521
AUCi - context	0.00	1.00	.982	1.17	1.00	.280
AUCi - session	2.35	1.00	.126	51.87	1.00	<.001*
AUCi - context x session	0.28	1.00	.598	0.18	1.00	.670

Note. Overview over main and interaction effects of our omnibus models as well as of AUCi analyses for our physiological stress measures. We provide ATS-statistics, degrees of freedom (df) and *p*-values. Significant effects are marked with an asterisk. sAA=salivary alpha-amylase; ATS=ANOVA-type statistics; AUCi=Area under the Curve with Respect to Increase.

compared to negative emotions (M=4.10, SD=1.48, p_{Holm}<. 001, r_b=5.20) while this difference was not significant for women (p_{Holm}>.05). Men further showed significantly lower levels of affective state empathy for negative emotions in comparison to women's levels of affective state empathy for negative emotions (M=4.80, SD=1.63, p_{Holm}=.002, r_b=3.10) and positive emotions (M=4.83, SD=1.74, p_{Holm}=.004, r_b=2.90).

4. Discussion

The current preregistered study investigated whether the TSST-OL can be used for robust stress induction across different contexts in subjective (i.e., PANAS, VAS) and physiological (i.e., cortisol, sAA) measures (H1; H2.a; H2.b). Additionally, we tested to what extent exposure to the TSST-OL affected cognitive and affective state empathy using the MET-core-2 (H3.a-H3.f). Unlike prior efforts to validate the TSST-OL, this study comprehensively assessed stress outcome parameters of all relevant dimensions of the stress response using a sufficient sample size and a control condition. It also explored cognitive and affective state empathy along with the effects of context (home vs. laboratory) to reconcile the inconsistent data on empathy under stress and the influence of contextual variables in online experiments. In general, our results show that the TSST-OL has stress-inductive potential in terms of subjective and physiological stress and affect measures. Results regarding cognitive and affective state empathy are influenced by an interplay stimulus between valence, sex of the participant, and stress induction.

Concerning subjective stress and affect measures, the TSST-OL led (as compared to the fTSST-OL) to a decrease in positive affect, coupled with an increase in negative affect and self-reported stress, irrespective of context. These results are in line with our preregistered hypothesis H1 and existing literature concerning online versions of the TSST (Eagle et al., 2021; Gunnar et al., 2021; Harvie et al., 2021; Meier et al., 2022) and the in-person TSST.

Regarding physiological stress measures, we found both the TSST-OL and the fTSST-OL to increase sAA activity as a marker of SNS activation. sAA reactivity in response to the TSST-OL was in line with existing literature (Gunnar et al., 2021; Meier et al., 2022). Though different to our hypothesis H1, sAA activity towards the non-stressful fTSST-OL is not surprising. Increased sAA activity is regularly reported after control conditions of the in-person TSST (e.g., fTSST: Wiemers et al., 2013; Wiemers and Wolf, 2015, and pTSST: Het et al., 2009). As a result, the prediction made in our hypothesis failed to take into account that social interactions in general lead to an increase in arousal. In this context, it was argued that sAA may represent an indicator of activation parameters rather than a stress marker per se. The fTSST-OL of the current study involved speaking in front of a panel which - even though the panel was friendly and interactive - likely resulted in a certain arousal. Importantly, since most studies on the TSST-OL to date (e.g., Gunnar et al., 2021; Meier et al., 2022) did not include a control condition, the current evidence is important in showing that sAA activity can also occur in response to online variants of established control conditions of the TSST. Finally, it seems worth mentioning that in our experimental design, participants were not requested to stand up during the TSST-OL or the fTSST-OL which was required in previous publications (e.g., Gunnar et al., 2021). Indeed, several publications suggest that body posture affects physiological activity during rest as well as in response to stress (e.g., Acharya et al., 2005; Goto et al., 2020; Hackford et al., 2019; Tulen et al., 1999). Of note, however, we still asked our participants to take an upward position and to place both of their feet on the floor. In line with hypothesis H1, our data further confirm the stress-inductive potential of

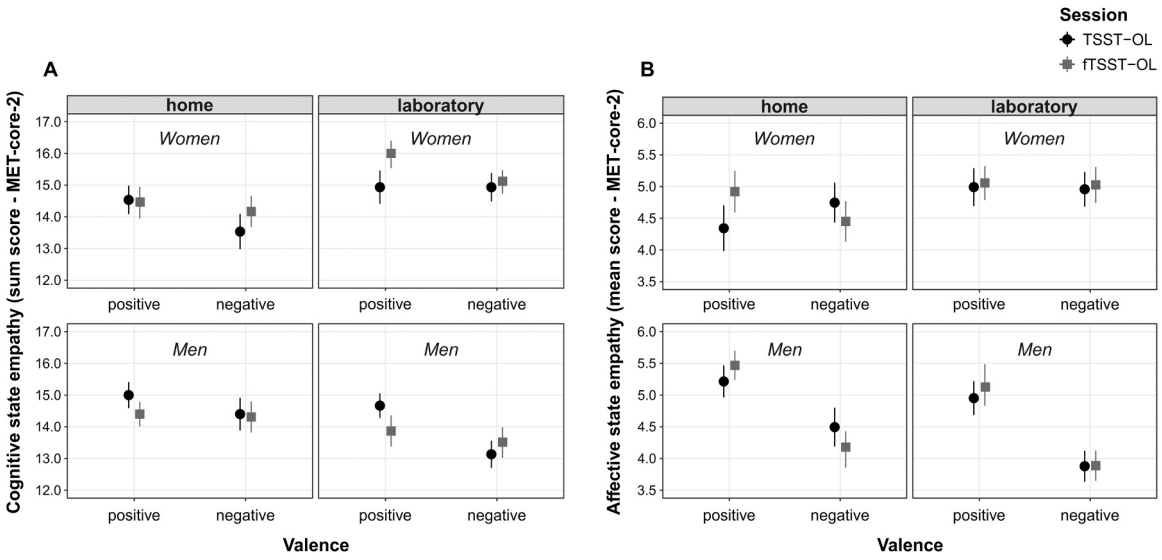

Fig. 4. State empathy. Note. This figure illustrates mean values (and standard errors) of sum scores of the cognitive empathy task of the MET-core-2 (Panel A) and of mean scores of the affective state empathy task of the MET-core-2 (Panel B) separately for the sex of the participant, the two contexts (home vs. laboratory) as a between-subjects factor. Emotions are of positive or negative valence which is illustrated on the x-axis. The two different curves per panel represent the two sessions (TSST-OL vs. fTSST-OL) that were undergone by all participants as a within-subjects factor. The maximum sum score for cognitive state empathy per valence (for every one of the four panels) during the MET-core-2 is 20. The maximum mean score for affective state empathy per valence (for every one of the four panels) during the MET-core-2 is 9. Note that not all participants were in the analysis (and the figure) either due to missing data points or because they were categorized as outliers. MET-core-2=Multifaceted Empathy Test Condensed and Revised; TSST-OL=Trier Social Stress Test (online); fTSST-OL=friendly Trier Social Stress Test (online).

Table 5
Overview over main and interaction effects for cognitive and affective state empathy.

	Cognitive state empathy (MET-core-2)			**Affective state empathy (MET-core-2)**		
	Statistic	*df*	*p*	*Statistic*	*df*	*p*
Model 1 (accounting for context)						
Context	0.00	1.00	.946	0.00	1.00	.946
Session	0.04	1.00	.844	2.01	1.00	.156
Valence	6.69	1.00	.010*	15.04	1.00	<.001*
Context x session	2.35	1.00	.126	0.40	1.00	.528
Valence x session	0.07	1.00	.786	8.81	1.00	.003*
Context x valence	0.90	1.00	.342	0.01	1.00	.907
Context x session x valence	0.22	1.00	.641	5.88	1.00	.015*
Model 2 (accounting for sex)						
Sex	2.90	1.00	.088	0.56	1.00	.456
Session	0.05	1.00	.822	2.00	1.00	.157
Valence	6.73	1.00	.001*	17.33	1.00	<.001*
Sex x session	5.44	1.00	.020*	0.05	1.00	.826
Valence x session	0.07	1.00	.790	8.39	1.00	.004*
Sex x valence	0.17	1.00	.684	17.97	1.00	<.001*
Sex x session x valence	2.52	1.00	.113	0.00	1.00	.977

Note. Overview over main and interaction effects of the two models (mode 1: session x valence x context; model 2: session x valence x sex) that were run for cognitive and affective state empathy as assessed by means of the MET-core-2 (cognitive state empathy: sum score, affective state empathy: mean score). We provide ATS-statistics, degrees of freedom (df) and *p*-values. Significant results are marked with an asterisk. ATS=ANOVA-type statistics; MET-core-2=Multifaceted Empathy Test Condensed and Revised.

the TSST-OL through cortisol reactivity, which is consistent with previous studies (Gunnar et al., 2021; Meier et al., 2022; Shields et al., 2024). Although cortisol levels increased after the TSST-OL, the absolute reactivity was relatively small, with only 25.83 % of participants meeting the 1.5nmol/l criterion for responders. Using the 15.5 % criterion, 47.50 % of participants were identified as responders, suggesting cortisol effects may stem from relative changes. While, according to the 1,5nmol/l criterion, Gunnar et al. (2021) (63 %) and Meier et al. (2022) (64 %) reported higher responders for their studies, estimates of Shields et al. (2024) (43 %) rather resemble the numbers also found in the current study. Of note, compared to the in-person TSST, variants of the TSST, for instance as adapted for virtual reality, typically induce smaller cortisol reactivity, possibly due to reduced social-evaluative threat (Dickerson and Kemeny, 2004) and less immersion (Helminen et al., 2019). We aimed to mitigate these factors by mandating a minimum screen size of 13 in. in our study. Finally, one may discuss whether the relatively fasted state (i.e., as described in the Appendix A.1, participants were requested to take their last meal as well as their last drink other from water around 90 min. before the experiment) may have impeded cortisol reactivity. Indeed, several studies (e.g., Kirschbaum et al., 1997; von Dawans, Zimmer, and Domes, 2021, but see Rüttgens and Wolf, 2022) suggest that sugary drinks affect cortisol reactivity as mediated via glucose and in some research designs glucose drinks are purposely given to facilitate stress hormone reactivity (e.g., Henze et al., 2020).

Regarding hypothesis H2.a, administering the TSST-OL at home resulted in significantly lower cortisol increases but did not affect positive affect, negative affect, subjective stress, or sAA. This suggests that the TSST-OL elicits changes in these variables regardless of the context, supporting the validity of online adaptations for stress research and in comparison to in-person applications (see Heyers et al. 2024 for important considerations on sAA in remote settings). Interestingly, context influenced cortisol reactivity, with participants in the laboratory showing higher levels compared to those at home, although AUCi analyses did not confirm this effect as cortisol reactivity towards the stressor was present in both contexts. Higher cortisol levels in the laboratory context may reflect increased overall cortisol output, possibly due to

anticipatory stress at the first timepoint, or heightened unpredictability and uncontrollability in the unfamiliar laboratory context, thereby supporting hypothesis H2.b. This result bears important implications for stress research as it shows that a noticeable portion of cortisol output measured at the laboratory is likely not produced by experimental stress induction per se but rather by the laboratory testing context. This underscores the impact of the testing context on cortisol levels, suggesting that remote stress induction procedures, like home-based application of the TSST-OL, may buffer anticipatory responses. With that, the TSST-OL might be of particular interest in order to investigate experimentally induced stress reactivity. Moreover, the TSST-OL generally allows to test participants that are not able to come to local research facilities as it is the case for cross-cultural or vulnerable samples (Pfeifer et al., 2021).

Concerning state empathy, the current study revealed mixed results. Our online application of the MET-core-2 may be considered valid in that performance in absolute numbers was comparable to results as achieved with the MET-core-2 in regular laboratory contexts (Drimalla et al., 2019; Gamsakhurdashvili et al., 2021), thereby indicating its suitability for remote use. Of note, online assessment of empathy may be increasingly relevant as emotional interactions shift to digital platforms.

Cognitive state empathy was, in contrast to hypothesis H3.a, not higher after the TSST-OL. Hence, acute stress did not lead to overarching changes in the cognitive state empathy facet. In some previous studies, cognitive state empathy was enhanced after stress exposure (Deckers et al., 2015; Domes and Zimmer, 2019). Other studies did not replicate this finding (Graumann et al., 2021; Wingenfeld et al., 2018). Inconsistencies between studies may be attributed to the choice of empathy task (Nitschke and Bartz, 2023): studies investigating cognitive state empathy have often only focused on one element of cognitive empathy (e.g. empathy for pain: Gonzalez-Liencres et al., 2016, recognition of basic emotions: Deckers et al., 2015; Domes and Zimmer, 2019) instead of capturing complex emotions.

Furthermore, neither context (hypothesis H3.c), nor participant sex (hypothesis H3.d) were identified as overarching influential factors. Interestingly, however, and in line with hypothesis H3.e, participants were better at recognizing positive compared to negative emotions across sessions. A similar pattern was observed by Wolf et al. (2015). Furthermore, and in line with hypothesis H3.f, participant sex might represent a confounding factor as women outperformed men in identifying emotions correctly after the fTSST-OL, while this advantage was not noticeable after the TSST-OL. As a consequence, men may have benefited more strongly from rising cortisol levels than women demonstrating that effects of acute stress on cognitive state empathy may be stratified by sex. Other variables such as the attentional focus of participants during the task (Gu and Han, 2007) may create further interference.

Contrary to hypothesis H3.b, affective state empathy did not uniformly increase after stress. In contrast, interaction effects emerged. Participants exhibited higher levels of affective empathy for positive emotions compared to negative emotions after the fTSST-OL, whereas this difference was absent after the TSST-OL. Indeed, this effect was due to a decrease in affective state empathy for positive emotions after stress. Such findings allow for two different, although not exclusive, explanations. One might argue that it is easier for participants to feel mood-congruent emotions, which might explain why affective state empathy was decreased for positive emotions after the TSST-OL. Alternatively, an impaired self-other differentiation after stress might have led participants to transport negative affective states experienced after the TSST-OL onto the pictures. It has been reported that men's, but not women's, ability to differentiate between oneself and others decreases after stress (Tomova et al., 2014).

Regarding affective state empathy, neither sex of participant (hypothesis H3.d) nor context (hypothesis H3.c) exerted significant effects across sessions. Considering context in the interaction with valence of stimuli helped to unravel further effects. Participants joining from home indicated higher affective state empathy for positive emotions compared to negative emotions after the fTSST-OL. Moreover, participants joining from the laboratory expressed higher affective state empathy for positive emotions after the fTSST-OL compared to negative emotions after the TSST-OL.

Consistent with hypothesis H3.e, participants showed a preference for empathizing with positive over negative emotions across all contexts, a pattern supported by existing literature, at least in men (Wolf et al., 2015). Of note, we found that across sessions, men reported lower levels of affective state empathy for negative emotions in comparison to women (for both positive and negative emotions) suggesting that this effect is sex-specific to a certain degree. Still, in contrast to hypothesis H3.f and the findings for cognitive state empathy, we did not find that men benefited more strongly from rising cortisol levels compared to women with respect to affective state empathy. In general, however, it seems worth mentioning that assuming absolute dosage of cortisol to be relevant, we might have found stronger cortisol-induced effects in the different empathy-related research questions, applying another perhaps stronger stressor. Indeed, as outlined above, the TSST-OL produced cortisol reactivity that undercut in-person applications of the TSST, for instance.

Remote research settings offer advantages but also bear methodological challenges. Conducting experiments online with participants sitting at their own homes is more frequently accompanied by technical issues resulting in exclusion of participants and requires compromises concerning experimental control. For instance, in our study, participants completed the MET-core-2 on their own screens out of view of the researchers, requiring trust in their adherence to instructions. Of note, participants in the current study likely executed the MET-core-2 with care as performance was in line with previous studies conducted at the laboratory (Drimalla et al., 2019). Additionally, logistical challenges, like postal delivery of testing material, require careful management. Researchers must assess whether a remote design is suitable for their research questions, weighing both its advantages and challenges.

5. Conclusion

Overall, subjective, and physiological measures confirm the efficacy of the TSST-OL in inducing acute stress compared to a control condition. Of note, stress induction succeeded regardless of context. Still, context affected cortisol levels, with laboratory-based participants showing higher overall cortisol output compared to participants tested at home. This finding underscores the attractiveness of home contexts for stress research since a familiarized context seems to buffer anticipatory stress responses. Nonetheless, the TSST-OL induced cortisol reactivity with reduced magnitude compared to in-person variants. Moreover, future studies may explore the validity of the TSST-OL in further contexts such as field settings. With that, the TSST-OL may represent a crucial advancement in stress research, especially when aiming to include more diverse samples and context-independent application. Concerning cognitive and affective state empathy, our study showed that both constructs are not universally affected by acute stress. Instead, factors such as stimulus valence or participant sex modified effects. Further research is needed to comprehend the nuanced interplay between acute stress and state empathy.

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Declaration of Competing Interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2024.107211.

References

Acharya, R.U., Kannathal, N., Mei Hua, L., Mei Yi, L., 2005. Study of heart rate variability signals at sitting and lying postures. J. Bodyw. Mov. Ther. *9* (2), 134–141. https://doi.org/10.1016/j.jbmt.2004.04.001.

Allen, A.P., Kennedy, P.J., Dockray, S., Cryan, J.F., Dinan, T.G., Clarke, G., 2017. The trier social stress test: principles and practice. Neurobiol. Stress *6*, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001.

Baez, S., Flichtentrei, D., Prats, M., Mastandueno, R., García, A.M., Cetkovich, M., Ibáñez, A., 2017. Men, women…who cares? A population-based study on sex differences and gender roles in empathy and moral cognition. PloS One *12* (6), e0179336. https://doi.org/10.1371/journal.pone.0179336.

Cuff, B.M., Brown, S.J., Taylor, L., Howat, D.J., 2016. Empathy: a review of the concept. Emot. Rev. *8* (2), 144–153. https://doi.org/10.1177/1754073914558466.

Deckers, J.W.M., Lobbestael, J., van Wingen, G.A., Kessels, R.P.C., Arntz, A., Egger, J.I. M., 2015. The influence of stress on social cognition in patients with borderline personality disorder. Psychoneuroendocrinology *52*, 119–129. https://doi.org/10.1016/j.psyneuen.2014.11.003.

DeJoseph, M.L., Finegood, E.D., Raver, C., Blair, C.B., 2019. Measuring stress reactivity in the home: Preliminary findings from a version of the Trier Social Stress Test (TSST-H) appropriate for field-based research. Cent. Open Sci. https://doi.org/10.31234/osf.io/5qapw.

Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. Psychol. Bull. *130* (3), 355–391. https://doi.org/10.1037/0033-2909.130.3.355.

Domes, G., Zimmer, P., 2019. Acute stress enhances the sensitivity for facial emotions: a signal detection approach. Stress (Amst., Neth.) *22* (4), 455–460. https://doi.org/10.1080/10253890.2019.1593366.

Dorris, L., Young, D., Barlow, J., Byrne, K., Hoyle, R., 2022. Cognitive empathy across the lifespan. Dev. Med. Child Neurol. *64* (12), 1524–1531. https://doi.org/10.1111/dmcn.15263.

Drimalla, H., Landwehr, N., Hess, U., Dziobek, I., 2019. From face to face: the contribution of facial mimicry to cognitive and emotional empathy. Cogn. Emot. *33* (8), 1672–1686. https://doi.org/10.1080/02699931.2019.1596068.

DuPont, C.M., Pressman, S.D., Reed, R.G., Manuck, S.B., Marsland, A.L., Gianaros, P.J., 2022. An online Trier social stress paradigm to evoke affective and cardiovascular responses. Psychophysiology 59 (10), e14067. https://doi.org/10.1111/psyp.14067.

Dziobek, I., Preissler, S., Grozdanovic, Z., Heuser, I., Heekeren, H.R., Roepke, S., 2011. Neuronal correlates of altered empathy and social cognition in borderline personality disorder. NeuroImage *57* (2), 539–548. https://doi.org/10.1016/j.neuroimage.2011.05.005.

Dziobek, I., Rogers, K., Fleck, S., Bahnemann, M., Heekeren, H.R., Wolf, O.T., Convit, A., 2008. Dissociation of cognitive and emotional empathy in adults with Asperger syndrome using the Multifaceted Empathy Test (MET). J. Autism Dev. Disord. *38* (3), 464–473. https://doi.org/10.1007/s10803-007-0486-x.

Eagle, D.E., Rash, J.A., Tice, L., Proeschold-Bell, R.J., 2021. Evaluation of a remote, internet-delivered version of the trier social stress test. Int. J. Psychophysiol. *165*, 137–144. https://doi.org/10.1016/j.ijpsycho.2021.03.009.

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav. Res. Methods *39* (2), 175–191. https://doi.org/10.3758/BF03193146.

Gamsakhurdashvili, D., Antov, M.I., Stockhorst, U., 2021. Sex-hormone status and emotional processing in healthy women. Psychoneuroendocrinology *130*, 105258. https://doi.org/10.1016/j.psyneuen.2021.105258.

Gonzalez-Liencres, C., Breidenstein, A., Wolf, O.T., Brüne, M., 2016. Sex-dependent effects of stress on brain correlates to empathy for pain. Int. J. Psychophysiol. *105*, 47–56. https://doi.org/10.1016/j.ijpsycho.2016.04.011.

Goodman, W.K., Janson, J., Wolf, J.M., 2017. Meta-analytical assessment of the effects of protocol variations on cortisol responses to the Trier Social Stress Test. Psychoneuroendocrinology *80*, 26–35. https://doi.org/10.1016/j.psyneuen.2017.02.030.

Goto, Y., Hu, A., Yamaguchi, T., Suetake, N., Kobayashi, H., 2020. The influence of a posture on the autonomic nervous system and stress hormones in saliva. Health *12* (02), 118–126. https://doi.org/10.4236/health.2020.122010.

Graumann, L., Duesenberg, M., Metz, S., Schulze, L., Wolf, O.T., Roepke, S., Otte, C., Wingenfeld, K., 2021. Facial emotion recognition in borderline patients is unaffected by acute psychosocial stress. J. Psychiatr. Res. *132*, 131–135. https://doi.org/10.1016/j.jpsychires.2020.10.007.

Gu, X., Han, S., 2007. Attention and reality constraints on the neural processes of empathy for pain. NeuroImage *36* (1), 256–267. https://doi.org/10.1016/j.neuroimage.2007.02.025.

Gunnar, M.R., Reid, B.M., Donzella, B., Miller, Z.R., Gardow, S., Tsakonas, N.C., Thomas, K.M., DeJoseph, M.L., 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.

Hackford, J., Mackey, A., Broadbent, E., 2019. The effects of walking posture on affective and physiological states during stress. J. Behav. Ther. Exp. Psychiatry *62*, 80–87. https://doi.org/10.1016/j.jbtep.2018.09.004.

Håkansson Eklund, J., Summer Meranius, M., 2021. Toward a consensus on the nature of empathy: a review of reviews. Patient Educ. Couns. *104* (2), 300–307. https://doi.org/10.1016/j.pec.2020.08.022.

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 (Amst. Neth.) *24* (6), 1023–1032. https://doi.org/10.1080/10253890.2021.1995714.

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.

Henze, G.-I., Konzok, J., Kreuzpointner, L., Bärtl, C., Peter, H., Giglberger, M., Streit, F., Kudielka, B.M., Kirsch, P., Wüst, S., 2020. Increasing deactivation of limbic structures over psychosocial stress exposure time. Biol. Psychiatry Cogn. Neurosci. Neuroimaging *5* (7), 697–704. https://doi.org/10.1016/j.bpsc.2020.04.002.

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., Wolf, O.T., 2009. Neuroendocrine and psychometric evaluation of a placebo version of the 'Trier Social Stress Test. Psychoneuroendocrinology *34* (7), 1075–1086. https://doi.org/10.1016/j.psyneuen.2009.02.008.

Heyers, K., Pfeifer, L.S., Walusiacki, K., Reinke, P., Moser, D., Ocklenburg, S., Wolf, O.T., 2024. Stability and durability of salivary alpha-amylase across different storage conditions. Psychoneuroendocrinology *161*, 106929. https://doi.org/10.1016/j.psyneuen.2023.106929.

Joëls, M., Baram, T.Z., 2009. The neuro-symphony of stress. Nat. Rev. Neurosci. *10* (6), 459–466. https://doi.org/10.1038/nrn2632.

Kirschbaum, C., 2021. Why we need an online version of the trier social stress test. Psychoneuroendocrinology *125*, 105129. https://doi.org/10.1016/j.psyneuen.2021.105129.

Kirschbaum, C., Gonzalez Bono, E., Rohleder, N., Gessner, C., Pirke, K.M., Salvador, A., Hellhammer, D.H., 1997. Effects of fasting and glucose load on free cortisol responses to stress and nicotine. J. Clin. Endocrinol. Metab. *82* (4), 1101–1105. https://doi.org/10.1210/jcem.82.4.3882.

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* (1-2), 76–81. https://doi.org/10.1159/000119004.

Krohne, H.W., Egloff, B., Kohlmann, C.-W., & Tausch, A. (1996). PsycTESTS Dataset. https://doi.org/10.1037/t49650-000.

Kudielka, B.M., Hellhammer, D.H., Kirschbaum, C., Harmon-Jones, E., Winkielman, P., 2007. Ten years of research with the Trier Social Stress Test—revisited. Soc. Neurosci.: Integr. Biol. Psychol. Explan. Soc. Behav. *56* (83).

Kudielka, B.M., Schommer, N.C., Hellhammer, D.H., Kirschbaum, C., 2004. Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of day. Psychoneuroendocrinology *29* (8), 983–992. https://doi.org/10.1016/j.psyneuen.2003.08.009.

Lazarus, R.S., Folkman, S., 1984. Stress, Appraisal, and Coping. Springer Publishing Company.
Liu, J.J.W., Ein, N., Peck, K., Huang, V., Pruessner, J.C., Vickers, K., 2017. Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): a meta-analysis. Psychoneuroendocrinology *82*, 26–37. https://doi.org/10.1016/j.psyneuen.2017.04.007.
Lorentz, K., Gütschow, B., Renner, F., 1999. Evaluation of a direct alpha-amylase assay using 2-chloro-4-nitrophenyl-alpha-D-maltotrioside. Clin. Chem. Lab Med. *37* (11-12), 1053–1062.
Meier, M., Haub, K., Schramm, M.-L., Hamma, M., Bentele, U.U., Dimitroff, S.J., Gärtner, R., Denk, B.F., Benz, A.B.E., Unternaehrer, E., Pruessner, J.C., 2022. Validation of an online version of the Trier Social Stress Test in adult men and women. Psychoneuroendocrinology 1 (8), 142. https://doi.org/10.1016/j.psyneuen.2022.105818.
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* (9), 832–840. https://doi.org/10.1097/PSY.0000000000000002.
Mogg, K., Mathews, A., Bird, C., Macgregor-Morris, R., 1990. Effects of stress and anxiety on the processing of threat stimuli. J. Personal. Soc. Psychol. *59* (6), 1230–1237. https://doi.org/10.1037/0022-3514.59.6.1230.
Nitschke, J.P., Bartz, J.A., 2023. The association between acute stress & empathy: a systematic literature review. Neurosci. Biobehav. Rev. *144*, 105003. https://doi.org/10.1016/j.neubiorev.2022.105003.
Nitschke, J.P., Pruessner, J., Bartz, J.A., 2022. Stress and stress-induced glucocorticoids facilitate empathic accuracy in men but have no effects for women. Psychol. Sci. *33* (10), 1783–1794. https://doi.org/10.1177/09567976221101315.
Noguchi, K., Gel, Y.R., Brunner, E., & Konietschke, F. (2012). nparLD: An R software package for the nonparametric analysis of longitudinal data in factorial experiments.
Peirce, J., Gray, J.R., Simpson, S., MacAskill, M., Höchenberger, R., Sogo, H., Kastman, E., Lindeløv, J.K., 2019. Psychopy2: experiments in behavior made easy. Behav. Res. Methods *51* (1), 195–203. https://doi.org/10.3758/s13428-018-01193-y.
Pfeifer, L.S., Heyers, K., Ocklenburg, S., Wolf, O.T., 2021. Stress research during the COVID-19 pandemic and beyond. Neurosci. Biobehav. Rev. *131*, 581–596. https://doi.org/10.1016/j.neubiorev.2021.09.045.
Pfeifer, L.S., Heyers, K., Wolf, O.T., Stockhorst, U., Güntürkün, O., Merz, C.J., Ocklenburg, S., 2024. Using the online version of the Trier Social Stress Test to investigate the effect of acute stress on functional lateralization. Sci. Rep. *14* (1), 20826. https://doi.org/10.1038/s41598-024-71668-w.
RStudio Team (2021). *RStudio* [Computer software]. RStudio Team. http://www.rstudio.com/.
Rüttgens, T., Wolf, O.T., 2022. The influence of a glucose administration on stress responsivity and memory after a socially evaluated cold pressor test. Psychoneuroendocrinology *142*, 105803. https://doi.org/10.1016/j.psyneuen.2022.105803.
Shields, G.S., Hunter, C.L., Trudell, E.V., Gray, Z.J., Perkins, B.C., Patterson, E.G., Zalenski, P.K., 2024. Acute stress influences the emotional foundations of executive control: Distinct effects on control-related affective and cognitive processes. Psychoneuroendocrinology *162*, 106942. https://doi.org/10.1016/j.psyneuen.2023.106942.
Shields, G.S., Sazma, M.A., Yonelinas, A.P., 2016. The effects of acute stress on core executive functions: a meta-analysis and comparison with cortisol. Neurosci. Biobehav. Rev. *68*, 651–668. https://doi.org/10.1016/j.neubiorev.2016.06.038.
Smeets, T., Dziobek, I., Wolf, O.T., 2009. Social cognition under stress: differential effects of stress-induced cortisol elevations in healthy young men and women. Horm. Behav. *55* (4), 507–513. https://doi.org/10.1016/j.yhbeh.2009.01.011.
Tomova, L., Dawans, B., von, Heinrichs, M., Silani, G., Lamm, C., 2014. Is stress affecting our ability to tune into others? Evidence for gender differences in the effects of stress on self-other distinction. Psychoneuroendocrinology *43*, 95–104. https://doi.org/10.1016/j.psyneuen.2014.02.006.
Tulen, J.H., Boomsma, F., Man in 't Veld, A.J., 1999. Cardiovascular control and plasma catecholamines during rest and mental stress: effects of posture. Clin. Sci. (Lond. Engl.: 1979) *96* (6), 567–576.
Ulrich-Lai, Y.M., Herman, J.P., 2009. Neural regulation of endocrine and autonomic stress responses. Nat. Rev. Neurosci. *10* (6), 397–409. https://doi.org/10.1038/nrn2647.
von Dawans, B., Spenthof, I., Zimmer, P., Domes, G., 2020. Acute psychosocial stress modulates the detection sensitivity for facial emotions. Exp. Psychol. *67* (2), 140–149. https://doi.org/10.1027/1618-3169/a000473.
von Dawans, B., Strojny, J., Domes, G., 2021. The effects of acute stress and stress hormones on social cognition and behavior: current state of research and future directions. Neurosci. Biobehav. Rev. *121*, 75–88. https://doi.org/10.1016/j.neubiorev.2020.11.026.
von Dawans, B., Zimmer, P., Domes, G., 2021. Effects of glucose intake on stress reactivity in young, healthy men. Psychoneuroendocrinology *126*, 105062. https://doi.org/10.1016/j.psyneuen.2020.105062.
Wiemers, U.S., Schoofs, D., Wolf, O.T., 2013. A friendly version of the trier social stress test does not activate the HPA axis in healthy men and women. Stress (Amst. Neth.) *16* (2), 254–260. https://doi.org/10.3109/10253890.2012.714427.
Wiemers, U.S., Wolf, O.T., 2015. Cortisol broadens memory of a non-stressful social interaction. Psychopharmacology *232* (10), 1727–1733. https://doi.org/10.1007/s00213-014-3808-7.
Wingenfeld, K., Duesenberg, M., Fleischer, J., Roepke, S., Dziobek, I., Otte, C., Wolf, O.T., 2018. Psychosocial stress differentially affects emotional empathy in women with borderline personality disorder and healthy controls. Acta Psychiatr. Scand. *137* (3), 206–215. https://doi.org/10.1111/acps.12856.
Wingenfeld, K., Kuehl, L.K., Janke, K., Hinkelmann, K., Dziobek, I., Fleischer, J., Otte, C., Roepke, S., 2014. Enhanced emotional empathy after mineralocorticoid receptor stimulation in women with borderline personality disorder and healthy women. Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol. *39* (8), 1799–1804. https://doi.org/10.1038/npp.2014.36.
Wolf, O.T., 2009. Stress and memory in humans: twelve years of progress? Brain Res. *1293*, 142–154. https://doi.org/10.1016/j.brainres.2009.04.013.
Wolf, O.T., Schulte, J.M., Drimalla, H., Hamacher-Dang, T.C., Knoch, D., Dziobek, I., 2015. Enhanced emotional empathy after psychosocial stress in young healthy men. Stress (Amst. Neth.) *18* (6), 631–637. https://doi.org/10.3109/10253890.2015.1078787.
Zhao, D., Ding, R., Zhang, H., Zhang, N., Hu, L., Luo, W., 2022. Individualized prediction of females' empathic concern from intrinsic brain activity within general network of state empathy. Cogn. Affect. Behav. Neurosci. *22* (2), 403–413. https://doi.org/10.3758/s13415-021-00964-z.