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Gender Difference in the Effect of Examination Stress on Brain Oscillations during Memory Tasks

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Abstract

***Background*: Researches revealed that increased levels of stress hormones lead to enhancement of memory consolidation in rodents. Recently, it became apparent that the brain oscillations reflect cognitive aspects and information processing in the brain. Quantitative electroencephalogram (qEEG) provides an objective assessment of the electrical activity of the brain *via* many techniques such as power spectral analysis and coherence. *Aim:* To assess the effect of gender on the qEEG oscillations during the delayed memory retrieval after exposure to acute stress. *Subjects and Methods:* This prospective (longitudinal) study was applied on 34 healthy undergraduate medical students from both genders (17 males and 17 females) in the Faculty of Medicine, Suez Canal University. The qEEG analysis was done in neurology department, Suez Canal University Hospital using the relative power (RP) during memory tasks. Serum cortisol was analyzed by enzyme linked immunosorbent assay (ELISA) as a measure for stress. *Results:* The results revealed insignificant effect of gender on the quantitative analysis of EEG oscillations regarding the mean relative power in all frequency bands (delta "δ": 2.36±0.72; 2.04±0.46, theta "ϴ": 13.72±3.13; 12.72±3.58, alpha "α": 12.72±3.58; 18.50±3.81 and beta "β": 33.26±3.23; 32.72±4.47 for males and females respectively during the examination period). Whereas, there was a positive correlation between the serum cortisol level and the mean relative power of delta (r: 0.53, p=0.03), and theta (r: 0.55, p=0.02) and a negative correlation with beta (r: -0.51, p= 0.04) bands in males only. *Conclusion:* There are positive correlations for delta and theta bands and negative correlation for beta band between the quantitative electroencephalographic analysis (RP) with the hormonal measure of the stress in spite of the insignificant changes in the EEG oscillations in the non-stress and stress periods.**

***Keywords*:** electroencephalogram, cortisol, qEEG

Introduction

Stress is known to increase the activity of the hypothalamic adrenal axis. This in turn leads to increase in the release of glucocorticoids from the adrenal cortex(1). Animal studies showed that stress hormones could enhance or impair memory. Corticosterone administration and stress lead to impairment in delayed memory retrieval. Several studies failed to find an effect of stress on memory. However other studies with different methodologies revealed an effect of stress on memory(2-5).

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Brain imaging techniques had a major impact on the research field concerning the cognitive aspects and neural bases of human memory. One of these aspects is the retrieval processing, when the subject try to retrieve information during a memory test[6]. There are evidence that EEG activity can reflect cognitive and memory performance[7]. Multiple recent researches showed asymmetry in the involvement in the prefrontal area in information processing. The left prefrontal cortex is found to be involved more in encoding, while the right is more involved in retrieval process. This is called hemispheric encoding and retrieval asymmetry "HERA model"[8]. The gender difference regarding the effect of stress of learning and memory is not completely clear[9]. There is a suggestion from epidemiological as well as experimental studies in elderly subjects that postmenopausal women are more susceptible to the impairing effects of elevated cortisol levels on memory than elderly men[10].

Subjects and Methods

Prospective (longitudinal) study applied on undergraduate students, studying in faculty of medicine, Suez Canal University. The actual number of students on which the statistical analysis was applied was 34 students (17 male and 17 females). Seven students dropped out during the study due to variable reasons. *Inclusion criteria:* Healthy undergraduate students from both sexes. Their ages ranged between 18 and 23 years. *Exclusion criteria:* Subjects were excluded from the study if there is a history of psychiatric disorders, if they are on systemic corticosteroid therapy for any reason, for females: if they are pregnant, or on hormonal contraceptives [2]. If they are smoking more than 10 cigarettes/day[11], working overnight shift or having unusual sleep patterns[3], performed physical exercise 2 hours before the test sessions, there are other major stressors rather than the examinations during the two weeks prior to the study time (e.g. severe illness or death of a first degree relative, moving, etc.)[12]. If they are left-handed, they have uncorrected errors of refraction, they are having endocrine abnormalities (e.g. Cushing syndrome, diabetes mellitus, etc.)[13]. Females having irregular menstrual cycles (<24 or >36 days)[1]. Preparatory phase lasts from July 2011 to December 2011.

Constructing the word list to be used in the word-recognition memory task.

A list of 254 words was created .These words were chosen from the pool of Arabic words from *Mukhtar Us-Sahah* Arabic lexicon, verified according to emotional valence and word frequency in daily life of students. Two parallel lists were then created from this pool of words. Each list contained 30 words (20 for the first learning session and 10 to be introduced as new words in the recognition memory task).

Preparing the photos for photo-recognition memory task

The photos were digitally manipulated to show the face, hair, and ears only. All the clothes and accessories cues were removed. All photographed subjects showed neutral or close to neutral facial expressions. All photos were of the same size, the used photos were of front to intermediate view.

Implementation of the study

In non-examination period, the students went through an encoding session, in which the students learned a list of 20 words and 13 faces of male and female children. Two weeks later, the students were asked to attend to the EEG assessment unit for EEG recording during memory task, in which the students retrieve and recognize the learned words and photos from newly introduced ones. The two weeks delay aimed to separate encoding phase and consolidation phase from retrieval phase; this is in order to be able to draw a conclusion about the effect of stress on a distinct memory phase[1]. The retrieval session was within 48 hours prior to the students' periodic examinations.

Recognition tasks and EEG recording session

Electrical signals were recorded with SCAN LT^{TM} *(Neuro Scan Medical System, Neurosoft, Inc., 2001).* The high pass filter was 1 Hz and the low pass filter was 30 Hz. The sensitivity was between 100-200 µv. The traces were saved for later off-line analysis. During recording sessions, subjects sat, semi-reclined with eyes open and neck and arms supported. Nineteen electrodes (Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, P3, P4, Pz, T5, T6, T7, T8, O1, O2) were positioned according to the international 10/20 system and were referenced to electrodes linked to the earlobes (A1, A2). An electrode was placed as a ground[14].

Quantitative analysis of the EEG

In off-line quantitative analysis of EEG; one minute free of artifacts for each task (word recognition and photos recognition tasks) was chosen. Each minute was segmented into 3 time epochs; each of which is 20 seconds [15] for calculating the relative power, *"which is the ratio of power in a band divided by the total power in all bands combined"* for each band (delta, theta, alpha and beta) and the peak power frequency, *"which is the frequency in the spectrum that displays the highest power in a particular epoch"* for electrodes (C3, C4, O1, and O2)[18,19], C3, C4, O1, and O2 were specifically chosen to facilitate finding the effects of stress on EEG oscillation[16]. The Default band widths on the SCAN LT^{TM} system are: Delta: 0.5 to 4.0 Hz, Theta: 4.0 to 8.0 Hz, Alpha: 8.0 to 14.0 Hz, Beta: 14.0 to 35.0 Hz[17]. A blood sample of 3cm venous blood was drawn after finishing EEG recording and recognition tasks to assess the serum cortisol level in both sexes analyzed by enzyme linked immunosorbent assay (ELISA) as a hormonal measure of stress[2, 17].

Statistical analysis

The results analyses were done by using SPSS statistical package version 10.0., and 17 and Microsoft Excel 2010. Data were tabulated and proper statistical analyses were performed. Descriptive data was expressed as mean±SD and ranges as appropriate. The difference between both genders was assessed by student t-test (independent or paired according to the data required). Qualitative analysis of non-parametric data wherever applicable, e.g., Chi-square test. P values of < 0.05 were regarded as significant and $P < 0.01$ as strongly significant.

Ethical Considerations

The study was approved by the ethics committee of the scientific research of the Faculty of Medicine, Suez Canal University. A written consent was obtained from all participants. The data of the researchers and the deputy of them; including the names and the telephone numbers were included in the consent. The purpose and the procedures of the study were explained to the participants. No personal data were allowed to be published. The participants were free to quit from the study at any time without explaining the reasons and without any negative consequences on them.

Results

Demographic Data

The age of the students ranged between 18-23 years (mean 21.35±0.86 and 20.76 ±1.25 years in males and females respectively). Most of the students were in the 6^{th} and 5^{th} years representing 41.18% and 32.35% respectively of the study sample. Only one student was in the first year (2.94%).

Hormonal Measure of Stress (serum cortisol level)

A comparison between the serum cortisol levels in each sex separately during non-examination and examination periods revealed a highly significant rise in the serum cortisol level in both sexes in the examination period (males: 10.72±1.90, females: 9.53±1.92) compared to non-examination period (males: 7.42±2.52, females: 6.35±2.01) reflecting increased level of stress; (Table 1). (The normal value of the serum cortisol for males and females [AM] 3.95-27.23 µg/dl).

Table 1: Serum cortisol levels during the non-examination and the examination periods in relation to gender

Test period	Serum cortisol (µg/dl)	
	Males (n=17)	Females (n=17)
Non- Examination Period	7.42 ± 2.5	6.35 ±2.0
Examination Period	10.72 ± 1.9	9.53 ±1.9
P value	0.01	< 0.001

Data are presented as mean±SD

Results of Quantitative Electroencephalogram Analyses

On assessing the differences in the mean scores of relative power recorded during word-recognition memory task for each band in both sexes during the examination and non-examination periods, there was no significant difference (p value >0.05) between males and females regarding the relative power in the four EEG frequency bands (δ, Θ, α and beta ß). Regarding the relative power recorded during photo-recognition memory task for each band in the examination and non-examination periods in each sex, we found no significant difference among males or females regarding the relative power of the four EEG frequency bands (δ, Θ, α and ß) in examination compared to non-examination periods (Table 2).

Correlation Analyses

A correlation between the mean serum cortisol level and the mean scores of relative power recorded during word and photo- recognition memory tasks was done in both sexes using a bivariate correlational analysis.

During the "non-examination" period, the analysis showed insignificant correlation (p >0.05) between the mean scores of serum cortisol level and the mean scores of relative power in the frequency bands (δ, Θ, α and ß) during word and photo-recognition tasks in both sexes.

During the "examination" period, the results revealed a positive correlation between the serum cortisol level and the relative power of delta band in quantitative EEG in males only during word-recognition task in the examination period (r=0.53, p=0.03). A positive correlation between the serum cortisol level and the relative power of theta band in quantitative EEG was found in males only during word-recognition task in the examination period (r=0.55, p=0.02). There was a negative correlation between the serum cortisol level and the relative power of beta band in quantitative EEG in males only during word-recognition task in the examination period (r= -0.5, p=0.04) (Table 3).

Table 2: The relative power recorded during photo- recognition memory task in each band during non-examination and examination periods

Test period	Relative power in each EEG band during photo-recognition task		
	EEG Band	Males (n=17)	Females (n=17)
Non-exam. Period	δ	2.37 ±0.30	2.26 ±0.63
Exam. Period	δ	2.29 ±0.66	2.15 ±3.73
Non-exam. Period	ϴ	13.58 ±2.08	13.11 ±3.35
Exam. Period	ϴ	13.35 ±2.67	13.47 ±2.99
Non-exam. Period	α	17.69 ±4.39	17.61 ± 4.26
Exam. Period	α	16.91 ±3.64	17.85 ± 5.17
Non-exam. Period	ß	33.46 ±2.68	33.45 ±3.65
Exam. Period	ß	33.87 ±2.84	33.21 ±3.85

Data are presented as mean ±SD

Table 3: A correlation between serum cortisol level and the mean relative power in examination period in both genders

Task type	Frequency band	Serum cortisol (μg/dl)			
		Males (n=17)		Females (n=17)	
		R	P-value	r	P-value
Word-recognition memory task	δ	0.53	0.03*	-0.01	0.96
	ϴ	0.55	0.02*	-0.07	0.79
	α	0.37	0.14	-0.16	0.55
	ß	-0.51	0.04*	0.06	0.83
Photo-recognition memory task	δ	0.38	0.13	-0.96	0.72
	ϴ	0.48	0.05	-0.07	0.78
	α	0.30	0.24	0.13	0.63
	ß	-0.42	0.09	0.024	0.93

Significant values and p< 0.05

Discussion

In the current study, we investigated the gender effect on the EEG oscillations during memory retrieval process after exposure to acute stress. Data analysis revealed insignificant gender differences in the electroencephalographic oscillations regarding the relative power of EEG in the four bands (delta, theta, alpha, and beta) in the examination period compared to the non-examination period. Both sexes showed elevated levels of cortisol in examination compared to non-examination sessions. This is in line with the findings of other researchers[(18,19)]. These similarities in the results of the previous studies and the current one may reflect that different types of stressors can have the same implications on cortisol level. Additionally; in line with *what Domes et al*[(21)] documented**,** which is both serum (used in the current study) and salivary cortisol levels (used in the other studies) are considered good indicators of stress[(20)] Females in the current study showed stronger significant elevation than males in cortisol levels during the examination compared to the non-examination period. This implies that females have a higher level of examination stress than males. Many studies documented that females are being affected more than males by examination stress. These studies attributed this to the higher level of emotionality among females although they have the same level of worry as males[21,22].

One of the major findings of the present study is the presence of selective gender differences in the correlation between mean relative power of delta, theta, and beta frequency bands, and the serum cortisol level during word recognition memory task. The results showed positive correlation between serum cortisol level and theta and delta powers in males only. These positive correlations indicate that with the elevation of serum cortisol level; relative power of delta and theta bands becomes higher. On the other side, there was a negative correlation with beta power in males only. This negative correlation indicates that with the elevation of serum cortisol level; relative power of beta band becomes lesser. McNaughton et al[23] reported that theta brain rhythmicity is reduced by all anxiolytics˙ While Gold et al[24] reported that suggesting frontal midline theta as a biomarker for anxiety is still unclear. Peer et al[25] reported that there is a correlation between the slow and fast activities which becomes enhanced in people with anxiety and in those with high cortisol level. In their study they found that this correlation is not due to the change in the mean power (either decrease or increase) of the slow and fast activities (delta and beta waves)[25]. This implies that this correlation could be due to the coherence between the activities of the neural systems underlying these oscillations rather than the change in the activity of these systems. The findings of these previous studies indicate that the relation between the emotional and psychological state of the individual and the cortical activity reflected by brain oscillations is still in debate and in need for further studies. Other researchers found conflicting results regarding the EEG activities in stress compared to non-stress conditions. Hayashi et al[26] found that beta activity was higher in the frontal and temporal areas in non-stress than in stress condition under emotionally unpleasant stimuli. They induced stress by audio-visual stimuli. The results of the current study are consistent with Hayashi et al[26], in spite of the use of different types of stressors. This may suggest that the type of the stressor does not play the key role on affecting the brain activities. On the other hand, Seo and Lee[17] quantified the effects of chronic stress using EEG. They found significant relationship between salivary cortisol level and high beta activity in eye-closed but not in eye-open condition at both anterior temporal sites and a tendency to a similar relationship over the right occipital region. Their results are not concordant with the present study. This could be related to the duration of application of the stressor under the study. In the current research we studied the effect of acute stress (examination stress), while in Seo and Leo[17] study; they studied the effect of chronic stress.˙ Lewi et al[13] reported a relatively greater left frontal activity during period of low examination stress which was shifted to greater left frontal activity during high examination stress. Lewi and colleagues results were interesting as they found no change in cortisol level under high and low examination stress, and found no evidence that cortisol is the mediator of the relationship between examination stress and the right frontal asymmetry[13] of the discrepancy between Lewi et al. and our results may be because they focused on the frontal region of the brain and they didn't use memory tasks during the experiment. Owing to the scarcity, to our knowledge, in the published studies that correlate the cortisol level to the quantitative changes in different EEG bands under conditions similar to the current study; these conflicting results can support the view of Tops et al[27]. Their suggestion was the effect of cortisol on cortical activity is influenced by the testing condition.

Conclusion

From this study, we conclude that for the quantitative analysis of EEG; it is not a necessity to show significant changes in all parameters (relative power and peak power frequency) under the same circumstances. A correlation between the quantitative analysis of EEG with one or more of the measures of the stress (hormonal and psychological) could be present although there are insignificant changes in the EEG oscillations in the non-stress and stress periods. When a correlation is present with one of the stress measures and the EEG oscillations, this does not necessarily mean that a similar correlation should be present between all stress measures and EEG oscillation. As regarding the luteal phase in females, there are insignificant changes in the EEG oscillation in stress compared to non-stress periods. This could be due to phase effect only and could be due to the gender difference in general.

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