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Are salivary gonadal steroid concentrations influenced by acute psychosocial stress? A study using the Trier Social Stress Test (TSST)

Daniela Schoofs *, Oliver T. Wolf

Department of Cognitive Psychology, Ruhr-University Bochum, Germany

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ABSTRACT

It is well documented that acute stress activates the sympathetic nervous system (SNS) and the Hypothalamus–Pituitary–Adrenal (HPA) axis. Results regarding the hypothalamus pituitary gonadal (HPG) axis, in contrast, are less consistent. Stress-associated increases as well as decreases have been reported for testosterone and estradiol. In the present study, healthy young male (n = 39) and female participants (n = 44, all tested in the luteal phase) were randomly assigned to a well-evaluated psychosocial stress protocol ("Trier Social Stress Test", TSST) or to a non-stressful control condition ("Placebo-TSST"). Salivary concentrations of cortisol, alpha-amylase, testosterone, progesterone, and estradiol were measured immediately before and twice (10 and 25 min) after the treatment. As was to be expected, cortisol- and sAA-concentrations increased in response to the stressor. Stressed men showed a more pronounced increase of cortisol than stressed women. In contrast, acute stress did not affect testosterone-, progesterone-, and estradiol-concentrations. The results of the present study suggest that an acute psychosocial laboratory stress or has no strong rapid effects on salivary gonadal steroids. In line with several previous studies the findings might suggest that stress-induced changes in gonadal steroids occur in response to physical stressors, to competitive stressors or to more severe stressors only.

1. Introduction

The term stress is used to describe experiences that put a high demand on emotional and physiological processes (McEwen, 2007). Physiological processes include secretion of glucocorticoids (GCs, in humans primarily cortisol) and catecholamines (adrenaline and noradrenaline) to facilitate adaption. The release of these stress messengers is promoted by an activation of the sympathetic nervous system (SNS) and the Hypothalamus–Pituitary–Adrenal (HPA) axis. The response of the HPA- axis is initiated by the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) in the hypothalamus. Higher CRH-concentrations stimulate secretion of the pituitary adrenocorticotropic hormone (ACTH), which in turn activates the release of cortisol by the adrenal glands (Charmandari et al., 2005). The described processes launch an immediate enhancement (among other effects) of oxygen and glucose availability (de Kloet et al., 2005; Sapolsky et al., 2000), both of which provide energy for adaptive mechanisms. However, considering the limited energy resources of an organism, it would make sense to reduce energetically expensive processes that are not directly related to the adaptive response (e.g. digestion, growth, reproductive behaviour; Sapolsky et al., 2000). Indeed, based on his observations, Selye had assumed that stress disrupts reproductive behaviour in animals as early as 1939 (Selye, 1939). It seems reasonable that these effects are modulated by interactions between the HPA-axis and the Hypothalamus–Pituitary–Gonadal (HPG) axis. The HPG-axis orchestrates the release of sex steroids, including testosterone (T), estradiol (E2), and progesterone (PROG), from the gonads and the adrenals (Rivier and Rivest, 1991; Williamson et al., 2005).

The assumption of a close interaction between both axes is supported by studies reporting evidence that exogenous GCs suppress the release of gonadotropins (LH and FSH) from the pituitary in different animal species (Breen and Karsch, 2006). Further studies employing acute or chronic stress protocols (e.g. immobilization, foot shock, sleep deprivation etc.) found stress-induced changes in T-, E2-, and PROG-concentrations in animals (Andersen et al., 2004; Chichinadze and Chichinadze, 2008; Shors et al., 1999). In regard to male rodents, most studies reported significant decreases in T and E2, while corticosterone (as a main GC in rodents) and PROG usually increased after stress-induction (Andersen et al., 2004; Dong et al., 2004; Orr et al., 1994). In contrast, one study observed higher E2 levels in female rodents after exposure to an acute stressor, although the magnitude of the effect was additionally modulated by the specific cycle stage (Shors et al., 1999).

In addition, it should be noted that the release of sex steroid hormones in male as well as in female laboratory animals seems to depend on the distinct type of the stressful experience and the associated corticosterone response to the specific stressor since some types of stressors did induce lower or none stress-dependent changes of those hormones (Andersen et al., 2004; Shors et al., 1999). One

* Corresponding author at: Department of Cognitive Psychology, Ruhr-University Bochum, Universitätsstr. 150, D-44780 Bochum, Germany. Tel.: +49 234 32 29233; fax: +49 234 32 14308.
E-mail address: Daniela.schoofs@rub.de (D. Schoofs).

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study systematically investigating the influence of stress on gonadal steroids submitted rats to five different chronic stress groups for four days (Andersen et al., 2004). The stressors were applied either twice a day for periods of 1 h (restraint stress, footshock, cold, and forced swimming) or for 96 h (paradoxical sleep deprivation; PSD). While PSD and footshock resulted in significantly lower T- and E2-concentrations and higher PROG-levels, cold and restraint stress induced solely lower T- and lower E2-levels, respectively. However, it should be noted that significant corticosterone changes were observed only in those groups exposed to PSD and footshock. In line with the previously mentioned results Shors et al. (1999) observed no changes of estradiol after an acute swim stressor in female rats but in contrast to prior observation made in male rats found elevated E2 levels in females after tailshock. The results did not depend on corticosterone changes since both stressors induced significant corticosterone elevations.

Possible mechanisms explaining the described influences of stress and/or GC on the HPG-axis are still under discussion, but existing results suggest that stress might affect the HPG axis on three levels (Charmandari et al., 2005; Rivier and Rivest, 1991). On the first level, stress might inhibit secretion of the gonandotropin releasing hormone (GnRH) by the hypothalamus, while on the second level, it could interfere with the GnRH-induced release of the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) by the pituitary. Finally, stress might alter responsiveness of the gonads for gonadotropins.

Only few studies have investigated the effects of acute stress on sex steroid-concentrations in humans (Gerra et al., 2000; Heinz et al., 2003) — the influence of gender on the magnitude of the response of the HPA-axis on the other hand has attracted much more attention. Regarding this issue, the majority of studies employing standardized acute laboratory stressors (for example the Trier Social Stress Test (TSST); Kirschbaum et al., 1993) have shown significantly larger stress-induced salivary cortisol-concentrations in male compared to female participants (Kajantie and Phillips, 2006; Kudielka et al., 2009). In addition, the response of the HPA-axis in women seems to depend on the distinct menstrual cycle stage, with women in the luteal phase displaying similar stress-induced cortisol-levels as men and higher concentrations compared to women in the follicular phase and to those taking oral contraceptives (Kajantie and Phillips, 2006; Kudielka and Kirschbaum, 2005). Some authors have suggested that sex differences in the HPA-axis-response might be generated by protective effects of circulating estrogens (Bowman et al., 2001; Charney, 2004; Luine, 2002). However, dimorphisms in brain function, differences in corticosteroid-binding globulin-levels, and gonadal and adrenal interactions at the genomic and cellular levels are also discussed as possible mechanisms (Chichinadze and Chichinadze, 2008; Handa et al., 1994; Viau, 2002).

To examine effects of HPA- axis activation on sex steroids in humans many researchers have studied competitive situations such as sport tournaments (Bateup et al., 2001; Kivlighan et al., 2005; Suay et al., 1999) or cognitive competitions (e.g. Japanese chess or computer games; Gladue, 1989; Hasegawa et al., 2008; Mazur, 1997). In men a variety of sports competitions (e.g. rowing, judo; Kivlighan et al., 2005; Suay et al., 1999) as well as cognitive competitive situations, which lack the physical component, seems to increase anticipatory (Mazur, 1997) or post-competition cortisol and T concentrations (Gladue, 1989; Hasegawa et al., 2008). The results are less homogeneous for women. Studies employing sport tournaments observed enhanced post-competition cortisol- and T-levels (Bateup et al., 2001), others merely found significant cortisol increases and unchanged T concentrations (Kivlighan et al., 2005), while one study using a cognitive competition reported no changes at all (Mazur, 1997). In reality, the issue is even more complicated, since the magnitude of hormonal changes seems to depend on further psychological variables such as the individual experience in the specific competitive situation, winning or losing the competition, and the strength of team bonding (Gladue, 1989; Kivlighan et al., 2005). In comparison to the number of studies examining sex differences in the endocrine stress-response and studies investigating the impact of competitive situations on changes in HPA- and HPG-activity, it is striking that only very few studies have employed standardized laboratory stressors to address the question of how stress affects sex steroid concentrations in humans.

Existing results for male participants are rather heterogeneous. It was found that metabolic stress (glucodeprivation, Elman and Breier, 1997) and anticipatory stress before a one day clinical research protocol (Schulz et al., 1996) significantly increased cortisol- and decreased T-levels while a public oral presentation on a scientific conference (Heinz et al., 2003) and a combined laboratory stressor (mental arithmetics, Stroop-task, and public speaking; Gerra et al., 2000) didn't induce T-changes in healthy participants. However, it should be noted that the latter study tested peripubertal male participants, whose endocrine state and response pattern probably differ considerably from male adults. Regarding the influence of stress on PROG it was reported that, consistent with results from animal studies, metabolic stress induced a rise of PROG-concentrations (Elman and Breier, 1997). In contrast to PROG, E2-levels were not influenced by a stressful public speaking situation (Heinz et al., 2003). Only one study regarding female participants was found in the literature. However, in this study, no stress induction was employed, but hydrocortisone was administered for several days to healthy young women (Saketos et al., 1993).This treatment lead to decreased PROG-levels, while having no effect on E2 concentrations.

In summary, animal and human studies on male and female individuals suggest that stress and/or enhanced GC concentrations can influence the HPG-axis. However, results in animals are relatively homogeneous. In rodents most studies found stress induced decreases in T and E2 and an enhancement of corticosterone and PROG (Andersen et al., 2004; Dong et al., 2004; Orr et al., 1994). In human studies the picture is less consistent and results seem to be additionally modulated by various psychological variables (Gladue, 1989; Kivlighan et al., 2005). One obvious explanation might be the lack of studies using well standardized stressors, which reliably induce a robust endocrine stress response. One laboratory stressor which meets this criterion is the TSST, a well-evaluated psychosocial stressor which reliably induces significant activation of the HPA-axis and the SNS (Dickerson and Kemeny, 2004; Kirschbaum et al., 1993). Thus, we were interested in examining the effects of the TSST (as one of the most employed psychosocial laboratory stressors in humans) on physiological stress markers and sex steroids in young male and female adult humans. The TSST can be characterized as a paradigm which combines motivated performance with uncontrollability and social evaluative threat (Dickerson and Kemeny, 2004). It induces feelings of anxiety and shame (Dickerson et al., 2008) and can be described as a situation of experimentally induced failure. Further on, a well matched control situation for the TSST exists: the Placebo-TSST (Het et al., 2009), which does not induce a cortisol response. Based on this conceptualisation and on results from animal studies we expect decreased T-levels in response to the TSST, while PROG is expected to increase (Andersen et al., 2004; Elman and Breier, 1997; Schulz et al., 1996). Regarding E2,various studies have yielded no consistent results (Andersen et al., 2004; Saketos et al., 1993; Shors et al., 1999), therefore it is hardly possible to predict the direction of a potential stress effect.

2. Methods

2.1. Participants

Eighty-three healthy young male ($n = 39$; average age $\pm$ SD $= 24.85 \pm 4.06$) and female ($n = 44$; average age $\pm$ SD $= 24.73 \pm 3.90$) participants participated in the experiment. All participants took part in one of two studies investigating the effects of stress on memory performance in a working memory (Schoofs et al., 2008b) or a declarative memory

task (Schoofs and Wolf, 2009). None of the participants indicated acute or chronic disease or regular medication intake. All participants were normal weighted (BMI ± SD: 22.62 ± 2.42 kg/m^2) and provided written informed consent before their participation. The female participants were naturally cycling with menstrual cycles between 24 and 36 days and were tested in their luteal phase (4th to 8th day before the onset of the new menstrual cycle). To ensure a regular cycle female participants were asked to specify the dates of at least two complete previous menstrual cycles (first day of menses). In addition, the menstrual cycle phase was validated by mean concentrations of PROG and E2. The results confirmed that the female participants were really tested in their luteal phase (mean pg/ml ± SEM; estradiol: 3.31 ± 0.31; progesterone: 180.56 ± 19.87, IBL standards for the luteal phase: 0.8–10.8 pg/ml for E2 and 127–446 pg/ml for PROG). The study was approved by the national ethic committee of the German Psychological Association (DGPs).

2.2. Procedure and tests

2.2.1. Procedure

The study was a group comparison design and participants were randomly assigned to the TSST (male = 18; female = 23) or a non-stressful control situation (Placebo-TSST; Het et al., 2009; male = 21; female = 21). The physiological stress response was assessed by measuring cortisol and salivary alpha-amylase (sAA), with the latter providing an indirect marker for the activation of the SNS (Nater and Rohleder, 2009). The individual sessions were conducted between 10.00 h and 12.30 h to control the diurnal cycle of cortisol (Horrocks et al., 1990) and sAA (Rohleder et al., 2004). After arrival in the laboratory, participants were given a resting phase of 25 min before the first samples for cortisol, sAA, T, PROG, and E2 were taken (baseline). The saliva was collected in two different collection devices: Salivette collection devices were used for cortisol and sAA (Sarstedt, Nuembrecht, Germany), while T, PROG, and E2 were collected in SaliCap devices (IBL, Hamburg, Germany). Participants always started with the sampling of the sex steroids before they provided the saliva for the analysis of stress markers. Immediately after the baseline sampling, participants attended either the TSST or the Placebo-TSST with an average duration of approximately 18 min. Ten minutes (sample +10) and 25 (sample +25) minutes after cessation of treatment, participants provided two further saliva samples for the endocrine measurement. To assure a frequent monitoring of the stress response, an additional saliva sample (sample +01) was collected for the stress markers cortisol and sAA immediately after the treatment (TSST or Placebo-TSST) since previous studies had demonstrated a rapid response of the SNS with peak concentrations immediately after completion of the TSST (Nater et al., 2005; Schoofs et al., 2008b). In addition, participants filled out the Positive and Negative Affect Schedule (Watson et al., 1988) once immediately before and once after the treatment.

2.2.2. TSST and Placebo-TSST

The stress induction (TSST) or the control situation (Placebo-TSST) was administered 30 min after the arrival of the participants at the laboratory. The TSST consists of a short preparation time (5 min), a video-taped free speech (5 min), and a subsequent demanding mental arithmetic task (5 min) in front of a committee (two members, one man and one woman). The committee acts with a reserved attitude and gives no verbal or non-verbal feedback regarding the performance of the participant. The Placebo-TSST was relatively similar in physical and mental demand (speech and less demanding math task) but lacked the stress-inducing components of the TSST since no other person (except the participant) was present in the room during the performance (Dickerson et al., 2008; Het et al., 2009).

2.2.3. Cortisol, sAA and sex steroid assessment

Participants were requested to abstain from eating, drinking, physical exercise or smoking during the hour preceding the beginning of the testing session. Saliva was collected using Salivette collection devices for cortisol and sAA and SaliCap collection devices for the measurement of T, PROG, and E2. All saliva samples were analysed in the laboratory of Prof. Dr. C. Kirschbaum (Dresden, Germany). Cortisol was measured using an immunoassay (IBL, Hamburg, Germany). For sAA a quantitative enzyme kinetic method was used as described elsewhere (van Stegeren et al., 2006). For some samples the amount of saliva collected was insufficient for the analysis of both markers. In such cases the analysis of cortisol was preferred. Therefore, cortisol levels were obtained from 81 participants, while for sAA the concentrations of 73 participants could be determined. Inter- and intra assay variations did not exceed 10%. Testosterone, PROG, and E2 were measured using commercially available competitive chemiluminescence immunoassays (IBL, Hamburg, Germany). Cotton swab-based sampling was avoided because it has been shown that this salivary sampling technique leads to incorrect results for some sex steroids measured out of saliva (Shirtcliff et al., 2002). The sensitivities for the assays are 0.3 pg/ml for the E2 assay, 2.6 pg/ml for the PROG assay, and 2.5 pg/ml for the T assay. Inter and intra assay coefficients of variation for the three assays were below 12%.

2.2.4. Affect measurement

In order to assess the effects of the stressor on negative and positive affect, participants filled out the PANAS (Watson et al., 1988) at baseline and immediately after cessation of the treatment. The PANAS consists of 10 items for positive affect (e.g. interested, enthusiastic) and 10 items for negative affect (e.g. upset, ashamed). Participants have to rate the items on a five point scale based on the current strength of emotion from 1 = "very slightly or not at all," to 5 = "extremely". The pre- and post-ratings of the positive and negative items were averaged to a positive and negative affect score, respectively. Subsequently, a delta score was created. For this purpose, the values of the pre-ratings were subtracted from the post-treatment scores for negative and positive affect scores separately.

2.3. Statistical analysis

All statistical analyses were calculated using PASW Statistics 18.0. Unless indicated, descriptive data in the text are shown as means ± standard deviations (SD), while mean values ± standard error mean (S.E.M.) are depicted for figural illustrations.

First, an exploratory data analysis was used to identify individual values, which appear to deviate markedly from the data obtained from the entire sample. Based on the expected differences in sex steroid concentrations in male and female participants, the analysis was conducted separately for both sex groups. In PASW, outliers are defined as individual measurements that are at least 1.5 Inter-Quartil-Ranges above the upper or beneath the lower quartile respectively. The analysis revealed that some participants showed considerably deviating hormone concentrations in one or more endocrine parameters compared to the values of their specific comparison-group (2–4 participants per analysis). Therefore, all hormone samples of participants with conspicuous baseline values (outliers) were excluded from the respective analyses. Thus, the number of participants included in the analyses for the different hormones varies, which is why "n" is specified at the beginning of each analysis.

The influence of stress on the dependent variables (salivary stress markers, sex steroids, and affect) was evaluated with a mixed model analysis of variances (ANOVA) with the repeated measurement factor TIME (2–4 levels depending on the measure used) and the between group factors TREATMENT (TSST vs. Placebo-TSST) and SEX (men vs. women). Greenhouse-Geisser-corrected p values were used when appropriate.

Table 1
Descritptive data for cortisol- and sAA-concentration separately for male and female participants.

	Treatment		base	+01	+10	+25
a. Cortisol (nmol/l)						
Women	Control (n = 20)	Mean ± SD	9.75 ± 5.50	8.04 ± 4.77	7.21 ± 3.80	6.07 ± 3.21
	TSST (n = 22)	Mean ± SD	10.80 ± 6.22	12.52 ± 9.03	14.78 ± 9.33	10.04 ± 5.81
Men	Control (n = 21)	Mean ± SD	15.02 ± 7.22	13.41 ± 6.64	10.92 ± 5.33	8.53 ± 3.95
	TSST (n = 18)	Mean ± SD	12.81 ± 6.47	17.10 ± 7.71	19.22 ± 8.26	16.81 ± 7.27
b. sAA (U/l)						
Women	Control (n = 19)	Mean ± SD	28.11 ± 24.59	53.07 ± 50.33	39.29 ± 41.18	50.31 ± 52.79
	TSST (n = 21)	Mean ± SD	30.72 ± 22.79	84.83 ± 70.47	55.39 ± 57.52	62.56 ± 56.41
Men	Control (n = 18)	Mean ± SD	38.03 ± 15.64	51.82 ± 32.63	41.51 ± 23.90	37.98 ± 28.09
	TSST (n = 15)	Mean ± SD	38.37 ± 27.38	82.46 ± 71.14	52.32 ± 36.37	50.84 ± 30.06

Descriptive data for cortisol (a) and sAA (b) for men and women, separately. The ba sample was taken immediately before treatment, while the other sampling labels (+01, +10, +25) indicated the time point of measurement after the cessation of the treatment.

3. Results

3.1. Affect measurement

A significant TIME-by-TREATMENT interaction was found for the negative affect scale of the PANAS ($F(1,79) = 31.307$; $p < 0.001$). While both groups did not differ in negative affect before treatment (stress group: 1.33 ± .36 vs. control group: 1.44 ± .48) they did differ significantly after treatment, with stressed participants reporting more negative affect (stress group: 1.66 ± .51 vs. control group: 1.26 ± .56). The between-subject factor SEX (men vs. women) did not influence negative mood in the stress- or control-condition. No significant effects were shown regarding positive affect, both for TREATMENT and SEX on the positive affect scale of the PANAS.

3.2. Endocrine stress parameters: cortisol and sAA measurement

44 female (21 controls) and 39 male (21 controls) participants were included in the analysis of cortisol levels, while 2 female participants had to be excluded due to missing values. As expected, the repeated measurement ANOVA including the repeated measurement factor TIME (baseline, +01, +10, and +25) and the between group factors TREATMENT (TSST vs. Placebo-TSST) and SEX (men vs. women) showed significant main effects for TIME ($F(3, 231) = 9.561$; $p < .001$), TREATMENT ($F(1, 77) = 11.595$; $p = .001$) and SEX ($F(1, 77) = 11.257$; $p = .001$). Furthermore, significant TIME*TREATMENT ($F(3, 231) = 22.293$; $p < .001$) and TIME*TREATMENT*SEX ($F(3, 231) = 4.134$; $p < .05$) interactions were observed. The ANOVA yielded no significant results for the TIME*SEX ($F(3, 231) = .573$; $p > .05$) and TREATMENT*SEX ($F(1, 77) = .009$; $p > .05$) interactions. Post-hoc independent sample *t*-tests demonstrated that men displayed significantly higher cortisol concentrations for the +25 measurement in the stress condition compared to the female participants ($t(38) = 1.890$; $p < .01$). Overall, female participants showed lower cortisol concentrations compared to male participants (Table 1).

For further investigation of the observed TIME*TREATMENT*SEX interaction and a possible influence of the gonadal steroids on the cortisol response three separate ANCOVA's were calculated with the baseline concentrations of each sex hormone (T, E2, and PROG) as covariate, respectively. The results showed neither a significant main effect of sex hormones nor any significant interaction (all p's > .05). Importantly, results revealed that the TIME*TREATMENT*SEX interaction was still significant when baseline sex steroid concentrations were taken into account.

To examine sAA, 40 women (19 controls) and 33 men (18 controls) were analysed, while 10 participants had to be excluded due to an insufficient amount of saliva provided in the samples (Table 1). The repeated measurement ANOVA yielded a significant main effect for TIME ($F(3, 207) = 21.591$; $p < .001$) and a significant TIME*TREATMENT interaction ($F(3, 207) = 4.104$; $p < .05$). No further main effects or interactions were significant (all $p > .05$). Post-hoc independent t-tests showed a significant difference between stressed and control participants for the sAA-concentration at the +01-measurement ($t(71) = 1.55$; $p < .05$). No significant differences between male and female participants were found. This was also the case for both control and stressed participants (Table 1).

3.3. Sex hormones: E2, T, and PROG

To analyse E2 (n = 42♀ and 37♂; excluded n = 4), T (n = 40♀ and 39♂, excluded n = 4), and PROG (n = 42♀ and 37♂; excluded n = 4), repeated measurement ANOVAs with the repeated measurement factor TIME (baseline, +10 and +25) and the between group factors TREATMENT (TSST vs. Placebo-TSST) and SEX (men vs. women) were calculated. All three analyses revealed a significant main effect for the between group factor SEX (all $p < .001$). The directions of the observed effects were consistent with the expected results. In all three of the sampling points (baseline, +10, +25), men displayed significantly higher overall T-levels compared to women, while women showed significantly higher concentrations of E2 and PROG. Regarding all three of the sex steroids, neither the factor TREATMENT nor the TREATMENT-by-SEX interaction was significant (or approaching significance). However, concerning PROG, a trend for a main effect of TIME was observed ($F(2, 150) = 2.974$; $p = .054$). Exploratory paired *t*-tests showed a significant increase of PROG concentrations between the +10 and the +25 measurement ($t(78) = -2.179$; $p < .05$; averaged difference: 28.29 ± 115.39 pg/ ml), which, however, was independent of treatment (TSST or control condition) (Fig. 1).

In addition, to examine the internal consistency for T, PROG, and E2 between the three points of measurement Cronbach's alpha was calculated for each of the three hormones. The analysis revealed Cronbach's $\alpha \geq .75$ for each of the three gonadal steroids. This indicates a high consistency/stability of the obtained salivary gonadal steroid measure over the course of the study. The results of the analyses did not change when calculated for the control- and stress-group separately.

4. Discussion

The objective of the study was to investigate the influence of a standardized psychosocial stressor, the TSST, on salivary concentrations of T, E2, and PROG in young, healthy men and women. As was to be expected, the results showed a significant influence of the TSST on psychological and physiological stress markers. The stress response was reflected in an increase of negative mood, cortisol, and sAA in participants after attending the TSST. This indicates successful stress-induction in both male and female participants (Dickerson and Kemeny, 2004; Kirschbaum et al., 1993; Nater and Rohleder, 2009).

Regarding a possible influence of sex on the stress response, results showed a significant difference between men and women in the cortisol response, with males displaying higher concentrations

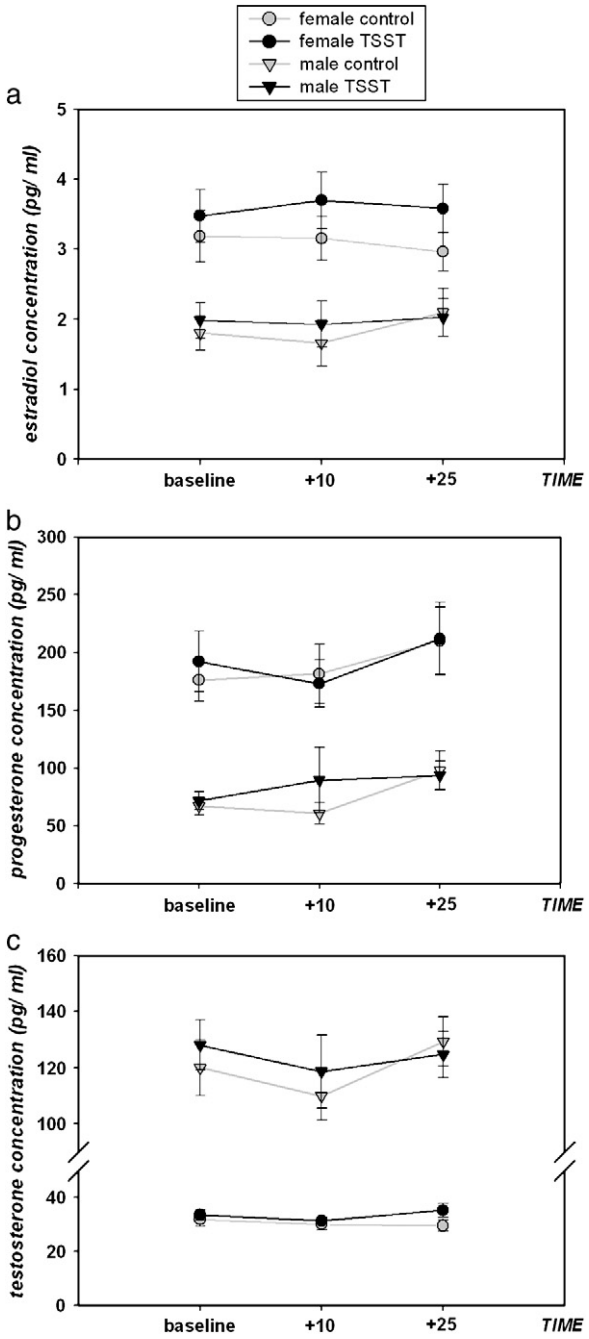

Fig. 1. Mean concentrations ± S.E.M. for the sex steroids E2 (a), PROG (b), and T (c) separately for male and female participants in the TSST and the non-stressful control condition (P-TSST). While sex steroids differed, as to be expected, substantially between the sexes no influence of the stressor were apparent.

compared to female participants. This result is in line with previous studies reporting higher post-stress cortisol concentrations in men compared to women (Kajantie and Phillips, 2006; Kudielka et al., 2009; Kudielka and Kirschbaum, 2005). However, it should be noted that in contrast to our results, some studies have observed a similar cortisol response in men and free-cycling women in their luteal phase (Kirschbaum et al., 1999; Rohleder et al., 2001). In contrast to the findings on cortisol, no sex differences were observed for the sAA or mood changes. Only few previous studies have investigated possible sex differences in sAA levels and results have been heterogeneous (Nater et al., 2006; Takai et al., 2004; van Stegeren et al., 2008).

Based on previous human and animal studies, which altogether have yielded a rather inhomogeneous pattern with respect to possible stress effects on sex steroids, we assumed that stress should decrease T- and increase PROG-levels (Andersen et al., 2004; Elman and Breier, 1997; Schulz et al., 1996), while no clear prediction could be made for E2 in light of inconsistent previous results (Andersen et al., 2004; Saketos et al., 1993; Shors et al., 1999). However, the analyses of our data yielded no support for an effect of acute psychosocial stress on salivary sex steroids. Merely the expected overall sex differences in sex steroid concentrations became apparent.

4.1. Possible effects of the time of hormone measurement

One of the explanation which comes to mind for the missing stress effects on salivary sex steroid concentrations is the comparatively short post-treatment sampling period (30 min) employed for measuring sex steroid concentrations. Since hormonal responses are relatively slow and cortisol usually reaches its peak concentration in saliva 10 minutes after completion of the TSST (Kirschbaum et al., 1993; Kirschbaum and Hellhammer, 1989), it is possible that the interaction effects between the HPA- and the HPG-axis might take longer to become apparent. However, several animal and human studies found an influence of stress even though they used similar or even shorter sampling periods. In a study by Dong et al. (2004), for example male mice were exposed to immobilization stress. Fifteen minutes after the onset of the stressor, animals displayed significant increases of corticosterone which were accompanied by significant decreases in T measured 30 min after onset of the stressor. Similar rapid stress effects on sex steroid concentrations were reported in female rats, which showed significant changes in PROG- and E2-concentrations after 20 min of swim-stress or 30 min of tail-shock stress (Shors et al., 1999). Since the sampling points in the just mentioned studies (Dong et al., 2004; Shors et al., 1999) were defined by the onset of the stressors, the reported samples roughly correspond to the + 10 measurement in our experiment.

Concerning human studies Gladue (1989), employing a non-physical competitive situation, could show that T increases within 20 min after the beginning of a laboratory reaction-time task in which wins and losses of male participants were manipulated. Another study examining a possible influence of psychological stress on the HPG-axis investigated the effects of movies with different emotional contents (neutral, aggressive, sexually stimulating and stressful) on salivary T-levels in young men (Hellhammer et al., 1985). All movies were presented for 30 min and saliva was collected before, 15 min after the beginning and 15 min after the end of the movie. Results showed no changes of T for the neutral and aggressive movies, a significant increase in the sexually arousing condition and a significant decrease of T-levels for the stressful movie. Interestingly, the changes in T-concentrations only became apparent in the saliva samples taken 15 min after the beginning of the movie, but not in the samples collected after the end of the movie. Unfortunately, in this study, no cortisol levels were measured, thus it is not known if and to which extent the movie presentation influenced the HPA-axis. In sum, previous studies suggest that stress can affect the HPG-axis within 30 min of the beginning of a stressor. Therefore, the sampling points employed in the present study should be sufficient to detect rapid stress-induced sex steroid changes.

4.2. Possible effects of situational and psychological variables

Another reason for the inhomogeneous results in the literature concerning possible stress effects on sex steroids might be the different types of stressors employed. In animal studies, it has already been shown that different stressors promote different changes in sex steroid levels (Andersen et al., 2004; Shors et al., 1999). While chronic sleep deprivation and footshocks induced significant changes of T, PROG, and E2 in male rats, no stress effects were found for a forced swim stressor (Andersen et al., 2004). Similar results were reported for female rats by Shors et al. (1999), who observed significant increases of PROG after exposing the animals to tail shocks, but not

after swim stress, although both stressors significantly enhanced GCs. The results suggest that besides the physiological stress response and the accompanying changes regarding corticosterone, other factors that are not yet well understood seem to affect the interaction between the HPA- and HPG-axis.

Previous human studies also suggest that besides situational factors, additional variables might be of importance. In contrast to animal studies employing stress protocols, human competition studies have often found increases of T-levels in participants before and after participation in the competitions. However, the magnitude of the response seems to depend at least partially on psychological variables such as motivation, individual experience with competitions and their specific outcome (winning vs. losing; Chichinadze and Chichinadze, 2008; Suay et al., 1999). With regard to the importance of motivational factors one study investigating male judo players observed an anticipatory rise of T solely in those participants reporting a high motivation to win the competition, while participants with lower motivation did not show a preceding T response (Salvador et al., 2003). Furthermore, as mentioned before, the endocrine reaction as a response to the competition itself seems to be influenced by the outcome of the contest. Here, studies have reported that winning was associated with higher T-levels in male participants compared to those who lost the competition (Gladue, 1989; Mazur et al., 1992; McCaul et al., 1992), although it should be noted that not all studies have observed similar associations in female (Bateup et al., 2001) and male participants (Hasegawa et al., 2008; Mazur, 1997).

In contrast to competitive situations, which are often associated with social rank and dominance behaviour (Chichinadze and Chichinadze, 2008; Mazur and Booth, 1998), the TSST with its inherent social evaluative threat can be understood as an experimentally induced failure, and it is known that such situations are usually accompanied with an increase of negative mood, feelings of shame, and anxiety (Dickerson et al., 2004, 2008; Scholz et al., 2009). While in the literature, positive associations have been reported between dominance behaviour and T-concentrations (at least in men; Mazur and Booth, 1998), no relationship has been observed between anxiety and T in healthy participants (Herbert et al., 1986). Therefore, the unchanged sex steroid levels after the TSST in our study might reflect a different emotional response (e.g. shame; Dickerson et al., 2004, 2008) to the social self-threatening component of the TSST compared to that to competitive situations.

4.3. Anticipatory vs. acute endocrine response

Another reason for the heterogeneous results in previous studies might be based on the different points of time at which sex steroids were measured. Studying the literature, it becomes apparent that the endocrine response of both the HPA- and HPG- axis seems to consist of two different components: an anticipatory reaction before the beginning of the specific situation on the one hand and an acute response to the competition or stressful condition on the other (Khaksari et al., 2005; Salvador et al., 2003; Schulz et al., 1996; Suay et al., 1999).

Indeed, numerous studies have confirmed an anticipatory change of cortisol- and sex steroid concentrations preceding competitive or stressful situations (Khaksari et al., 2005; Mazur, 1997; Preuss et al., 2010; Rohleder et al., 2007; Schoofs et al., 2008a; Suay et al., 1999).

However, the literature suggests that the magnitude and direction of the HPA- and HPG-reactions again depends on specific situational demands and on the gender of the participant. In competitive situations, the anticipatory response is often reflected in an enhancement of cortisol- and T-concentrations. This has been repeatedly shown for male participants with varying types of physiological and psychological competitions (Mazur, 1997; Suay et al., 1999). Regarding women, some studies have confirmed an anticipatory T-increase (Bateup et al., 2001), while others have found no sex steroid changes (Mazur, 1997; Mazur and Booth, 1998).

Various studies have suggested that not only the expectance of a competition elicits an increase of cortisol, but that an upcoming stressor also induces higher anticipatory cortisol levels (Khaksari et al., 2005; Preuss et al., 2010; Schoofs et al., 2008a). However, contrary to the observed rise of T prior to competitive situations, the expectance of a stressor seems to elicit the opposite effect on T, with lower concentrations preceding a stressful situation (Khaksari et al., 2005; Schulz et al., 1996). For example, Schulz et al. (1996) found higher cortisol-, but decreased T-levels at night before a one-day stressful clinical research protocol. Similar results have been reported for male students before an academic examination (Khaksari et al., 2005). Here, too, females in their luteal phase only displayed a significant increase of cortisol, but no changes of T- and E2-concentrations (Khaksari et al., 2005). Summarized, the literature shows that the anticipation of a competitive or stressful situation seems to elicit a specific anticipatory response depending on the specific characteristic of the situation (competition vs. stressor).

In our study, we were not able to test possible anticipatory responses, since all participants (control- and stress-group) were informed that there was a 50% chance of being assigned to a stressful situation containing a free, evaluated speech in front of a committee. Therefore, it is probable that the expectations in both groups are comparable at the point of the baseline saliva measurement and should result in similar endocrine changes based on the anticipation of the stressor.

Regarding the acute response of cortisol and sex steroids in the course of the specific condition, it should be noted that the majority of competition studies employed situations comprising a high physiological demand (e.g. sport competitions like rugby-games, rowing, or judo). In light of this fact, it is possible that the physical activity itself could have contributed to the changes observed in sex steroid levels. This assumption is supported by studies demonstrating that physiologically demanding activity without a competitive component is also capable of inducing changes in sex steroids (Suay et al., 1999). In one study (Suay et al., 1999), the increase of T and cortisol during a judo competition and a non-competitive ergometry session were indeed virtually similar, which suggests that physical demand itself is capable of provoking a T- and cortisol-rise.

In contrast to results reported from rather physical competitions, those few studies using psychological stressors have generated less consistent results (Gerra et al., 2000; Heinz et al., 2003; Hellhammer et al., 1985). In accordance to our study, Heinz et al. (2003) examined cortisol-, E2-, and T-concentrations in male participants before and after an academic oral presentation and found no acute response of E2- and T-levels to the stressor. A similar study showed no effect of a combined laboratory stressor (TSST, Stroop-task, and mental arithmetic) on T-concentrations in healthy male adolescents (Gerra et al., 2000). However, in contrast to both of the described studies, Hellhammer et al. (1985) observed an acute decrease of T due to presentation of stressful video material.

The reviewed literature suggests an anticipatory response of both axes (HPA- and HPG-axis), which, however, appears to be influenced by multiple situational factors as well as by the sex of the participants. Regarding possible acute responses of sex steroids, studies employing stressors with characteristics comparable to the TSST (psychological or psychosocial stressors) could not unambiguously confirm an influence of stress on sex hormones. Indeed, the endocrine response seems to depend (at least partially) on different factors like the physical demand of the task and the motivational state of the participants.

4.3.1. Sex differences in HPA stress responsivity: what role for sex steroids?

Previous work has established that the hormones of the HPG-axis can influence the response to the TSST. For example changes over the menstrual cycle have been established (Kudielka et al., 2009; Kudielka and Kirschbaum, 2005) and even stronger effects of oral contraceptives have been reported (Kirschbaum et al., 1999). In addition, experimental

evidence has been provided by applying estradiol to young (Kirschbaum et al., 1996) or older (Kajantie and Phillips, 2006; Komesaroff et al., 1999; Kudielka et al., 1999) participants. In the current study we did not observe strong associations between the measured salivary gonadal hormones and the cortisol stress response to the TSST. Adding the baseline sex steroid concentrations as a covariate into an ANCOVA model did not influence the interaction between sex, treatment and time observed in the initial ANOVAs. This might, however, reflect a lack of power or a reduced variance due to the fact that all women were studied in a particular menstrual phase. While we certainly cannot rule out activational influences of sex steroids alternative explanations need to be considered as well. Organisational influences of sex steroids on brain and HPA development need to be considered and more attention should be paid to psychological and societal influences (Dedovic et al., 2009). *For example* the assessment of sex related traits might be of interest in future stress studies (Cahill et al., 2004).

4.4. Limitations

Finally, some limitations of our study need to be addressed. One critical point is the method of gonadal steroid measurement employed in the present study. Monitoring hormones in saliva has several advantages over measuring hormones in blood serum or plasma. The collection of saliva is a non-invasive sampling method and therefore does not induce additional stress in participants (Hellhammer et al., 2009; Kirschbaum and Hellhammer, 1989). On the other hand, previous studies have raised the question if and to which extent saliva hormone concentrations of E2, T, and PROG actually reflect free levels of these hormones in blood samples (Shirtcliff et al., 2002; Wood, 2009). A recent review from Wood (2009) summarizes different studies investigating steroid hormone concentrations (amongst others E2, T, and PROG) in blood and saliva. The article reports that salivary T-levels, and in this case especially in women, showed poor correlations with concentrations measured in blood samples. These results have been supported by another study, which found very similar results (Shirtcliff et al., 2002). These poor correlations might induce a substantial underestimation (even more pronounced in women) of hormone-behaviour associations. In addition, the review reports that E2-, PROG-, and T- levels in saliva showed greater fluctuations in concentrations compared to blood samples. Therefore, in future studies it seems desirable to employ additionally measurements of steroid hormones from blood samples (serum or blood spot analysis, Shirtcliff et al., 2002).

Besides hormones, a series of other studies has shown that implicit motives are capable of influencing HPG- as well as the HPA-axis (Brown et al., 2009; Stanton and Schultheiss, 2009; Wirth et al., 2006). One example is the implicit power motivation, which seems to be highly correlated with T-levels, particularly in men, while in women it is proposed that E2 might play a major role in dominance motivation (Stanton and Schultheiss, 2009). Therefore, in future studies, it would probably be worthwhile to measure implicit motives and thus get a better understanding of the complex interactional effects between endocrine, motivational, and psychological variables.

Furthermore, it would be interesting to incorporate two groups of participants who definitely know which group they will be assigned to. This approach would present the opportunity to closer examine possible anticipatory effects, since the literature has shown that an anticipated response might be a component (and perhaps the first) of a HPG response due to stress (Bateup et al., 2001; Suay et al., 1999).

In addition, in future experiments we suggest employing a longer overall post-treatment sampling period and simultaneously increasing the number of saliva measurements subsequent to the treatment. This monitoring at close intervals seems to be important to get a better idea of the temporal dynamic of sex steroid reactions, since it allows the detection of possible short-lived effects (Hellhammer et al., 1985) as well as slow interactional effects between both hormone axes.

Finally, our study was conducted in the morning. The meta-analysis of Dickerson and Kemeny, (2004) observed that the effect size for stress studies conducted in the afternoon was larger than for those conducted in the morning. However, an integrated analysis of five TSST studies indicated that the cortisol response (delta increase) to the TSST was similar in the morning and afternoon, despite the fact that baseline cortisol concentrations were higher in the morning. The latter study suggests that the impact of time of day on the HPA response to the TSST is not very strong. Future studies may wish to investigate the influence of the circadian rhythm on the HPA and HPG response to stress more explicitly.

5. Conclusion

In sum, the present experiment indicates that the Trier Social Stress Test, one of the most prominent psychosocial laboratory stressors in humans, does not induce substantial changes in salivary sex steroids in men or women. A stress-induced decrease in gonadal steroids might occur only after exposure to more physical or more severe stressors. Based on the reviewed literature, it seems plausible that besides the well-established psychosocial stress features (e.g. social evaluative threat, uncontrollability, motivated performance), an additional competitive component might be necessary to influence sex steroid levels. Therefore, an interesting question to investigate in future studies would be to assess whether the addition of a competitive component to the TSST would have the potential to induce changes in T- (and perhaps in E2- and PROG-) concentrations. Given the limitations discussed above, additional experimental studies in humans are needed to untangle the complex interaction between the HPA- and the HPG- axis.

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