

Analysis of the effects of anatomy committee exam stress on visual and auditory reaction time and cortisol level: A neuroperformance study

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Abstract

Objective: The present study aims to find out the effects of exam stress, which exists in every moment of education life, on visual and auditory reaction time (VRT, ART), cortisol level and stress perception.

Material and methods: A total of 66 students (36 males, 30 females) were included in the study. VRT and ART measurements were carried out with reaction timer by asking the participants to use their dominant hands. Reaction times were recorded on two occasions, namely 30 days before the committee exam (relaxed period) and, again, on the day of exam (stressed period). Additionally, students provided salivary samples and filled in State Trait Anxiety Inventory-I on both occasions.

Results: Median values of cortisol, STAI-I, VRT and ART scores of male and female students were higher during stress period than that of the relaxed period ($p < 0.05$). Correlation analysis showed a positive weak correlation between cortisol level and STAI-I scores of students in exam period (male: $r = 0.317$, $p < 0.05$; female: $r = 0.253$, $p < 0.05$). Moreover, cortisol levels were positively correlated with VRT (male: $r = 0.155$, $p < 0.05$; female: $r = 0.227$, $p < 0.05$) and ART (male: $r = 0.159$, $p < 0.05$; female: $r = 0.163$, $p < 0.05$) scores both male and female students.

Conclusion: The results show that stress increase was found to increase cortisol level and STAI-I scores. Increased cortisol level was associated with longer reaction times as measured by VRT and ART.

Keywords: stress, reaction time, saliva, cortisol, neuroperformance

Introduction

In our daily lives, stress is everywhere. Stress can balance behavioural and hormonal reactions [1,2] and radically change neural responses given to information received [3,4]. Acute stress has a considerable influence on various cognitive processes including attention, cognitive control, memory and social cognition [5,6].

Stressful encounters and also individuals' psychological reactions to these encounters activate the

hypothalamic-pituitary-adrenocortical (HPA) axis and the sympathetic nervous system, causing excessive levels of stress hormones, especially cortisol and catecholamines, to be secreted. When stress hormones are secreted, bodily functions, especially immune, cardiovascular and metabolic functions may undergo dysregulation [7]. One of the naturalistic stressor examples is academic exams. These exams are time-limited and generally considered to be aversive and they are seen as psychological and physiological

reactions to stressful encounters. Based on cognitive theories of stress, the present study analyses the effects of cognitive appraisals of stress in exam situation and test anxiety. According to cognitive approach, the individual who has coping sources to cope with a stressful situation evaluates the threat or challenge in the stressful situation through cognitive processing in stressful encounters like academic exams [8]. The coping strategies individuals use in the face of stressful situations affect their evaluations and determine the psychological (e.g., experienced stress, test anxiety) and physiological results of the encounter. In addition to cognitive aspects (i.e., worry) and the elements of emotional and physiological arousal (i.e., emotionality), test anxiety includes particular cognitive, emotional and physiological reactions caused by the stimuli of testing [9-11]. In every stage of our lives, we are faced with decisions that we have to make. The speed, quality and hit rates of the decision made are the most important parameters of success. The stressful environment an individual is in has negative influences on the individual's biological and psychological state. This stress experienced causes carelessness and low concentration, low performance and inefficiency [12-14].

Reaction time (RT) is the time between the presentation of a sensory stimulus and the following behavioural reaction [15,16]. RT is closely associated with the speed of making a decision [17]. For a move fit for the purpose, sensory and motor systems should be working in coordination. In humans, reaction speed is a direct indicator of nerve transmission speed. RT is different from reflex time, which is the automatical response given to an external stimulus. It is a completely voluntary situation in which central nervous system is enabled [18]. Characteristics such as the transmission speed of the nerves which have a role in the stimulus reaching the central nervous system and the response being carried to the effector organ and the effector muscle being a fast or slow muscle creates differences of milliseconds from person to person [18].

A great number of studies report RT being influenced by stress and anxiety [19,20]. Stress is present in every moment of our education life. The idea that stress can affect the reaction is the hypothesis of our study. This study aims to examine the effects of exam stress, which exists in every moment of students' educational life and influences students negatively most of the time, on visual and auditory RT (VRT-ART) and cortisol level.

Material and methods

Participants

The study includes 66 students (36 males, 30 females; mean age 19.4±1.8 years; range, 18 to 20 years) who were studying at İnönü University, Faculty of Medicine between May 2018 and June 2018 and who signed informed consent form.

Inclusion criteria consisted of the following: being physically healthy, not having any medical obstacles to prevent participation in the study, not having any disease, no previous history of orthopaedic surgery, not having performed resistance exercise at least six months before the start of the study, not using food supplement, such as keratin during the study. Antidepressant drug and herbal relaxant use before the stressed and relaxed periods were exclusion criteria.

Students using hearing aids and contact lenses and glasses were not included in the study. Female students in menstrual cycle or those to enter this cycle in three days were also excluded [21]. Malatya Clinical Researches Ethical Board (2018/110) approved the study protocol and the principles of the Declaration of Helsinki were followed. Participants were informed about the study and an informed consent form was signed.

The relaxed period was accepted as the period 30 days before the committee exam and the first RT measurements and saliva samples were taken then. The stressed period was the day of the committee exam and the second RT measurements and saliva samples were taken. Since we thought that students would have high levels of stress before the test, this period was considered stressed. State Trait Anxiety Inventory-I (STAI-I) and cortisol measurements were used to support this hypothesis. There was a period of 30 days between the relaxed period and the stressed period. All students were given STAI-I, a commonly used scale in the assessment of anxiety, during the relaxed and stressed periods. Öner and Le Compte conducted the validity and reliability of STAI-I's Turkish version [22]. Classification of the points was as follows in STAI-I scale: ≤ 36 as "no anxiety", 37-42 as "mild anxiety", ≥ 42 as "high anxiety".

RT measurements

Hubbard Scientific Reaction Timer (Model: 6027, USA) was used to make VRT and ART measurements. Two different warnings as visual (light) and auditory (sound) are obtained from Reaction Timer device. RT measurements were performed in a noise free environment with sufficient light between 09:30 and 11:00. Each subject was asked to put their dominant hands on a table in front of them which had a button and a Reaction Timer 10 cm away from the button. With "ready" command, either sound or light stimulant was given and the subjects were asked to press the buttons in shortest time in accordance with the stimulants. Each subject made 10 trials for sound and light stimuli, the first of which was taken as practice. The average of the last trials was determined as RT [23].

Analysis of cortisol in saliva

In order to minimize the impact of extra test components on cortisol levels, the subjects were told to avoid smoking, caffeine, and physical exertion for 3 hours before coming to their appointment, in the preliminary information provided, that all samples should be given at 10:00 (am). They were asked to eat a low fat and protein meal and not eat or drink for 1 hour before coming to their appointment. Passive drool method was used to collect saliva samples, as shown by Granger et al. [24]. Samples were kept in a laboratory freezer at -20 °C. After thawing, the samples were centrifuged at 4000 g for 10 min and supernatant was used for ELISA analyses. Samples were diluted 1:5 and assayed in triplicate with assay buffer. ELISA procedure; Cortisol-BSA stock solution (1 mg/mL) was diluted with carbonate buffer, pH 9.6 before adding to a 96-well microtiter plate at 200 μ L/well. For one night, the microtiter plate was incubated at +4 °C and washed five times with wash buffer and eight-channel pipette. With blocking buffer (200 μ L/well), binding places that had no coating antigen were blocked at 37 °C for 2 h. After washing, standard solutions or samples (40 μ L/well) and diluted 1st Ab (antiserum) (40 μ L/well) were put in duplicate and incubated for 45 min at 37 °C. Later, after the washing process, following the addition of biotinylated anti-Rabbit antibody (100 μ L/well), the plate was incubated for 30 min at 37 °C. After washing for 5 times, the plate was incubated at +4 °C for 15 min following the addition of streptavidin peroxidase solution (100 μ L/well). Next, after the plate was washed for another 5 times, substrate solution (150 μ L/well) was added and incubated in dark for 10 min. Stop solution (50 μ L/well) was added after incubation and microplate reader was used to measure absorbance at 450 nm. While intra-assay variation (CV) was found as 5.6%, inter-assay variation was found as 7.8%. Elisa test was made by the same expert in the same laboratory [21].

Statistical analysis

Normality assumption of the data was tested with Kolmogorov Smirnov test. The data without normal distribution were analysed with Wilcoxon paired samples test. Spearman Rho coefficient was used to calculate the correlations. Significance was considered at $p < 0.05$ level and IBM SPSS Statistics 22.0 for Windows program was used. In calculating the sample size, the 95% confidence interval in the G-Power 3.1.7 package program was calculated for the paired samples t test. For determining at least 1ms of change in RT, minimum sample size for $\alpha = 0.05$ and $1 - \beta = 0.80$ was calculated to be 52 with the effect size estimated as 0.40.

Table 1 Median (Min-Max) values of cortisol and STAI-I, Wilcoxon paired-samples test results between relaxed and stressed, Spearman rho correlation analysis results between cortisol level and STAI-I in exam period

Sex	Variables	Relaxed		Stressed		p	Exam Period	
		Median	Min-Max	Median	Min-Max		Correlation	Cortisol
Male	Cortisol	14.7	2.9-58.3	24.5	8.8-196	.013	STAI-I*	r: .317
	STAI-I*	46	32-70	55	31-76	.041		p: .025
Female	Cortisol	14.9	3.4-61.5	27.5	7.91-779.7	.019	STAI-I*	r: .253
	STAI-I	43	27-67	50	28-77	.042		p: .018

*STAI-I: State Trait Anxiety Inventory-I

According to the Wilcoxon paired-samples test results, both VRT and ART were found to increase statistically significantly during transition from relaxed period to the stressed period ($p < 0.05$), (Table 2). With these data, it can be concluded that VRT and ART increase as stress increases.

Table 2 Median (Min-Max) values of VRT and ART measurements and Wilcoxon paired-samples test results between relaxed and stressed

Sex	Variables	Relaxed		Stressed		p
		Median	Min-Max	Median	Min-Max	
Male	VRT*	31.6	24.6-44.3	35.6	28.3-54.6	.008
	ART**	37.6	27.6-51	41.3	27.3-60.6	.007
Female	VRT*	32.6	23.6-44	41.3	27.6-51.6	.003
	ART**	36.3	24.6-51	40.3	26.3-51.3	.011

*VRT: Visual reaction time

**ART: Auditory reaction time

Spearman's rho correlation analysis was performed to determine how VRT and ART values differed with Cortisol and STAI-I scores (Table 3). According to the results of the analysis conducted, a positive and weak correlation was found between VRT and ART and cortisol level and STAI-I scores in both male and female students (Table 3).

Table 3 Spearman rho correlation analysis results conducted on the VRT and ART scores

Variables	Test statistics	Male		Female	
		Cortisol	STAI-I	Cortisol	STAI-I
VRT*	r	.155	.314	.227	.348
	p	.048	.039	.046	.011
ART**	r	.159	.172	.163	.276
	p	.049	.042	.047	.035

*VRT: Visual reaction time

**ART: Auditory reaction time

Table 4 Change of individual numbers according to STAI-I score

Sex	Period	<36 (no anxiety)		37- 42 (mild anxiety)		>42 (high anxiety)	
		Count	%	Count	%	Count	%
Male	Relaxed	19	52.8%	7	19.4%	10	27.8%
	Stressed	3	8.3%	6	16.6%	27	75.1%
Female	Relaxed	16	53.3%	5	16.6%	9	30.1%
	Stressed	3	10%	4	13.3%	23	76.7%

Results

According to the results of the analysis we conducted, cortisol level and STAI-I score median values were found to increase in the stressed period when compared with the relaxed period. Wilcoxon paired-samples test was given to the data to determine whether there were changes in cortisol and STAI-I scores during transition from relaxed period to the stressed period. Analysis results showed that the increase in cortisol level and STAI-I scores of both men and women during transition from relaxed period to the stressed period was statistically significant ($p < 0.05$). According to Spearman rho correlation analysis of cortisol and STAI-I scores in exam period, a positive weak correlation was found in both male ($r: 0.317$, $p < 0.05$) and female ($r: 0.253$, $p < 0.05$) students (Table 1).

The number and percentage of the individuals in groups called no anxiety, mild anxiety and high anxiety during relaxed and stressed periods according to STAI-I scores were given in Table 4. According to these data, the number of individuals in high anxiety group were found to increase significantly in stressed period when compared with relaxed period in terms of both males and females (Table 4).

Discussion

In the current study, cortisol and STAI-I anxiety scores were higher in stressed period when compared with the relaxed period. In addition, there was a positive and weak correlation between cortisol levels and STAI-I scale scores of male and female during the stressed period. This result shows the accuracy of cortisol measurements and that participants are stressed during exams period. It was found that VRT and ART values of males and females who participated in the study increased in stressed period when compared with the relaxed period. It was found that stress had an adverse effect on the sense of VRT and ART. To the best of our knowledge, this is the first study in the literature. When previous studies are examined, the present study seems to be quite unique, which makes it difficult to discuss the results we found.

Stress affects our performance, how we feel and many physical functions (neuro-physiological) [25]. Individuals faced with stress respond with a physiological or psychological response [26]. When compared with relaxed individuals, it has been reported that it is easy for stressed individuals to make mistakes, they are more anxious and uneasy [27]. RT is a significant detail for duties requiring attention. In their studies, Welford [28] and Brebner and Welford [29] stated that individuals moved slower when they were under stress. In the study they conducted, Saha et al., [19] stated that noisy stress decreased VRT and ART.

A great number of researchers state that auditory reaction is faster than visual reaction and average auditory RT's are between 140 and 160 ms, while visual RT's are between 180 and 200 ms [30, 31]. It was stated that maybe the reason for this

was the fact that it took about 8-10 ms for an auditory stimulant to reach the brain [30, 32], while it took 20-40 ms for a visual stimulant [33]. Bellis (34) found VRT as 220 ms in men and as 260 ms in women, while ART was found as 190 ms in men and 200 ms in women.

In their study, Engel et al. [35], found ART in men as 227 ms and as 242 ms in women. Silverman [36] stated that since women drove more carefully recently and that they participated in fast-action sports more, it was possible for them to have a better VRT when compared with men. In their study with lacrosse players, Spierer et al. [37] found that VRT and ART had faster scores in men when compared with women. In a study conducted in Germany, it was stated that in general, women were better than men in handcrafts and ability [38]. In another study conducted, while no significant difference was found between men and women in RT's for visual stimulants, men were found to have faster movement time [39]. In their study, Binboğa et al. [40] found that although men had shorter auditory simple RT than women, the difference was not found to be statistically significant. Gürsoy et al. [41] compared sedentary men and women and found that while right hand visual simple RT of men were found to be significantly shorter, ($p < 0.05$), left hand visual simple RT of men were also found to be shorter, but not significantly ($p > 0.05$).

Gender is a significant factor in RT. Women have slower RT when compared with men. This time is 30% shorter in adolescence and maturity. In a study they conducted on 56 females and 57 males between 11 and 14 years of age, Taimela and Kujala concluded that women were slower than men in simple and optional RT's [42].

In every part of life, primarily in educational and business life, individuals who can cope with stress, those who have a high professional efficiency and a high percentage of making right decisions are preferred. Success shown in such situations will

put both individuals and institutions a step ahead. The present study has some limitations. First of all, sample size can be larger. However, since we made measurements during students' exam periods and since the measurements took long, we had some problems. Despite all difficulties, a suitable number of participants were included in the power analysis. Secondly, some of the participants in exam stress stated that they got sedatives before measurements in stressed periods and these students were excluded from the study.

Conclusion

As a conclusion, our study results showed that stress had a negative influence on VRT and ART. This study should be taken into consideration by educators, by individuals who experience exam stress, and by families who think that their children have too much stress in exams. The fact that no similar study was found in our literature research reveals the originality of this study. Exams exist in every moment of our lives and we are faced with important decisions that we have to make at every stage of our lives. The speed, quality and accuracy of the decisions we make are the most important parameters of our success. Considering the results of this study, we should change our perspective on life and remember that excessive stress will negatively affect us. We believe that the present study, which did not have much of a place in literature in detail previously, can be a source for future studies.

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