Cortisol titre increases with novelty of academic oral examinations

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Abstract

OBJECTIVE: In the present study we evaluated whether intensity and novelty of oral academic examinations are reflected in saliva cortisol titre.

DESIGN AND SETTING: 91 pupils completed questionnaires on sensation seeking according to Zuckerman as well as on stress coping strategies and rated their individual stress intensity upon minor oral examinations. 26 of these students donated saliva samples before as well as ten and 30 minutes after the examination to quantify cortisol using an immuno assay. Oral examinations during the school year, where students may re-adjust their grade on subsequent examinations, where regarded as minor oral examinations, whereas school exit examinations, where students do not have the chance to correct their grade anymore and may even risk a delay of several months in their academic career when they fail, were regarded as major examinations.

RESULTS: Minor oral examinations revealed either a moderate, but significant, increase in saliva cortisol titre by 1.1 fold or did not have a significant impact on cortisol titre. In contrast, school exit exams elevated cortisol titre 5.2 fold. In minor oral examinations, moderate correlations between cortisol titre and subsets in sensation seeking and coping strategies, respectively, were identified.

CONCLUSION: Intensity and novelty of an academic examination is significantly correlated to cortisol titre. Minor oral examinations, with little consequences on academic career, only have a minor impact on cortisol titre, whereas school exit examinations, which are novel to students and may have major consequences on the academic career, elicit a major increase in cortisol titre.

Introduction

The intensity score of oral examinations in school ranges from moderate, like interviews about the topic of the last lesson, to intense, like school exit examinations (A-level, "Matura"). These differences in the intensity of examinations may also be reflected in the titre of stress hormones, like cortisol. Tightly controlled studies of examinations – like challenges under laboratory conditions, like the Trier Social Stress Test (TSST), have shown that holding a presentation freely and solving mental arithmetic tasks in front of an audience elicits a robust activation of hypothalamic-pituitary-adrenal axis [2,8,10,11,12,13,21].

Despite these tightly controlled laboratory conditions, few studies have evaluated the neuroendocrine response to examinations in a real life context yet, like examinations at school, university, or driving schools to obtain a driving licence [1,16,25]. Using changes in cortisol titre as an indicator of stress intensity, most of the studies reported an increase in cortisol titre [1,14,16,17]. However, some of these studies reported on individuals, who don not show an increase but even show a decline in cortisol titre [4,16,25].

The reason for these inconsistencies in the magnitude of changes in cortisol titre may be habituation to a stressor [3,21], personality traits [18], or gender and personality specific appraisal of the identical challenging situation [18,23]. However, most studies failed to find a significant correlation between personality trait and cortisol response [7,18,20,24]. Only sensation seeking seems to correlate with the cortisol titre [[16,18].

The goals of the present study were (1) to quantify saliva cortisol, as an index of hypothalamo-pituitaryadrenal axis activity during familiar examinations throughout the school year and during an unfamiliar mandatory exit examination, (2) to evaluate the stability of the individual cortisol response profile upon examinations, (3) to estimate individual coping strategies, sensation seeking and self-reported stress levels, and (4) to correlate the personality states and traits, respectively, with saliva cortisol titres.

Material and methods

Participants

91 adolescent students (44 females and 47 males; aged 15 to 19 years; Caucasian ethnicity) from a local high school (grammar school, "Realgymnasium") in Salzburg, Austria, completed questionnaires on sensation seeking according to Zuckerman (SSS-V), on coping strategies (SVF-120) according to Janke (Janke et al., 1997), and rated their perceived stress level after minor oral examinations by labelling a number between zero ("not stressed at all") to four ("absolutely stressed"). SVF-120 identifies five factors: cognitive strategies (POS1), distraction (POS2), problem focus (POS3), avoidance (VERM), and seeking social support (BESOZU). 26 students donated saliva for cortisol quantification. Participation was voluntary and no payment was offered to the students.

Oral examinations

Oral academic examinations at school were used as stressors. These examinations differed in their novelty, amount of workload to prepare, and consequences. Minor oral academic examinations during the school year in front of their peers belong to daily hazels of pupils at school. These examinations are not announced and have only minor consequences on their grade as they still have the opportunity to repeat these examinations. In the present study, we used minor oral examinations in biology, physics, and Latin, taught by different teachers, as minor stressors. Oral examinations took between five and ten minutes. In contrast, oral school exit examinations (A-level, "Matura") are major examinations in front of a board of familiar and unfamiliar teachers. The students have to prepare an enormous amount of workload. Furthermore, students have no chance to correct their grade following the examination. When they fail the school exit examination, they have to wait several months to repeat it.

Saliva collection

Prior to the study, students were instructed how to use sterile cotton salivettes (Sarstedt, Wiener-Neudorf, Austria). Saliva sampling took place under the supervision of one of the authors (BH) in the familiar environment of the students' classroom. For saliva collection, each student was given coded salivette tubes. Three saliva samples were taken from each student upon each examination. The first sample was collected from all participants in the break before the lesson started, as it was unknown who would be examined. Ten and approximately thirty minutes after the examinations, samples from examinees were collected. Major school exit examinations consist of up to five oral interviews, lasting up to 10 minutes each. We collected saliva samples before and approximately 10 minutes after the first and at the end of the entire examination. All samples were stored on ice and processed within two hours. Saliva was extracted from Salivettes by centrifugation at 4 °C at 3.500 rpm for 15 minutes. Supernatants were aliquoted into Eppendorf tubes and stored at -20 °C until quantification of cortisol. Saliva was collected at minor oral examinations during the first semester of the school year, and at school exit examinations at the end of the school year.

Quantification of cortisol

Saliva cortisol was quantified using a luminescence immunoassay (LIA) from IBL (Hamburg, Germany) according to the recommendations of the supplier.

Statistical analysis

The data were analyzed using non-parametric statistical methods. We used U-test according to Mann and Whitney to examine gender and group differences in cortisol titres, sensation seeking behaviours, coping strategies and stress level intensities. To examine associations between hormone concentrations and sensation seeking behaviours, coping strategies or individual stress perceptions, we used Spearman correlation. We used the Statistical Package for the Social Science (SPSS, 13th edition) for statistical computations.

Results

Three distinct responses in saliva cortisol titre were identified upon oral examination

We quantified and compared the individual saliva cortisol titre before, ten, and 30 minutes after the examination. The cortisol responses resulting from the examination are documented in Figure 1. After minor oral examinations, modest changes in cortisol titre were detectable. We detected three distinct cortisol profiles upon the examinations (Fig. 2). In the type 1 profile, examination evoked an increase in cortisol titre at ten (1.1 fold) and 30 minutes post examination (N = 13). Type 2 showed a decline at ten but an increase at 30 minutes following the examination (N = 5). Type 3 (N = 8) revealed a continuous reduction in saliva cortisol titre. Since among the three cortisol profiles only type 1 was statistically significant (p < 0.05), we concluded that type 2 and type 3 were non-responsive as they showed no significant change in salivary cortisol titre.

Once the individual cortisol response profile of a participant has been documented, we evaluated whether this profile is stable on three independent examinations in different subjects or not. Only one out of eight students responded consistently with the same cortisol profile (type 1) to examinations by various teachers.

The paradigm between minor and major oral examinations was different. At minor examinations, the students did not know in advance that they are about to be examined, whereas at school exit examinations, the students were aware of the examinations. The magnitude of cortisol titre before these oral examinations was significantly different. Before minor oral examinations, the media of the cortisol titre was about 2.20 ng/ml, but before the school exit examinations the media of the cortisol titre was 11.37 ng/ml (p < 0.001) (Fig. 3). Furthermore, the cortisol titre after school exit examinations was significantly higher than after minor examinations. Compared to cortisol titre on days without an examination, school exit examinations increased saliva cortisol titre 5.2 fold (N = 12). After these school exit examinations, the cortisol titre continuously declined (Fig. 3).

Sensation seeking and saliva cortisol titre

Because personality traits, like sensation seeking or coping strategies may have an impact on the magnitude of hypothalamic-pituitary-adrenal-axis activation, we evaluated these traits using Zuckerman's sensation seeking scale and the SVF (120) according to Janke and coworkers [6]. Among the four subscales of the sensation seeking scale, thrill and adventure seeking, disinhibition, experience seeking, and boredom susceptibility, male students scored significantly higher in "disinhibition" (p < 0.05), and female students in "experience seeking" (p < 0.05) (Fig. 4). We could not identify a difference in total sensation seeking scale between female and male students.

Cortisol titre significantly correlated with "Boredom Susceptibility" subscale in female at 30 minutes after examination (p < 0.05) and between baseline saliva cor-



Fig.1: Minor oral examinations elicit a moderate elevation in saliva cortisol titre (N=26). Saliva samples have been taken at three consecutive time points. Pre = before the examination, post = approximately 10 minutes after examination, and end = approximately 30 minutes after the examination.



Fig.2: Cortisol response profiles of three representative students upon minor oral examinations. Type 1 is characterized by a moderate transient increase, type 2 by a minor transient decrease, and type 3 by a cortisol decline. Saliva samples have been taken during the break before the lesson, as well as ten and about 30 minutes after the examination.



Fig.3: Saliva cortisol titre at school exit examinations (N = 12). Baseline, Pre = before the examination, post = approximately 10 minutes after examination, and end = approximately 30 minutes after the examination. Baseline saliva cortisol has been estimated from saliva samples taken during the break before minor oral examinations (see Fig.1).

tisol titre and "total sensation seeking" scale (p < 0.05) in cortisol-response-type 1 (Table 1).

Participants of the present study were within the normative range of coping strategies. Female and male students employ different strategies to cope with stressors. Male students scored significantly higher in cognitive coping strategies (POS1), like to play down a problem, and on controlling of stressors (POS3), like controlling reaction, than female students (p < 0.05). Female students scored significantly higher on negative coping strategies (NEG), like flight, and on seeking for social support (BESOZU) than male students (p < 0.05).

Only few of these strategies correlated with cortisol titre and cortisol response profile, respectively (Table 2). In female students, a Spearman correlation revealed a significantly negative association between negative coping strategies (NEG) and cortisol titre 30 min after the stressor (p < 0.05). Male students had a positive association between seeking social support (BESOZU) and baseline cortisol titre (p < 0.05).

Individual stress perception levels during minor oral examinations were rated low by the students. Spearman correlation did not reveal a significant correlation between cortisol titre or cortisol profile and subjective stress intensity.

Discussion

In the present study we showed that (1) compared to minor oral examinations, major examinations elicited a dramatic increase in saliva cortisol titre, (2) individual cortisol response profile was not stable upon examinations in distinct subjects, (3) female and male students differed in their sensation seeking and coping strategies, (4) subsets in sensation seeking scores and coping



Fig.4: Comparison of SSS-V (Sensation seeking scale) scores in female (N = 44) and male (N = 47) students. Males scored significantly higher on subscale DIS (p < 0.05), and females on ES (p < 0.05). Subscales: DIS = Disinhibition, BS= Boredom Susceptibility, TAS = Thrill and Adventure Seeking, ES = Experience Seeking, and Total SSS-V scores.

strategies, respectively, correlated moderately with saliva cortisol titre or cortisol response profile.

Minor oral examinations during the school year induce small, but significant, fluctuations in saliva cortisol titre. In contrast, oral school exit examinations (A-level, "Matura") trigger large increases in saliva cortisol titre [16, present study]. Moderate fluctuations in saliva cortisol upon minor oral examinations may be due to habituation to this mode of examinations, as students are familiarized to their peers ("audience"), school room, as well as their teachers. Furthermore, these interviews do not have major consequences on their academic career, because they can easily improve their grade on subsequent examinations. Similarly, repeated exposure to psychological stressful challenges under laboratory conditions, like the TSST, has been reported to result in habituation in humans [3,9,21]. In these experimental settings, cortisol concentrations decreased upon successive trials. In contrast to minor oral examinations, school exit examinations represent a novel situation to students. E.g., examinations are more formal and take place in front of a board of known and unknown teachers, the grade of the examinations can not be corrected in additional examinations, and, furthermore, when students fail this examination, they have to wait for several months to repeat it. Taken together, the aspects of novelty and intensity upon school exit examinations may be the cause of the substantial elevation of the cortisol titre.

Association between saliva cortisol titre and personality states and traits, respectively, is controversal. Whereas some studies claim an association between personality traits and cortisol release, other studies did not support this notion [5,16,19]. In the present study, we found only a moderate correlation between cortisol titre and **Table 1:** Spearman correlation between female (N = 11) and male (N = 15) students, type 1 (N = 13), and type 2, 3 (N = 13) salivary cortisol concentrations before (pre), 10 minutes after (post), and about 30 minutes after examinations (end). Total SSS-V scores; subscales: DIS = Disinhibition, BS = Boredom Susceptibility, TAS = Thrill and Adventure Seeking, ES = Experience Seeking.

	-	female			male	_
	pre	post	end	pre	post	end
total	-0.192	-0.078	0.384	0.144	0.298	0.023
DIS	-0.333	-0.148	0.232	0.124	0.195	0.067
BS	0.151	0.156	0,708*	-0.308	-0.218	-0.437
TAS	0.143	0.119	0.454	0.038	0.219	-0.024
ES	-0.176	-0.376	0.023	0.141	0.201	0.036
		Type 1			Type 2,3	
	pre –	post	end	рге	post	end
total	pre 0,554*	post 0.341	end 0.271	рге -0.232	post -0.098	end 0.136
total DIS	pre 0,554* 0.305	post 0.341 0.075	end 0.271 0.047	рге -0.232 -0.470	post -0.098 -0.086	end 0.136 0.214
total DIS BS	pre 0,554* 0.305 0.261	post 0.341 0.075 -0.132	end 0.271 0.047 -0.314	рге -0.232 -0.470 0.235	post -0.098 -0.086 0.139	end 0.136 0.214 0.266
total DIS BS TAS	pre 0,554* 0.305 0.261 0.375	post 0.341 0.075 -0.132 0.255	end 0.271 0.047 -0.314 0.283	рге -0.232 -0.470 0.235 -0.231	post -0.098 -0.086 0.139 -0.501	end 0.136 0.214 0.266 -0.290

*p < 0.05

Table 2: Spearman correlation between female (N = 11) and male (N = 15) students, type 1 (N = 13), type 2 (N = 5), and type 3 (N = 8) salivary cortisol concentrations before (pre), 10 minutes after (post), and about 30 minutes after the examinations (end). Pos1 = cognitive coping strategies, pos2 = coping strategies characterize tendencies for distraction of burden; pos3 = coping strategies cover measures for controlling the stressor, pos = positive coping strategies, neg = negative coping strategies, besozu = need for social support.

	_	pos1	pos2	pos3	pos	neg	besozu
female	pre	0,050	-0,293	0,256	0,096	-0,091	0,248
	post	-0,027	-0,133	-0,073	-0,014	-0,100	-0,009
	end	0,215	0,133	0,407	0,278	-0,636*	0,212
male	pre	-0,172	0,336	0,324	0,298	-0,089	0,566*
	post	0,075	0,247	0,403	0,393	-0,200	0,400
	end	-0,057	0,080	0,138	0,145	-0,157	0,197
type 1	pre	-0,127	-0,160	0,126	0,016	-0,632*	0,500
	post	-0,218	-0,072	-0,121	-0,066	-0,407	0,470
	end	-0,105	-0,129	-0,110	-0,066	-0,412	0,215
type 2,3	pre	0,063	0,085	0,202	0,272	0,137	-0,205
	post	0,157	0,314	0,431	0,476	0,049	0,183
	end	0,342	0,435	0,608*	0,616*	-0,220	0,207

*p < 0.05

boredom susceptibility as well as type 1 cortisol profile and total sensation seeking (Table 1).

The relation between oral examination and cortisol titre and cortisol profile, respectively, could be explained with the appraisal and coping model according to Lazarus and Folkman [15]. In the present study we found that the cortisol titre between minor and major oral examina-

tions differed drastically and that students do not have a stable cortisol profile when examined several times. Our findings may indicate that students continuously appraise their intellectual capacities and preparation of the subject as well as intensity and consequences of examinations. In conclusion, our study indicates that novelty, like school exit exam, is a potent activator of the hypothalamic-pituitary-adrenal axis.

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