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SHORT COMMUNICATION

Examination of cortisol and state anxiety at an academic setting with and without oral presentation

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Abstract

Holding oral presentations in a university course is perceived as stressful and can increase stress hormone concentrations and state anxiety. In such a naturalistic setting, further attention should be paid to the relationship between psychological and hormonal measures of acute stress, as well as women's intake of hormonal contraceptives as a potential moderating variable. In the present study, 76 healthy students gave saliva samples before and after their oral presentations in a university course as well as on a second, control day in the same course without giving an oral presentation. Anticipatory state anxiety was rated on both days. Cortisol concentrations as well as state anxiety were substantially higher on the presentation relative to the control day. During the oral presentation, an increase in cortisol concentrations was observed, whereas a decrease occurred on the control day. Nearly the same picture emerged for both variables when looking at men, women taking hormonal contraceptives and free-cycling women separately. A positive correlation was found between the change in anticipatory state anxiety in the presentation compared to the control day and cortisol concentrations before and after the oral presentation. Concluding, oral presentations constitute a potent stressor and do not seem to be substantially different between men, free-cycling women and women taking hormonal contraceptives. Future studies may want to explore changes associated with specific menstrual cycle phases and with specific hormonal contraceptives.

Keywords

Glucocorticoids, hormonal contraceptives, naturalistic setting, real-life stress, sex differences, stress hormones

History

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Introduction

Students awaiting the beginning of their oral presentation in an academic course show typical signs of a stress response – but can scientific studies support this anecdotal evidence? Generally, acute stress activates the sympathetic nervous system (SNS) and provokes the release of (nor)epinephrine, leading to an acceleration of the heart rate, constriction of blood vessels and perspiration within a few seconds. A slower stress response can be observed as initiated by the hypothalamus–pituitary–adrenocortical (HPA)-axis. A hormonal cascade eventually drives the release of glucocorticoids (mainly cortisol in humans) from the adrenal cortex with a delayed peak of 20–30 min after stress onset (Dickerson & Kemeny, 2004).

Activation of the HPA-axis is elicited by real-life situations such as, e.g. competitive ballroom dancing (Rohleder et al., 2007) or parachute jumping (Deinzer et al., 1997). Apart from these rather rare situations in everyday life, academic performance situations at school or at university are frequently experienced and involve anticipatory social evaluation, a critical component of a stressor (Dickerson & Kemeny, 2004). Elevated cortisol concentrations and anxiety measures have been observed in written (Lovallo et al., 1986; Preuss et al., 2010) as well as in oral examinations (Herbert et al., 1986; Lacey et al., 2000; Preuss et al., 2010; Schoofs et al., 2008). Here, sex differences have been shown to emerge, with men exhibiting higher cortisol increases compared to women in written, but not in oral examinations (Frankenhaeuser et al., 1978; Khaksari et al., 2005; Spangler, 1997; Weekes et al., 2006). Furthermore, no relationship between psychological measures (e.g. trait and state anxiety) and stress hormones has been detected in these examination studies, in contrast to other field studies (Filaire et al., 2009; Rohleder et al., 2007). In the laboratory, inconsistent results have also been observed, including experiments which did not find any associations at all (Bohnen et al., 1991; Childs et al., 2014; Oswald et al., 2006; Schommer et al., 1999; van Eck et al., 1996; cf. Prüssner et al., 1997).

Menstrual cycle and the intake of hormonal contraceptives have been observed to influence stress responses in laboratory studies, in particular affecting free cortisol concentrations (Kirschbaum et al., 1999). Thus, a more careful discrimination between free-cycling women and women taking hormonal contraceptives is indicated in real-life settings. It is also important to conduct larger field studies on this topic in order to be able to compare the findings to laboratory studies (cf. Dickerson & Kemeny, 2004). Oral examinations

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differ from typical laboratory stressors in several important domains (familiarity, importance for the future career). Thus, an extrapolation from laboratory studies to field studies has to be made with caution.

In the present study, we included a relatively large sample size of 76 students and also obtained information about the usage of hormonal contraceptives (free-cycling women compared to women taking contraceptives). All participants rated their state anxiety at baseline and provided saliva samples for cortisol analyses before and after their oral presentations as well as on a control day in the same course while listening to their classmates' presentation. Previous findings indicate a contribution of state anxiety to the neuroendocrine stress response obtained in men under laboratory conditions (Jezova et al., 2013). Our approach extends these results in investigating men and women under high anticipatory state anxiety (before holding an oral presentation) compared to low state anxiety on a control day in the same academic setting. We hypothesize that cortisol concentrations and state anxiety should be higher on the presentation compared to the control day. We assume a positive association between anticipatory state anxiety and cortisol. Based on previous laboratory studies, we expect women using hormonal contraceptives to display a blunted cortisol stress response.

Methods

Participants and procedure

Master students of psychology at the Ruhr-University Bochum participating in the seminar ''Stress and memory'' were asked whether they would like to voluntarily participate in a real-life stress study. In total, 85 students volunteered to participate after the purpose and procedure had been described to them; they provided written informed consent prior to the study. Fifteen participants were smokers; five students reported suffering from hypothyroidism but were under stable medical substitution and 71 participants reported being free from acute or chronic disease. Nine students had to be excluded because of the intake of medication such as insulin, beta blockers or antidepressants. Thus, the final sample consisted of 76 students comprising 12 men, 39 women using hormonal contraception (HC), and 25 free-cycling women (FC). The mean age was 25.8 years (SEM = 0.6) and the averaged body-mass-index was 22.2 kg/m^2 (SEM = 0.3).

This study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

Participants rated their state anxiety before the beginning of their oral presentation, which was performed in front of their classmates and the lecturer in groups of two to three presenters. State anxiety was also assessed on a control day when students sat in the same university course and were listening to their classmates' presentation (usually a week later). Besides state anxiety, students collected saliva before and after the oral presentation while sitting ($M = 71$ min ($SD = 21$ min) between both points of time) as well as at comparable points of time on the control day. During oral presentations, students were standing and alternatingly presenting parts of their respective topic (concerning selected empirical research manuscripts on stress and memory) concluding with a joint discussion with the audience. Depending on group size, the individual talks lasted approximately 25–35 min. Data were consecutively collected over five years; the seminar was always held in the winter semester between 12 a.m. and 2 p.m. or between 2 p.m. and 4 p.m.

State anxiety

The German version of the State-Trait-Anxiety Inventory (STAI; Spielberger et al., 1970) was used to assess anticipatory state anxiety before the presentation as well as at the identical point of time on the control day. This questionnaire consists of 20 items; the mean of these items for both days was used as the dependent variable.

Salivary cortisol

The physiological stress response was measured using salivary cortisol as an index of activity of the HPA-axis. We instructed all participants to refrain from smoking, eating and drinking anything but water for at least 30 min before the beginning of the seminar. After arrival, participants filled out a questionnaire on demographic variables and gave written informed consent. Then, the first saliva sample was taken using Salivette collection devices (white-capped Salivettes; Sarstedt, Nümbrecht, Germany). After the oral presentation ($M = 71$ min later), a second saliva sample was obtained. The same procedure was applied on the presentation day as well as on the control day.

All of the four saliva samples were stored at $-20\,^{\circ}$C until assayed. Saliva samples were analyzed at the end of the respective university course each year by the same laboratory using the same assay. Free cortisol concentrations were analyzed without prior extraction using a commercial Chemoluminescence Immunoassay (CLIA; IBL International, Hamburg, Germany) according to the manufacturer instructions. Intra-assay and inter-assay coefficients of variations were below 10%.

Statistical analyses

We conducted analyses of variance (ANOVA) separately for state anxiety and cortisol including the repeated measurement factors day (presentation day versus control day) and time (first versus second sample; only for cortisol) as well as the between subjects factor sex/contraceptive usage (men versus HC women versus FC women). Bivariate correlation analyses (Pearson's r) were calculated to examine the potential association between state anxiety and cortisol concentrations. All analyses were also carried out separately for men, HC and FC women in order to take a closer look at the moderating impact of sex differences/contraceptive usage. Effect sizes (d) for differences in state anxiety and cortisol concentrations between the presentation and the control day were calculated according to Becker (1988) and in line with a meta-analysis comprising studies using laboratory stressors (Dickerson & Kemeny, 2004).

Statistical analyses were performed in IBM SPSS Statistics for Windows 22.0 (IBM Corp., Armonk, NY) and Greenhouse–Geisser correction was applied when needed. The statistical significance level was set to $\alpha = 0.05$. Since neither state anxiety nor cortisol concentrations were

normally distributed, data were transformed with the natural logarithm in order to attain the Gaussian distribution needed for the applied statistical tests. All figures depict raw instead of transformed data for illustration purposes.

Results

State anxiety

As expected, mean state anxiety was higher before the oral presentation compared to the same point of time on the control day (main effect day: $F_{(1,71)} = 117.87$; $p < 0.001$; $d = 1.73$). The main effect of sex/contraceptive usage ($F_{(2,71)} = 5.64$; $p = 0.005$) revealed that, in general, men reported less anticipatory state anxiety compared to HC women ($F_{(1,48)} = 9.50$; $p = 0.003$) and to FC women ($F_{(1,33)} = 10.58$; $p = 0.003$), whereas the two female groups did not differ from each other ($F_{(1,61)} = 0.001$; $p = 0.975$; cf. Figure 1). No interaction between sex/contraceptive usage and day occurred ($F_{(2,71)} = 0.70$; $p = 0.501$).

Separate analyses showed that state anxiety was increased on the presentation day compared to the control day in men ($t_{(10)} = 8.12$; $p < 0.001$; $d = 1.97$), HC women ($t_{(38)} = 8.19$; $p < 0.001$; $d = 1.64$) and FC women ($t_{(23)} = 5.93$; $p < 0.001$; $d = 1.86$).

Cortisol concentrations

Cortisol concentrations were higher on the presentation day compared to the control day (main effect day: $F_{(1,74)} = 103.83$; $p < 0.001$; $d = 1.42$) and declined from the pre- to the post-sample (main effect time: $F_{(1,74)} = 5.51$; $p = 0.022$). Additionally, a significant interaction between time and day was found ($F_{(1,74)} = 40.79$; $p < 0.001$), which was due to a slight increase of cortisol concentrations on the presentation day ($F_{(1,74)} = 4.12$; $p = 0.046$), but a pronounced decrease on the control day ($F_{(1,74)} = 54.81$; $p < 0.001$; cf. Figure 2). The factor sex/contraceptive usage did not reveal any significant main ($F_{(2,74)} = 1.56$; $p = 0.216$) or interaction effects (day × sex/contraceptive usage: $F_{(2,74)} = 1.97$; $p = 0.147$; time × sex/contraceptive usage: $F_{(2,74)} = 0.68$; $p = 0.508$; day × time × sex/contraceptive usage: $F_{(2,74)} = 1.09$; $p = 0.342$).

Separate analyses of the three sex/contraceptive usage groups showed that the overall pattern of results was also evident in men (main effect day: $F_{(1,11)} = 29.86$; $p < 0.001$; $d = 1.59$; time × day interaction: $F_{(1,11)} = 8.84$; $p = 0.013$), HC women (main effect day: $F_{(1,39)} = 40.29$; $p < 0.001$; $d = 1.00$; time x day interaction: $F_{(1,39)} = 27.42$; $p < 0.001$) and FC women (main effect day: $F_{(1,24)} = 45.69$; $p < 0.001$; $d = 1.17$; time × day interaction: $F_{(1,24)} = 8.81$; $p = 0.007$).

Furthermore, we also tested whether group differences concerning sex/contraceptive usage might occur when looking at the presentation day and the control day separately. During the control day, no effect of sex/contraceptive usage was observed ($F_{(2,74)} = 0.25$; $p = 0.777$), whereas a trend was found during the presentation day (main effect of sex/contraceptive usage: $F_{(2,74)} = 2.68$; $p = 0.076$): men exhibited overall higher cortisol levels compared to HC women on the presentation day ($F_{(1,50)} = 5.50$; $p = 0.023$), whereas no differences emerged between FC women and men ($F_{(1,35)} = 1.06$; $p = 0.310$) or HC women ($F_{(1,63)} = 1.74$; $p = 0.192$).

Relationship between state anxiety and cortisol concentrations

We were also interested in the question of how the relative change in mean state anxiety from the control compared to the presentation day is related to elevated cortisol concentrations. Therefore, we correlated the difference between mean state anxiety on the presentation day and the control day with cortisol concentrations before and after the oral presentation. Indeed, correlation analyses revealed that the higher the difference in state anxiety levels, the larger the cortisol

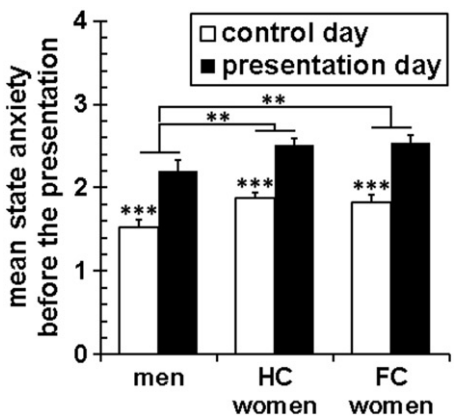

Figure 1. Mean (±standard errors of the mean) anticipatory state anxiety is presented for the control day and the presentation day for men, women taking hormonal contraceptives (HC) and free-cycling women (FC) separately. State anxiety was substantially higher on the presentation compared to the control day (***$p < 0.001$; presentation versus control day) and men reported overall lower state anxiety compared to HC and FC women (**$p < 0.01$; men versus HC women; men versus FC women).

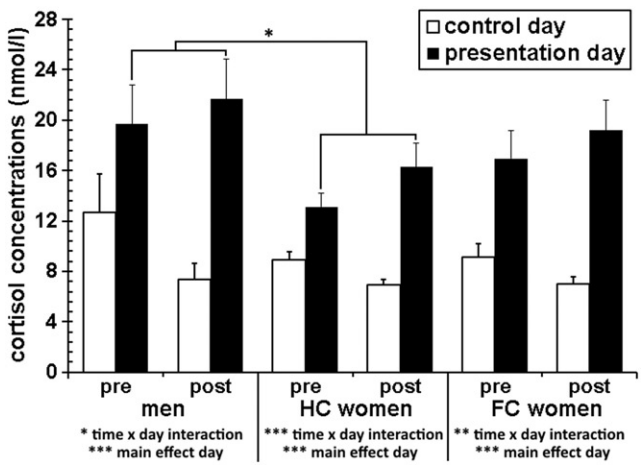

Figure 2. Cortisol concentrations before (pre) and after (post) the oral presentation are depicted as means ± standard errors of the mean. The bar chart is subdivided into the presentation day and the control day for men, women taking hormonal contraceptives (HC) and free-cycling women (FC) separately. Cortisol concentrations were higher on the presentation compared to the control day indicating that oral presentations constitute a potent acute stressor in the university context in all three groups (***$p < 0.001$; **$p < 0.01$). Differences between the three groups only occurred on the presentation day: Men showed overall higher cortisol levels compared to HC women (*$p < 0.05$).

concentrations before ($r = 0.33$; $p = 0.004$) as well as after the oral presentation ($r = 0.30$; $p = 0.009$).

Examining the three sex/contraceptive usage groups separately revealed significant correlations in HC women only before the presentation ($r = 0.38$; $p = 0.017$; after: $r = 0.26$; $p = 0.117$). In FC women, trends for associations between the change in state anxiety and cortisol concentrations could be observed before ($r = 0.35$; $p = 0.091$) as well as after the presentation ($r = 0.37$; $p = 0.076$), whereas no correlation could be detected in men (before: $r = -0.05$; $p = 0.896$; after: $r = 0.16$; $p = 0.640$).

Discussion

In this naturalistic study, we showed that holding oral presentations in the academic setting provokes significant differences in anticipatory state anxiety levels and cortisol concentrations. Our results are in line with prior research ascribing academic performance situations the status of a potent stressor (Herbert et al., 1986; Lacey et al., 2000; Lovallo et al., 1986; Preuss et al., 2010; Schoofs et al., 2008). Cortisol concentrations were increased on the presentation compared to the control day as indicated by an effect size of $d = 1.42$, comparable to our previous report in students undergoing an oral examination in a sample half as large as the present one (Schoofs et al., 2008). In contrast, laboratory stressors such as the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) do not elicit such strong cortisol releases (Dickerson & Kemeny, 2004), even though the TSST combines several facets of a stressor such as novelty, unpredictability, uncontrollability and social evaluation threat. A plausible reason for these differences to real-life situations might be the lower relevance for the participant's own future and the surprise character of the laboratory stressor (cf. Schoofs et al., 2008). Effect sizes in cortisol responses to the TSST are calculated from the peak concentration after the stressor relative to the baseline value (e.g. Dickerson & Kemeny, 2004) when participants do not know yet what to expect. In contrast, the present study included a separate control day to create more valid baseline cortisol concentrations. We assume saliva samples on the same day prior to the oral presentation to lead to a biased baseline in the naturalistic setting of a university course, because students can more easily and longer anticipate the upcoming stressful situation of their oral presentation compared to the TSST. Together with the pressure to get good grades, this circumstance might account for larger effect sizes in the cortisol response in comparison to laboratory conditions. In fact, the observed effect sizes presumably reflect that the anticipatory release of cortisol is the most critical factor in a situation with a potentially feared social evaluation. The oral presentation itself does not seem to have an effect additionally to the anticipatory one concerning cortisol levels.

The very large effect size of $d = 1.73$ for anticipatory state anxiety corroborates this view. In effect, the rise in anticipatory state anxiety from the control to the presentation day was associated with heightened cortisol concentrations before and after the oral presentation. Correlational evidence for a connection between subjective and HPA variables is scarce, which might in part be secondary to the time lag of the onset of arousal, and the delayed cortisol peak must be considered (Schlotz et al., 2008). Furthermore, the present study included an announced, familiar stressor for the students. The expected social evaluation led to heightened anticipatory anxiety and cortisol concentrations, and, in combination with the large sample size, possibly also gave rise to enough variance in order for correlations to occur.

Regarding sex differences and the impact of hormonal contraceptives, we found that both female groups generally reported more anxiety than men, confirming previous reports (McLean & Anderson, 2009). However, the present data indicate that the effect sizes of the cortisol response in HC women ($d = 1.00$) were lower compared to men ($d = 1.59$; FC women: $d = 1.17$), a finding which was especially evident when looking at the stressful presentation day. Here, HC women displayed significantly lower free cortisol concentrations than men, which is in line with prior research in the laboratory (Kirschbaum et al., 1999). In contrast, cortisol responses did not vary significantly between men, free-cycling women and women taking oral contraceptives during an oral examination (although descriptively in the same direction; Schoofs et al., 2008). Among others, it was argued that differences in free cortisol concentrations between hormone status groups might be restricted to laboratory stressors, because of their moderately stressful and surprising character. In the present study with almost double the sample size, we found significant differences between men and HC women, and thus, the discrepancy to the study by Schoofs and colleagues (2008) could be due to a lack of power in combination with a small effect size.

Prior research indicates that a combination of stressful somatic and mental stimuli in front of classmates induces cortisol increases in men during the afternoon, but not in the morning and not in women (Jezova et al., 2002). This result demonstrates that time of day and sex can impact cortisol responses to stress in real-life settings. Equally, laboratory studies conclude that the cortisol stress response is larger in the afternoon compared to the morning (cf. Dickerson & Kemeny, 2004). The present study involved seminar sessions starting after 12 p.m. and revealed large effect sizes; an extension to morning sessions would be desirable.

Comparable to prior field studies with oral examinations (Preuss et al., 2010; Schoofs et al., 2008), the limitations of this study comprise the inclusion of psychology students only and the inclusion of only two saliva samples per day. Furthermore, we did not assess trait anxiety, which has been linked to stress responsivity in both men and women (Duncko et al., 2006; Hlavacova et al., 2008; Jezova et al., 2004). Future studies should include state as well as trait measures. Another critical issue refers to the female subgroups as defined by contraceptive usage: we did not assess which exact hormonal contraceptive was used, and thus, a distinction between mono-, bi- or triphasic preparations was not possible in the HC women group. Furthermore, even though menstrual cycle phase-associated changes have been reported previously (Kirschbaum et al., 1999), we did not collect data on the exact stage of the menstrual cycle in FC women, so these results should be interpreted with caution. Our design, by setting the control day a week after the presentation day, has the advantage of controlling situational factors such as persons

present and time of day, but also has the disadvantage that the hormonal milieu in FC women might change. Future studies should realize within-subjects designs and measure circulating sex hormone concentrations. A major strength lies in the overall large sample size (with the accompanying power to detect group differences) and the distinction between groups of differing sex/contraceptive usage, even though the subsample of men consisted of 12 men only (explaining the lack of correlations in the male group).

Conclusions

In this study in the academic setting, we provide evidence for oral presentations evoking acute stress as revealed in elevated state anxiety and cortisol concentrations. In this naturalistic situation, state anxiety and stress hormones were positively related. No differences could be detected in cortisol release between men, free-cycling women and women taking hormonal contraceptives except for larger cortisol concentrations in men compared to HC women during the oral presentation. Future studies should incorporate direct measures of circulating sex hormones to further disentangle the underlying neurobiological mechanisms.

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Declaration of interest

Both the authors declare no conflict of interest.

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