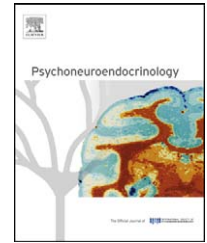




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The Trier Social Stress Test for Groups (TSST-G): A new research tool for controlled simultaneous social stress exposure in a group format

Bernadette von Dawans^a, Clemens Kirschbaum^b, Markus Heinrichs^{a,*}

^a Department of Psychology, Laboratory for Biological and Personality Psychology, University of Freiburg, Stefan-Meier-Strasse 8, D-79104 Freiburg, Germany

^b Department of Psychology, Biological Psychology, Technical University of Dresden, D-01062 Dresden, Germany

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Summary Psychological stress is an ubiquitous challenge across human cultures affecting mental and physical health. Recent evidence indicates that performance tasks combining elements of socio-evaluative threat and uncontrollability elicit reliable stress responses. The Trier Social Stress Test (TSST) is the most frequently used psychological protocol in stress research; however, to date it has only been available in a single-subject version. In particular, there is an increasing need in several emerging research fields such as stress research or social neurosciences for a standardized research tool to expose relatively large groups of subjects to controlled simultaneous stress. In search of a laboratory stressor that allows simultaneous stress exposure in a group format, we exposed a total of 25 healthy male participants to the Trier Social Stress Test for Groups (TSST-G; public speaking and mental arithmetic tasks in front of a panel of two evaluators in groups of six participants) and a specific control condition. Results showed that the TSST-G induced significant increases in cortisol, heart rate, and psychological stress responses. The TSST-G provides a novel, effective, and economical protocol for experimental paradigms requiring simultaneous stress induction in multiple participants.

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1. Introduction

Human beings are fundamentally and pervasively motivated to form and maintain enduring positive interpersonal

interactions (Baumeister and Leary, 1995). Depending on the circumstances, social interactions can be a source of stress, contributing to a wide spectrum of somatic, psychosomatic, and psychiatric disorders with major public health significance, or buffer against stress (Ruberman et al., 1984; House et al., 1988; Kirschbaum et al., 1995; Uchino et al., 1996; Heinrichs et al., 2003).

There is substantial evidence indicating that exposure to psychosocial stress alters hypothalamic-pituitary-adrenal

* Corresponding author. Tel.: +49 761 203 3029;
fax: +49 761 203 3023.

E-mail address: heinrichs@psychologie.uni-freiburg.de
(M. Heinrichs)

(HPA) axis function, which regulates the release of cortisol, an important hormone associated with psychological and physical health functioning (Chrousos, 2009). More specifically, a recent meta-analysis showed that motivated performance tasks combining elements of socio-evaluative threat and uncontrollability elicit robust and reliable psychological and biological stress responses (Dickerson and Kemeny, 2004). Socio-evaluative stress occurs when an aspect of the self could be negatively judged by others (Gruenewald et al., 2004). Uncontrollability refers to the inability of the individual to affect an outcome by a behavioral response (Thompson, 1981).

The Trier Social Stress Test (TSST; Kirschbaum et al., 1993) was developed for the induction of moderate psychosocial stress in a laboratory setting. As this stress paradigm combines uncontrollable and socio-evaluative elements in a highly standardized manner, it reliably leads to psychobiological stress responses (Dickerson and Kemeny, 2004), including 2–3-fold increases in HPA axis and cardiovascular stress responses. Due to large effect sizes and high reliability, the TSST has become a worldwide standard for psychological stress induction under controlled conditions. In brief, the original TSST protocol consists of a 5-min public speaking task (mock job interview) and a subsequent 5-min mental arithmetic task (serial subtraction) performed out loud in front of a panel of two unfamiliar evaluators and a conspicuous video camera. In addition to being used in studies on the deleterious effects of stress, the TSST has also been used as an experimental paradigm to investigate different stress buffering effects (e.g., social support, social attachment, physical contact, exercise, breast-feeding) (Heinrichs et al., 2001, 2003; Ditzen et al., 2007, 2008; Rimmele et al., 2007, 2009; Simeon et al., 2007; Storch et al., 2007; Robles et al., 2009). As the TSST is a single-subject method, the paradigm is unfortunately not applicable to experimental studies that require group testing, such as numerous study designs in social psychology, social neurosciences or behavioral economics. For economical experimental testing of relatively large groups of individuals and to avoid excessive expenses and infrastructures, a controlled simultaneous stress protocol for multiple individuals is required.

To date, there have been no experimental studies that directly address the development of a simultaneous group version of a psychosocial laboratory stressor in a randomized controlled study design. As a consequence, we undertook a controlled trial to develop and evaluate a new tool for standardized social stress exposure in a group format, which we hypothesized would significantly increase cortisol, heart rate, and subjective ratings. In addition, no or little changes of biological and psychological parameters were hypothesized in response to a specifically designed control condition containing all factors of the stress condition except for the psychosocially stressful components (i.e., socio-evaluative threat and uncontrollability).

2. Methods

2.1. Participants

Twenty-five healthy males with a mean age of 22.08 years ($SD = 3.08$) participated in the study. All participants were recruited via an online database at the University of Zurich. Exclusion criteria were prior participation in a stress

experiment, studying psychology, medication intake, reported medical illness, symptoms of psychopathology, substance abuse or smoking more than 5 cigarettes per day. Five of the original 30 participants did not meet the eligibility criteria and were therefore excluded from statistical analyses: one participant who met criteria for a mental disorder based on the Brief Symptom Inventory (Derogatis and Melisaratos, 1983), one with a BMI of 38.6, and three participants who participated in only one experimental condition so that no repeated measures were available. The study was approved by the institutional review board of the University of Zurich. Before participation, all participants provided written informed consent and were informed of their right to discontinue participation at any time. All participants were naïve to the applied stress procedure; participants within one group were not familiar with each other and no participant was familiar with the investigators. After completion of the experiment, participants were debriefed and were paid 100 Swiss francs for their participation.

2.2. Procedure

Participants were asked to have a standard breakfast and lunch at the two days of participation and were instructed to abstain from food 2 h prior to the afternoon session as well as from caffeine, alcohol, exercise, and any medication 24 h before the experiment. Participants were told that they would undergo two different stress tasks and underwent the stress and control conditions, separated by a 1-week interval, in a randomized balanced within-subject design. The 2.5-h sessions took place between 17:15 h and 19:45 h in order to control for diurnal variations of cortisol secretion (Pruessner et al., 1997). As depicted in Fig. 1A, the procedure included a preparation period (50 min), the task (TSST-G or control condition, 30 min), and a resting and debriefing period (60 min). After providing informed consent, participants had to draw a number (from 1 to 6), were instructed not to communicate with each other to provide anonymity, and were then guided to room A. They were then introduced to the experiment, a heart rate device was applied individually and saliva collection was explained within the preparation period of 50 min in room A. During this time, first psychometric and physiological measures were taken (see Assessments). The preparation as well as the resting and debriefing period were identical in the two conditions. Psychosocial stress was induced by the Trier Social Stress Test for Groups (TSST-G), which is based on the single-subject version, referred to as the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993). The TSST is a standardized psychosocial laboratory stressor consisting of a brief preparation period followed by a test period in which the subject is required to deliver a free speech concerning their suitability for employment in a mock job interview and to perform mental arithmetic in front of a panel of two evaluators (Foley and Kirschbaum, 2010).

TSST-G protocol – The TSST-G is a standardized motivated performance task protocol that combines high levels of socio-evaluative threat and uncontrollability in a group format. As depicted in Fig. 1A, the task consists of three phases: (i) an introduction, preparation, and anticipation phase of 10 min, (ii) a public speaking task (mock job

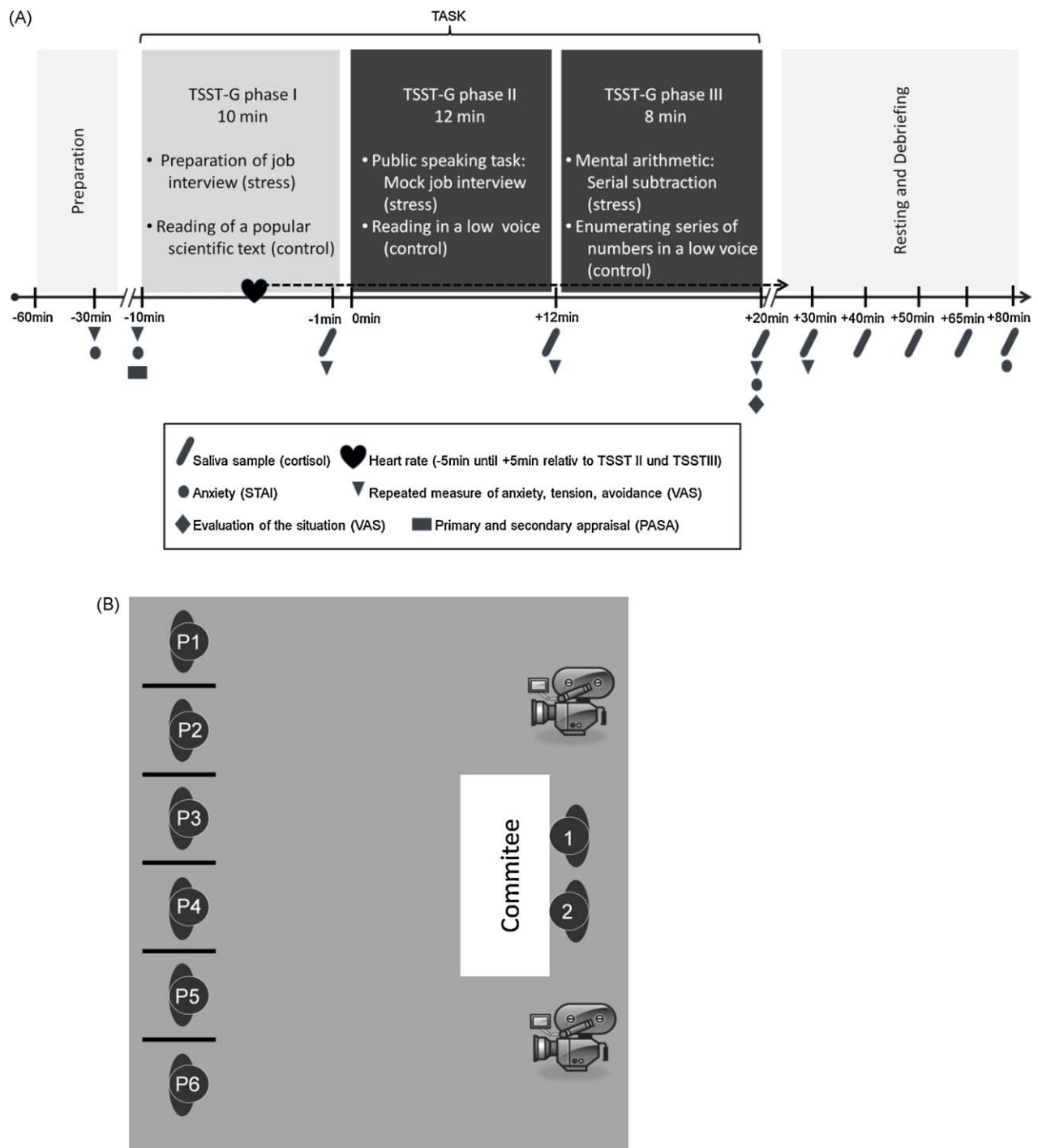


Figure 1 Study design. (A) Sequence of events and timeline. (B) Interior design of the stress exposure room (room B).

interview) of 12 min, and (iii) a mental arithmetic task (serial subtraction) of 8 min. After the preparation phase (–12 min relative to TSST-G), all participants received written instructions for the subsequent task. In particular, they were instructed to prepare an application for a job of their choice and to introduce themselves to the selection committee in a free speech of 2 min. They were asked to convince the committee that they were the most suitable candidates for this position. To increase task engagement, the job description was matched to each participant,

taking into consideration his own individual goals and aspirations.

As in the original TSST, the two members of the evaluation committee (one man and one woman wearing white laboratory coats; same evaluators for all participants) were trained to withhold verbal and non-verbal feedback and were presented as experts in the evaluation of non-verbal behavior. Moreover, the participants were told that a video analysis of their performance would be conducted. In addition, participants were informed that the panel could come back to them

at any time throughout the procedure to ask further questions. The written instructions ended with a remark about an unspecified second task that would follow the speech. After reading these instructions, the participants were given 10 min for preparation and were provided with a paper and pencil to outline their speech; however, they were not allowed to use their notes for their speech in room B.

After the preparation phase, all 6 participants were guided to room B and had to stand in a row in front of the committee, who sat behind a table, and two conspicuous video-cameras (see Fig. 1B). After giving a brief verbal summary of the forthcoming task, the investigator left the room. Participants were separated by mobile dividing walls that restricted any eye contact and social interaction with the other participants (see Fig. 1B). The committee called on each of the participants in random order to start their speech. Whenever a participant finished his speech in less than 2 min, the committee responded in a standardized way. First they told the subject "You still have some time left. Please continue!" Should the participants finish a second time before the 2 min were over, the committee was quiet for 20 s and then asked prepared standard questions. After all participants had given their 2-min speech (a total of 12 min), the committee asked the subject to serially subtract the number 16 from a given number as quickly and accurately as possible (e.g., 4878, 4862, etc.). Each participant received an individual starting number to avoid learning effects. If they made a mistake, they had to restart at their personal number with one member of the committee interrupting, "Stop. Please start again." Participants were interrupted and called upon several times, resulting in a total of 80 s of calculating for each participant (a total of 8 min). Finally, all participants were escorted back to room A and were instructed to rest quietly for 60 min.

Control protocol – The control setting was designed to guarantee specificity of the stress effects in a single-blind control condition containing all factors except for the psychosocially stressful components, i.e. socio-evaluative threat and uncontrollability. In order to control for orthostasis, effects of speech itself, and general cognitive load, the control condition kept all dimensions of the TSST-G constant but excluded any social evaluation or motivated performance. For the single-subject version of the TSST, Het and colleagues recently also reported a control condition ('placebo protocol') (Het et al., 2009). In the 10-min introduction, preparation, and anticipation phase, all participants had to read a popular scientific text and were explicitly told that their reading performance would not be evaluated. After this, they were guided to room B where the investigator gave a short summary of the task and left the room. Then all participants had to read out this text simultaneously in a low voice for 12 min and finally had to enumerate series of numbers in increments of 3, 5, 10, or 20 in a low voice for another 8 min (e.g., 5, 10, 15, etc.). Two individuals were present but they neither wore white laboratory coats nor interrupted the participants (the same two individuals were present for all participants). In addition, they did not observe or evaluate the participants nor did they ask any questions. There were also no video-cameras present. As in the TSST-G protocol, participants were separated by mobile walls that restricted eye

contact and social interaction with the other participants. Finally, all participants were escorted back to room A, where they rested quietly for 60 min. Both in the TSST-G and in the control protocol participants entered and left the laboratory individually in order to avoid any social interaction.

2.3. Questionnaires

The Brief Symptom Inventory (BSI) is a multidimensional self-report instrument designed to screen for a broad range of psychological problems and symptoms of psychopathology (Derogatis and Melisaratos, 1983). The trait anxiety scale of the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970) assessed participants' anxiety. The validated German versions of the questionnaires have been broadly used. The Cronbach's index of internal consistency shows good internal consistency for all questionnaires (between $\alpha = 0.63$ and 0.96). Psychological trait measures were obtained 1 week before the first experiment via an online questionnaire.

At baseline (–30 min relative to the onset of the stress/control condition), at anticipation of the experiment (–10 min), directly after the end of the stress/control condition (+20 min) and at the end of the experiment (+80 min), state anxiety was assessed using the state scale of the STAI (Spielberger et al., 1970). A set of visual analog scales (VAS) ranging from 0 (not at all) to 100 (maximum) was given six times during the protocol in order to assess subjective actual discomfort on the dimensions of anxiety, feeling of tension, and avoidance (desire to leave the situation) (at –30, –10, –1, +12, +20, and +30 min). At the beginning of the anticipation phase, we also measured anticipatory cognitive appraisal using the Primary Appraisal and Secondary Appraisal Questionnaire (PASA) (Gaab et al., 2005). At the end of the TSST-G or control condition, another set of 5 VAS was included to specifically evaluate the two conditions regarding strain, challenge, controllability, stress, and perceived seriousness of the task.

2.4. Endocrine stress responses

Recent studies have found the measurement of cortisol as an indicator for adrenocortical activity to be of high predictive value for psychosocial stress (Foley and Kirschbaum, 2010). Salivary free cortisol has been found to be highly correlated with the unbound cortisol concentration in plasma and is considered to be a reliable and valid indicator of the biologically active fraction of cortisol (Vining et al., 1983; Kirschbaum and Hellhammer, 1989, 1994). Eight saliva samples were collected immediately before (–1 min relative to the onset of the stress or control task), during (+12) and after the stress exposure or control condition (+20, +30, +40, +50, +65, +80 min) using a commercially available sampling device (Salivette[®]; Sarstedt[®], Nümbrecht-Rommelsdorf, Germany). After each experimental session, samples were stored at –20 °C. For biochemical analyses of free cortisol concentration, saliva samples were thawed and spun at 3000 rpm for 10 min to obtain 0.5–1.0 ml clear saliva with low viscosity. Salivary cortisol concentrations were determined by a

commercially available chemiluminescence immunoassay (CLIA; IBL Hamburg, Germany). Inter- and intrassay coefficients of variation were 8.4% and 4.6%, respectively.

2.5. Autonomic stress responses

Heart rate was assessed as a marker of engagement in the task and arousal by continuous recording for subsequent 60-s intervals from 5 min before the task until 5 min after cessation of the task using a wireless chest heart rate transmitter and a wrist monitor recorder (Polar RS800™, Polar Electro, Finland). Heart rate baseline was recorded over 5 min in an upright standing position to control for orthostatic effects. Specifically, the subjects were standing in an upright position during the baseline recording, the TSST-G phase II and TSST-G phase III. The rest of the study was conducted in a sitting position.

2.6. Statistical analyses

Cortisol, heart rate, and psychological data were analyzed using two-way analysis of variance (ANOVA) with repeated measurement (condition [2 conditions: stress condition and control condition] by time [repeated factor: 8 for cortisol, 32 for heart rate, 6 for VAS, 4 for STAI]). We verified repeated measures results with Greenhouse-Geisser corrections where the Mauchly test of sphericity determined heterogeneity of covariance. Paired *t*-tests were used for analyzing single time point evaluations of the two conditions. Data were analyzed using SPSS 16 (SPSS Inc., Chicago, IL). Data are presented as mean ± SEM. All analyses were two-sided, with the level of significance set at $p < 0.05$.

3. Results

3.1. General characteristics and manipulation checks

General trait anxiety (STAI: mean score = 38.36, SD = 8.43; Spielberger et al., 1970), symptoms of psychopathology (BSI: mean score T = 45.28, SD = 8.90; Derogatis and Melisaratos, 1983), and body mass index (BMI: 22.66 kg/m², SD = 2.09) were in the normal range of the general population.

Post-task ratings demonstrated that the TSST-G manipulation was successful. As Fig. 2 shows, participants in the TSST-G condition (immediately after cessation of exposure) stated that their strain ($t(24) = 7.08$, $p < 0.001$), challenge ($t(24) = 5.41$, $p < 0.001$), and stress was higher ($t(24) = 10.07$, $p < 0.001$), whereas subjective controllability was lower ($t(24) = -7.53$, $p < 0.001$) than in the control condition. Notably, the two conditions did not differ significantly in ratings of perceived seriousness of the task ($t(24) = 1.41$, $p = 0.16$), indicating that although only the TSST-G induced socio-evaluative stress and uncontrollability, participants perceived both settings as serious.

3.2. Cortisol responses to stress

Consistent with hypotheses, the TSST-G induced the expected significant increase in salivary free cortisol levels, whereas cortisol levels in the control condition followed the circadian decrease over time (main effect of time, $F(2.32,$

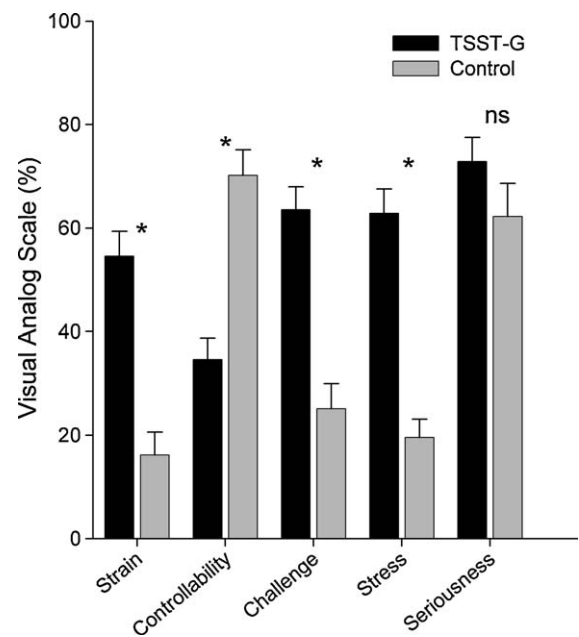


Figure 2 Manipulation check. Mean levels of strain, controllability, challenge, stress, and seriousness perception of the task following standardized psychosocial stress in a group format (Trier Social Stress Test for Groups, TSST-G) and control condition (assessed using visual analog scales immediately after cessation of exposure). Error bars are standard errors of the mean (SEM), asterisks indicate significant differences ($p < 0.001$).

$55.64) = 52.94$, $p < 0.001$; main effect of condition, $F(1, 24) = 56.68$, $p < 0.001$; condition × time interaction, $F(2.11, 5.60) = 53.92$, $p < 0.001$) (see Fig. 3). Cortisol levels did not differ between both conditions at baseline ($t = 0.51$, $p = 0.62$). Cortisol levels increased 3-fold over baseline levels with an average absolute rise in cortisol of 12.17 nmol/l (SEM = 1.46) in the TSST-G condition and a decrease of -1.62 nmol/l (SEM = 0.51) in the control condition (sample +30 min minus sample -1 min; $t(24) = 8.48$, $p < 0.001$), respectively. In order to control for potential effects of the serial position in which participants were called, we compared the first and second position against the fifth and sixth position. The serial position of the participants did not influence cortisol stress responses (condition by time interaction, $F(1.67, 26.67) = 0.45$, $p = 0.61$).

3.3. Heart rate responses to stress

A significant increase in heart rate was observed in both conditions (main effect of time, $F(6.47, 142.34) = 20.81$, $p < 0.001$). As depicted in Fig. 4, there was a significant main effect of condition and a time × condition interaction for heart rates (time × condition interaction, $F(6.30, 138.52) = 4.43$, $p < 0.001$; main effect of condition, $F(1, 22) = 11.18$, $p < 0.01$), with significantly higher elevations in the TSST-G condition. No significant baseline differences in heart rates were observed between both conditions ($t(22) = 0.123$, $p = 0.90$). The individual mean increase in heart rate response (baseline heart rate before stress compared to individual maximum heart rate during stress or

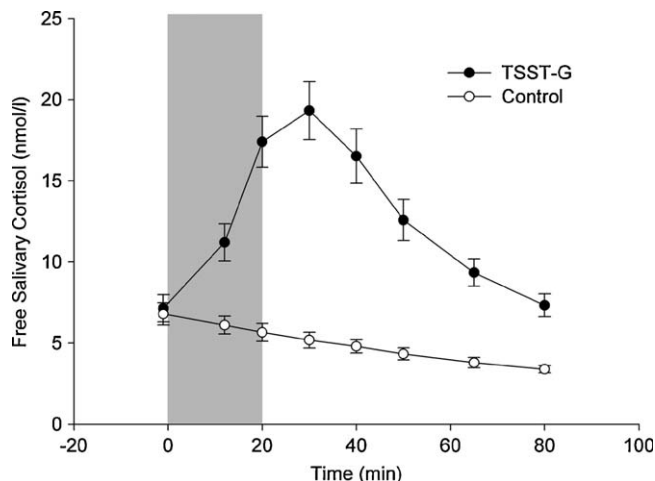


Figure 3 Mean salivary cortisol levels before, during (shaded area), and after a standardized psychosocial stressor in a group format (Trier Social Stress Test for Groups, TSST-G) and a control condition. Error bars are SEM.

control condition) was 38.26 beats/min in the TSST-G condition and 17.30 beats/min in the control condition ($t(22) = 5.40, p < 0.001$). Again, the serial position of participants did not influence heart rate responses to stress (condition \times time interaction, $F(5.68, 90.92) = 0.54, p = 0.77$).

3.4. Psychological responses to stress

In the TSST-G condition, participants showed the expected increase in psychological stress responses. Compared to the control condition, participants reported a significant increase in anxiety, tension, and the desire to leave the situation (see Fig. 5). Specifically, participants showed a significant increase in state anxiety (STAI) during the stress protocol (main effect of

time, $F(2.22, 53.31) = 10.71, p < 0.001$; main effect of condition, $F(1, 24) = 8.36, p < 0.01$; condition \times time interaction, $F(2.63, 63.17) = 7.82, p < 0.001$) (see Fig. 5A). In addition, the TSST-G protocol significantly increased anxiety (main effect of time, $F(3.38, 80.99) = 7.66, p < 0.001$; main effect of condition, $F(1, 24) = 26.00, p < 0.001$; condition \times time interaction, $F(2.91, 69.71) = 4.52, p < 0.01$), tension (main effect of time, $F(4.00, 96.07) = 7.33, p < 0.001$; main effect of condition, $F(1, 24) = 17.74, p < 0.001$; condition \times time interaction, $F(3.35, 80.37) = 3.32, p < 0.05$), and avoidance (main effect of time, $F(3.81, 91.41) = 3.02, p < 0.05$; main effect of condition, $F(1, 24) = 6.53, p < 0.05$; condition \times time interaction, $F(3.39, 81.40) = 3.50, p < 0.05$), as measured with visual analog scales (see Fig. 5B–D). No differences were observed between both

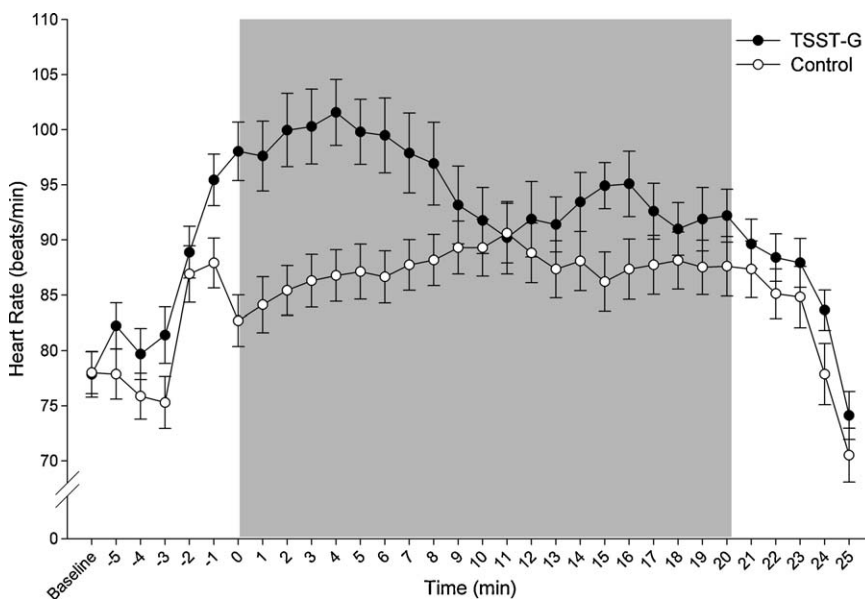


Figure 4 Mean heart rates before, during (shaded area), and after a standardized psychosocial stressor in a group format (Trier Social Stress Test for Groups, TSST-G) and a control condition. Error bars are SEM.

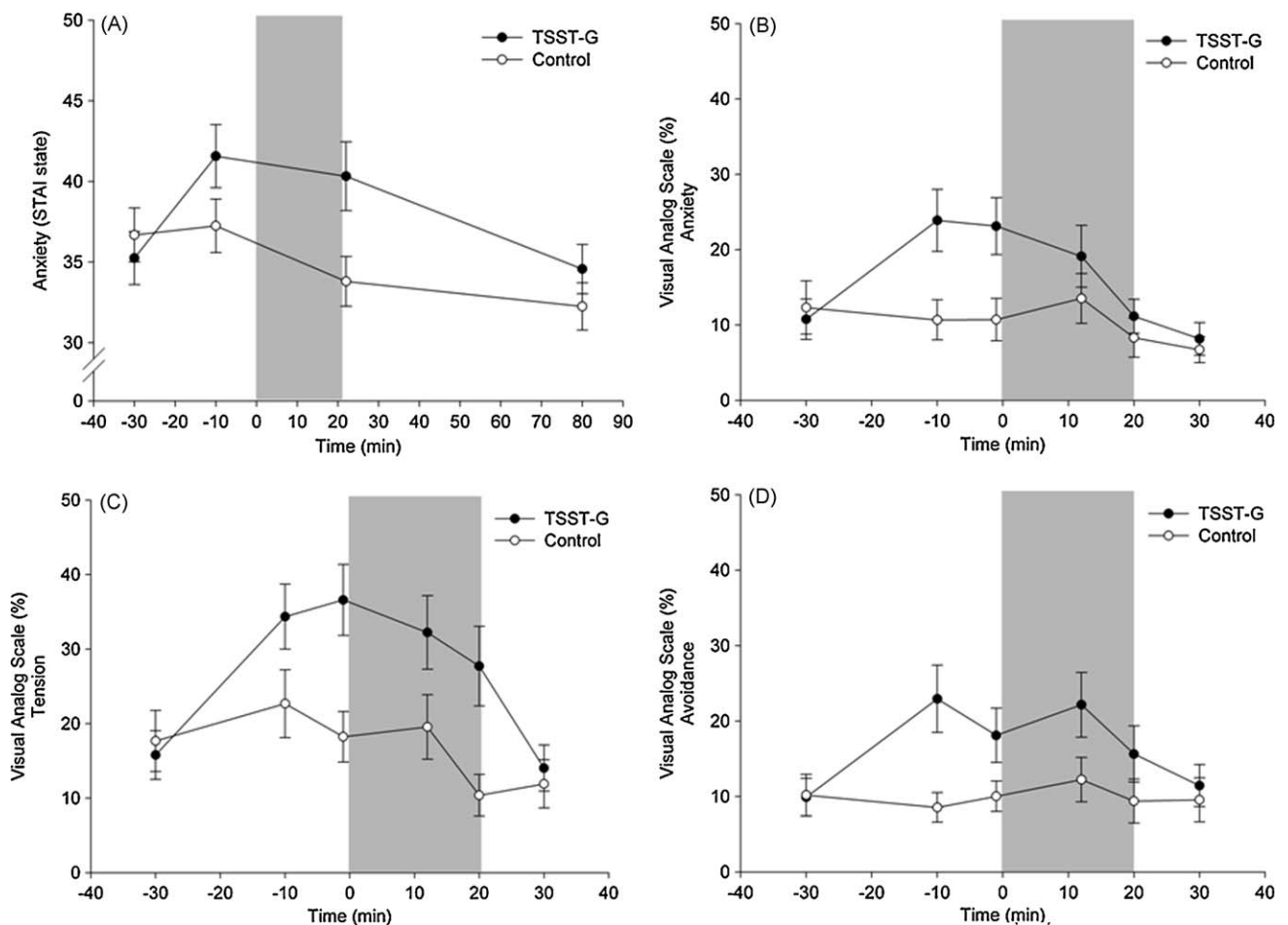


Figure 5 Mean levels (with SEM bars) of state anxiety (A), anxiety ratings (B), tension (C), and desire to leave the situation (avoidance) (D) before, during (shaded area) and after a standardized psychosocial stressor in a group format (Trier Social Stress Test for Groups, TSST-G) and a control condition.

conditions in these measures at baseline. In the anticipation phase, the TSST-G was rated as more threatening and challenging (PASA: subscales 'threat': $t(24) = 5.26$, $p < 0.001$; and 'challenge': $t(24) = 3.33$, $p < 0.05$) and the self-concept of own competence (PASA) ($t(24) = -3.79$, $p < 0.001$) was lower in the TSST-G condition compared to the control condition. There were no significant correlations between the appraisal and heart rate or cortisol measures in the stress condition (all $p > 0.05$).

4. Discussion

We here present and evaluate a newly developed standardized laboratory stress protocol that allows simultaneous psychosocial stress induction in a group format. In order to ensure specificity of stress effects using the 'Trier Social Stress Test for Groups (TSST-G)', we also designed a control condition containing all factors of the TSST-G (e.g., orthostasis, speech task, cognitive load, timeline) except for the psychosocially stressful components, i.e. socio-evaluative threat and uncontrollability. The TSST-G produced a more than 3-fold rise in cortisol and a significant increase in heart rates. Compared to the control condition, the TSST-G also significantly increased subjective stress and anxiety responses during stress exposure.

Immediately after cessation of stress, participants in the TSST-G condition reported significantly more strain, challenge, and stress, and less controllability than in the control condition. Importantly, in both conditions participants perceived similar seriousness of the task, indicating that although only the TSST-G induced socio-evaluative stress and uncontrollability, participants regard both setting as comparably meaningful and are equally motivated to complete the TSST-G and the control condition. Our findings replicate and extend a previous pilot study, which tested groups of two and three participants (Childs et al., 2006). Although this study was conducted without a control condition and with mixed-sex groups of participants without controlling for effects of menstrual cycle and different durations of the single and group versions, the authors found similar stress responses in the individual compared to the grouped stress tasks. Even though Childs and colleagues expected a stronger stress response in the group version compared with the single-subject version, only heart rate reactivity but not cortisol responses were higher in the group stressor (Childs et al., 2006).

A recent meta-analysis indicates that motivated performance tasks combining elements of socio-evaluative threat and uncontrollability elicit robust and reliable psychological and biological (cortisol, heart rate) stress responses (Dickerson and Kemeny, 2004). The Trier Social Stress Test

(Kirschbaum et al., 1993) has been the most frequently used naturalistic psychological stress protocol in stress research containing both factors; however, it has been restricted to a single-subject setting (Dickerson and Kemeny, 2004). Our results demonstrate that the group version of the TSST induces a similar pattern of results regarding psychological, endocrine (cortisol), and cardiovascular (heart rate) stress responses as the original single-subject version (e.g., Kirschbaum et al., 1993; Kudielka et al., 2004; Armbruster et al., 2009). Thus, the TSST-G allows for (i) simultaneous stress exposure of relatively large groups of individuals, as required within several emerging research fields, including stress research, social neurosciences or behavioral and neuroeconomics; and (ii) a far more economical means of testing multiple participants than six single experimental sessions.

Our manipulation was successful in inducing social-evaluative stress in the stress condition, and, most importantly, the TSST-G is also equipped with a specific and standardized control condition for use in controlled experimental study designs. However, some limitations of this study warrant comment. First, all the participants were men. It will be important for future studies to determine the degree to which these findings generalize to women, and, more specifically, whether sex steroids influence stress reactivity as in the single-subject version of the TSST (Kirschbaum et al., 1999). In addition, the TSST-G protocol should also be tested in mixed-sex groups. Furthermore, examinations of numerous factors that affect participants' responsiveness to social stress (e.g., social support, pharmacological treatments) have been fruitful for clarifying the specific mechanisms that can elicit or reduce physiological stress responses (Heinrichs et al., 2003; Soravia et al., 2006, 2009; Ditzen et al., 2007; Het and Wolf, 2007; Simeon et al., 2007; Storch et al., 2007; Robles et al., 2009); these approaches may also be useful for delineating the underlying mechanisms in standardized psychosocial stressors in a group format. Notably, in comparison to the single-subject version, the TSST-G might also cause additional error variance if individual stress responses interact with emotional responses by the other participants. Finally, our findings cannot be generalized directly to clinical populations.

Taken together, our results underscore the feasibility of simultaneous standardized psychosocial stress induction in a group format. The stress condition and the specific control condition of the TSST-G offer the opportunity to investigate stress interventions in a single-blind manner in groups of individuals. This procedure clearly reduces financial and personal expenses and infrastructures and offers a new experimental tool for numerous research fields.

Role of the funding sources

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Conflict of interest

The authors declare that they have no conflicts of interest.

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