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SHORT COMMUNICATION

A friendly version of the Trier Social Stress Test does not activate the HPA axis in healthy men and women

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Abstract

The Trier Social Stress Test (TSST) is a well-established laboratory stressor leading to a robust activation of the hypothalamus–pituitary–adrenal (HPA) axis. Existing control conditions are often not adequate to investigate participants' behavior during the situation as participants are often left alone in the room. This present study aimed to evaluate a friendly version of the TSST as control condition, the friendly-TSST (f-TSST). We expected that the f-TSST would not activate the HPA axis or increase the negative affect (NA). Forty-eight healthy male and female students (24 males) aged between 18 and 30 years were randomly exposed to either the TSST or the f-TSST. The latter features a similar structure and similar cognitive demands as in the TSST, and a social interaction with a committee. The main difference lies in the friendly and warm behavior of the committee opposed to the neutral and reserved behavior in the TSST, typically inducing social-evaluative threat. Salivary cortisol, salivary α -amylase (sAA), and affect were measured to evaluate the stress response to the respective procedure. As expected, the f-TSST neither activated the HPA axis nor increased the NA. The TSST by contrast led to an increase in both measures. A comparable and significant increase in the sAA-concentrations occurred in both conditions. The f-TSST could be useful as a standardized control condition for future stress studies. On a conceptual level our data indicate that mere social performance in the absence of social-evaluative threat and performance pressure does not activate the HPA axis.

Keywords: *Affect, α -amylase, cortisol, friendly-TSST, social evaluative threat, TSST*

Introduction

The hypothalamus–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS) are crucially involved in successful adaptation to stress (Ulrich-Lai and Herman 2009). Social evaluative threat, especially in combination with uncontrollability and motivated performance, has been postulated to be a major trigger for the HPA axis, and laboratory stressors characterized by these factors are associated with the largest cortisol responses (Dickerson and Kemeny 2004).

The Trier Social Stress Test (TSST; Kirschbaum et al. 1993) is a laboratory stressor that combines (1) social evaluative threat induced by a non-responsive committee acting neutral and reserved, and a video camera, (2) uncontrollability induced by the unfamiliar procedure, the unknown outcome, and the

standardized responses of the committee, which are independent of the actual performance of the participant, and (3) motivated performance induced by the fictitious job interview followed by mental arithmetic calculations. This paradigm reliably induces a robust HPA axis response (Kirschbaum et al. 1993; Dickerson and Kemeny 2004). Importantly, when participants have to perform a free speech or reading task on their own (Tollenaar et al. 2008; Het et al. 2009; Almela et al. 2011) no cortisol response is provoked. Likewise performing a speech in a room with an inattentive confederate who works in the field of vision of the participant but does not take account of the participant (Dickerson et al. 2008) provokes no cortisol response. Thus, the removal of the committee abolishes the cortisol response to

public speaking paradigms. According to the current state of research, performance in a social setting per se, not necessarily accompanied by threat, could also explain the response of the HPA axis during TSST-like laboratory stressors. This issue becomes even more interesting due to a recent study by Taylor and colleagues (2010). They reported that a supportive TSST audience caused a larger cortisol stress response than a non-supportive (classical) audience or no audience (Taylor et al. 2010); the supportive audience here was characterized as giving rather positive feedback by non-verbal communication (e.g., nodding). The HPA response in the study of Taylor et al. (2010) could suggest that social support might amplify stress instead of reducing it, especially if social support is given by strangers. Performance in a social setting without an activation of the HPA axis might for some research questions, however, be necessary, for example to evaluate behavior during a stressful condition compared with a non-stressful condition. Thus, a condition as similar as possible to the TSST including interaction with a committee is required. The placebo-TSST (p-TSST) (Het et al. 2009) is indeed a possible control condition for the TSST, however not including a social interaction with a committee as in the TSST. We therefore tested whether a friendly non-threatening committee without a video camera and with the explicit description of the task as a control condition would prevent an HPA axis stress response. In contrast to Taylor et al. (2010), we informed participants about being in the control condition before their preparation time in order to reduce anticipatory stress. Furthermore, participants were not videotaped to reduce the impression that

videotapes might be analyzed afterwards, which could induce performance pressure.

Here, we evaluated the friendly-TSST (f-TSST), which we hypothesized not to activate the HPA axis, but possibly to activate the SNS. We further expected negative affect to increase in the TSST, but not in the f-TSST group. We examined sex differences in an exploratory fashion, as some, but not all previous laboratory stress studies have observed sex differences in basal or stress-induced cortisol and salivary α -amylase (sAA) concentrations (Dickerson and Kemeny 2004; Het et al. 2009).

Methods

Participants

Forty-eight German-speaking healthy adults (24 males) between the ages of 19 and 30 years participated in this study. They were recruited via flyers in the university and an online job board. Exclusion criteria were a body mass index (BMI) < 18 or > 30 , being under medical treatment, taking medication known to influence the HPA axis, smoking, and former participation in the TSST. Pregnant women and women taking oral contraceptives were excluded as well. The testing of women was scheduled outside the time of menses. According to self-reports, 15 women were in their luteal phase, 2 in their follicular phase, 2 were ovulating, and 5 reports were missing. There were no differences in menstrual cycle phase between groups by a χ^2 -test ($\chi^2(4) = 0.27$, $p = 0.99$; Table I). The study was approved by the local ethical committee of the Faculty of Medicine and the Declaration of Helsinki was followed.

Table I. Demographic and affect values.

	TSST ($N = 23$)	f-TSST ($N = 24$)	Total group ($N = 47$)	p -value group differences
Mean age, years (\pm SD)	23.9 (± 2.45)	23.9 (± 2.38)	23.9 (± 2.40)	0.96
Males ($N = 11$)	24.0 (± 1.79)	24.0 (± 2.30)	24.0 (± 2.02)	
Females ($N = 12$)	23.8 (± 3.04)	23.8 (± 2.56)	23.9 (± 2.75)	
Mean BMI (\pm SD)	22.0 (± 2.44)	21.6 (± 2.67)	21.8 (± 2.54)	0.6
Males ($N = 11$)	23.1 (± 2.12)	22.1 (± 2.81)	22.6 (± 2.50)	
Females ($N = 12$)	21.00 (± 2.32)	21.1 (± 2.54)	21.0 (± 2.38)	
N menstrual cycle phase				0.99
Follicular phase	1	1	2	
Luteal phase	8	7	15	
Ovulation	1	1	2	
Missing data	2	3	5	
Log NA (mean \pm SE)				
Before (f-)TSST	0.12 (± 0.02)	0.10 (± 0.02)	0.11 (± 0.01)	0.51
After (f-)TSST	0.17 (± 0.04)	0.04 (± 0.01)	0.10 (± 0.02)	0.002
PA (mean \pm SE)				
Before (f-)TSST	2.79 (± 0.16)	3.03 (± 0.15)	2.91 (± 0.11)	0.27
After (f-)TSST	2.55 (± 0.18)	3.00 (± 0.18)	2.78 (± 0.13)	0.09

TSST, Trier Social Stress Test; f-TSST, friendly-Trier Social Stress Test; the procedure took approx. 15 min; significances refer to comparisons between TSST and f-TSST group, group differences are analyzed with two-sample t -tests for age, BMI, and for mean values of PA and NA, and with a χ^2 test for menstrual cycle phase.

Procedure

After arrival, participants signed informed consent forms and performed a task irrelevant for the current report. Afterwards, they rated their current affect (pre) and delivered the first saliva sample (baseline). Next, participants took part in the TSST or f-TSST. Group assignment was random. After completion of the procedure, participants delivered salivary cortisol samples at designated measurement times (+1, +10, and +25 min after the end of the procedure) and rated their current affect (post). Between times of measurement, participants completed questionnaires and a cognitive task. At the end of testing, the committee and the experimenter debriefed the participants (Figure 1).

Material

Salivary cortisol and sAA assays. Participants were advised to refrain from eating or drinking anything but water and brushing their teeth 1 h before testing, and also from taking medication, drinking alcohol, or doing excessive sports the day before. Cortisol and sAA assessment were carried out using four saliva samples per participant per test collected with Salivettes® (Sarstedt, Germany). Samples were deep-frozen at -18°C and sent to the laboratory of Prof. Kirschbaum at the University of Dresden for analyses. Cortisol was analyzed by an immunoassay

(IBL, Hamburg, Germany), and a quantitative enzyme kinetic method was used for sAA as described elsewhere (Rohleder and Nater 2009). Inter- and intra-assay variabilities were below 10%. Assay sensitivity for cortisol was 0.16 ng/ml, and for sAA 4 U/ml. As cortisol and sAA release follow a circadian rhythm, all testing was carried out in the afternoon starting between 14:00 and 15:30 h.

Affect measurements. Participants rated their current affect using the “Positive and Negative Affect Scale” (PANAS; Watson et al. 1988), in which 10 positive emotions (e.g., alert, proud, and enthusiastic) and 10 negative emotions (e.g., ashamed, distressed, and scared) are rated for current intensity on a five-point scale. The 20 items are subdivided, resulting in a positive affect (PA) and a negative affect (NA) score. The German version is marked by a good reliability with a Cronbach’s α of 0.85 for the PA and 0.86 for the NA scale (Krohne et al. 1996). In our sample internal consistency of PA was good with Cronbach’s $\alpha = 0.88$ and acceptable for NA with Cronbach’s $\alpha = 0.76$ for the pre-measurement.

Stress procedure

TSST. The procedure for the TSST was as follows: participants were brought into a room with two (1 male

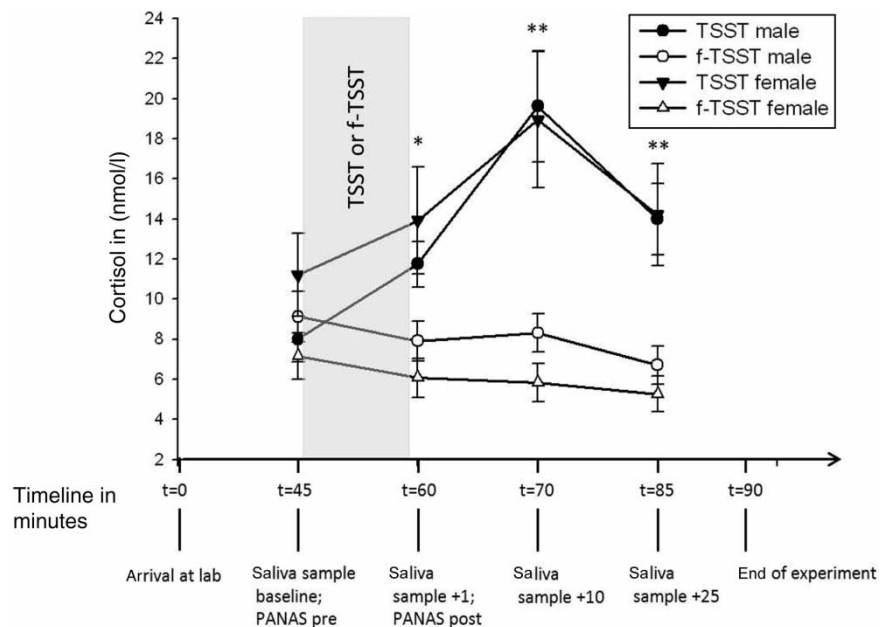


Figure 1. Mean values (\pm standard error of mean) of salivary cortisol concentration (nmol/l) separated into groups and by sex directly before (baseline) and 1 (+1), 10 (+10), and 25 (+25) min after the end of the procedure. PANAS, Positive and Negative Affect Schedule; TSST, Trier Social Stress Test; f-TSST, friendly-Trier Social Stress Test; the procedure itself took approx. 15 min; significant differences refer to comparisons between TSST ($N = 23$, 11 males) and f-TSST group ($N = 22$, 11 males): $**p < 0.001$; $*p < 0.01$; repeated-measures ANOVA with TIME of measurement as within-subject factor (baseline, +1, +10, and +25) and GROUP as between-subjects factor (TSST vs. friendly-TSST) showed a significant GROUP \times TIME interaction effect as well as a significant main effect of TIME and of GROUP, TSST participants showed higher cortisol values than f-TSST participants at all three times of measurement after the procedure; there were no significant interactions or main effects of SEX (all $p > 0.10$); the timeline at the bottom of the figure describes the procedure for the study.

and 1 female) committee members acting, neutral and reserved and wearing white laboratory coats, who were introduced as laboratory employees trained in analyzing behavior. Participants were instructed that they would have to give a free speech about their personal characteristics distinguishing themselves for a job. During the first 5 min, participants filled in a questionnaire about their personal estimation of their performance in the fields of general intelligence, presentation skills, mathematical skills, and personal presence on a 6-point scale. The questionnaire was given to the committee for examination after the preparation. The questionnaire was not analyzed but the fact that the committee examined it and made notes on it during the speech was aimed to increase ego threat to the participants. During the remaining preparation time participants prepared their speech. Next, each participant gave a free speech about their personal characteristics in a fictitious job interview for 8 min in front of the committee acting neutral and reserved. This procedure was videotaped. If participants paused in their speech the committee waited for about 20 s before they asked the participant to continue. If pauses occurred repeatedly the committee started to ask questions. At the end, participants were instructed to read aloud 30 words as part of a related study. The test modifications (e.g., omission of the mental arithmetic part) were because of requirements for a related study where the arithmetic part was not included.

Friendly-TSST. In order to reduce stress, participants were informed directly before entering the room for the f-TSST that they were taking part in a control condition. The committee, not wearing white laboratory coats, was introduced to the participants as laboratory employees with whom participants should talk for a while. It was explicitly stated that there was no videotaping during the procedure. During a 5 min preparation time, participants made

notes about their curriculum vitae (CV), career aspirations, hobbies, and favorite book or movie. To further relax the participants, one committee member left the room during the preparation time. After the preparation time, participants stood in front of the committee and talked freely about their life and career aspirations for 8 min. The committee reacted in a friendly way by nodding and smiling to give participants a feeling of safety and the feeling that they were not being negatively judged. The committee was instructed to avoid pauses in the conversation during the talk by asking follow-up questions. After the talk, participants were also instructed to read aloud 30 words. Table II compares the similarities and differences among the different TSST procedures.

Statistical analyzes

All dependent variables were examined for violations of normality. If normality was not reached, data were log transformed. Demographic group differences were examined with two-sample *t*-tests. Salivary cortisol and α -amylase data were analyzed with a repeated-measures analysis of variance (ANOVA) with TIME of measurement as within-subject factor (baseline, +1, +10, and +25 min) and GROUP as between-subjects factor (TSST vs. f-TSST). PA and NA were analyzed with a repeated-measures ANOVA with TIME of measurement as within-subject factor (pre and post) and GROUP (TSST vs. f-TSST) as between-subjects factor.

Results

Study sample

Analyzes were based on 47 participants (23 males). One male had to be excluded due to insufficient language proficiency. The TSST group comprised 23 participants (11 males), the f-TSST group was made up of 24 participants (12 males). Two-sample

Table II. Summary of modifications of the different Trier Social Stress Tests.

	TSST	p-TSST	f-TSST
Participants information	Not explicitly informed about condition	Not explicitly informed about condition	Explicitly informed about being in control condition before entering it
Committee behavior	Neutral, reserved, wearing laboratory coats, leaving speech pauses	No committee, participant alone in the room	Friendly, supportive, wearing no laboratory coat, leaving no speech pauses
Videotaping	Yes	No	No
Preparation time	Committee apparently observing	No committee	One committee member, no apparent observation
Speech topic	Job interview	Holiday or favorite book	CV and career aspiration
Possible applications	Stress induction	Control condition for assessing cognitive functions before or after a stressful or non-stressful procedure	Control condition to measure behavior during the procedure or memory for the procedure

CV, curriculum vitae; TSST, Trier Social Stress Test (modified – no mental arithmetic); p-TSST, placebo-Trier Social Stress Test (Het et al. 2009); f-TSST, friendly-Trier Social Stress Test.

t-tests showed that groups did not differ in age ($t(45) = 0.05$, $p = 0.96$) or BMI ($t(45) = 0.53$, $p = 0.60$; Table I).

Salivary cortisol concentration

Salivary cortisol samples from 45 participants were analyzed: two participants did not provide enough saliva for analysis. A repeated-measures ANOVA with TIME of measurement as within-subject factor (baseline, +1, +10, and +25 min) and GROUP as between-subject factor (TSST vs. f-TSST) was conducted. As Mauchly's test revealed a violation of sphericity ($\chi^2(5) = 76.06$, $p < 0.001$), Greenhouse Geisser corrected p -values ($\epsilon = 0.49$) are reported. Cortisol concentrations increased in the TSST group but not in the f-TSST group. This was reflected in a significant TIME \times GROUP interaction effect $F(3,129) = 21.90$, $p < 0.001$ and a significant main effect of TIME $F(3,129) = 15.07$, $p < 0.001$, as well as a significant main effect of GROUP $F(1,43) = 18.10$, $p < 0.001$. Post hoc *t*-tests with Bonferroni–Holm corrections revealed no significant differences for salivary cortisol concentrations between the groups at baseline ($p > 0.10$), but significant differences at +1 min, $t(43) = 3.60$, $p = 0.001$ (corr.), +10 min, $t(43) = 5.36$, $p < 0.001$ (corr.), and +25 min, $t(43) = 4.85$, $p < 0.001$ (corr.).

There were no interactions or main effects of SEX ($p > 0.10$; Figure 1). In the TSST group 4 out of 23 (17%) were non-responders based on the criterion of an increase from baseline to the peak at +10 min of less than 2.5 nmol/l (Wuest et al. 2000). In the f-TSST group, 2 out of 22 (9%) were responders with an increase higher than 2.5 nmol/l cortisol.

Salivary α -amylase

Samples from 39 participants for sAA were analyzed. Cortisol and sAA were analyzed from the same samples and priority was given to analysis of cortisol samples. Thus samples from eight participants did not contain enough saliva to be analyzed for sAA. Due to violation of normality, sAA data were log transformed. A repeated-measures ANOVA with the same factors as above was conducted. As Mauchly's test revealed a violation of sphericity ($\chi^2(5) = 25.84$, $p < 0.001$), Greenhouse Geisser corrected values ($\epsilon = 0.67$) are reported. In both groups, we observed a strong increase followed by a rapid decrease in sAA concentrations. This was reflected in a significant main effect of TIME $F(3,111) = 22.18$, $p < 0.001$. However, there was neither a significant GROUP main effect nor a TIME \times GROUP interaction (both $p > 0.10$). Again, no interactions or main effects of SEX were observed ($p > 0.10$; Figure 2).

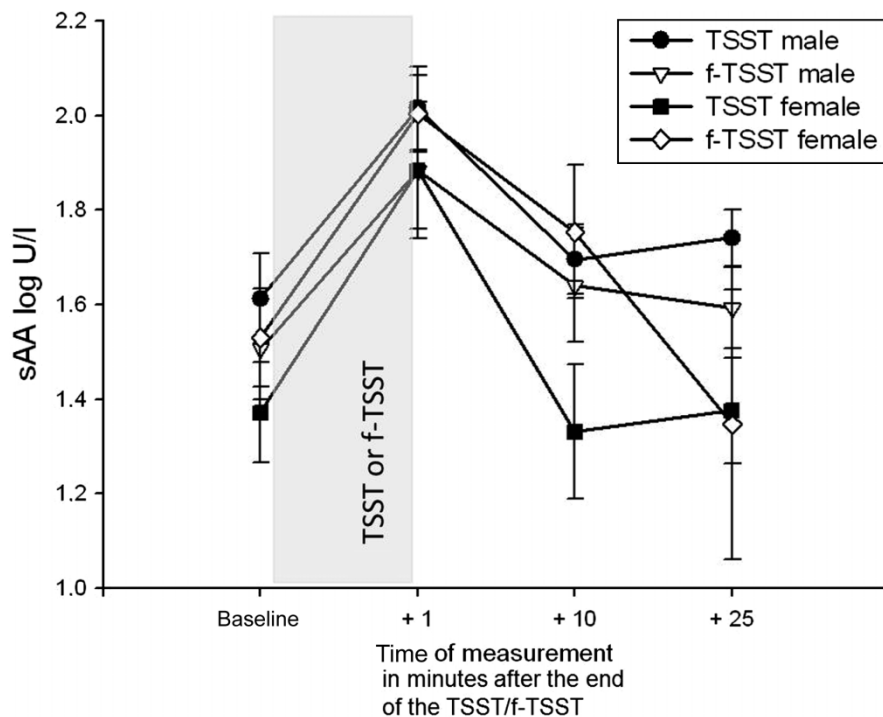


Figure 2. Log-transformed mean values (\pm standard error of mean) of sAA concentrations (log U/l) separated into groups and by sex directly before (baseline) and 1 (+1), 10 (+10), and 25 (+25) min after the end of the procedure. TSST, Trier Social Stress Test; f-TSST, friendly-Trier Social Stress Test; sAA, salivary α -amylase; the procedure itself took approx. 15 min; significant differences refer to comparisons between TSST ($N = 20$, 11 males) and f-TSST group ($N = 19$, 9 males); a repeated-measures ANOVA with TIME of measurement as within-subject factor (baseline, +1, +10, and +25 min) and GROUP as between-subjects factor (TSST vs. f-TSST) showed a significant main effect of TIME ($p < 0.001$); there was no significant interaction or main effect of GROUP or SEX ($p > 0.10$).

Positive and negative affect

As PA and NA are discrete variables, they were analyzed in separate analyses (mPA and mNA). Due to violation of normality, mNA values were log-transformed. Repeated-measures ANOVA with TIME of measurement as within-subject factor (pre- and post-TSST/f-TSST) and GROUP (TSST vs. f-TSST) as between-subjects factor was conducted. Stressed participants responded with increased NA to the TSST whereas participants in the f-TSST did not. This was reflected in a TIME \times GROUP interaction effect $F(1,45) = 8.68, p = 0.005$ and a main effect of GROUP $F(1,45) = 7.12, p = 0.011$, but no main effect of TIME ($p > 0.10$). Stressed participants reported more NA than participants who had not been stressed ($p = 0.002$, corr.) after the procedure but not before the procedure ($p = 0.51$). There were no effects of SEX ($p > 0.10$).

Analysis of mPA revealed no significant effects ($p > 0.10$). mPA values were not significantly different before the procedure for both groups or after the procedure. Again, no SEX effects were observed ($p > 0.10$; Table I).

Discussion

This study describes observations made with a friendly version of the well-known Trier Social Stress Test. A committee responding in a friendly way, no videotaping, and explicit mentioning of the fact that participants were part of a control situation resulted in the complete absence of an HPA response to this public speaking task. Previous studies have reported a missing cortisol response to a speech conducted alone in a room without any experimenter being present (Het et al. 2009) or to a speech conducted in the presence of an inattentive experimenter who did not overtly observe participants' performance but sat at a computer doing something else (Dickerson et al. 2008). We report that even a speech in front of an attentive committee consisting of two unfamiliar experimenters does not stimulate the HPA axis. The absence of an HPA response to the f-TSST is remarkable given that this situation might still be perceived by the participant as novel and somewhat unpredictable, two factors described by Mason as typically leading to an HPA response (Mason 1968). This makes the f-TSST a useful control condition for the TSST in an appropriate setting. For example, studies evaluating behavior or speech patterns during the TSST and its control condition or studies assessing memory for the respective situation are definitely in need of an attentive committee. This differentiates the f-TSST from the p-TSST (Het et al. 2009) and makes it for certain situations more suitable. Further differences and similarities between the TSST, p-TSST, and f-TSST are outlined in Table II, to

help experimenters find the appropriate conditions for future studies. It has to be emphasized that the f-TSST differs in several respects from the regular TSST (Table II). Participants were told at the beginning of the preparation period that they are taking part in a control condition. This could have reduced performance pressure typically associated with an oral presentation. Moreover the committee was not only acting friendly but also helped actively in avoiding pauses, thus reducing the potential awkwardness of the situation. Finally, the committee was dressed differently than for the TSST and no videotaping took place. The combination of these modifications might explain why our current findings differ from those of Taylor and colleagues (2010). These authors reported that a supportive audience providing positive feedback caused an increase in the stress rather than a blunted cortisol response to the TSST (Taylor et al. 2010). In that study participants were unaware of the experimental condition they were to receive during the preparation period, which might have caused an anticipatory stress response (Wirtz et al. 2006; Starcke et al. 2008). Moreover, during the supportive and non-supportive condition a difficult mental calculation task had to be performed, which might have been stressful as well. In line with this are the affect ratings observed in the Taylor study (2010) indicating that the supportive and non-supportive audience did both result in a similar increase in NA. This is in contrast with our current findings. The apparent discrepancy between our current results and the data reported by Taylor and colleagues (2010) calls for additional research aimed at further specifying the crucial factors causing an HPA response in laboratory stress paradigms like the TSST.

In contrast to the f-TSST group, the TSST group showed a quite pronounced significant cortisol response to the stressor, indicating that the minor modifications made to the paradigm (e.g., removal of the arithmetic part) did not weaken its power. Salivary AA data did not differ between f-TSST and TSST participants. Both groups showed an increase in sAA concentration as a response to the procedure. We thus conclude that there was a SNS response merely because an attentive committee was present or due to the physical demands of the task (speaking and standing upright). Others have also shown a sAA response to emotional arousal or mild physical effort (Het et al. 2009; Nater and Rohleder 2009). This is interesting from a conceptual perspective. The presence of an attentive, but not socially threatening committee did not lead to a HPA response while it still led to a SNS response.

None of the collected endocrine or psychometric stress measures showed sex differences. Most notably, in the TSST women showed a similar (and strong) cortisol response to that of men. Sex differences in

cortisol responses to the TSST have been shown before with some studies reporting men producing a stronger cortisol response to the TSST than women (Kudielka and Kirschbaum 2005; Kajantie and Phillips 2006; Schoofs and Wolf 2011). The absent sex difference here could be due to the modifications of the TSST: either the elongated speech period or the omission of the mental arithmetic part could underlie the absence of sex differences in cortisol reactions. At the least, the data indicate that the omission of mental calculation does not reduce the stress induced by the TSST for women.

In sum, the newly developed friendly version of the TSST neither increases salivary cortisol concentrations nor does it increase the NA. It does, however cause an increase in sAA concentrations. The current findings indicate that mere performance in a social context is not a trigger for the HPA axis if it takes place in a friendly setting and performance pressure is reduced. Depending on the specific research question of interest, the f-TSST could be an attractive alternative to the p-TSST (Het et al. 2009) as a control condition for the TSST, as it encompasses similar cognitive demands and situational conditions. Furthermore, the current results could be a starting point in characterizing further the stressful elements of the TSST.

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