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Multimodal stress assessment: Connecting task-related changes in self-reported stress, salivary biomarkers, heart rate, and facial expressions in the context of the stress response to the Trier Social Stress Test

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A R T I C L E I N F O

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A B S T R A C T

When we are stressed, do we show it? Although the answer to this question may feel intuitive, prior work on the topic does not provide a clear answer. To address this gap, we present the results of the first study that examined physiological and psychological stress responses and facial expressions using a validated acute stress task and control condition in a within-subjects design. Participants ($N = 105$; 59 women; 22.36 ± 3.52 years of age) underwent the Trier Social Stress Test (TSST) and the friendly TSST (f-TSST) on consecutive days and provided self-reports via the Short Stress State Questionnaire in German (SSSQ-G), saliva samples, and heart rate. Participants were further filmed during both conditions, allowing us to examine their observable emotional displays using their facial muscle movement data (Action Units, AUs). As expected, the TSST elicited higher SSSQ-G scores and greater cortisol and heart rate increases than the f-TSST. Additionally, the trajectory of cortisol was influenced by the order in which the conditions were presented. The total score, along with the *Self-evaluation* and *Worry* subscale scores of the SSSQ-G, correlated with the cortisol maximum increase in response to the TSST, as did heart rate. We found no evidence for displays of common emotions during the manipulation, but we did observe more friendly expressions in the f-TSST (compared to the TSST). Individual AUs neither predicted physiological outcomes nor self-reported stress state scores. Together, these results highlight both the complexity of the stress response in relation to observable emotions and the importance of multimodal stress assessment.

1. Introduction

With the rise of stress-related diseases and disorders, and the associated societal burden, there is a growing need for a deeper understanding of the mechanisms that shape individual stress responses (Chrousos, 2009; Cohen et al., 2016; Engert et al., 2018; Rohleder, 2019). The fact that prolonged or repeated exposure to stressful life-events impacts health negatively is undisputed (Chrousos, 2009; Miller et al., 2007). Theoretical models suggest that different stress response systems—cognitive, autonomic, and endocrine—are typically orchestrated in response to environmental demands, and that their coordination plays a key role in adaptive functioning (Cohen et al., 2016; Kemeny, 2003; McEwen, 1998). However, while our understanding of the physiological processes surrounding stress has grown over the past decades, many questions remain regarding the potential interplay between physiological, behavioral, and psychological stress responses (Engert et al., 2018; Schlotz et al., 2008). It is worth noting that our perspective aligns with the specificity of the stress response. Not all

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demands on the body and mind necessarily result in a uniform stress response (Kemeny, 2003). Social-evaluative stressors, however, have been shown to reliably activate multiple stress response systems (Man et al., 2023). In theory, indicators of stress—such as self-report, heart rate (HR), and cortisol—reflect the same underlying construct and result from the same central processing; In some contexts, they should therefore covary to some degree. In practice, however, findings are mixed, and relatively few studies have systematically examined the interplay between psychological and physiological stress responses, particularly in designs capable of disentangling within- and between-person variation (Campbell and Ehlert, 2012; Man et al., 2023; Schlotz et al., 2008). This points to a central challenge in current stress research: Integrating these multifaceted processes into a coherent understanding of stress reactivity to then allow for more targeted prevention and intervention (Engert et al., 2018; Man et al., 2023; Rohleder, 2019).

To disentangle the intricate ways in which stress-related processes are linked, it is necessary to adopt a multidimensional approach. According to the most widely accepted model of stress (e.g., Cohen et al., 2016; McEwen, 1998; Rohleder, 2019) individuals experience potentially stressful situations, which elicits cognitive appraisals that initiate a cascade of affective and motivational responses. These stress responses in turn influence both behavioral processes and biological stress systems, such as the autonomic nervous system (ANS)—comprised of the sympathetic and parasympathetic branches—and the hypothalamus-pituitary-adrenal (HPA) axis. Stress-responsive measures indicative of ANS reactivity include HR and salivary alpha-amylase (sAA), with HR reflecting the dynamic interplay between sympathetic and parasympathetic input, while sAA is more specifically associated with sympathetic-adrenal-medullary (SAM) system activation. Stress-related HPA axis responses are shown well via changes in (salivary) cortisol.

Relations between biological and psychological measures of stress are often less strong than might be expected (Schlotz et al., 2008). For example, an analysis of all 49 studies published before 2012 showed that only 25 % of studies obtained a significant correlation between subjective emotional stress and cortisol responses (Campbell and Ehlert, 2012). Conversely, another study found a positive association between negative affect and sAA, but a negative association between negative affect and cortisol responses (Het et al., 2012). Compounding this confusing literature, stress appears to exert its strongest effects on the two indices that are often uncorrelated: A recent meta-analysis of 61 studies including various emotional and physiological responses to the Trier Social Stress Test (TSST; (Kirschbaum et al., 1993) found that the strongest indicators of acute stress were responses in negative mood and cortisol (Man et al., 2023). Taken together, evidence suggests that stress influences both psychological and biological processes, but it is unclear whether these effects are related as strongly as might be expected intuitively.

An important caveat to the above is that most studies that examine both psychological and biological stress responses focus on neighboring constructs, such as affect or anxiety, but do not explicitly examine stress state changes in response to a task. However, cognitive and affective processes interact in complex ways, which together may muddy psychological stress responses if they are only assessed via adjacent constructs (Denson et al., 2009; Lazarus, 1982; Pessoa, 2008). Recently, we addressed this issue for future German-speaking studies by translating and validating the Short Stress State Questionnaire into German (SSSQ-G; Ringgold et al., 2024), which explicitly measures task-related changes in self-reported stress. The SSSQ-G is sensitive to stress-specific changes that further differ with stressor severity, making it an ideal measure for detecting changes from pre- to post-acute-stress-task.

Another measure that has been largely neglected in stress research, but may offer valuable insights into emotional dynamics, are facial expressions, as they can reflect affective states through expressive behavior—yet their potential has rarely been systematically explored. Recent advances have permitted automatic quantification of the appearance of “action units” (AUs), which represent distinct facial muscle movements. Historically, these AUs are grouped into distinct emotional states, such as “happy” or “angry” (Barrett et al., 2019; Ekman et al., 1987). However, more recent research has questioned the practice of categorizing muscle movements into distinct emotions, as muscle activations can convey different meanings in different contexts (Mayo and Heilig, 2019; Blasberg et al., 2023). Facial expressions communicate affective states that are theorized to be influenced by internal and external factors, as well as genetic variations (Mayo and Heilig, 2019). Additionally, they vary along the valence dimension (pleasant vs. unpleasant) in response to emotional stimuli; for example, pleasant images elicit significantly stronger positive emotion intensities, whereas neutral and unpleasant stimuli do not differ significantly in expression intensity (Höfling et al., 2020). Given that the TSST consistently elicits higher negative affect compared to control conditions, it is reasonable to expect a corresponding facial response during the task. (Het et al., 2012; Richer et al., 2024; Wiemers et al., 2013). Very few studies have analyzed the association of facial expressions, measured by AUs, and the physiological stress response (Mayo and Heilig, 2019). Some prominent findings are that individuals that display “fear” on their face also have higher cardiovascular and cortisol responses, while more “anger” or “disgust” lead to lower responses in these outcome measures (Lerner et al., 2007). In another study, self-reported emotions did not correlate with emotion expressions, although increases in self-reported fear—assessed by two items of the Positive and Negative Affect Schedule (Watson et al., 1988)—predicted a blunted cortisol response, whereas greater anger expressions predicted higher cortisol in men (Lupis et al., 2014). Findings from a recent study investigating the relationship between the physiological stress response with facial expressions, point towards some AUs being connected to self-report and physiological data measured in the context of participants completing the TSST (Blasberg et al., 2023). Higher cortisol showed an association with the occurrence of AU05 (“upper eyelid raiser”) and AU10 (“upper lip raiser”), while intensity of AU06 (cheek raiser) and AU12 (“lip corner puller”) was linked to lower cortisol reactivity, and AU07 intensity (“eyelid tightener”) was associated with more worry, tension, and nervousness (Blasberg et al., 2023). What is more, women showed more smiling intensity in response to stress, compared to men.

To date, no study has investigated the connection between the physiological stress response, task-related changes in self-reported stress states, and facial expressions using a validated acute stress induction task and a control condition utilizing a within-subjects design. Our study aims to address this gap by examining both psychological and biological stress-responsive indicators using a within-subjects, crossover design. We hypothesized that the Short Stress State Questionnaire (SSSQ-G) would replicate its six-factor structure and show stronger stress-induced changes in response to the TSST compared to an adapted version of the friendly TSST (f-TSST), with no effects of condition order, body position, or sex. We expected a stronger cortisol response to the TSST, particularly when administered first, and anticipated sex differences (lower responses in women) but no effects of body position. We hypothesized that self-reported stress would be associated with cortisol reactivity in the TSST, and that these associations would vary across SSSQ-G subscales. For salivary alpha-amylase (sAA) and heart rate, we expected no overall differences between conditions, but predicted order effects (stronger sAA reactivity when TSST came first) and a body position effect on heart rate (higher when standing), with no sex differences. We expected sAA to correlate with subjective stress and heart rate. Regarding facial expressions, we hypothesized that sex differences would emerge in the expression of specific action units (AUs), with women showing greater activity in AUs associated with affiliative emotions (e.g., AU06, AU12) and men showing more activity in AUs linked to anger and confrontation (e.g., AU04). We further expected that individual AUs and AU composites related to discrete emotional expressions (e.g., anger, contempt, fear, disgust) would be significantly associated with physiological stress markers, including cortisol, salivary alpha-amylase (sAA), and heart rate. Finally, we anticipated at least one significant difference in AU activity between the TSST and f-TSST conditions, with sex moderating this effect. The hypotheses outlined above reflect a summary of our preregistered predictions. For the full set of detailed hypotheses, please refer to our preregistration (https://doi.org/10.17605/OSF.IO/854ED).

2. Methods

2.1. Transparency and openness

This study was preregistered[1] before data was viewed by the main authors of the manuscript (VR, GSS, NR). Four authors of the manuscript (LA, MK, VM, RR) carried out the preprocessing of the primary data, and thus were not involved in the preregistration or analyses to ensure all data analysis was conducted without prior knowledge of the data. This study is part of the primary data of large-scale studies within the framework of the Collaborative Research Centre (CRC) “EmpkinS” (Empatho-Kinaesthetic Sensor Technology), funded by the Deutsche Forschungsgemeinschaft (DFG). Data corresponding to this manuscript will be made accessible via https://osf.io/2u4jh/.

2.2. Participants

Our sample consisted of 105 healthy participants (Age in years: $M = 22.36 \pm 3.52$, range = 18–34; BMI: $M = 22.11 \pm 2.39$, range = 18.03–29.59), of which 59 were women. Due to the influence of sex hormones on cortisol responsivity (Kirschbaum et al., 1999), we excluded women who were pregnant, in menopause, or using hormonal contraceptives, and ensured all participating women were in the luteal phase of their cycle. The exclusion criteria[2] were taken from previous studies (e.g., (Janson and Rohleder, 2017), as well as from recommended stress study exclusion criteria (e.g., (Shields, 2020). All participants gave written informed consent prior to participation and were compensated either monetarily (50€ via bank-transfer) or via course credit (up to 5 h for psychology students). All studies were approved by the ethics committee at the Friedrich-Alexander-Universität Erlangen--Nürnberg (protocol #493_20 B) and conducted in accordance with the Declaration of Helsinki.

One person dropped out of the study before providing the first saliva sample and three participants only completed day one of the two-day experiment, resulting in TSST data for 103 participants and f-TSST data from 102 participants. Due to these dropouts, technical issues (e.g., with the ECG sensor during recording), mistakes made during data collection (e.g., forgetting to administer post-questionnaires), or non-valid salivary samples (e.g., not enough saliva in the synthetic fiber swabs), the final sample varied slightly between the different measures (e.g., HR vs. cortisol), as well as for specific variables derived from them (e.g., for HR_Mean_tsst vs. HR_Mean_ftsst). All analyses were conducted using the available data for each outcome. The exact number of participants included per marker are specified in the corresponding subsections of the *Measures* section.

2.3. Stress induction and control condition

The TSST (Kirschbaum et al., 1993) reliably activates the HPA axis (Allen et al., 2014) and consists of a 5-min preparation phase, a 5-min speech task, and a 5-min mental arithmetic task. In this study, the TSST was performed as described in detail elsewhere (Janson and Rohleder, 2017; Richer et al., 2024). For better comparability with the TSST, the friendly-TSST (Wiemers et al., 2013) was adapted by shortening the speech task to 5 min—compared to the 8-min speech task in the original f-TSST (Wiemers et al., 2013)—and adding a 5-min mental arithmetic task during which participants had to alternate between summing the numbers 10 and 20. The two judges responded warmly to the participant and speech pauses were avoided by asking follow-up questions. If a participant made an error during the math task, they were corrected in a friendly manner and continued from the corrected number.

Participants either underwent the TSST or the modified version of the f-TSST on day one, and vice versa on day two, with various data collected before and after the respective experimental procedure. The preregistration for the main project investigating the effect of acute psychosocial stress on micro- and macroscopic body movements using Empathokinesthetic Sensors (https://doi.org/10.17605/OSF.IO/YC5DJ) provides an overview over all data that was collected.

Due to the need to test novel technical equipment relevant to the primary study, speech pauses and changes in body position (sitting vs. standing) were introduced into the (f-)TSST. Thus, the (f-)TSST consisted of a 5-min preparation phase, followed by a 1-min pause, a 5-min speech phase that was divided into two 2.5-min intervals, with a 30-s pause in between. After the speech phase followed another 30-s pause, two 2.5-min mental arithmetic phases, with a 30-s pause in between, before the (f-)TSST was concluded by a 1-min pause. This resulted in a total length of 18.5 min for the (f-)TSST. Half of the participants underwent the (f-)TSST in a sitting condition, while the other half completed it in the regular standing position. The (f-)TSST committee consisted of one male and one female experimenter for the first 45 participants and was female-only for all subsequent participants.

2.4. Procedure

Participants were recruited through advertisements on social media among the student body of Friedrich-Alexander-Universität Erlangen-Nürnberg. The recruitment and the study were conducted in German language. First, participants were asked to complete an online screening. If eligible, they were invited to the laboratory on two consecutive afternoons between 13.00 and 19.00—with testing times chosen to minimize circadian variations in cortisol (Smyth et al., 1997)—for 2–3 h (4–6 h total). Participants were instructed to wake up at least three hours before partaking in the study, avoid strenuous physical activities as well as food at least one hour before the study, and refrain from consuming alcohol in the 24 h before participating in the study. Once participants arrived at the lab, the experimenter verified that they had adhered to these instructions, before they were asked to complete the informed consent form (day 1 only). To infer sAA concentrations and HPA axis activity from cortisol concentrations in saliva, eight saliva samples were collected throughout the course of one experimental session, resulting in a total of 16 saliva samples per participant. Before providing the first sample using salivettes (Sarstedt AG & Co. KG, Nümbrecht, Germany), participants were instructed to place the synthetic fiber roll from the salivette into their mouths and move it around for two minutes, refraining from chewing on it. The first sample (S0) was collected 40 min

[1] At the advice of a helpful reviewer, we revised our analytic strategy from what we specified in our preregistration. The analytic strategy now taken in the manuscript is that recommended by one of our reviewers.

[2] Age below 18 or above 40 years, diagnosis of acute and/or chronic somatic disease, use of prescription medications (especially beta blockers or glucocorticoid drugs), regular drug use or smoking > 5 cigarettes per day, prior experience with laboratory stress protocols or knowledge about the procedure of a laboratory stress test, for female participants: hormonal contraceptives, pregnancy or menopause, individuals with a body mass index < 18 or > 30 kg/m^2, a score > 22 in the Allgemeine Depressionsskala based on a scale ranging from 0 to 3 (ADS; Hautzinger et al., 2012), Pathologies: Any malfunction of the nervous system, visual system, cardiovascular system, digestive system; known inflammation; diseases related to liver, gallbladder, pancreas, skeleton, muscles, connective tissues, kidneys, urinary tract, genital organs, blood; any infectious or parasitic disease; cancer; mental disorders, Intake of specific medication: e.g. for the treatment of high blood pressure, heart disease, cardiac arrhythmias, heart attacks; any anticoagulant or anti-inflammatory medication; insulin, pain medication, antidepressants, psycho-stimulants, hormones, cholesterol-lowering medication

before stressor onset. To avoid large variations in blood sugar levels (Zänkert et al., 2020) participants were given 200 ml of grape juice to drink after providing this initial saliva sample. Next, participants were asked to fill out a set of questionnaires, including the SSSQ-G (Ringgold et al., 2024), before they were equipped with sensors for the assessment of cardiopulmonary parameters. Afterwards, the experimenter led the participants to the room where the TSST was conducted, with the panel members already being seated behind the desk. The experimenter explained the next steps, before the participants were sat at a table for the preparation phase. After three minutes, the panel asked the participants to fill out the Primary Appraisal Secondary Appraisal questionnaire (Gaab, 2009), and after two more minutes they were asked to move to the designated spot–sitting or standing, depending on the condition and given the instruction to wait and be still until given the signal to start ("Now you have some time to mentally prepare for the task. We will give you a sign when you can start with your presentation. Remember to remain sitting/standing as still as possible."). After half of the time allocated for the speech task were up, participants were given the instruction to think about their personal characteristics ("Now please take some time to silently reflect on your personal attributes. We will give you a signal when you can continue with your presentation."). After a 30-s break they were asked to continue. The speech task was followed by another 30-s break before participants were asked to begin with the math task – either counting down in steps of 17 from 2043 (TSST) or alternating between summing the numbers of 10 and 20 starting at 0 (f-TSST). Just as the speech task, the mental arithmetic task was divided in two, with a 30-s break in between in which the participant was asked to silently continue with the task and on a prompt by the panel give the number at which they had arrived. After the math task, participants were asked to remain sitting/standing quietly until they received the signal to leave the room. The prompts for the breaks were very similar with regard to content and just differed with regard to friendliness, comparable to the instructions for the (f-)TSST. Once participants had left the room where the (f-)TSST was conducted, the experimenter took them to another room, where they first gave another saliva sample (S2), before they filled out a set of post-questionnaires and provided five more saliva samples (peak and recovery), with the sensors being removed in between S3 (+10 min post-(f-)TSST) and S4 (+20 min post-(f-)TSST). After the first day of the two-day experiment participants were reminded about their upcoming second appointment, while after the second day they were debriefed and given further information as well as the documents to receive their course credit or monetary compensation. The timeline and full procedure of the study, as well as the expected temporal dynamics of the dependent variables can be seen in Fig. 1.

2.5. Measures

2.5.1. Short Stress State Questionnaire in German

The Short Stress State Questionnaire in German (SSSQ-G; Ringgold et al., 2024) measures self-reported affective states and appraisals that are stress-related. It consists of 24 items, with the pre- and post-version of the questions being adapted to tense (e.g., "I want to succeed on the task" vs. "I wanted to succeed on the task"). Items are rated on a 5-point likert scale (1 = *Not at all* to 5 = *Extremely*). For the 24-item solution Cronbach's α is.85, while Cronbach's α for the subscales (*Distress, Worry, Confidence, Negative Affect, Motivation,* and *Self-evaluation)* ranges between .73 and .75. for all subscales except Self-evaluation, which exhibits a Cronbach's α of .56. Higher scores on the *Distress, Worry, Self-evaluation, and Negative Affect* subscales indicate greater psychological stress, whereas higher scores on the *Confidence* and *Motivation* subscales reflect lower stress. For the computation of the total SSSQ-G score, the Confidence and Motivation subscales are reverse-coded so that higher total scores consistently represent greater psychological stress. Example items for the subscales are: Feeling "dissatisfied" or "irritated" *(Distress),* "I am/was worried about what other people think of me" *(Worry),* "I expect to perform/performed proficiently on this task" *(Confidence),* feeling "depressed" or "sad" *(Negative Affect),* "I want/wanted to succeed on the task" *(Motivation),* and "I'm reflecting/reflected about myself" *(Self-evaluation)*. Due to dropouts and issues during data collection the final sample for the SSSQ-G consisted of 103 participants assessed during the TSST and 100 participants assessed during the f-TSST with data of 99 participants for both conditions.

The confirmatory factor analysis constraining the model to the six factors observed when validating the SSSQ-G (Ringgold et al., 2024) showed that the fit index for absolute fit was acceptable (RMSEA =.076); however, the fit indices for incremental fit were slightly below the acceptable threshold (CFI =.832; TLI =.803). The correlations between several, yet not all, factors were substantial, which supports the use of the total scale with 24 items.

2.5.2. Salivary alpha-amylase and cortisol

Sampling times for saliva samples before participating in the (f-)TSST were –40 min before stressor onset (baseline; S0) and –1 min before task onset (S1). Following the (f-)TSST, additional saliva samples were collected at + 1 (stress-phase; S2), and + 10, + 20, + 30, + 45, and + 60 min (peak and recovery samples; S3-S7). After data collection, saliva samples were stored at −18°C. Immediately before determination of cortisol and sAA concentrations, samples were centrifuged for 10 min at 2000 g and 20°C. For sAA determination an in-house enzyme kinetic assay was used as previously described (Rohleder and Nater, 2009). Concentrations of salivary cortisol were determined in duplicate using chemiluminescence immunoassay (CLIA, IBL, Hamburg, Germany). Intra- and inter-assay coefficients of variation for both sAA and cortisol were below 10 %. After excluding participants with elevated cortisol baseline values (indicative of anticipatory stress and thus unlikely to mount a HPA axis response; Roos et al., 2019), as explained in the preregistration, we also excluded participants with missing values at critical sampling points—immediately before the (f-)TSST (S1; −1 min), immediately after (S2; +1 min; the expected sAA peak), or at the expected cortisol peak (S3; +10 min; or S4; +20 min)—from analyses for the respective condition to avoid biased estimations of physiological responses. In the salivary data, six outliers (three each in cortisol and sAA) with z-scores beyond ±3.29 were winsorized to the nearest value within that range. Thus, the final sample for sAA consisted of 98 full datasets (i.e., data from both conditions with no missing values in S1 or S2), including 100 participants from the TSST and 101 from the f-TSST. For cortisol, the final sample included 89 full datasets (i.e., data from both conditions with no missing values in S1, S2, S3, or S4), with 92 datasets for the TSST and 95 for the f-TSST.

2.5.3. Heart rate

During the experiment the electrocardiogram (ECG) of participants was recorded at 256 Hz using a wearable ECG sensor node (Portabiles GmbH, Erlangen, Germany) recording a 1-channel ECG according to Lead I of Einthoven's triangle. To reduce noise, the raw ECG signal was filtered with a second-order FIR bandpass filter (3–45 Hz), before R-peaks were detected using the QRS detection algorithm proposed by Hamilton (2002) and RR intervals were computed. During preprocessing, outliers (≥2.576σ), differences of successive RR intervals of ≥ 1.96σ, and RR intervals that corresponded to a HR of < 45 bpm or > 200 bpm were removed and imputed by linear interpolation (Happold et al., 2021). The processing was performed using the BioPsyKit Python package (Richer et al., 2021). As a final step, data were split into sample periods for a 5-min baseline measurement, the preparation phase of the (f-)TSST and the two main phases of the (f-)TSST, namely speech phase and math phase. Further, we normalized the three phases of the (f-)TSST to baseline by subtracting the baseline value from the respective means for further analyses. Due to technical issues during recording, HR data was available for 85 participants in total, including complete data for both conditions in 70 participants, f-TSST data in 80 participants, and TSST data in 75 participants. One outlier (z > ±3.29) was identified and

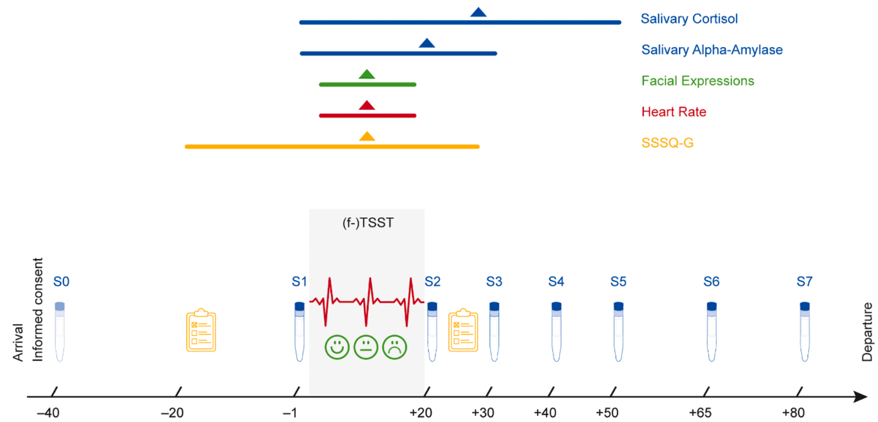

Fig. 1. Timeline, procedure, and expected temporal dynamics of the study. The icons depict the respective measure, namely saliva samples, questionnaires, electrocardiogram, and facial expressions. The timeline is measured in minutes. The lines above the image correspond to the timeframe in which the markers are expected to change and the triangle shows the expected peak of the changes; (f-)TSST = (friendly) Trier Social Stress Test; SSSQ-G = Short Stress State Questionnaire in German; S0–S7 = Saliva Samples 0 (Baseline) to 7.

adjusted using winsorization to reduce its influence on the analysis.

2.5.4. *Facial expressions*

During the experiment participants were recorded with a red-green-blue (RGB) camera (Sony SRG-300H). These recordings were then segmented into the two phases of the (f-)TSST, excluding the speech pauses that were introduced to test the novel technical equipment. To derive AUs from the videos, the open-source software package *Python Facial Expression Analysis Toolbox*[3] (*pyfeat*, version 0.6.1; py-feat.org) was used. Pyfeat extracts 20 AUs and seven emotions (neutral, anger, disgust, fear, happiness, sadness, surprise) on a frame-by-frame basis. For each frame, the algorithm assigns a value between 0 (not activated) and 1 (fully activated) to each AU to indicate the intensity of each AU. The mean intensity reflects the average proportion of AU activation across all frames in the recorded video, where values range between 0 and 1, representing the relative presence of each AU over time. Only frames where the detected face had an accuracy above 95 % were included in analyses. For the scope of this work, we used the mean intensity of the AUs for the math and speech part of the (f-)TSST per participant. Due to technical difficulties during recording or issues during preprocessing, data for 93 participants for both conditions was available for analyses. Fig. 2 shows a simplified illustration of the facial muscle movements corresponding to the AUs analyzed in this work.

2.6. *Statistical analyses*

All analyses were conducted using R (v4.4.2; R Core Team, 2024) and RStudio (v2024.12.0 +467; R Core Team, 2024) utilizing the open-source packages afex (v.1.3–1; Singmann et al., 2024) and lavaan (v.0.6–18; Rosseel, 2012). As cortisol and sAA were not normally distributed, we applied square root transformation to sAA data and logarithmized cortisol data, using the natural logarithm. For indices that we computed (e.g., maximum increase), we did not test for normal distribution or use transformations. Data were analyzed using linear mixed-effects models to account for repeated measures and individual variability. Growth curve modeling was applied where appropriate. Repeated-measures ANOVAs were used for analyses of self-reported stress state changes, and peak response values (e.g., maximum increase). If the assumption of sphericity was violated, the Greenhouse-Geisser correction was applied.

Model estimation was conducted using maximum likelihood estimation and model fits were assessed using Comparative fit index (CFI), Tucker-Lewis index (TLI), and the root mean square error of approximation (RMSEA). For CFI and TLI values closer to 1.0 denote a better fit, with above 0.90 being considered acceptable, and for the RMSEA, values closer to zero indicate a better fit, with below.08 being considered acceptable (Fabrigar et al., 1999; Hu and Bentler, 1999).

Because the primary study (i.e., the study from which this study was derived) was preregistered, we were unable to conduct an *a priori* power analysis for this work.

3. Results

3.1. *Short Stress State Questionnaire in German*

The repeated measures ANCOVA using the change score of the 24-item scale with the between-subject factor order (TSST_first; f-TSST_first), the within-subject factor of condition (TSST; f-TSST), and the covariates sex (Women; Men) and body position (Sitting; Standing) showed a significant effect for condition ($F(1, 95) = 116.76$, $p < .001$, generalized $\eta^2 = .42$), order ($F(1, 95) = 13.93$, $p < .001$, generalized $\eta^2 = .05$), and sex ($F(1, 95) = 7.36$, $p = .01$, generalized $\eta^2 = .03$). All other effects were n.s. (smallest $p = .07$). These results indicate that there was a significant difference in self-reported stress states between the f-TSST and TSST, with higher scores in the TSST, compared to the f-TSST for the 24-item total score. Further, women had higher SSSQ-G scores, and the order of the two conditions influenced peoples' self-reported stress response. The differences in subscales across conditions can be seen in the Supplementary Figure 1 in Appendix A. We further assessed the extent to which change scores in each condition significantly differed from zero, as well as whether these scores differed across stress induction types, namely TSST and f-TSST (Table 1).

3.2. *Salivary alpha-amylase and cortisol*

We next examined sAA responses using a linear mixed-effects model with square-root-transformed sAA values as the outcome. Time was centered at 20 min (S2), immediately following the (f-)TSST, to model change relative to the expected peak in sAA response. The analysis included samples collected at –1 min before task onset (S1), and at + 1 min (S2) and + 10 min (S3) after the (f-)TSST. The model included fixed effects for centered time (linear and quadratic terms), experimental condition (TSST; f-TSST), condition order (TSST_first; f-TSST_first), interactions of condition × condition order × time^2, as well

[3] Deviating from the pre-registration, we did not use OpenDBM (https://aicure.github.io/open_dbm) for the preprocessing of the videos, as the video data was processed within a complex analysis pipeline in the context of the primary study for which *pyfeat* proved to be the better option.

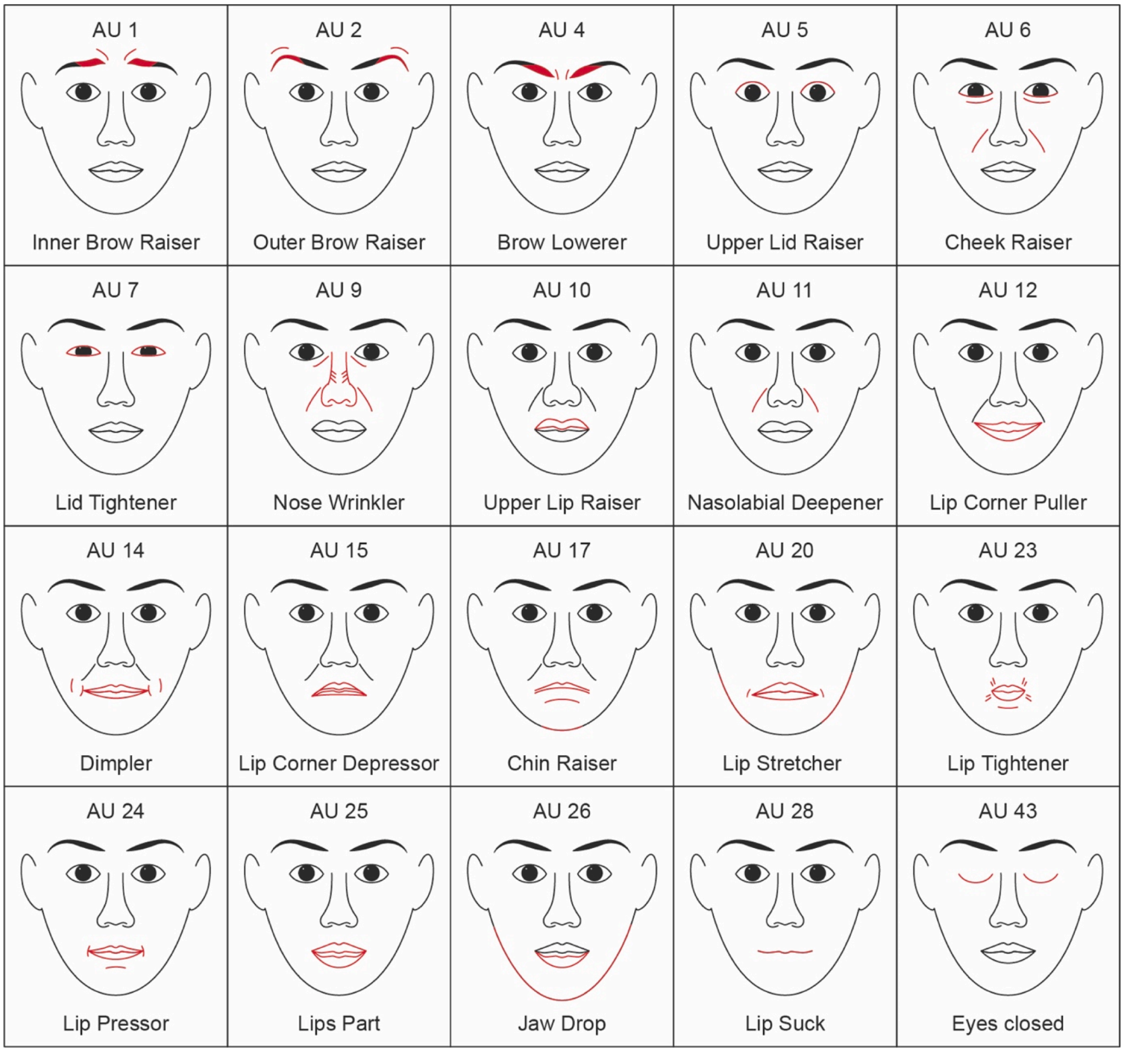

Fig. 2. Graphical illustration of the action units analyzed in this study by Veronika Ringgold. A reusable version of this figure is available at https://osf.io/3dv9b/ under a CC-BY 4.0 license.

Table 1
T-tests using SSSQ-G change scores of TSST and f-TSST (one-sample) and comparing both conditions (paired).

	t statistic		
	TSST	f-TSST	f-TSST – TSST
24-item Total Score	10.60***	–5.39***	–10.93***
Distress	11.91***	–1.08	–11.17***
Worry	8.25***	–1.89^{+}	–6.53***
Confidence	–5.38***	6.09***	7.76***
Negative affect	5.97***	–2.94**	–7.91***
Motivation	–1.25	3.68**	3.72***
Self-evaluation	1.74^{+}	–2.06*	–2.74**

Note. SSSQ-G = Short Stress State Questionnaire in German; (f-)TSST = (friendly) Trier Social Stress Test; Items from the Confidence and Motivation scales were reverse coded in the 24-item total score. $^{+}$p < .10, *p < .05, **p < .01, ***p < .001.

as covariates for sex (Women; Men), and body position (Sitting; Standing). Random intercepts and slopes for linear time and condition were included at the subject level. We found that sAA values followed a significant linear decrease over time relative to the peak (b = –0.111, p < .001), with a significant quadratic effect (b = –0.008, p < .001), indicating an initial increase before the peak followed by a decelerating decline. There was no main effect of condition (TSST vs. f-TSST) or condition order, nor was there a significant interaction between time and condition. However, we observed a significant interaction between condition and condition order (b = 0.86, p = .047), indicating that participants who started with the TSST showed higher sAA levels. Additionally, significant interactions between the quadratic time term and both condition (b = –0.002, p = .047) and condition order (b = –0.002, p = .03) suggest that the curvature of the sAA response trajectory varied depending on these factors. We further conducted a repeated measures ANCOVA with sAA increase as the outcome (calculated as S2 minus S1), the between-subjects factor order (TSST_first; f-TSST_first) and the within-subjects factor of condition (TSST; f-TSST), covarying sex (Women; Men) and body position (Sitting; Standing). All effects were n. s. (smallest p = .06).

We then examined HPA axis responses using a linear mixed-effects model with log-transformed cortisol values. Time was centered at 30 min (S3), to model change relative to the expected peak in cortisol response. The model included fixed effects for linear and quadratic centered time, condition (TSST; f-TSST), condition order (TSST_first; f-TSST_first), and their interactions, covarying for sex (Women; Men) and body position (Sitting; Standing). It also included random intercepts and random slopes for linear time and condition at the subject level. Cortisol levels showed a significant curvilinear trajectory over time, with a positive linear effect (b = 0.004, p < .001) and a negative quadratic effect (b = –0.0002, p < .001). Levels were higher in the TSST condition compared to the f-TSST ($b = 0.30$, $p < .001$). Women exhibited lower cortisol responses than men ($b = –0.20$, $p = .04$), and standing participants had higher cortisol levels than those sitting ($b = 0.27$, $p = .01$). The significant interactions between linear time and condition ($b = 0.008$, $p < .001$) and quadratic time and condition ($b = –0.0002$, $p < .001$) indicate that participants exhibited a more pronounced cortisol response in the TSST, compared to the f-TSST. Additionally, a significant interaction between linear time and condition order ($b = –0.004$, $p = .02$) and a three-way interaction between linear time, condition, and condition order ($b = 0.005$, $p = .01$) suggest that the trajectory of cortisol over time depended not only on the condition but also on the order in which conditions were presented. In a repeated measures ANCOVA with cortisol maximum increase as the outcome (calculated as the highest value of S2, S3, and S4 minus S1) with the between-subjects factor order (TSST_first; f-TSST_first) and the within-subjects factor of condition (TSST; f-TSST) as well as the covariates sex (Women; Men) and body position (Sitting; Standing), we found main effects of sex ($F(1, 85) = 6.21$, $p = .02$, generalized $\eta^2 = .05$), with men showing a stronger cortisol increase than women, and condition ($F(1, 85) = 45.42$, $p < .001$, generalized $\eta^2 = .16$), indicating that the TSST produced a greater cortisol increase than the f-TSST. All other effects were n.s. (smallest $p = .08$). Fig. 3 provides an overview over the physiological stress response, depicting cortisol, sAA and HR.

3.3. Heart rate

The model included fixed effects for linear and quadratic time, condition (TSST; f-TSST), condition order (TSST_first; f-TSST_first), and their interactions with the quadratic time term. It also covaried for sex (Women; Men) and body position (Sitting; Standing). Random intercepts and random slopes for linear time and condition were modeled at the subject level. Heart rate showed a significant linear increase over time ($b = 3.22$, $p < .001$) and a significant quadratic decrease ($b = –0.23$, $p < .001$), indicating a curvilinear trajectory during the session. Heart rate was significantly higher in the TSST condition compared to the f-TSST ($b = 7.25$, $p < .001$). Participants standing had higher heart rates than those sitting ($b = 5.93$, $p < .001$). Similarly, in a repeated measures ANCOVA with HR maximum increase as the outcome (calculated as the highest heart rate value observed during the math and speech tasks minus the baseline heart rate value), we observed main effects of body position ($F(1, 66) = 19.13$, $p < .001$, generalized $\eta^2 = .18$) and condition ($F(1, 66) = 41.87$, $p < .001$, generalized $\eta^2 = .14$), indicating that participants' HR was overall higher in the TSST than the f-TSST, and that participants standing had higher heart rates than those sitting. All other effects were n.s. (smallest $p = .26$).

3.4. Facial expressions

Mean intensity rates per condition and phase for all AUs are presented in the Supplementary Table 1 in Appendix A. AU28 ("lip suck") showed a mean and standard deviation close to zero and was dropped from subsequent analyses. An overview of the AUs across conditions is given in the Supplementary Figure 2 in Appendix A.

As the preregistered confirmatory factor analysis (CFA) model clustering AUs into basic emotions according to the Facial Action Coding System (FACS; (Barrett et al., 2019; Ekman and Friesen, 1978) failed to converge, we conducted exploratory factor analyses (EFA) on all available AUs, as well as excluding speech-related AUs (Meng et al., 2019), to investigate the underlying factor structure. The number of factors was determined using a scree plot of eigenvalues and parallel analysis, which identified five factors as the most appropriate for the full set of AUs, while excluding the speech-related AUs resulted in a two-factor solution. The EFA was conducted with a promax rotation to allow for correlations among factors. Fit indices, however, were not acceptable.[4] Thus, we increased the number of factors until we obtained an acceptable fit, which resulted in 10 factors for the full set of AUs and four factors for the set excluding speech-related AUs, with substantial cross-loadings and multiple factors consisting of only one AU. Thus, we had to conclude that the facial expressions from this study cannot be meaningfully grouped into factors, and certainly not into the preregistered emotion categories proposed elsewhere.

Because we did not observe the expected emotion categories in our data, we examined individual Action Units (AUs) as well as composite scores representing *friendly* (AU06, AU07, AU12) and *confrontational* (AU04, AU05, AU07, AU09) facial activity, as recommended by prior research (Blasberg et al., 2023; Mayo and Heilig, 2019). We used linear mixed-effects models to analyze these outcomes, including fixed effects for condition (TSST; f-TSST), sex (Men; Women), their interaction, condition order (TSST_first; f-TSST_first), and body position (Sitting; Standing). Random intercepts for participants accounted for repeated measures across conditions. After applying Benjamini-Hochberg correction for multiple comparisons, neither the main effect of sex nor the sex × condition interaction was significant for any AU. In contrast, the main effect of condition remained significant for all AUs (largest adjusted p = .04). Estimated AU values were higher during the TSST for AU01, AU02, AU04, AU05, AU15, AU17, and AU23, whereas AU06, AU07, AU09, AU10, AU11, AU12, AU20, AU25, AU26, and AU43 showed higher values during the f-TSST. Effect sizes ranged from –0.14–0.03. In both models using the composites, we found significant main effects of condition: Participants showed less friendly facial expressions ($b = –0.28$, $p < .001$), but also less confrontational facial expressions ($b = –0.10$, $p < .001$) in the TSST compared to the f-TSST. Body position also showed main effects in both models, with standing participants displaying more friendly ($b = 0.13$, $p = .032$) and more confrontational facial expressions ($b = 0.07$, $p = .007$) compared to those sitting. All other effects were nonsignificant (*ps* >.06).

3.5. Associations of stress response measures and facial expressions

Bivariate correlations between biomarkers, HR, self-reported stress state changes and facial expressions in the TSST data can be found in Table 2.

We used a theory-driven, nested model comparison approach with linear mixed-effects models. Models were fit sequentially to assess the incremental contribution of experimental conditions, interaction terms, and facial action units (AUs) to the respective outcome variables (sAA, cortisol, HR, and SSSQ-G total scores), with model comparisons conducted using likelihood ratio tests. As the factor analyses yielded no acceptable results, we considered AUs not confounded by speech as independent variables (AU01, AU02, AU04, AU05, AU06, AU07, AU09, AU12), which specifically included variables that have been found to predict stress outcome measures in previous research (Blasberg et al., 2023; Mayo and Heilig, 2019). For HR we incorporated an interaction term between condition and phase (Math, Talk). Across all models, the inclusion of AUs did not significantly improve model fit. For full model details, see Supplementary Table 2. This suggests that, within this modeling framework, AUs were not reliable predictors of acute stress responses.

However, exploratory correlations indicated that cortisol maximum increase was positively associated with AU17 ("chin raiser") and

[4] EFA with all AUs using TSST data: RMSEA = .17, 90 % CI [.151,.19], TLI = .687, empirical $\chi^2(86, N = 97) = 75.78$, $p < .78$; EFA without speech-related AUs using TSST data: RMSEA = .14, 90 % CI [.094,.198], TLI = .862, empirical $\chi^2(13, N = 97) = 27.51$, $p < .01$.

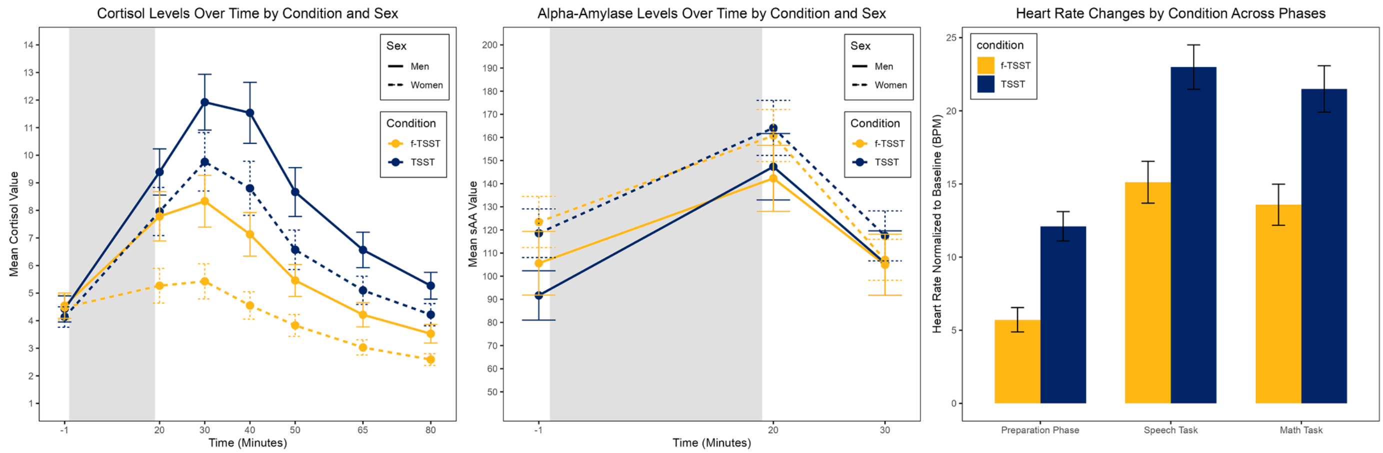

Fig. 3. Physiological stress response to the (f-)TSST. Salivary markers show the untransformed values of both men and women at baseline as well as 1, 10, 20, 30, 45, and 60 min after the (friendly) Trier Social Stress Test [(f-)TSST]. Heart rate values are shown normalized to baseline. Graphs show the mean and standard error of the mean. The gray shaded area indicates the (f-)TSST.

Table 2
Bivariate correlations between all outcome measures in the TSST data.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1. sAA (increase)																														
2. Cortisol (max increase)	.16																													
3. HR Preparation Phase	.04	.53***																												
4. HR Talk Phase	.11	.56***	.67***																											
5. HR Math Phase	.18	.54***	.47***	.79***																										
6. SSSQ-G Total Scale	.10	.26*	.15	.18	.20																									
7. SSSQ-G Distress	.06	.08	.13	.25	.34**	.66***																								
8. SSSQ-G Worry	.08	.28*	.10	.04	.10	.66***	.14																							
9. SSSQ-G Confidence	−.13	−.25	−.32*	−.28*	−.17	−.64***	−.30*	−.25																						
10. SSSQ-G Negative Affect	.09	.01	−.11	−.01	.01	.60***	.54***	.16	−.18																					
11. SSSQ-G Motivation	.07	.16	.11	<.01	<.01	−.38**	−.12	−.13	.25	−.18																				
12. SSSQ-G Self-evaluation	−.01	.34**	.09	.02	−.02	.56***	.19	.45***	−.21	.20	.07																			
13. AU01	.09	.20	.10	.18	.30*	.04	.13	.02	−.08	−.15	.13	.11																		
14. AU02	−.01	.15	.17	.19	.23	.11	.27*	.05	−.13	−.12	.08	.02	.85***																	
15. AU04	.14	−.02	−.13	.08	.07	.10	−.07	.09	−.09	.22	−.02	.06	−.09	−.23																
16. AU05	.29*	−.01	−.18	−.28*	−.20	.10	.11	.08	−.08	<.01	.16	.17	.18	.09	−.07															
17. AU06	−.02	.08	.14	.18	.23	−.24	−.21	−.14	.21	−.24	−.15	−.13	.09	.07	−.21	−.49***														
18. AU07	−.02	.01	.05	.06	.23	−.22	−.13	−.12	.22	−.20	−.09	−.16	.25	.21	−.41**	−.38**	.79***													
19. AU09	−.05	.03	.15	.13	−.03	.05	.02	−.10	−.09	.28*	<.01	−.06	−.20	−.12	.15	−.32*	.05	−.07												
20. AU10	.01	.06	.09	−.03	.10	−.15	−.20	−.03	.18	−.18	−.06	.05	.18	.03	−.27*	−.24	.74***	.79***	.03											
21. AU11	−.10	−.01	.08	.06	.17	−.15	−.13	−.02	.15	−.23	−.12	−.12	.26*	.32*	−.24	−.38**	.76***	.84***	−.04	.65***										
22. AU12	−.16	−.01	.17	.00	−.03	−.19	−.20	−.13	.13	−.15	−.16	−.14	−.16	−.04	−.32**	−.53***	.82***	.68***	.04	.58***	.71***									
23. AU14	−.16	.13	.05	−.05	−.01	−.08	−.15	<.01	.15	−.13	−.13	.10	−.10	−.02	−.60***	−.35**	.63***	.76***	.03	.68***	.64***	.72***								
24. AU15	.14	<.05	.06	.02	.02	.17	.10	.11	−.20	.18	.07	.06	.23	.20	.46***	.16	−.38**	−.52***	.23	−.36**	−.42***	−.55***	−.71***							
25. AU17	.06	.30**	.09	.20	.12	.26*	.15	.24	−.18	.05	−.03	.19	−.02	.09	−.10	.17	−.34**	−.47***	.12	−.45***	−.45***	−.42**	−.26*	.35*						
26. AU20	−.22	−.19	.01	−.24	−.36**	−.11	−.11	−.17	−.01	.11	.03	−.14	−.31*	−.16	−.04	−.26*	.08	.05	.21	.06	.08	.50***	.25	−.34**	−.48***					
27. AU23	−.05	−.09	−.09	−.33*	−.43***	.15	−.03	.11	−.15	.18	−.16	.02	−.52***	−.34**	−.09	−.17	−.29*	−.32*	.23	−.20	−.37**	.07	.09	<.01	.18	.47***				
28. AU24	−.05	.21	−.01	<.01	.06	.24	.01	.33**	−.13	−.07	−.25	.17	−.05	−.02	.07	.05	−.08	−.17	−.30*	−.18	.05	−.02	−.10	.07	.32*	−.34**	−.04			
29. AU25	.01	−.33**	−.02	−.17	−.21	−.19	−.03	−.29*	.01	.12	.14	−.29*	−.02	−.04	.29*	−.04	.03	−.07	.20	−.02	−.03	.13	−.25	.08	−.58***	.62***	.07	−.50***		
30. AU26	.04	−.17	−.09	−.03	.09	−.16	.05	−.34**	.05	.09	.12	−.13	.32*	.25	.12	−.04	.18	.26*	.16	.21	.11	.06	.01	.09	−.42***	.29*	−.10	−.68***	.62***	
31. AU43	−.16	.09	.17	.36**	.45***	−.16	<.01	−.16	.10	−.17	−.05	−.21	.44***	.46***	−.16	−.54***	.57***	.65***	−.01	.38**	.57***	.37**	.34**	−.21	.19*	−.09	−.36**	−.16	−.06	.29*

Note. sAA = Salivary Alpha-Amylase; HR = Heart Rate; SSSQ-G = Short Stress State Questionnaire in German; AU = Action Unit; $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

negatively associated with AU25 (“lips part”) in the TSST, which may reflect that participants who talk more during the stress task exhibit lower cortisol reactivity. Additionally, cortisol maximum increase also showed positive associations with the SSSQ-G total score as well as with the subscales *Worry* and *Self-evaluation*.

Overall, these findings suggest that individual facial action units, aggregated across the TSST and f-TSST, offer limited incremental predictive value for stress biomarkers and self-reported stress.

A model using HR during the preparation, math, and talk phases as a latent predictor of cortisol maximum increase in the TSST showed acceptable fit, $\chi^2(2) = 4.11$, $p = .13$, CFI $= .985$, TLI $= .954$, SRMR $= .034$, RMSEA $= .12$, 90 % CI [0.000, 0.294]. While RMSEA was slightly above the conventional cutoff of .08, other indices indicated good model fit. In this model, HR significantly predicted cortisol increase ($\beta = 0.55$, $p < .001$, Fig. 4).

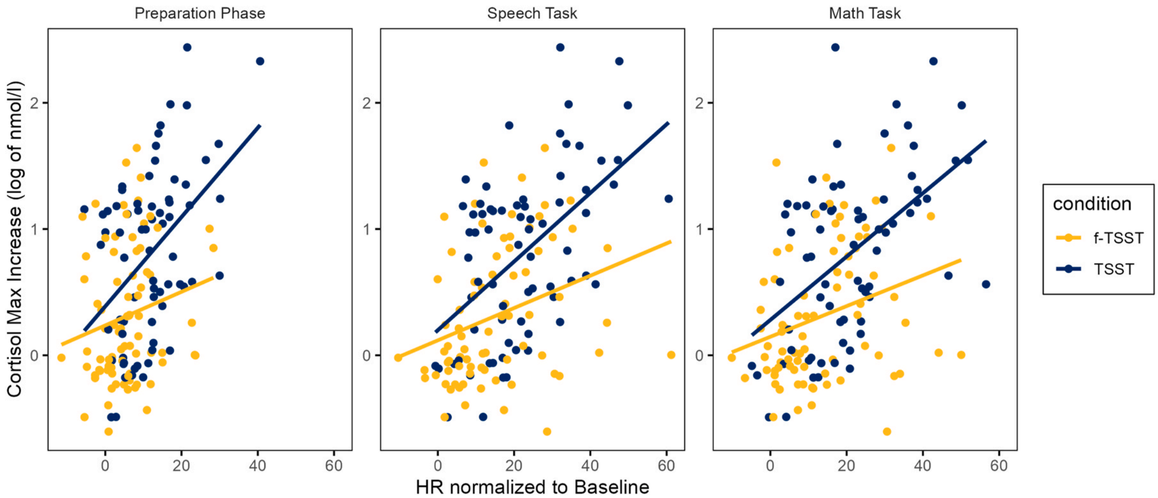

Fig. 4. Scatterplot of heart rate and cortisol maximum increase by condition. HR = Heart Rate. HR is shown normalized to baseline and cortisol maximum increase shows the maximum increase using log-transformed values of nmol/l.

4. Discussion

In this study, we set out to investigate the dynamics of different outcome measures of the acute stress response by examining psychological, physiological, and behavioral changes, both separately and in conjunction. Participants underwent the TSST and its (modified) friendly control condition in a within-subjects, crossover design, obtaining cortisol and alpha-amylase from saliva samples, heart rate from ECG, questionnaire data, and AU mean intensity from video data.

4.1. Summary and discussion of results

With regard to the SSSQ-G (Ringgold et al., 2024), our data support the factor structure of the SSSQ-G with the 24-item total scale and the six subscales. The somewhat poorer fit indices observed in the current study may be due to our smaller sample size and differences in stress induction tasks. Importantly, though, we confirmed the expected differences in stress-induced increases in self-reported stress between the TSST and f-TSST, with higher total scores in the TSST. Change scores differed from zero and, more importantly, they differed between the two conditions (Table 1). For the TSST, *Distress, Worry,* and *Negative Affect* increased, while *Confidence* decreased between pre- and post-stress. The f-TSST triggered an increase in *Confidence* and *Motivation* and a decrease of *Negative Affect* and *Self-evaluation*. These results highlight the SSSQ-G's usefulness in assessing task-related changes in self-reported stress permitting the quantification of not only affective processes but also (task-related) motivational and cognitive ones.

Regarding the salivary markers, we found a significant sAA increase in response to the TSST and the f-TSST. However, consistent with (Wiemers et al., 2013), we observed no difference in sAA responses between the two conditions. For cortisol, we found the expected larger increase following the TSST. Cortisol responses to the f-TSST were markedly smaller than those to the TSST, although, as expected from an active control, a response was still observed. While women showed the anticipated pattern—a clear cortisol peak after the TSST and little response to the f-TSST—men exhibited a more pronounced cortisol response to the f-TSST. The observed sex differences may stem from switching to an all-female panel approximately halfway through data collection, which was necessitated by recruitment constraints. An all-female panel would present less of a social-identity threat to women, with evidence pointing towards a heightened reactivity to the opposite sex (Goodman et al., 2017; Labuschagne et al., 2019). We also found that participants in the standing condition exhibited higher cortisol levels than those in the sitting condition. It is possible that the difference in body position between the sitting panel and the standing participants accentuated the perceived gap, possibly heightening participants' sense of threat. Additionally, we found that the order of the conditions had a significant effect on sAA and cortisol responses. Participants showed significantly higher overall sAA values in the f-TSST if it was experienced first, while the responses to the TSST were comparable regardless of the condition order. Cortisol trajectories did not just differ by condition, but also by the order in which they were presented, with a more marked response to the f-TSST when it was experienced first. Regarding associations with self-report, we did not find a significant correlation for sAA with the SSSQ-G. However, in the TSST data, the total score, as well as the subscales *Worry* and *Self-evaluation* showed small but significant positive correlations with the maximum increase in cortisol. Further research is necessary to confirm these associations. However, they are theoretically plausible, given that uncontrollability and social-evaluative threat are key drivers of cortisol responses, which could translate to more self-reported worry and self-evaluation during tasks such as the TSST (Dickerson and Kemeny, 2004).

For heart rate (HR), we found higher HR in the TSST compared to the f-TSST, indicating stronger sympathetic nervous system activation during the stress task. We also found higher HR in the standing condition compared to the sitting condition. We did not find associations between sAA and HR. However, HR did predict maximum cortisol increase in the TSST, suggesting a link that warrants further exploration. As wearables become more common, detecting high HR can help individuals understand their immediate stress responses and use techniques to mitigate adverse effects early on. Our results suggest that in certain contexts, interventions lowering HR might affect subsequent cortisol responses, which could be worth exploring in future research.

Regarding facial expressions, the key finding is that we were unable to replicate the factor structure of the basic emotions (anger, contempt, happiness, fear, and disgust). This may indicate that these emotional categories are not suitable for capturing the facial expressions people display during acute stress in real-life, context-dependent situations (Barrett et al., 2019). Additionally, we found that all AUs, as well as the composites of friendly and confrontational expressions, differed by condition. Notably, sex did not appear to influence the composite or individual AU measures once multiple testing corrections were applied. We also analyzed the relationship between stress reactivity and facial expressions. Given the absence of distinct emotion categories in the AU data, we restricted our predictive models to facial action units unlikely to be confounded by speech. This decision reflects procedural differences between conditions, as the panel was instructed to maintain continuous conversation without pauses during the f-TSST, which likely increased facial movements associated with speech. Facial expressions did not significantly improve model fit for any of the stress markers. These results do not support previously reported associations between facial expressions and cardiovascular or cortisol responses. However, discrepancies may stem from differences in preprocessing pipelines or the specific aspects of AU data analyzed, e.g., occurrence vs. intensity, or the use of specific AU composites (Blasberg et al., 2023; Lerner et al., 2007; Lupis et al., 2014). Overall, our results suggest that facial expressions, as nonverbal displays of affective states, differ between contexts, such as the TSST and f-TSST and that a more fine-grained approach is necessary to capture the dynamic nature of these markers. Additionally, although not the focus of the present study, it is worth considering that that acute stress has been shown to enhance *response inhibition*—the ability to suppress a prepotent response, such as the display of facial expression—while it impairs *cognitive inhibition,* which involves resisting distracting internal or external information and thus may result in rumination or worry (Shields et al., 2016). In the presence of strangers and in an unfamiliar, evaluative setting, participants may have actively inhibited facial expressions of affect, either consciously or as an automatic regulatory response, potentially masking emotional signals that might otherwise be expressed in less constrained environments. Further exploration of how facial expressions relate to physiological stress markers in the context of various disorders could inform future therapeutic interventions, such as the treatment of major depressive disorder through biofeedback (Keinert et al., 2024).

4.2. Strengths and limitations

Although this study has several strengths, including a within-subjects, crossover design, a relatively large sample, and the integration of psychological, physiological and behavioral markers, some limitations should be noted. First, the adaptation of the f-TSST to include both a math and a speech phase allows for a direct comparison with the TSST, but the detailed impact of this change on participants' stress responses remains unexamined and should be explored in future research, as it lay outside of the scope of this work. Second, the study protocol was adjusted to test novel technical equipment for contact-free measurement of physiological parameters, which required incorporating speech pauses, as well as different body postures, during the TSST and f-TSST. The pauses specifically may have influenced participants' responses, as they could have used them to compose themselves or prepare further for the task. Although we excluded these pauses from the analysis of the video data, they may nonetheless have contained predictive information and could be worth exploring in future research. Third, the timing of outcome assessments, data aggregation, and the integration of various data types are crucial when connecting multiple stress-related measures. In this study, we aggregated facial expression intensity across (f-)TSST phases and assessed self-reported stress before and immediately after the task. However, as subjective stress peaks during the task itself (Hellhammer and Schubert, 2012), future studies should include real-time subjective stress ratings to better understand the processes driving individual stress responses. Regarding facial expressions, future analyses could preserve the temporal resolution of the data by modeling AU trajectories over time, to capture moment-to-moment fluctuations. Moreover, as has been pointed out elsewhere, facial expression occurrence, as opposed to intensity, may show a stronger link to the physiological and psychological stress response (Blasberg et al., 2023). However, intensity, as a continuous measure averaged across frames, may capture more nuanced variations, whereas occurrence, a binary measure, reflects only the proportion of activated frames, potentially leading to a trade-off.

4.3. Future directions

By adding to the literature on the connectivity between stress-related outcomes such as biomarkers, heart rate, self-reported stress, and facial expressions, our study offers valuable extensions to existing findings. Specifically, we replicated established stress effects using a within-subjects design and highlighted the influence of condition order. We also improved the comparability of the f-TSST to the standard TSST by incorporating a math task. In addition, we introduced the SSSQ-G as a multidimensional measure capturing affective, motivational, and cognitive aspects of stress responses. Finally, we identified condition-dependent differences in facial expressions which, when aggregated, did not predict common stress outcomes—suggesting their potential lies in more fine-grained or temporally sensitive analyses. These findings point to several promising directions for future research on the behavioral and physiological dynamics of stress.

First, the link between self-reported worry and self-evaluation with cortisol may be important for interventions, especially given the role of rumination and worry in anxiety and depressive disorders (McLaughlin et al., 2007). If individuals, in response to uncontrollable or evaluative stressful situations, react with heightened worry, (negative) self-evaluation, less confidence, and potentially higher cortisol in connection to that, this might result in more negative health outcomes for people already suffering from affective disorders, such as anxiety or depression.

Next, we want to emphasize the necessity of assessing self-reported stress states not only before and after a stress-inducing task, but also during, to capture the dynamic nature of subjective stress parallel to physiological and behavioral markers, in line with previous work (Campbell and Ehlert, 2012; Schlotz et al., 2008). This poses challenges for future study setups, as such assessments might disrupt the study flow.

Additionally, we seek to highlight the crucial role of integrating multiple physiological outcome measures in studies using established stress protocols—with close attention to context-specificity, including stressor characteristics, timing, and the distinct mechanisms underlying physiological, psychological, and behavioral responses. In our sample, we found that HR during the TSST predicted the subsequent cortisol response. A recent meta-analysis identified cortisol as the most reliable biomarker of stress (Man et al., 2023). However, the relative contribution of other physiological outcome measures is less understood, as fewer studies have examined them alongside cortisol, and warrants further exploration. Also, the connection between self-reported stress states and biomarkers needs to be explored more rigorously. Specifically, assessment timing and frequency are critical to obtaining a complete profile of the acute stress response, and will help researchers uncover interdependencies, e.g., how cortisol influences later affective outcomes (Campbell and Ehlert, 2012; Man et al., 2023). It is also possible that the stress response is primarily functional, with no strong or consistent coordination among its components. In such a case, stress response metrics may not show clear patterns of association, as individual components vary depending on the specific demands of a situation. We therefore urge the stress research community to utilize a multitude of outcome measures, specifically ones that depict the complexity of the psychological stress response, such as self-report measures that allow the quantification of cognitive, emotional, and motivational changes in response to stress. A multi-systemic approach is crucial to understanding a potential interplay between biological, psychological, and behavioral processes in stress.

Finally, we believe that our results speak to the implications and possibilities for future research on stress-related facial expressions. We did not find support for the commonly used emotion categories (anger, contempt, happiness, fear, disgust), which, despite their widespread application, have been previously questioned (Barrett et al., 2019; Barrett and Satpute, 2019; Mayo and Heilig, 2019). While facial expressions are valuable for conveying individual experiences in a social setting (Crivelli and Fridlund, 2018), they may not be universal, but instead, highly variable and context-dependent (Barrett and Satpute, 2019). We propose challenging the use of fixed emotional categories without first considering their feasibility in specific contexts and datasets. Future studies should apply more sophisticated statistical methods to analyze facial expressions with greater temporal resolution, accounting for individual differences and situational factors. This more granular approach would allow researchers to map both individual and situational variations, providing a deeper understanding of how these markers reflect the human stress response. Aggregating facial expression data over longer periods may overlook the complexity and variability of these markers, especially in stress-related contexts (Mayo and Heilig, 2019).

5. Conclusion

Although it may feel like we have a single "stress response," a growing body of work highlights a complex set of interrelated but distinguishable stress responses. However, few studies have explored these dynamics by simultaneously assessing the responses of salivary biomarkers, heart rate, and self-reported subjective states to acute psychosocial stress. Further, the relationship of these responses to facial expressions had not yet been explored in a within-subjects design, which enables inferences at both the intra- and inter-individual levels. Given that cognitive and emotional processes are key antecedents of the physiological and behavioral stress response to acute psychosocial stress (Cohen et al., 2016), and the relationship between them is bidirectional (Schlotz et al., 2008), understanding these interrelations is crucial for addressing stress-related affective disorders and dysregulations (Man et al., 2023). Our study establishes links between self-reported cognitive stress components—worry and self-evaluation—and cortisol, as well as heart rate and cortisol, while no significant relationship was found between salivar alpha-amylase and other markers. The facial expression results reveal that these behavioral markers vary between the stress and control condition. However, individual facial muscle movements were not predictive of biological stress responses. In sum, our findings provide valuable insights into the complex interplay between the mind and body in response to acute psychosocial stress.

Publication ethics

The study was conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent before testing. The Ethics Committee of the Friedrich-Alexander-University Erlangen-Nürnberg approved the study (ethical approval code 493_20 B).

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CRediT authorship contribution statement

Veronika Ringgold: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Felicitas Burkhardt:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Luca Abel:** Methodology, Investigation, Data curation, Conceptualization. **Miriam Kurz:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Victoria Müller:** Software, Data curation. **Robert Richer:** Writing – review & editing, Software, Project administration, Methodology, Investigation, Funding acquisition. **Bjoern M. Eskofier:** Project administration, Methodology, Funding acquisition. **Grant S. Shields:** Writing – review & editing, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **Nicolas Rohleder:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT (GPT-4o; OpenAI, San Francisco, CA, USA) in order to enhance readability and optimize code in R. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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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.2025.107560.

Data availability

Data and Code used for the analyses in this manuscript can be found at https://osf.io/2u4jh/

References

Allen, A.P., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., 2014. Biological and psychological markers of stress in humans: focus on the Trier Social Stress Test. Neurosci. Biobehav. Rev. 38, 94–124. https://doi.org/10.1016/j.neubiorev.2013.11.005.

Barrett, L.F., Adolphs, R., Marsella, S., Martinez, A.M., Pollak, S.D., 2019. Emotional expressions reconsidered: Challenges to inferring emotion from human facial movements. Psychol. Sci. Publ. Inter. A J. Am. Psychol. Soc. 20 (1), 1–68. https://doi.org/10.1177/1529100619832930.

Barrett, L.F., Satpute, A.B., 2019. Historical pitfalls and new directions in the neuroscience of emotion. Neurosci. Lett. 693, 9–18. https://doi.org/10.1016/j.neulet.2017.07.045.

Blasberg, J.U., Gallistl, M., Degering, M., Baierlein, F., Engert, V., 2023. You look stressed: a pilot study on facial action unit activity in the context of psychosocial stress. Compr. Psychoneuroendocrinology 15, 100187. https://doi.org/10.1016/j.cpnec.2023.100187.

Campbell, J., Ehlert, U., 2012. Acute psychosocial stress: does the emotional stress response correspond with physiological responses? Psychoneuroendocrinology 37 (8), 1111–1134. https://doi.org/10.1016/j.psyneuen.2011.12.010.

Chrousos, G.P., 2009. Stress and disorders of the stress system. Nat. Rev. Endocrinol. 5 (7), 374–381. https://doi.org/10.1038/nrendo.2009.106.

Cohen, S., Gianaros, S., Manuck, S.B., 2016. A stage model of stress and disease. Perspect. Psychol. Sci. A J. Assoc. Psychol. Sci. 11 (4), 456–463. https://doi.org/10.1177/1745691616646305.

Crivelli, C., Fridlund, A.J., 2018. Facial displays are tools for social influence. Trends Cogn. Sci. 22 (5), 388–399. https://doi.org/10.1016/j.tics.2018.02.006.

Denson, T.F., Spanovic, M., Miller, N., 2009. Cognitive appraisals and emotions predict cortisol and immune responses: a meta-analysis of acute laboratory social stressors and emotion inductions. Psychol. Bull. 135 (6), 823–853. https://doi.org/10.1037/a0016909.

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.

Ekman, P., Friesen, W.V., 1978. Facial action coding system: a technique for the measurement of facial movement. Consulting Psychologists Press, Palo Alto.

Ekman, P., Friesen, W.V., O'Sullivan, M., Chan, A., Diacoyanni-Tarlatzis, I., Heider, K., Krause, R., LeCompte, W.A., Pitcairn, T., Ricci-Bitti, P.E., 1987. Universals and cultural differences in the judgments of facial expressions of emotion. J. Personal. Soc. Psychol. 53 (4), 712–717. https://doi.org/10.1037/0022-3514.53.4.712.

Engert, V., Kok, B.E., Puhlmann, L.M.C., Stalder, T., Kirschbaum, C., Apostolakou, F., Papanastasopoulou, C., Papassotiriou, I., Pervanidou, P., Chrousos, G.P., Singer, T., 2018. Exploring the multidimensional complex systems structure of the stress response and its relation to health and sleep outcomes. Brain Behav. Immun. 73, 390–402. https://doi.org/10.1016/j.bbi.2018.05.023.

Fabrigar, L.R., Wegener, D.T., MacCallum, R.C., Strahan, E.J., 1999. Evaluating the use of exploratory factor analysis in psychological research. Psychol. Methods 4 (3), 272–299. https://doi.org/10.1037/1082-989X.4.3.272.

Gaab, J., 2009. PASA – Primary appraisal secondary appraisal. Verhaltenstherapie 19 (2), 114–115. https://doi.org/10.1159/000223610.
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.
Hamilton, P., 2002. Open source ECG analysis. Comput. Cardiol. 101–104. https://doi.org/10.1109/CIC.2002.1166717.
Happold, J., Richer, R., Kuderle, A., Gabner, H., Klucken, J., Eskofier, B.M., Kluge, F., 2021. Evaluation of orthostatic reactions in real-world environments using wearable sensors. Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual International Conference 2021, 6987–6990. https://doi.org/10.1109/EMBC46164.2021.9630842.
Hautzinger, M., Bailer, M., Hofmeister, D., Keller, F., 2012. Allgemeine Depressionsskala (ADS). Hogrefe, Göttingen.
Hellhammer, J., Schubert, M., 2012. The physiological response to Trier Social Stress Test relates to subjective measures of stress during but not before or after the test. Psychoneuroendocrinology 37 (1), 119–124. https://doi.org/10.1016/j.psyneuen.2011.05.012.
Het, S., Schoofs, D., Rohleder, N., Wolf, O.T., 2012. Stress-induced cortisol level elevations are associated with reduced negative affect after stress: indications for a mood-buffering cortisol effect. Psychosom. Med. 74 (1), 23–32. https://doi.org/10.1097/PSY.0b013e31823a4a25.
Höfling, T.T.A., Gerdes, A.B.M., Föhl, U., Alpers, G.W., 2020. Read my face: automatic facial coding versus psychophysiological indicators of emotional valence and arousal. Front. Psychol. 11, 1388. https://doi.org/10.3389/fpsyg.2020.01388.
Hu, L.-T., Bentler, P.M., 1999. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. Struct. Equ. Model. A Multidiscip. J. 6 (1), 1–55. https://doi.org/10.1080/10705519909540118.
Janson, J., Rohleder, N., 2017. Distraction coping predicts better cortisol recovery after acute psychosocial stress. Biol. Psychol. 128, 117–124. https://doi.org/10.1016/j.biopsycho.2017.07.014.
Keinert, M., Schindler-Gmelch, L., Rupp, L.H., et al., 2024. Facing depression: evaluating the efficacy of the EmpkinS-EKSpression reappraisal training augmented with facial expressions – protocol of a randomized controlled trial. BMC Psychiatry 24, 896. https://doi.org/10.1186/s12888-024-06361-3.
Kemeny, M.E., 2003. The psychobiology of stress. Curr. Dir. Psychol. Sci. 12 (4), 124–129. https://doi.org/10.1111/1467-8721.01246.
Kirschbaum, C., Kudielka, B., Gaab, J., Schommer, N.C., Hellhammer, D., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Psychosom. Med. 61 (2), 154–162. https://doi.org/10.1097/00006842-199903000-00006.
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.
Labuschagne, I., Grace, C., Rendell, P., Terrett, G., Heinrichs, M., 2019. An introductory guide to conducting the Trier Social Stress Test. Neurosci. Biobehav. Rev. 107, 686–695. https://doi.org/10.1016/j.neubiorev.2019.09.032.
Lazarus, R.S., 1982. Thoughts on the relations between emotion and cognition. Am. Psychol. 37 (9), 1019–1024. https://doi.org/10.1037/0003-066X.37.9.1019.
Lerner, J.S., Dahl, R.E., Hariri, A.R., Taylor, S.E., 2007. Facial expressions of emotion reveal neuroendocrine and cardiovascular stress responses. Biol. Psychiatry 61 (2), 253–260. https://doi.org/10.1016/j.biopsych.2006.08.016.
Lupis, S.B., Lerman, M., Wolf, J.M., 2014. Anger responses to psychosocial stress predict heart rate and cortisol stress responses in men but not women. Psychoneuroendocrinology 49, 84–95. https://doi.org/10.1016/j.psyneuen.2014.07.004.
Man, I.S.C., Shao, R., Hou, W.K., Xin Li, S., Liu, F.Y., Lee, M., Wing, Y.K., Yau, S.-Y., Lee, T.M.C., 2023. Multi-systemic evaluation of biological and emotional responses to the Trier Social Stress Test: a meta-analysis and systematic review. Front. Neuroendocrinol. 68, 101050. https://doi.org/10.1016/j.yfrne.2022.101050.
Mayo, L.M., Heilig, M., 2019. In the face of stress: Interpreting individual differences in stress-induced facial expressions. Neurobiol. Stress 10, 100166. https://doi.org/10.1016/j.ynstr.2019.100166.
McEwen, B.S., 1998. Stress, adaptation, and disease: allostasis and allostatic load. Ann. N. Y. Acad. Sci. https://doi.org/10.1111/j.1749-6632.1998.tb09546.x.
McLaughlin, K.A., Borkovec, T.D., Sibrava, N.J., 2007. The effects of worry and rumination on affect states and cognitive activity. Behav. Ther. 38 (1), 23–38. https://doi.org/10.1016/j.beth.2006.03.003.
Meng, Z., Han, S., Tong, Y., 2019. Listen to your face: Inferring facial action units from audio channel. IEEE Trans. Affect. Comput. 10 (4), 537–551. https://doi.org/10.1109/TAFFC.2017.2749299.
Miller, G.E., Chen, E., Zhou, E.S., 2007. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol. Bull. https://doi.org/10.1037/0033-2909.133.1.25.
Pessoa, L., 2008. On the relationship between emotion and cognition. Nat. Rev. Neurosci. 9 (2), 148–158. https://doi.org/10.1038/nrn2317.
Richer, R., Koch, V., Abel, L., Hauck, F., Kurz, M., Ringgold, V., Müller, V., Küderle, A., Schindler-Gmelch, L., Eskofier, B.M., Rohleder, N., 2024. Machine learning-based detection of acute psychosocial stress from body posture and movements. Sci. Rep. 14 (1), 8251. https://doi.org/10.1038/s41598-024-59043-1.
Richer, R., Küderle, A., Ullrich, M., Rohleder, N., Eskofier, B., 2021. BioPsyKit: A Python package for the analysis of biopsychological data. J. Open Source Softw. 6 (66), 3702. https://doi.org/10.21105/joss.03702.
Ringgold, V., Shields, G.S., Hauck, F., Kurz, M., Schindler-Gmelch, L., Abel, L., Richer, R., Eskofier, B.M., Rohleder, N., 2024. The short stress state questionnaire in German (SSSQ-G): a multi-study validation. Eur. J. Health Psychol. 31 (4), 189–200. https://doi.org/10.1027/2512-8442/a000160.
Rohleder, N., 2019. Stress and inflammation - the need to address the gap in the transition between acute and chronic stress effects. Psychoneuroendocrinology 105, 164–171. https://doi.org/10.1016/j.psyneuen.2019.02.021.
Rohleder, N., Nater, U.M., 2009. Determinants of salivary alpha-amylase in humans and methodological considerations. Psychoneuroendocrinology 34 (4), 469–485. https://doi.org/10.1016/j.psyneuen.2008.12.004.
Roos, L.G., Janson, J., Sturmbauer, S.C., Bennett, J.M., Rohleder, N., 2019. Higher trait reappraisal predicts stronger HPA axis habituation to repeated stress. Psychoneuroendocrinology 101, 12–18. https://doi.org/10.1016/j.psyneuen.2018.10.018.
Rosseel, Y., 2012. lavaan: An R Package for Structural Equation Modeling. J. Stat. Softw. 48 (2), 1–36.
Schlotz, W., Kumsta, R., Layes, I., Entringer, S., Jones, A., Wüst, S., 2008. Covariance between psychological and endocrine responses to pharmacological challenge and psychosocial stress: a question of timing. Psychosom. Med. 70 (7), 787–796. https://doi.org/10.1097/PSY.0b013e3181810658.
Shields, G.S., 2020. Stress and cognition: a user's guide to designing and interpreting studies. Psychoneuroendocrinology 112, 104475. https://doi.org/10.1016/j.psyneuen.2019.104475.
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.
Singmann, H., Bolker, B., Westfall, J., Aust, F., & Ben-Shachar, M.S. (2024). afex: Analysis of Factorial Experiments (R package version 1.3-1) [Computer software]. https://doi.org/10.32614/CRAN.package.afex.
Smyth, J.M., Ockenfels, M.C., Gorin, A.A., Catley, D., Porter, L.S., Kirschbaum, C., Hellhammer, D.H., Stone, A.A., 1997. Individual differences in the diurnal cycle of cortisol. Psychoneuroendocrinology 22 (2), 89–105. https://doi.org/10.1016/S0306-4530(96)00039-X.
Watson, D., Clark, L.A., Tellegen, A., 1988. Development and validation of brief measures of positive and negative affect: the PANAS scales. J. Personal. Soc. Psychol. 54 (6), 1063–1070. https://doi.org/10.1037/0022-3514.54.6.1063.
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 16 (2), 254–260. https://doi.org/10.3109/10253890.2012.714427.
Zänkert, S., Kudielka, B.M., Wüst, S., 2020. Effect of sugar administration on cortisol responses to acute psychosocial stress. Psychoneuroendocrinology 115 (104607), 104607. https://doi.org/10.1016/j.psyneuen.2020.104607.