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The stressed student: Influence of written examinations and oral presentations on salivary cortisol concentrations in university students

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Abstract
Laboratory research has demonstrated that social-evaluative threat has an influence on the hypothalamus pituitary adrenal axis (HPA). In two studies using independent samples, we evaluated the anticipatory cortisol response to a written university examination ($n = 35$) and to an oral presentation ($n = 34$). Saliva samples were collected before and after the examinations and on a control day. Additionally, saliva samples were collected on the day before the written examination and a control day. Results revealed significantly elevated cortisol concentrations on the day prior to the examination; however, this effect occurred only in those participants who had their control day after the examination. Cortisol concentrations were elevated on the examination day, with increased concentrations before but not after the examination. For the oral presentation study, the results revealed substantially elevated cortisol concentrations before and after the oral presentation. Taken together the results indicate that written examinations cause a mild anticipatory HPA response while oral presentations induce a strong HPA response. These findings appear to support the idea that social-evaluative threat is an important factor determining the size of the HPA response to laboratory stressors as well as to real-life stressors.

Keywords: *Cortisol, salivary samples, socio-evaluative threat, stress, university students*

Introduction

In the case of threat or challenge, the body answers with an adaptive reaction to cope with the situation. This stress response includes an enhanced activity of the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis. Activation of the HPA axis leads to an increased secretion of glucocorticoids (particularly cortisol in humans) from the adrenal cortex (de Kloet et al. 2005). This hormone can be measured in saliva (Kirschbaum and Hellhammer 1989). It has been repeatedly shown that the two stress systems influence cognitive and affective processes (de Kloet et al. 2005; Lupien et al. 2007; Wolf 2008). In addition, multiple target systems in the periphery (cardiovascular system, immune system, glucoregulatory system) are influenced by these stress mediators (McEwen 1998).

It has long been conceptualised that in humans the situational factors novelty and uncontrollability in combination with ego involvement lead to psychological stress (Mason 1968). More recently, it has been hypothesised that a threat to the social self (e.g. status, reputation) is especially stressful (Dickerson and Kemeny 2004) for humans as social individuals. Indeed by analysing over 100 laboratory studies Dickerson and Kemeny (2004) showed that social-evaluative threat was a potent predictor for the stress response of the HPA axis (Dickerson and Kemeny 2004). Thus, social-evaluative threat seems to be an important prerequisite to cause an HPA response in the laboratory.

However, it remains controversial whether the findings from studies with controlled experimental stressors can be predictive of cortisol reactions to real-life stressors (van Eck et al. 1996; Cohen and Hamrick

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2003; Kamarck and Lovallo 2003). To heighten ecological validity the physiological response to naturalistic stressors can be assessed. University life contains several different stressors for students and examinations are often used as real-life stressors (Stowell 2003). There are different forms of examinations and two kinds are very popular for studies evaluating university stress. On the one hand there are oral examinations (Schoofs et al. 2008) and on the other hand written examinations (Ng et al. 2003; Gaab et al. 2006). While the results for oral examinations are mostly homogenous, the empirical picture is less clear for written examinations. Oral examinations were found to be associated with increases in cortisol secretion (Herbert et al. 1986; Lacey et al. 2000; Schoofs et al. 2008). For example, we recently observed that salivary cortisol concentrations of university students were increased by more twofold immediately before undergoing an oral examination (Schoofs et al. 2008). In written examinations, results are less consistent. While some studies report stress effect (Lovallo et al. 1986; Loft et al. 2007), other studies failed to find effects on cortisol secretion (Spangler 1997).

It is obvious that the results in the field of examination stress are heterogeneous and a consensus about the influence of examination stress on the neuroendocrine stress response has not been reached. There are several possible reasons for these inhomogeneous results. First there are large differences in the written examinations used in the different studies (Stowell 2003). While some authors investigated a single written examination (Frankenhaeuser et al. 1978), other authors assessed the hormonal stress response during a whole examination period (Loft et al. 2007). During a period of examinations, anticipatory effects might exert additional influences on stress responses. Additionally, the impact of the evaluated examination on the student's life also differs between the studies. While some studies evaluated matriculation examinations (Frankenhaeuser et al. 1978), other studies evaluated the impact of final examinations in medical school (Zeller et al. 2004) or written examinations in undergraduate students (Ng et al. 2003; Gaab et al. 2006). These differences might account for the heterogeneity in the field of stress research on written examinations, but there is still need for further investigation. Especially, the impact of examination stress on the anticipatory stress response needs additional attention.

A reason for the finding that the results for oral examinations are more homogeneous than the results for written presentations might be that these two kinds of examinations differ in social-evaluative threat. While oral examinations always contain a social-evaluative threat because of the listening and judging audience, written examinations lack this component. This lack of social-evaluative threat, which is an important factor in laboratory research (Dickerson and Kemeny 2004), might also influence the magnitude of the stress response during real-life stress.

Although examinations are popular real-life stressors, other stressors in the university context exist. Examples are oral presentations in university courses. These situations contain performance pressure and social-evaluative threat induced by the listening audience and therefore can be compared with oral examinations. However, the impact on the students' overall grade level is typically smaller for oral presentations. Moreover, the factor 'uncontrollability' (Dickerson and Kemeny 2004) appears to be less present during oral presentations, since the students can determine most of the action, in contrast to examinations, where the students have to respond to the questions raised. However, the impact of social-evaluative threat in university situations with mild performance pressure, low uncontrollability but high social-evaluative threat has not been investigated yet. Thus, the characterisation of the HPA response to oral presentations is of interest.

In light of the heterogeneity of previous findings it becomes clear that the influence of university stress on the HPA axis needs further investigation. The aim of the present study therefore was to evaluate the impact of stress on the cortisol response in university students. Therefore, two studies were conducted. In a first study, we assessed the influence of a single written examination on the cortisol stress response. Additionally, daytime cortisol concentrations were assessed on the day before the examination and the day before the control day. A second study was conducted to evaluate the influence of an oral presentation on the cortisol stress response. For the written examination, we hypothesised an anticipatory stress response with higher cortisol concentrations on the day before the examination compared to the day before the control day. We expected both the written examination and the oral presentation to elicit a significant stress response because both types of performance tests are potential stressors for students. Because of the stronger impact of social-evaluative threat in oral presentations we hypothesised that in the oral presentation study a stronger cortisol response would be observable compared to the written examination study.

Materials and methods

Written examination study

Thirty-five undergraduate psychology students (4 males: mean age = 25.0 ± 1.12 years; 31 females: mean age = 23.42 ± 1.11 years (mean ± SEM)) participated in this study. The averaged body mass index (BMI) was 20.97 ± 0.39 kg/m^2 for the females and 21.59 ± 1.92 kg/m^2 for the males. Twenty-three

female participants used oral contraceptives (OCs). No information about the stage of the menstrual cycle was collected. Four participants suffered from hypothyroidism but were under stable medicament substitution. Eight participants were smokers. Smokers reported to smoke between 0.5 and 18 (7.5 ± 2.36) cigarettes per day. Subjects gave written informed consent and were paid for participating. The study was approved by the national ethic committee of the German Psychological Association.

Oral presentation study

Thirty-seven students (28 females, 9 males) participated in the study. Three participants showed salivary cortisol concentrations above 100 nmol/l (indicative of sample contamination and/or acute disease) and therefore were excluded from the analyses. Data for 34 students (7 males, 27 females) were analysed. The mean age of the males was 25.28 ± 1.79 years. Mean age of the females was 24.96 ± 1.25 years. One female participant suffered from hypothyroidism but was under stable medicament substitution. Sixteen of the 28 female participants used OCs. The averaged BMI for males was 24.67 ± 2.02 kg/m^2 and for the females 22.06 ± 0.68 kg/m^2.

Experimental procedure

Written examination study

Subjects took part in written examinations at the end of the winter semester (February or March; $n = 20$) or at the end of the following summer semester (July; $n = 15$). All examinations started in the morning at 09:00 or 10:00 h and lasted about two and a half hours. Participants took part in a control day between 4 and 8 days after or before the examination. For practical reasons, participants could choose on which day they would participate in the control day. Nine participants took part in the control session before the examination and the remaining 26 after the examination. This session started at the same time as the examinations did (09:00 or 10:00 h, respectively) and participants solved some filler tasks in a seminar room of the university. Time of awakening was recorded on both days.

Saliva samples

In total, participants collected 10 saliva samples. Participants were told not to smoke, eat or drink anything (except water) for at least 30 min prior to each saliva collection. Two salivettes were handed out to the participants in front of the examination room. One sample was collected immediately before the students entered the examination room and the second one was given to the students with the instruction to collect saliva immediately after finishing the examination. Students wrote down the time when they collected the second saliva sample. On the control day, the saliva samples were collected at the same times as on the examination day. The first saliva sample was collected at 09:00 or 10:00 h and the second one at the same time as students collected the sample on the examination day. Students who took part in the control day before the examination were asked to collect the second saliva sample on the control day at the time when the examination would probably end. Saliva samples were kept refrigerated and delivered on the examination or control day, respectively.

Additional to the saliva samples on the control and examination day, participants were asked to collect three saliva samples on the day before the examination and control days at 10:00, 16:00 and 21:00 h, respectively. This was done to evaluate the daytime cortisol concentrations on the day before a written examination in order to detect possible early anticipatory cortisol increases. Saliva samples were kept refrigerated and were brought by the participants to the university on the examination or control day, respectively.

Saliva was collected using salivette collection devices (Sarstedt, Nuembrecht, Germany). Cortisol was measured using a commercially available immunoassay (IBL, Hamburg, Germany). Inter- and intra-assay variations were below 10%. Analyses were carried out at Professor Kirschbaum's laboratory at the Technical University of Dresden, Germany.

Oral presentation study

All participants gave an oral presentation in a university course. Saliva was collected before the beginning of the presentation and immediately after finishing it. Correct sampling was controlled by one of the authors (O. T. Wolf). Additionally, participants collected data after their presentation at the identical times while listening to a different presentation in the same university course. Saliva was collected and cortisol was analysed as for the above study. Mean (+SEM) duration of the oral presentation was 43:58 (± 2:54) min. The oral presentations started between 10:00 and 16:00 h (mean 11:54 h ± 0:20).

Statistical analysis

Differences in cortisol levels between academic assessments and control days were tested by mixed models using a repeated measures design with an unstructured error covariance matrix. Effects of interest were: average cortisol concentration differences between academic assessment and control day (main effect day), average differences between assessment times within days (main effect time) and the difference in the time course of cortisol concentration

between academic assessment and control day (interaction day × time). Analyses of written examinations were adjusted for sex, smoking status, season, OC use and order of measurement days (examination day or control day first), and time of getting up (out of bed) was included as a time-varying covariate. Analyses of oral presentations were adjusted for sex, OC use, time of day when the presentation was given and duration of the presentation. All analyses were done using SPSS Statistics v17 (SPSS, Inc., Chicago, IL, USA). We used an alpha level of 0.05 for all statistical tests.

Results

Written examination study

Cortisol concentrations on the day prior to the written examination (daytime cortisol concentrations). Participants reported waking up at 08:28 h (± 0:11 min) on the day prior to the examination day and at 8:42 h (± 0:12 min) on the day prior to the control day. The wake up times were not significantly different from each other ($t(31) = -0.775$, $p = 0.44$).

Cortisol measurements in the morning, afternoon and late evening on the day preceding the examination (or the control day) are presented in Figure 1a,b. Results revealed the expected decline of cortisol levels between 10:00 and 21:00 h (($F[2,33.9] = 159.1$; $p < 0.001$). Average cortisol concentrations were higher on the day before the examination ($F[1,31.9] = 5.8$; $p = 0.022$). Although the test of differential cortisol time-courses fell short of significance ($F[2,33.5] = 2.4$; $p = 0.106$), the differences in average cortisol concentrations seemed to be largely due to differences in the 16:00 h cortisol values (16:00 h diff = 1.4 nmol/l; $F[1,34.4] = 11.7$; $p = 0.002$; 10:00 h diff = 0.9 nmol/l, $F[1,31.9] = 1.1$; $p = 0.313$; 21:00 h diff = 0.4 nmol/l; $F[1,33.4] = 2.2$; $p = 0.147$). In contrast to the cortisol concentrations on the examination day (see below), overall cortisol concentrations were higher in participants who did the control and examination measurements during winter ($F[1,30.9] = 7.9$; $p = 0.009$), and there was a trend towards higher cortisol levels in women ($F[1,32.3] = 3.7$; $p = 0.064$). OC usage and smoking had no significant effect. Participants had higher cortisol levels when they reported getting up later on that day ($F[1,43.4] = 7.0$; $p = 0.011$). Overall cortisol levels were higher in participants who did the control measurements before the examination measurements ($F[1,31.9] = 5.8$; $p < 0.001$). Including the interaction of order with examination in the model showed a borderline significant effect ($F[1,30.7] = 4.1$; $p = 0.053$; Figure 1a,b). Mean cortisol concentrations on the day before the examination were higher than those on the control day only if the control day measurements were done after the examination (diff = 1.2 nmol/l; $F[1,36.3] = 9.1$; $p = 0.005$; Figure 1a) but not when they were done before the examination (diff = 0.3 nmol/l; $F[1,46.6] = 0.3$; $p = 0.617$; Figure 1b).

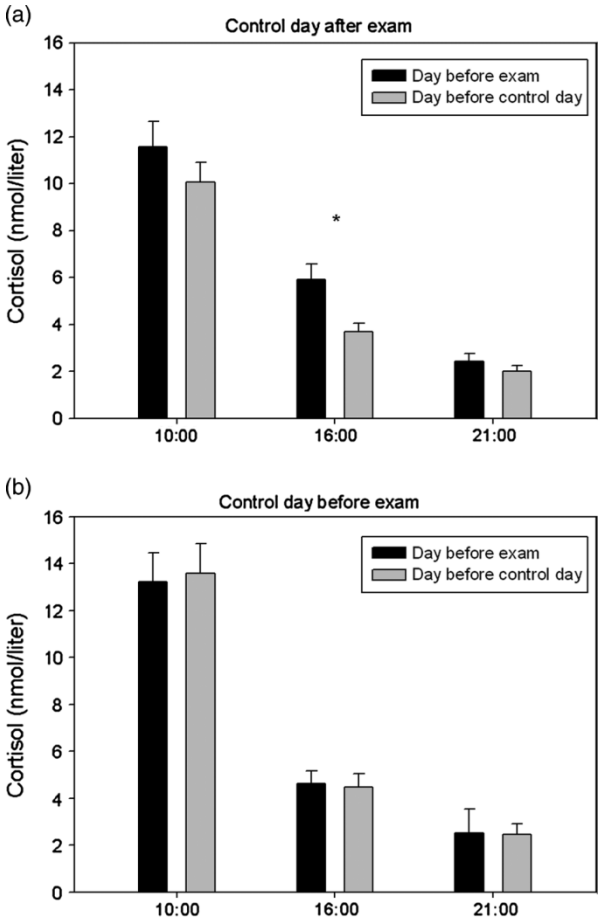

Figure 1. Salivary cortisol concentrations on the day before the written examination and the day before the control day for 10:00, 16:00 and 21:00 h for (a) participants with the control day after the examination ($n = 26$) and (b) participants with the control day before the examination ($n = 9$). Data are mean ± SEM. Mixed model analysis revealed that cortisol concentrations were significantly elevated on the day preceding the examination. Further analyses showed that this effect was due to differences at 16:00 h, but only in those participants who took part in the control day after the written examination (there was a trend towards an interaction between examination day and order). * indicates $p < 0.05$.Exam: examination.

Cortisol concentrations on the examination day. Participants reported to wake up at approx. 06:52 h (± 0:07 min) on the examination day and at approx. 07:10 h (± 0:06 min) on the control day. This difference (although relatively small) was statistically significant ($t(33) = 2.075$, $p < 0.05$).

Cortisol concentrations on the day of the examination and the control day are shown in Figure 2. Mean cortisol concentrations were slightly higher on the day of the examination ($F[1,32.4] = 5.1$; $p = 0.030$). Cortisol concentrations on average were smaller at

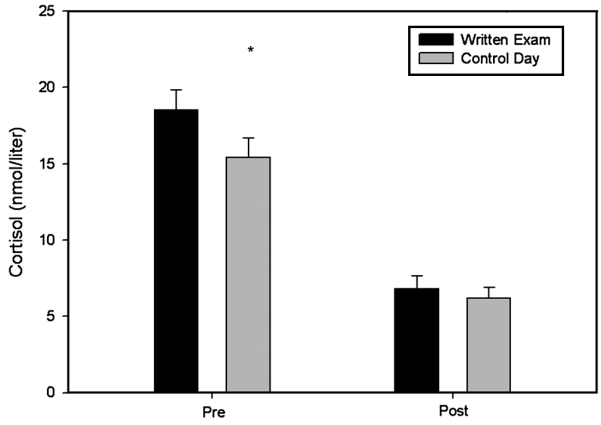

Figure 2. Salivary cortisol concentrations before and after a written examination and a control day ($n = 35$). Data are mean ± SEM. The examinations lasted on average about two and a half hours and started in the morning between 09:00 and 10:00 h. Mixed model analysis revealed that cortisol concentrations were significantly elevated on the examination day. This effect was largely driven by the pre-examination measurement. * indicates $p < 0.05$.

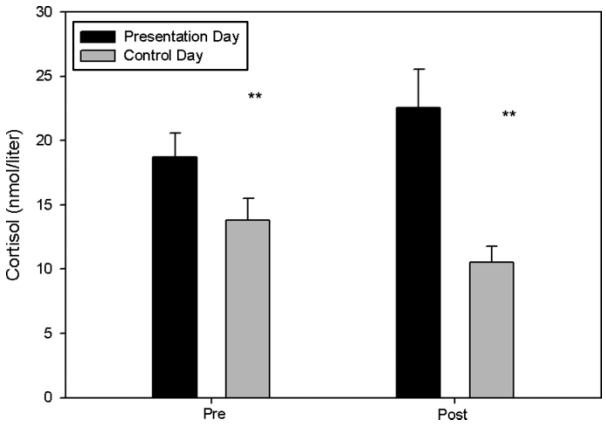

Figure 3. Salivary cortisol concentrations before and after an oral presentation and on a control day ($n = 34$). Data are mean ± SEM. Mixed model analysis revealed that cortisol concentrations were significantly elevated before (Pre) and after (Post) the oral presentation. ** indicates $p < 0.01$. In this study, all participants collected the salivary samples for the control day after the oral presentation.

the second sampling point (post examination or post control day; $F[1,33.0] = 86.7$; $p < 0.001$), and this decline tended to be more pronounced on the examination day ($F[1,33.0] = 3.6$; $p = 0.067$) due to a higher cortisol concentration before the examination (diff = 3.5 nmol/l; $F[1,34.0] = 6.1$; $p = 0.019$). Similar to the analysis of the daytime cortisol concentrations, participants tended to have higher overall cortisol concentrations when they reported getting up later on that day ($F[1,53.1] = 3.1$; $p = 0.085$) and when they did the control day before the examination day ($F[1,23.0] = 3.3$; $p = 0.084$). However, the order of the measurement day did not interact with day and time, indicating that the cortisol differences between examination and control day were similar in both conditions. In this analysis season, smoking, sex and OC usage had no significant effect.

Oral presentation study

Results for the cortisol measurements before and after the oral presentation and the control day are shown in Figure 3. The comparison of cortisol changes on these days showed opposite trends, resulting in a negligible overall time effect ($F[1,31.0] = 0.9$; $p = 0.764$) but a highly significant day × time interaction ($F[1,31.0] = 7.5$; $p = 0.010$). In addition, overall cortisol levels were clearly higher on the day of the oral presentation ($F[1,31.0] = 19.0$; $p < 0.001$). While there was already a significant difference before the oral presentation (difference pre = 5.1 nmol/l; $F[1,31.0] = 12.4$; $p = 0.001$), the difference increased until after the presentation (difference post = 12.0 nmol/l; $F[1,31.0] = 16.4$; $p < 0.001$). This was due to a significant decrease on the control day (diff pre–post = 3.1 nmol/l; $F[1,31.0] = 11.0$; $p = 0.002$) and a marked increase, with higher variability, on the day of the oral presentation (diff pre–post = −3.8 nmol/l; $F[1,31.0] = 3.1$; $p = 0.089$). There was no significant influence of the time or duration of the presentation. Men showed higher average cortisol levels ($F[1,27.0] = 4.9$; $p = 0.035$), but OC usage had no significant effect.

Discussion

The aim of the present study was to characterise the cortisol stress response to different forms of stress in university students. Therefore, two studies were conducted and the cortisol stress response to a written examination and an oral presentation in two samples of students was assessed. Additionally, we assessed the cortisol concentrations on the day before the written examination and the day before the control day to evaluate possible stress effects of anticipating the written examination.

For the written examination, the results revealed significantly higher salivary cortisol concentrations on the day preceding the examination. This effect was modulated by order, since it could only be detected in participants who participated in the control day after the written examination. With respect to the examination day itself participants showed again higher cortisol concentrations and this was most pronounced before the examination. For the oral presentation, the results revealed substantial differences before the presentation which further increased until the post presentation measurement.

The finding of a hormonal stress response to academic stressors is in line with findings of several studies investigating the influence of examinations on cortisol release (Frankenhaeuser et al. 1978;

Lovallo et al. 1986; Al-Ayadhi 2005; Lindahl et al. 2005; Schoofs et al. 2008). However, there are some studies reporting no significant influence of examinations on cortisol release (Frankenhaeuser et al. 1978; Malarkey et al. 1995; Spangler 1997) or even a decrease (Loft et al. 2007). These inhomogeneous effects might be in part due to the differences in the experimental designs. As Stowell (2003) pointed out there are some problems in comparing different studies in the field of examination stress. Studies reporting significant elevated cortisol responses to examination stress used different designs. While some studies assessed the hormonal response to one discrete examination (Spangler 1997; Schoofs et al. 2008) other studies evaluated the cortisol concentrations during longer periods of examinations (Weekes et al. 2006; Loft et al. 2007). Comparability of the studies is furthermore limited because of the fact that some studies do not report if the examinations were oral or written examinations (Lovallo et al. 1986; Malarkey et al. 1995; Al-Ayadhi 2005). While results for written examinations are inhomogeneous, oral examinations have been repeatedly found to produce a strong stress response (Herbert et al. 1986; Schoofs et al. 2008). For example, a recent study of ours evaluated the impact of an oral examination on the acute release of cortisol and found substantially (two to threefold) elevated mean cortisol concentrations on the examination day (Schoofs et al. 2008). A characteristic of oral examinations is the experience of social threat. In laboratory studies, social threat is known to be a strong factor in determining the size of the HPA response (Dickerson and Kemeny 2004) but its impact on naturalistic stressors has not yet been evaluated sufficiently. In our oral examination study, grade pressure was combined with social-evaluative threat triggered by the presence of an auditor (the professor as the main examiner present at the oral examination) and a co-auditor (a co-worker taking notes; Schoofs et al. 2008). Written examinations on the other hand also induce grade pressure, but the experience of social threat is much weaker. Students in written examinations have to show their knowledge about a special topic but the written examination is graded afterwards without the students being present. Therefore, the experience of social threat is weaker compared to an oral examination where the performance is graded and assessed directly when the students are present. In line with these arguments, the present written examination study revealed a significant but small effect of the examination on salivary cortisol concentrations.

An interesting finding was observed by evaluating cortisol concentrations 1 day before the written examination. Cortisol concentrations of the students were already elevated on the day prior to the examination, indicative of an anticipatory HPA response. A recent study in ballroom dancers (Rohleder et al. 2007) observed anticipatory HPA responses several hours before the start of the tournament. Our findings replicate and extend these observations by demonstrating elevated cortisol concentrations on the day preceding the examination. It has to be noted that an anticipatory HPA response on the day preceding the examination could only be detected in those participants in whom the control day was after the examination day. Thus, students who were approximately 1 week away from a written examination might already show elevated cortisol concentrations, which would support the idea that examination periods are characterised by elevated cortisol concentrations (Weekes et al. 2006; Loft et al. 2007). In contrast, students who have completed an examination about 1 week previously appear to show already a normal (lower) HPA activity when measured during the course of the day. This finding emphasises the need to pay close attention to the issue of order as well as temporal distance between the examination and control condition in future studies on this issue (Stowell 2003).

An interesting side finding was that cortisol concentrations were overall higher when the sampling took place during the winter months. This is in line with other studies suggesting that HPA activity is stronger in winter months (Walker et al. 1997; King et al. 2000) and indicates that seasonal factors are able to influence HPA activity in humans.

Another important modulating variable was wake up time. Participants who reported waking up later displayed higher cortisol concentrations in both parts of the written examination study. This highlights the need to pay close attention to inter- as well as intra-individual alterations in wake up time when conducting studies investigating HPA activity/reactivity.

The second study was conducted in order to evaluate another form of university stress in students. Students had to perform oral presentations in university courses in front of the class. Results of the oral presentation study showed that the oral presentations elicited a strong cortisol stress response, with elevated salivary cortisol concentrations before and after the oral presentation. The cortisol elevations were much more pronounced when compared to the written examination. However, compared to our previous oral examination study (Schoofs et al. 2008) the effects were smaller. While a direct comparison of these different studies is problematic, the three studies discussed were highly similar with respect to sample size, gender distribution and OC use.

It seems that the social-evaluative threat in oral presentations makes these situations more stressful (with respect to HPA activation) than written examinations. However, oral presentations seem to be less stressful than oral examinations, maybe because students experience an oral presentation

as less uncontrollable than an oral examination. It is known from laboratory research that social-evaluative threat in combination with uncontrollability is highly potent stressors (Dickerson and Kemeny 2004). From the present results, this also seems to be the case for a real-life situation. Nevertheless, it has to be mentioned that of course social-evaluative threat is not the only factor that differs between oral presentations, oral examinations and written examinations. Other factors may be novelty, preparedness or predictability (Mason 1968).

We found some evidence for sex differences in the two current studies, but the effects were not consistent. Moreover, they reflected higher overall cortisol concentrations in women (examination study) or men (oral presentation study) rather than differences in cortisol reactivity. Because of the small number of male participants in the current studies, these findings should be interpreted with caution.

The present results revealed no influence of OC use in the written examination study and the oral presentation study. In laboratory stress studies, the use of OCs is known to alter the cortisol stress response in women, which might be in part mediated by an increased production of the cortisol binding globulin. Women using OCs show a blunted free salivary stress response to psychosocial laboratory stressors like the Trier Social Stress Test (Kirschbaum et al. 1999). Our recent oral examination study found no influence of OCs on the cortisol stress response (Schoofs et al. 2008). Similarly in the present studies no impact of OC use was detected. It appears that the inability of women using OCs to mount a free cortisol response is restricted to moderate and surprising stressful events induced in the laboratory, which most likely reflect a single brief HPA activation. In contrast, the anticipatory HPA response to social-evaluative threat appears to start hours (Rohleder et al. 2007) or days (see daytime cortisol concentrations of the written examination study) ahead of the event and might thus allow the organism to assure a robust free cortisol increase via a feedback based repeated activation of the axis.

There are several limitations of our two studies. We investigated the effects of a written examination and an oral presentation in two different samples of students. This limits the comparability between the two situations. Ideally, we would have tested the same students in those two different real-life stressors. In the context of this study such an approach was not feasible due to organisational issues, since most students we had access to had to take part in a written examination or an oral presentation. However, at least at a descriptive level (sample size, male female ratio, percentages of women using OCs) the two study groups were quite similar.

The sample size was modest ($n = 35$ for the written examination study and $n = 34$ for the oral presentation study) and was restricted to psychology students. Although we conducted analysis for the influence of OCs, it was not feasible to control for the phase of the menstrual cycle. Additionally, only four males participated in the written examination study and seven males in the oral presentation study, but this mirrors the fact that many more females study psychology in Germany, so male students are harder to find. Thus, the potential influence of sex (or gender) could not be addressed with an adequate power in this study.

The HPA response to academic stressors might be influenced by anxiety and preparedness. It thus would have been desirable to measure these constructs with the current study.

Another aspect is the fact that we did not have any information about the performance of the participants in the written examination. Although it would be interesting to assess possible relationships between academic performance and the cortisol response, we did not collect information about performance for reasons of privacy.

For the assessment of daytime cortisol concentrations, participants were instructed to collect saliva during the day on their own, without any control of compliance. An ambulatory compliance assessment as described by Broderick et al. (2004) would have been desirable, but was not feasible in the present study. At least the correct sampling of saliva before and after the examination as well as before and after the oral presentation and the respective control days was controlled by the investigators.

Another limitation is that we did not assign participants randomly to the control condition before or after the written examination. While such an approach would have been desirable from a methodological point of view this was not feasible in the framework of the current study since participation was expected to have decreased further. This is an example of the sort of compromise that has to be made for a field study. A possible anticipatory stress effect might have influenced the cortisol concentrations on the control day, when it took place before the examination, which is exactly what we observed. Daytime cortisol concentrations were only elevated on the day preceding the examination in the participants who had their control day after the examination day. An alternative explanation could be that participants with a better preparation for the examination or with lower anxiety chose the control day before the examination, while participants with higher anxiety and a worse stress management chose the day after the examination. Since we did not randomise the order of examination and control days, we cannot decide between these different explanations for our empirical findings.

We did not control for additional examinations around the time of the assessed written examination

or the control day. An examination period could be associated with chronic stress leading to altered HPA activity which might influence the stress response to an acute stressor (Loft et al. 2007). Additionally, chronic examination stress might influence cortisol concentrations on the control day as well (Weekes et al. 2006), which in our study was about 1 week apart from the examination itself. Thus, in future studies an additional assessment of baseline cortisol concentrations several weeks before the examination period would be desirable. In addition, measurements of chronic stress should be included.

Cortisol secretion is known to show a strong circadian rhythm and HPA reactivity might differ between the morning and the afternoon (Dickerson and Kemeny 2004), but see Kudielka and Kirschbaum (2005). The written examinations always took place in the morning, while the oral presentations took part in the morning, at noon or in the afternoon. Even though we found no evidence that the response to the oral presentation was modulated through time of day, we can nevertheless not exclude the possibility that the more pronounced response to the oral presentations is in part mediated by the fact that they differed in their starting time from the written examinations.

An additional factor that can influence HPA reactivity is the individual's chronotype. Morning types show higher cortisol morning levels than evening types (Bailey and Heitkemper 1991, 2001; Kudielka et al. 2006, 2007; Griefahn and Robens 2008). Moreover, an examination in the morning may be more stressful for evening types than for morning types because the conditions are less optimal for the evening type (May and Hasher 1998). Therefore, future studies on this topic might collect information about the chronotype of their participants.

In sum, the present studies report an anticipatory cortisol stress response to a written examination. Salivary cortisol concentrations on the day before the examination were elevated, indicating an anticipatory HPA response. Similarly, cortisol concentrations were elevated immediately prior to the examination. Additionally, in a second sample of students a cortisol stress response was observed to giving an oral presentation. Cortisol concentrations were elevated before and after the presentation and the effects were larger than those observed in response to the written examination. When compared to our previous oral examination study (Schoofs et al. 2008) the current data appear to indicate that written examinations are weaker stressors than oral examinations at least for their impact on the HPA axis. Oral presentations by contrast seem to have less impact on the HPA axis than oral examinations but more impact than written examinations. A reason for this might be that oral examinations and oral presentations contain social-evaluative threat but that written examinations lack this factor. This interpretation of the current findings would support the notion that social-evaluative threat is a major determinant of the acute HPA response to challenge. However, this hypothesis needs to be substantiated with additional studies comparing cortisol responses to different real-life stressors, ideally within the same participants.

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