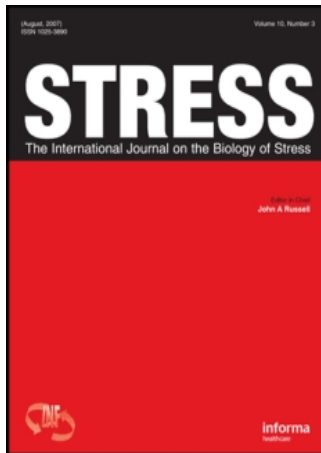


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Neuroendocrine stress responses to an oral academic examination: No strong influence of sex, repeated participation and personality traits

D. Schoofs^a; R. Hartmann^a; O. T. Wolf^a

^a Department of Psychology, University of Bielefeld, Bielefeld, Germany

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RESEARCH REPORT

Neuroendocrine stress responses to an oral academic examination: No strong influence of sex, repeated participation and personality traits

D. SCHOOF, R. HARTMANN & O. T. WOLF

Department of Psychology, University of Bielefeld, Postfach 10 01 31, D-33501 Bielefeld, Germany

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Abstract

Public speaking tasks have been widely used as laboratory stressors in human research. Fewer studies have investigated similar real life situations like oral examinations and results have been inconsistent. The present study investigated salivary cortisol (as a marker of hypothalamus–pituitary–adrenal (HPA) activity) and salivary alpha-amylase (sAA as a marker of sympathetic nervous system (SNS) activity) within the context of a university examination.

Subjects were 40 undergraduate students who participated in an oral examination. Of these, 20 also participated in a second examination within a few weeks. Cortisol and sAA were measured immediately before and after the examination and on a control day. Additionally, subjects filled out personality questionnaires.

A strong anticipatory increase in salivary cortisol and sAA as well as more modest further increases between the pre- and post-measurements were detected during the examination. Sex or oral contraceptive use had no influence on either measure. In addition, no significant differences between the first and second examination were observed.

The findings indicate the neuroendocrine stress responses to laboratory stressors and to heralded real life stressors as well as the modulatory variables involved differ from each other.

Keywords: *Oral examination, salivary alpha-amylase (sAA), salivary cortisol, sex differences, stress*

Introduction

Stressful events lead to an enhanced activity of the sympathetic nervous system (SNS) and the hypothalamic–pituitary–adrenal (HPA) axis (de Kloet et al. 2005). The SNS acts via the catecholamines adrenaline and noradrenaline and triggers a first rapid response. Alpha-amylase (sAA) has been used as a non-invasive salivary marker of SNS activity. SAA is a protein of importance for enzymatic digestion, but its secretion is initiated by the sympathetic branch of the autonomic system. Several studies have shown that the activity of sAA is increased by acute stress (Chatterton et al. 1996; Rohleder et al. 2004; van Stegeren et al. 2006), that this increase can be prevented with beta receptor blockers (van Stegeren et al. 2006) and that this increase correlates with other measures of SNS

activity (Chatterton et al. 1996; Rohleder et al. 2004; van Stegeren et al. 2006).

A somewhat slower second response is the activation of the HPA axis which leads to adrenal secretion of glucocorticoids (GCs, particularly cortisol in humans; de Kloet et al. 2005). These two stress systems influence a number of target tissues in the periphery and in the brain. For example, alterations of the HPA axis and the SNS modulate cognitive and affective processing (de Kloet et al. 2005; Roozendaal et al. 2006; Wolf 2006).

Two different strategies have been used to investigate stress. Firstly, stress might be induced in the laboratory (Kirschbaum et al. 1993; Sita and Miller 1996). One laboratory stressor, the Trier Social Stress Test (TSST; Kirschbaum et al. 1993), appears to be especially potent. It consists mainly of a public speech and mental arithmetic in front of an audience

Correspondence: O. T. Wolf, Department of Psychology, University of Bielefeld, Postfach 10 01 31, D-33501 Bielefeld, Germany. Tel: 49 521 1064433. Fax: 49 521 1066422. E-mail: oliver.wolf@uni-bielefeld.de

(Kirschbaum et al. 1999). Dickerson and Kemeny (2004) demonstrated that the efficiency of laboratory stressors depends on the combination of psychological factors such as ego involvement, social evaluative threat and uncontrollability. These factors can also be elements of real life stressors—the second major group of stressors investigated. Often, oral or written examinations presented in school or university settings are studied (Frankenhaeuser et al. 1978; Herbert et al. 1986; Spangler 1997). While real life stressors have a higher external validity they bear the problem of less standardized conditions and the influence of multifaceted variables. These factors could be responsible for the inhomogeneous results (Stowell 2003).

In addition to differences related to the stressor, other variables modulate the neuroendocrine stress response. Laboratory stress studies have reported effects of sex steroid fluctuations over the menstrual cycle and hormonal contraceptive use (Traustadottir et al. 2003; Kudielka and Kirschbaum 2005; Kajantie and Phillips 2006). Results of real life stressors revealed larger urinary and plasma cortisol responses on the examination days in male high school or male medical students (Frankenhaeuser et al. 1978; Khaksari et al. 2005). Also the anticipatory cortisol increase before an examination revealed sex differences, but results have been conflicting (Spangler 1997; Lacey et al. 2000).

Psychological laboratory stressors also stimulate the SNS (Biondi and Picardi 1999; van Stegeren et al. 2006). Here again inconsistent results are reported for stress-induced sex-specific differences in the response of the catecholamines (Davidson et al. 1984; Stoney et al. 1988; Stoney et al. 1990; Litschauer et al. 1998; Ross et al. 2001). The results for SNS activation from studies using real life stressors are heterogeneous as well (Frankenhaeuser et al. 1978; van Doornen and van Blokland 1987; Bosch et al. 1996). Therefore, in the present study we were interested in illustrating the influence of sex or oral contraceptive use on the HPA and SNS stress response.

Personality traits might help to understand the substantial inter-individual differences in the stress response. High self esteem, high extroversion, low trait anxiety, and low neuroticism have been reported to be associated with a reduced neuroendocrine stress response (Pruessner et al. 1997; LeBlanc et al. 2004; Oswald et al. 2006). However, repeated results are not uniform and a substantial number of studies failed to observe associations (Bohnen et al. 1991; van Eck et al. 1996; Schommer et al. 1999). In the current study we, therefore, employed several personality questionnaires.

While the stress response appears to be influenced by trait factors like sex and personality, several state factors are also of relevance. One of them is novelty and the related effect of habituation. Laboratory studies reported strong habituation effects

for stress-induced responses of the HPA axis (Kirschbaum et al. 1995b; Schommer et al. 2003). However, repeated exposure to important real life stressors might not be associated with HPA habituation (Rohleder et al. 2007). Concerning the SNS, Schommer et al. (2003) observed a uniform activation pattern of adrenaline and noradrenaline after repeated exposure to a laboratory stressor and concluded that the HPA axis but not the SNS might habituate to repeated exposure to a similar stressor. It has been suggested that a failure to habituate to repeated stress exposure causes allostatic load and an increased disease risk (McEwen 1998). Therefore, the present study investigated the neuroendocrine stress response of the participants in a second examination with the same examiner.

In sum, the objective of our study was to characterize the response of the SNS and HPA axis to an oral academic examination. Salivary alpha-amylase and salivary cortisol concentrations were assessed before and after the examinations and compared with control days. Special interest was paid to the presence of sex differences and the impact of oral contraceptive use. Moreover, the occurrence of habituations and possible relationships with personality traits were explored.

Methods

Subjects

Forty undergraduate psychology students from the University of Duesseldorf (11 males, 29 females; mean age \pm SEM = 21.50 \pm 0.32 years) participated. Two female students were under thyroxine substitution for chronic hypothyroidism. All other participants reported being free from acute or chronic disease. Three subjects were smokers and 18 women took oral contraceptives (OCs). The averaged body-mass-index (BMI) was 21.31 \pm 2.75 kg/m².

Subjects had volunteered to take part in the study after the purpose and procedure had been described to them. The study was approved by the national ethical committee of the German Psychology Association (DGPs) and all subjects provided written informed consent.

Twenty students participated in a second examination, which took place in the same examination period between one to four weeks apart from the first examination. In this second examination students were tested by the same examiner but on another topic.

Examination

All subjects took part in an oral examination (in social psychology or developmental psychology) for their “Vordiplom” (comparable to the bachelor degree) at the end or the beginning of a semester. In total seven

examinations of this kind have to be taken by the students over a period of one and a half to two years. During the examinations subjects sat opposite the professor in his office at a small desk. In addition, a third person, sitting at another table, was taking notes for the protocol.

The examinations commenced at times varying between 9:00 a.m. and 4:20 p.m. (mean time \pm SEM = 11:54 a.m. \pm 0:22 min) for the first period of examinations and between 9:30 a.m. and 3:20 p.m. (mean time \pm SEM = 12:23 p.m. \pm 0:27 min) for the second one.

Experimental procedures

Cortisol and sAA measurements. On the examination day students collected saliva samples, immediately before and directly after the oral examination which lasted approximately 30 min. At the time of the second sampling the students were not yet aware of their examination results. In addition, participants had to collect two saliva samples on a control day within 7 days before or after the examination (mean number of days before/after the examination \pm SEM = 4.58 \pm 0.24). One sample had to be taken at the time of the beginning of the oral examination and one 30 min afterwards (comparable to the end of the examination). The students were instructed to keep the samples refrigerated and to bring them on the day of their oral examination or later to the laboratory. Those 20 students who attended the second oral examination had another control day comparable to the first one.

Saliva was collected using Salivette collection devices (Sarstedt, Nuembrecht, Germany). Free cortisol levels were measured using an immunoassay (IBL, Hamburg, Germany). Inter- and intra-assay variations were below 15%. 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, sAA levels were obtained from 37 subjects in the first and from 16 subjects in the second examination.

Questionnaires. The students had to fill out demographic and five mood and personality questionnaires. Completion took place either at the university or at the students' homes. Questionnaires were selected based on previous studies (Herbert et al. 1986; Bosch et al. 1996; Pruessner et al. 1997, 2005; Gaab et al. 2006) suggesting that those personality measures might influence the HPA stress response.

Questionnaire of competence and control (FKK) (Krampen 1991). The FKK is a 32-item questionnaire and assesses generalized expectancies.

The items belong to four different scales: (1) positive self-concept (2) internality (3) powerful others and (4) chance. Each scale consists of eight items.

General depression scale (ADS-L; Hautzinger and Bailer 1992). The ADS-L is a revised German translation of the "Center for Epidemiological Studies Depression Scale" (CES-D) originally developed by Radloff (1977). The ADS-L is designed as a screening instrument to assess the existence, frequency and duration of depressive symptoms. Subjects have to rate 20 statements about their feelings during the previous seven days.

State-trait-anxiety inventory (STAI; Laux et al. 1981). A German version of the STAI (Spielberger et al. 1970) was utilized to assess the level of general anxiety (subscale: trait-anxiety). The subjects had to rate 20 items, 13 of these statements included references to anxiety while seven statements were control items.

Personality questionnaire (Neo-FFI; Costa and McCrae 1992). The NEO Five Factor Inventory with 60 items was used to assess scores for five domains of adult personality: neuroticism, extroversion, openness, agreeableness and conscientiousness.

Rosenberg self-esteem scale (Rosenberg 1965). This scale is a one-dimensional measure of self-esteem. Subjects had to rate 10 items. The items represent a continuum of self-worth statements ranging from statements that are endorsed even by individuals with low self-esteem to statements that are endorsed only by persons with high self-esteem.

Data analysis

Cortisol and alpha-amylase measurements were analysed with analysis of variance (ANOVA) for repeated measurements with the two factors examination (examination day vs. control day) and time (pre- vs. post-examination). Possible influences of sex and/or the use of hormonal contraception were analysed by including, in two separate sets of analyses, the factors sex or hormonal status (males, naturally cycling women, hormonal contraception users). Greenhouse-Geisser corrected *p* values were used when appropriate. Bilateral tests were performed for all analyses and *p* was set to 0.05.

Due to the fact that endocrine data often show right-skewed distribution, both cortisol and sAA concentrations were tested for normal distribution. With the exception of the sAA post measurement on the control day all data were normally distributed.

All calculations were also done with log-transformed data. Since the major results were not affected by this procedure, only the results obtained from the analysis of the raw data are presented in the following section.

Relationships between the personality questionnaires and cortisol or sAA levels were examined by Pearson's correlation coefficients. Because sex and circadian rhythm have an influence on cortisol and sAA secretion, partial correlations controlling for sex and time of day were calculated (Dickerson and Kemeny 2004; Rohleder et al. 2004; Kudielka and Kirschbaum 2005). In order to reduce the number of correlations the averaged pre- and post-concentrations of cortisol as well as sAA were used. All statistical analyses were conducted using SPSS 11.0 statistical software.

In addition, effect sizes (*d* Hedges) were calculated for the main significant findings and several power analyses were performed for selected non-significant findings (Dickerson and Kemeny 2004; Het et al. 2005). These procedures are described below.

Results

Academic performance

In both oral examinations all subjects passed. The average grade of all subjects in the first examination was 1.43 ± 0.083 (mean \pm SEM, the German grade "1" is comparable to the North American grade "A", the grade "2" is equivalent to "B" and so on). Male and female students showed no obvious difference in their examination outcome (men: 1.44 ± 0.156 , women: 1.43 ± 0.100). For the second examination the grade point average was 1.52 ± 0.157 . A separated analysis for each sex was not performed, because the number of male students was too small (18 women and 2 men).

Neuroendocrine stress response

Possible influence of sex. The oral examination caused significant elevated concentrations of cortisol and sAA. Neither cortisol- nor sAA showed significant sex differences with regard to their concentration on the control and examination days. A repeated measurement ANOVA with the between-subject factor sex and the two within-subject factors examination (examination vs. control) and time (pre vs. post) was performed. The analysis of cortisol responses included 11 male and 29 female subjects, whereas 10 men and 27 were analysed for the sAA concentrations. All ANOVA results are shown in Table I.

ANOVA revealed a significantly greater average cortisol concentration on the examination day compared to the control day (main effect of examination). The averaged cortisol concentration (mean \pm SEM)

Table I. ANOVA results summary of the influence of sex on salivary cortisol- and alpha-amylase-responses to an oral examination.

Effect	<i>n</i>	<i>F</i>	<i>p</i>
<i>Cortisol</i>			
Exam \times sex	40	0.546	0.465
Time	40	5.339	0.026
Time \times sex	40	0.018	0.895
Exam \times time	40	6.847	0.013
Exam \times time \times sex	40	0.130	0.909
<i>Alpha-amylase</i>			
Exam	37	22.245	<0.001
Exam \times sex	37	0.433	0.515
Time	37	20.513	<0.001
Time \times sex	37	0.304	0.585
Exam \times time	37	13.657	0.001
Exam \times time \times sex	37	0.151	0.700

The ANOVA model contained the between group factor sex and the within group factors examination (exam vs. control) and time (pre vs. post).

was 11.11 ± 1.30 nmol/l on the control day compared to 26.57 ± 1.71 nmol/l on the examination day. Moreover, a significant examination-by-time interaction was observed. Cortisol concentrations increased during the examination, while decreasing on the control day. No influence of the between-subject factor sex could be found (neither main effect nor interaction). See Figure 1a for means and SEMs of male and female subjects (the group of female subjects is further divided into women taking hormonal contraceptives or not; see second set of analyses below).

Two effect sizes (Hedges unbiased effect sizes) were calculated. The first one was for the anticipatory increase (by comparing the pre-examination cortisol levels with the corresponding levels on the control day). The second one was for the increase during the examination (by comparing the post- with the pre-examination levels). Analysis was performed using *G** power (Erdfelder et al. 1996) and in a similar fashion as reported by Dickerson and Kemeny (2004). The effect size for the anticipatory rise was 1.60 (e.g. the mean for the baseline cortisol levels on the examination day was 1.6 SDs above the mean on the control day). This indicates a very strong effect. According to Cohen (1988) effect sizes of 0.80 or larger can be considered large. The effect size for the increase during the examination was 0.41, which can be considered small to medium.

A similar pattern was observed for sAA (Figure 1b). The analysis of the within subject factors for both examination and time showed significant results. They revealed an increase of the average alpha-amylase concentration between the control and examination day from 23.75 ± 4.04 to 53.95 ± 6.79 U/ml and a significant increase between the pre- and post-measurement. In addition, a significant examination-by-time interaction was observed. The between

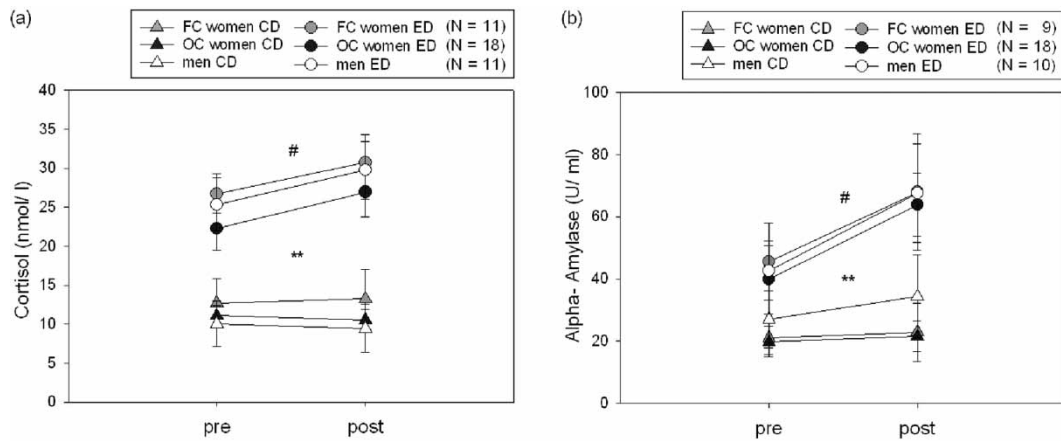


Figure 1. Significant main effect of examination (ED) vs. control (CD; ** $p < 0.001$); significant main effect for time (pre vs. post; # $p < 0.05$); and significant interaction between examination *time ($p < 0.05$) for cortisol (Figure 1a) and sAA (Figure 1b). No significant influence of hormonal status (free cycling women (FC); women using oral contraceptives (OC); men) for Cortisol or sAA. Data presented as group mean \pm SEM.

subject factor sex again did not reach significance. See Table I for all ANOVA results. Effect sizes were calculated in an analogous fashion to those for cortisol. The anticipatory increase again indicated a large effect size ($d = 0.90$). The increase during the examination was again medium ($d = 0.60$).

Possible influence of hormonal status. Because women using hormonal contraception show especially blunted free cortisol responses to laboratory stressors (Kudielka and Kirschbaum 2005; Kajantie and Phillips 2006) we computed further ANOVAs with hormonal status as between group factor. A repeated measurement ANOVA with the factor hormonal status (males, $n = 11$ cortisol/ $n = 10$ sAA; naturally cycling women, $n = 11$ cortisol/ $n = 9$ sAA; hormonal contraception users, $n = 18$ cortisol/ $n = 18$ sAA) and the within subject factors examination and time for cortisol, as well as sAA, was performed. Results were similar to the preceding analysis. The between subject factor hormonal status had no statistically significant influence (Table II), whereas the within subject factors examination and time and the interaction examination-by-time showed a significant change of the cortisol- and alpha-amylase-concentration, respectively. See Figure 1a,b for means and SEMs.

A power calculation was performed using G^* power in order to estimate the power to detect an effect of OC use on cortisol reactivity. Based on data presented in the second experiment in Kirschbaum et al. (1995a) we calculated the expected effect size for OC use (in comparison to non OC-using women) on the cortisol stress response. The effect size for OC use reported in the Kirschbaum et al. (1995a) paper was 0.73 indicating a medium to large effect. The present study containing 18 OC users and 11 naturally cycling women had a limited power of 0.60 (1-beta).

Repeated oral examination. Those subjects who underwent two oral examinations showed very similar neuroendocrine responses to both examinations. Neither the pre- nor the post-cortisol and sAA concentrations differed between the first and second examination (Figure 2a,b).

A repeated measurement ANOVA was performed for cortisol and alpha- amylase, respectively, with the within-subject factors number of examination (examination 1 vs. examination 2) and time (pre vs. post). The analysis revealed a significant main effect for the factor time ($F(1,19) = 11.08$, $p < 0.01$) which emerged from greater cortisol concentrations at the post-measurement (25.89 ± 2.25 nmol/l) when compared with the pre-measurement (22.71 ± 1.98 nmol/l). The main effect of

Table II. ANOVA result summary of the influence of hormonal status on salivary cortisol- and alpha-amylase-responses to an oral examination.

Effect	<i>n</i>	<i>F</i>	<i>p</i>
<i>Cortisol</i>			
Exam	40	60.475	<0.001
Exam \times hormonal status	40	0.349	0.708
Time	40	6.545	0.015
Time \times hormonal status	40	0.017	0.983
Exam \times time	40	7.284	0.010
Exam \times time \times hormonal status	40	0.100	0.905
<i>Alpha-amylase</i>			
Exam	37	28.173	<0.001
Exam \times hormonal status	37	0.247	0.782
Time	37	20.921	<0.001
Time \times hormonal status	37	0.155	0.857
Exam \times time	37	16.117	<0.001
Exam \times time \times hormonal status	37	0.085	0.918

The ANOVA model contained the between group factor hormonal status (males, naturally cycling women, hormonal contraceptive users) and the within group factors examination (exam vs. control) and time (pre vs. post).

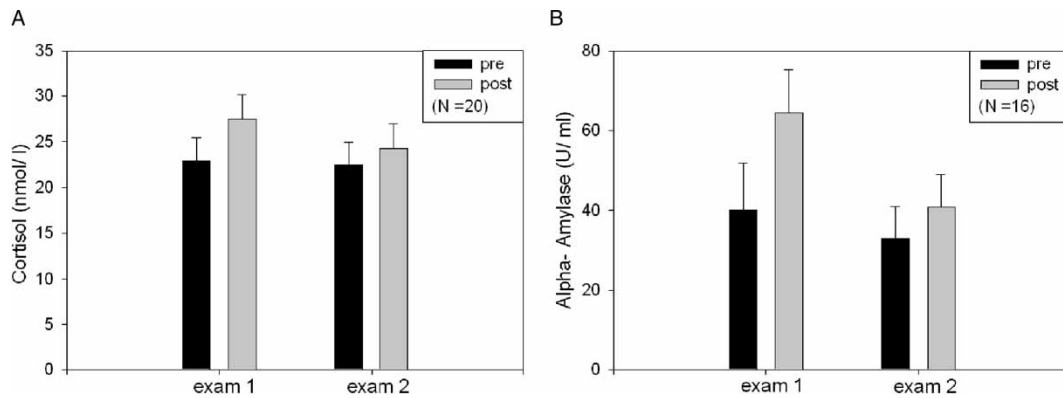


Figure 2. Mean cortisol (A) and sAA (B) concentrations for those participants who also took part in the second oral examination. Results revealed no significant differences between first and second examinations for both measures (main effect as well as interactions). Data presented as group mean \pm SEM.

number of examination ($F(1,19) = 0.388$, $p = 0.541$) and the examination-by-time interaction ($F(1,19) = 3.02$, $p = 0.098$) were not significant.

We found similar results for the analysis of sAA. The repeated measurement ANOVA showed no significant main effect for number of examination ($F(1,15) = 2.06$, $p = 0.172$) and a significant main effect of time ($F(1,15) = 10.22$, $p < 0.01$), caused by the elevated sAA levels at the post measurement (pre-measurement: 36.40 ± 7.32 U/ml; post-measurement: 52.50 ± 7.67 U/ml). Finally, no significant interaction of examination number and time was observed ($F(1,15) = 1.30$, $p = 0.273$).

Associations with personality measures. Relationships between the different personality variables and the averaged values of the pre- and post-cortisol and alpha-amylase concentrations on the first examination day were analysed. Partial correlations controlling for sex and time of day were calculated. All correlations between the averaged cortisol concentrations and the subscales of the six personality questionnaires were not significant (all p s > 0.08 , data not shown).

For sAA a positive correlation for the subscale neuroticism of the NEO-FFI ($r = 0.41$, $p = 0.015$) and the subscale trait-anxiety of the STAI ($r = 0.37$, $p = 0.029$) were observed. Thus higher neuroticism and higher trait anxiety were associated with greater sAA concentrations on the examination day.

Associations with academic performance. Partial correlations controlling for sex and time of day were also performed for the associations between the averaged cortisol and sAA levels and the academic performance (received grade). However, neither for cortisol nor for sAA was a significant association with the received grades detected (both $p > 0.10$).

Discussion

The objective of this study was to characterize the response of the HPA axis and SNS to an oral academic examination. Additionally, the impact of sex and use of OCs were considered as well as the occurrence of habituation and possible relationships with personality traits. In the following our results will be compared to previous findings from real life stress studies and laboratory stress studies.

Anticipatory and acute response pattern

A strong anticipatory rise (with large effect sizes) in cortisol and sAA concentrations was observed on the examination day. During the examination concentrations continued to rise moderately. For the anticipatory increase in sAA our results are in line with other studies using an examination situation. Medical and dental students revealed increased SNS activity before examinations (Herbert et al. 1986; Bosch et al. 1996). The observed additional sAA increase between the pre- and post-examination measure is well in line with different studies using laboratory stressors (e.g. Rohleder et al. 2004; Nater et al. 2006).

For cortisol, the strong anticipatory increase before the examination is also in line with several previous studies on this topic, which found that cortisol concentrations before anticipated examinations were higher than those on days without an examination (Herbert et al. 1986; Lacey et al. 2000; Martinek et al. 2003, but see Spangler 1997; Vedhara et al. 2000 for contrary results). In contrast to real life stressors, anticipatory increases are relatively rarely observed in the laboratory. The TSST, like most laboratory stressors, can be conceptualized as an unfamiliar "surprise stressor" while examinations are announced, anticipated and familiar stressors. This might be able to account for missing anticipatory stress responses in the laboratory. There are of course additional

differences: whereas the achievement in the laboratory is not relevant for one's future career, a negative performance in an examination might have negative consequences.

Influence of sex and OC use

Cortisol and sAA levels were neither influenced by sex nor by hormonal status (OC use). Former studies, which investigated the SNS stress response via noradrenaline/adrenaline measurements, resulted in no consistent picture regarding the influence of sex and OC use. Dependent on the catecholamine measuring method and the kind of stressor used, studies found either no sex differences (Stoney et al. 1990; Litschauer et al. 1998) or higher catecholamine concentrations in men (Frankenhaeuser et al. 1978; van Doornen and van Blokland 1987; Ross et al. 2001). Several of those studies used urinary catecholamine measures (e.g. Frankenhaeuser et al. 1978; van Doornen and van Blokland 1989), a method which might reflect the tonic response but is less suited to capture the acute SNS response.

A further variable that might add to the variance is the potential influence of sex hormones. Some authors found no changes of SNS stress reactivity over the menstrual cycle (Litschauer et al. 1998; Kirschbaum et al. 1999). Others observed variations of catecholamine responsivity across the menstrual cycle (Collins et al. 1985). The use of OCs seems to diminish the SNS stress response (Luecken et al. 1997), but so far not enough studies have investigated the influence of hormonal contraceptive intake on the catecholamine stress reactivity. In our study we did not find evidence for an impact of OC use on the sAA response to the examination stress.

With respect to cortisol, previous examination studies investigating sex differences revealed mixed results. While some studies observed evidence for a stronger response in male students (Frankenhaeuser et al. 1978; Khaksari et al. 2005; Weekes et al. 2006) others did not find sex differences (Martinek et al. 2003). These inconsistencies could again be caused by multiple variables like differences in the studied population and the investigated examination type. In addition, physiological factors like menstrual cycle phase (Frankenhaeuser et al. 1978; Armario et al. 1996) or the use of hormone-containing contraceptives (Frankenhaeuser et al. 1978) are rarely taken into account in field studies.

A critical factor in examining the acute stress response represents the design of the study (sampling time points). Several previous studies investigated responses to academic stress by using a sampling schedule that was less well suited to detect acute stress responses of the HPA and the SNS system. Assessment during the examination period reflecting the days or weeks of preparation before the examination,

e.g. (Vedhara et al. 2000; Weekes et al. 2006) might lead to findings, which differ from assessments during the examination itself (Stowell 2003).

At first sight, for cortisol the missing influence of sex seems to be in contrast to studies which found that the cortisol response to the TSST is stronger for males (Kudielka and Kirschbaum 2005; Kajantie and Phillips 2006). However, in their large meta-analysis Dickerson and Kemeny (2004) failed to find an influence of sex on the average cortisol response. This suggests that sex differences are not reliably found in laboratory stress studies and might be related to specifics of the stress procedure used.

Changes in HPA axis response over the menstrual cycle have been detected in some studies (Altemus et al. 1997; Kirschbaum et al. 1999; Kajantie and Phillips 2006). More pronounced is the substantially blunted free cortisol response in women using OCs (Kirschbaum et al. 1995a, 1999). Ethinyl estradiol-containing OCs produce an increased production of corticosteroid binding globulin (Wiegratz et al. 1995). This increase is compensated by elevated basal total cortisol levels. The acute stress response in OC users during the TSST is characterized by a comparable ACTH and total cortisol stress response in the face of a blunted free cortisol response (Kirschbaum et al. 1999). Thus, the robust anticipatory cortisol response in OC using women in this examination study was remarkable. It appears that the inability of OC-using women to mount a strong free cortisol response is restricted to moderate and surprising stressful events. In contrast, an examination is a more severe and anticipated stressor so that there is enough time to orchestrate a HPA response which also results in substantially increased free cortisol levels. In addition, the magnitude of the response might also be an explanation; in the current study the cortisol increase was stronger than in most previous TSST studies. The calculated effect size was almost twice as large as that calculated for the most effective laboratory stressors in a recent meta-analysis (Dickerson and Kemeny 2004). Our data suggest that in the case of a strong stressor, and/or for a stressor anticipated well in advance, salivary cortisol concentrations are no longer modified by OC use.

Response to the second examination

No habituation effects for those students also participating in the second examination, neither for the HPA nor for the SNS response, were detected. Both examinations had to be taken by the same examiner and all students performed well at the first examination. This could have increased their self-confidence and should have reduced the influence of novelty. Even though a somewhat reduced increase in salivary cortisol and sAA levels during the second examination were observed this difference was not

significant with the small sample studied. However, the initial anticipatory increase, reflected in the pre-examination measure, was similar at both examinations for both neuroendocrine markers.

Concerning the SNS response after repeated stress, other studies reported contradicting results with increasing, decreasing and unchanged catecholamine responses (Dobrakovova et al. 1993; Gerra et al. 2001; Schommer et al. 2003). The absent habituation effect for the HPA axis is contrary to laboratory studies that reported strong habituation effects (Kirschbaum et al. 1995b; Schommer et al. 2003). But not all studies showed habituation of HPA activity and different characteristics of the stressor, e.g. intensity and frequency, appear to play a critical role in development of habituation (Gerra et al. 2001). For example, in competitive ballroom dancers results provided no evidence for habituation of the cortisol stress response (Rohleder et al. 2007). This study together with our current findings indicates that there is no or a slow habituation for real life stressors with high intensity and high self relevance.

Associations with personality traits

We found no close relationships between personality measures and the cortisol response. Previous studies on this topic have lead to inconclusive results (van Eck et al. 1996; Pruessner et al. 1997; Schommer et al. 1999). A lack of statistical power might be responsible for some of the negative findings. In addition, situational factors might mask associations between personality traits and the cortisol stress response (Pruessner et al. 1997) With respect to sAA we found positive correlations that suggested an association between higher scores on the neuroticism and trait anxiety scale with higher sAA concentrations on the examination day. Results should be interpreted with caution since no alpha adjustments for multiple comparisons were performed. For neuroticism, previous studies revealed no correlation with the SNS stress response (Herbert et al. 1986), or even a negative correlation (LeBlanc et al. 2004). For trait anxiety the observations were also inconsistent (Netter 1987; Jezova et al. 2004). The comparability of these previous studies is limited by the use of different types of stressor and different groups of subjects.

Limitations

In our study several limitations need to be addressed. Firstly, the group of subjects was restricted to psychology students and thus not representative of the entire student community. In addition, the group had a medium size ($n = 40$) and, therefore, non-significant results might in part reflect a lack of power. This is especially the case for the subgroup analysis that was conducted (sex, OC use, habituation). For female

participants the phase of the menstrual cycle could not be controlled for, which might have contributed to the variance in this group. Furthermore, for practical reasons, only two samples for the cortisol and the sAA analysis were collected on the examination day. More samples before the beginning of the examination could be of interest in order to better characterize the start of the anticipatory response (Rohleder et al. 2007).

With our design the question as to whether or not this strong anticipatory HPA response is adaptive or maladaptive remains unanswered. Experimental studies investigating the effects of stress or cortisol treatment on memory retrieval have consistently reported an impairing effect of stress on retrieval (Roosendaal et al. 2006; Wolf 2006). However, in our study we did not find associations between cortisol and sAA levels and the grade received. Reasons for this could be the small variance in the grades (ceiling effect), differences in the individual preparation for the examination or differences in general intelligence. Other recent findings in contrast pinpoint towards a beneficial or stress protective effect of elevated cortisol levels on mood and anxiety during acute stress exposure (Soravia et al. 2006; Het and Wolf 2007). Thus, the anticipatory HPA response could lead to reduced anxiety during the examination. Future studies are needed to assess academic achievements but also the subjective response to the examination in more detail.

Conclusion

In sum, the neuroendocrine stress response to an oral examination is characterized by a marked anticipatory response of the SNS and the HPA. No effect of sex, OC use or repeated examination was detected. In addition, no strong associations with personality factors occurred. Possible modulatory variables thus appear to differ between such an important real life stressor announced well in advance, and acute laboratory stressors that have an element of surprise. Because of these differences the external validity of laboratory stressors might be restricted to unexpected moderately stressful real life events. For the future the development of a laboratory stressor for the investigation of the anticipatory stress response appears to be desirable.

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