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## Effect of Examination Stress on Brain Oscillations During Memory Tasks in Human Females During the Luteal Phase

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**Abstract:**

Stress is known to increase the activity of the hypothalamic adrenal axis. Some researchers were unable to find an effect of stress on memory retrieval in females. A quantitative electroencephalogram (qEEG) provides an objective assessment of the electrical activity of the brain via many techniques such as power spectral analysis and coherence. The current study was conducted to assess the effect of examination stress on qEEG oscillations during delayed memory retrieval in females during the luteal phase. A Prospective longitudinal study was applied to 11 healthy females. qEEG analysis was done using the relative power (RP) and the peak power frequency (PPF) during memory tasks. Serum cortisol was analyzed as a measure of stress. Serum estrogen and progesterone were assessed to validate the phase of the menstrual cycle. All hormonal analyses were done by enzyme-linked immunosorbent assay (ELISA) technique. The results revealed insignificant effects on the quantitative analysis of EEG oscillations during the luteal phase regarding the mean relative power in all frequency bands (delta "δ"; P-value: 0.38 and 0.41, theta "θ"; P-value: 0.26 and 0.87, alpha "α"; P-value: 0.69 and 0.85 and beta "β"; P-value: 0.91 and 0.74 during the word and photo recognition tasks; respectively). Regarding the peak power frequency EEG analysis; there were no significant differences in (δ, θ, α, and β) EEG bands during the word and photo- recognition tasks over the central (C3 and C4) and occipital (O1 and O2) sites in stress compared to non-stress periods; P-values were: C3: 0.37 and 0.55, C4: 0.33 and 0.11, and were: O1: 0.13 and 0.13 and O2: 0.9 and 0.29 during the word and photo recognition memory tasks; respectively. It is concluded that gonadal steroids have no effect on brain oscillation during both resting and stress conditions.

**Keywords:** qEEG, Peak power frequency, Spectral analysis, Stress, Memory, Luteal phase.

## INTRODUCTION

Students are always experiencing stress during academic courses (Joshi, 2018). Stress leads to an increase in the release of glucocorticoids from the adrenal cortex (Coluccia *et al.*, 2008). Previous researches revealed conflicting results regarding the effect of stress on memory (Kuhlmann *et al.*, 2005; Maki *et al.*, 2002).

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 process when the subject tries to retrieve information during a memory test (Buchanan, 2006). There are pieces of evidence that EEG activity can reflect cognitive and memory performance (Wolf *et al.*, 2002). Multiple pieces of research showed asymmetry in the involvement of 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 the retrieval process. This is called hemispheric encoding and retrieval asymmetry "HERA model" (Babiloni *et al.*, 2004).

Female sex hormones are largely fluctuating throughout the female life and across the menstrual cycle as well. Female gonadal hormones and their metabolites affect mood and behavior by affecting multiple brain regions and neurotransmitter systems (Río *et al.*, 2018). Yet, it is crucial to confirm the expected hormone level in order to validate the menstrual cycle phase (Rugg *et al.*, 2000). Females react to stressors in an exaggerated manner as well as they are liable to show variation in stress reactivity and performance in memory tasks across the phases of the menstrual cycle. During the luteal phase, females have a greater tendency to overreact to stressors (Liu *et al.*, 2017; Iida *et al.*, 2018). There is scarcity in the human studies which had applied memory assessment test batteries during EEG recording in females using neutrally valenced stimuli

during resting and stress periods. This is besides the special nature of the study in the faculty of medicine, which in turn increases the level of stress on students. Therefore, we suggested that these multiple factors could affect brain oscillations. This study was conducted to investigate the effect of examination stress on qEEG oscillations during delayed memory retrieval in female medical students in the luteal phase of the menstrual cycle.

## MATERIALS AND METHODS

A prospective (longitudinal) study has been applied to undergraduate female students, studying in the faculty of medicine, Suez Canal University. Students have been recruited via announcements in the faculty related social media forums. Twenty female students have been volunteered to share in the study. Six of them have been excluded as they were in the follicular phase during the sessions and 3 of them have withdrawn during the sessions. So, the statistical analyses of qEEG were done on 11 students only.

Subjects were excluded from the study if they have reported irregular menstrual cycles (<24 or >36 days) (Río *et al.*, 2018), they have a history of psychiatric disorders, they have been on systemic corticosteroid therapy for any reason, pregnant or had been on hormonal contraceptives (Luethi *et al.*, 2009), they have performed physical exercise 2 hours before the test sessions, or have expressed 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....) (Schoofs and Wolf, 2009), they were left-handed, or with uncorrected errors of refraction, they have suffered from endocrine abnormalities (e.g. Cushing syndrome, diabetes mellitus, etc...), they have reported unusual sleep patterns (Knott *et al.*, 2001; Xiong *et al.*, 2015).

### **Preparatory phase**

**A-** Constructing the word list to be used in the word-recognition memory task. A list of 254 words has been created. These words have been chosen from the pool of Arabic words from Mukhtar Us-Sahah Arabic lexicon, and have been verified according to emotional valence and the word frequency in daily life by medical students who haven't shared in the implementation stage of the study. Two parallel lists have been created from the pool of neutral words only. Each list contained 30 words: 20 for the first learning session and 10 have been introduced as new words (distractors) in the recognition memory task.

**B-** Preparing the photos for photo-recognition task. The photos have been digitally manipulated to show the face, hair, and ears only. All the clothes and accessories cues have been removed. All photographed subjects have shown neutral or close to neutral facial expressions. All photos have been of the same size, and of front to intermediate view.

### **Implementation of the study**

During the non-examination period: the students have gone through an encoding session, in which the students have learned a list of 20 words and thirteen neutrally valenced faces of male and female children. Two weeks later; the students have been asked to attend to the EEG assessment unit for EEG recording during the memory recognition task, in which the students have been asked to retrieve and recognize the learned words and photos from newly introduced distractors. The two weeks delay aimed to separate encoding and consolidation phases from the retrieval phase; to draw a conclusion about the effect of stress on a distinct memory phase (retrieval) (Wolf *et al.*, 2002). The retrieval session was within 48 hours prior to the students' periodic examinations. The

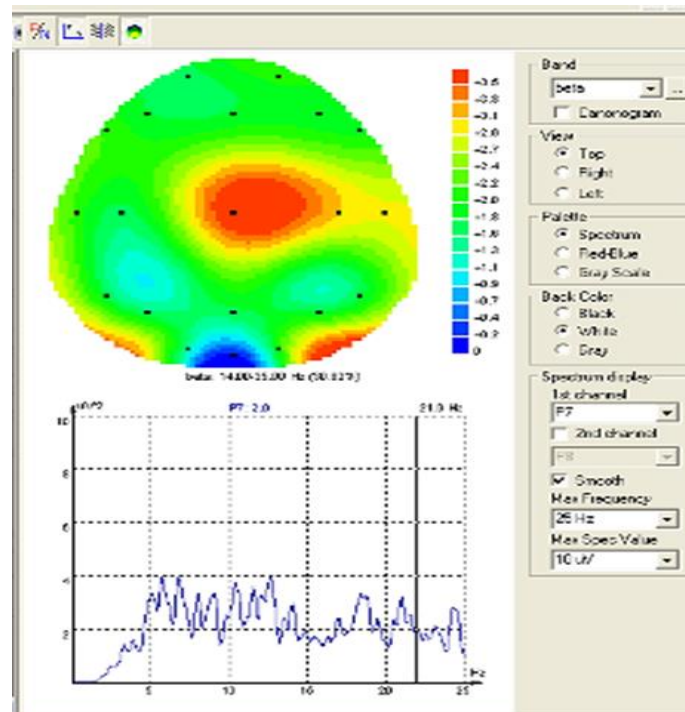
students have been informed to avoid writing or studying words after the learning session to avoid the effect of rehearsal on memory.

### **Recognition tasks and EEG recording session**

Electrical signals have been recorded with SCAN LT™ (Neuro Scan Medical System). The filters have been set as, high pass at 1 Hz and low pass filter at 30 Hz. The sensitivity has been between 100-200  $\mu$ v. The traces have been saved for later off-line analysis. During recording sessions, subjects have 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) have been positioned according to the international 10/20 system and have been referenced to electrodes linked to the earlobes (A1, A2). An electrode was placed as a ground (User's guide, 2001; Tharawadeepimuk and Wongsawat, 2017).

### **Quantitative analysis of the EEG:**

In an off-line quantitative analysis of EEG; one minute free of artifacts for each task (word recognition and photos recognition tasks) has been chosen. Each minute has been segmented into 3-time epochs; each of which is 20 seconds 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). C3, C4, O1, and O2 have been specifically chosen to facilitate finding the effects of stress on EEG oscillation (Seo and Lee, 2010; Bian *et al.*, 2014); (Figure 1). The Default bandwidths on the SCAN LT™ 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 (Stowell *et al.*, 2003, Bian *et al.*, 2014).



**Fig. 1.** A sample screenshot obtained from one of the female students involved in the current study. The shot displays the analysis of RP for the beta band in the upper section. The RP value appears below the topographic map. The analysis of PPF appears in the lower section. The PPF is calculated by identifying the highest power ( $\mu\text{V}^2$ ) and then identify the bband (HZ) with the highest power.

### Hormonal assays:

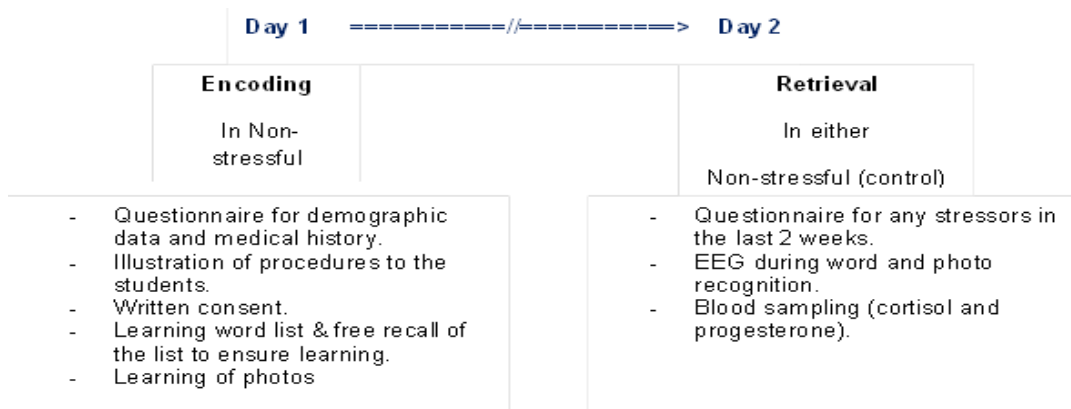
To assess the serum cortisol level as a hormonal measure of stress, a blood sample of 3 c.c. venous blood has been drawn after finishing EEG recording and recognition tasks, to avoid any extra stress on the students by the invasive technique (Conneely and Hughes, 2010; Kuhlman and Wolf, 2005). Estrogen and progesterone have been assessed using Estradiol EIA (Enzyme Immunoassay) kits (Cayman chemical company, Canada/USA) and progesterone ELISA (Enzyme-Linked Immunosorbent Assay) kits (Neogen corporation; Canada/USA); respectively in females for validating the menstrual cycle phases (analyses were done according to the instructions of the producers). Analyses have been done according to the instructions of the

producers. The normal value of the serum cortisol (A.M) is 3.95-27.23  $\mu\text{g/dl}$ .

The normal level of estrogen is 20-280  $\text{pg/ml}$ ; and for progesterone is 0.5-13  $\text{ng/ml}$  (Maki *et al.*, 2002).

### Ethical Considerations

The purpose and the procedures of the study have been clarified to the participants. Written consent was taken from them. The protocol of the study was accepted by the ethics committee of scientific research, faculty of medicine, Suez Canal University. The participants were free to quit from the study at any time without explaining the reasons to the researcher and without any negative consequences for them.



## RESULTS

The average age of students ranged between 18 and 23 years, with the mean age is  $20.6 \pm 1.4$  years. Based on the medical history and the hormonal profiles for estrogen and progesterone; 11 females have been found to be in the luteal phase of the cycle in the non-examination and academic examination sessions, while only 5 females have been in the follicular phase during both test periods. Therefore, the qEEG analysis has been done only for those in the luteal phase and the females in the follicular phase have been excluded.

### Hormonal assay

Serum cortisol, estrogen, and progesterone levels have been assessed for 11 volunteered students: the mean cortisol level during the non-examination period was  $6.3 \pm 2.1$   $\mu\text{g/dl}$ , while it was  $9.3 \pm 1.6$   $\mu\text{g/dl}$  during the examination period. Paired sample student t-test revealed a highly significant increase in the cortisol level in the examination compared to the non-examination period (p-value: 0.001). All females have shown normal estrogen (non-exam. period:  $27.8 \pm 26.4$   $\text{pg/ml}$ ; exam. period:  $69.9 \pm 27.1$   $\text{pg/ml}$ ) and progesterone levels (non-

exam period:  $4.3 \pm 1.9$   $\text{ng/ml}$ ; examination period:  $4.8 \pm 1.1$   $\text{ng/ml}$ ).

### Quantitative Electroencephalogram Analyses

The results revealed insignificant differences in the mean RP recorded from off-line analysis of EEG in non-stress compared to stress periods, for the four EEG ( $\delta$ ,  $\Theta$ ,  $\alpha$ , and  $\beta$ ) bands during both word- and photo-recognition tasks; p-value > 0.05 (Table 1).

On the other hand, for analyzing the peak power frequency, we calculated the percentage of subjects expressed a specific band in each occipital and central electrode sites. All students expressed  $\Theta$  and  $\alpha$  during word-recognition tasks in an insignificant manner during the stress and non-stress periods; p-value > 0.05 (Table 2). The same was for the expression of bands during the photo-recognition task, all females expressed  $\Theta$  and  $\alpha$ , although there was an insignificant difference between stress and non-stress periods; p-value > 0.05 (Table 3). None of the students expressed the  $\delta$  band during word- & photo-recognition tasks. Regarding the  $\beta$  band, although it was not significant, only 18.2% of females expressed it over the O1 site during both the word- and photo-recognition tasks in the stress-period only (Table 2, 3).



**Table 1.** A comparison of the mean relative power in females in the luteal phase in both test periods (N= 11 females).

EEG band	Test period	Mean $\pm$ SD relative power for each EEG band in females in the luteal phase in both test periods			
		During word-recognition task	P-value	During photo-recognition task	P-value
$\delta$	Non-exam. Period	2.08 $\pm$ 0.35	0.38	2.19 $\pm$ 0.41	0.41
	Exam. Period	2.36 $\pm$ 0.92		2.39 $\pm$ 0.53	
$\Theta$	Non-exam. Period	12.96 $\pm$ 3.28	0.26	13.55 $\pm$ 3.04	0.87
	Exam. Period	14.69 $\pm$ 4.03		13.79 $\pm$ 3.03	
$\alpha$	Non-exam. Period	18.82 $\pm$ 3.56	0.69	17.46 $\pm$ 4.70	0.85
	Exam. Period	18.17 $\pm$ 4.39		17.96 $\pm$ 5.22	
$\beta$	Non-exam. Period	32.47 $\pm$ 3.36	0.91	33.35 $\pm$ 3.38	0.74
	Exam. Period	32.47 $\pm$ 4.27		33.82 $\pm$ 4.14	

**Table 2.** Comparison of the percentage of subjects expressing peak power frequency in the luteal phase in both test periods during the word-recognition memory task (N= 11 females).

Test period	The percentage of subjects expressing the different frequency bands in each electrode during the word-recognition memory task															
	C3				C4				O1				O2			
	$\delta$	$\Theta$	$\alpha$	$\beta$	$\delta$	$\Theta$	$\alpha$	$\beta$	$\delta$	$\Theta$	$\alpha$	$\beta$	$\delta$	$\Theta$	$\alpha$	$\beta$
Non-exam period	-	45.5	54.5	-	-	90.9	9.1	-	-	2.3	72.7	-	-	54.5	45.5	-
Exam Period	-	45.5	54.5	-	-	63.6	36.4	-	-	9.1	72.9	18.2	-	18.2	81.8	-
$\chi^2$	0.79				0.96				4.09				2.79			
P-value	0.37				0.33				0.13				0.09			

**Table 3.** Comparison of the percentage of subjects expressing peak power frequency in females in the luteal phase in both test periods during the photo-recognition memory task (N= 11 females).

Test period	The percentage of subjects expressing the different frequency bands in each electrode during the photo-recognition memory task															
	C3				C4				O1				O2			
	$\delta$	$\Theta$	$\alpha$	$\beta$	$\delta$	$\Theta$	$\alpha$	$\beta$	$\delta$	$\Theta$	$\alpha$	$\beta$	$\delta$	$\Theta$	$\alpha$	$\beta$
Non-exam period	-	45.5	54.5	-	-	63.6	36.4	-	-	45.5	54.5	-	-	54.5	45.5	-
Exam Period	-	45.5	54.5	-	-	63.6	36.4	-	-	18.2	63.6	18.2	-	36.4	63.6	-
$\chi^2$	0.36				2.52				4.01				1.09			
P-value	0.55				0.11				0.13				0.29			

## DISCUSSION

The current study investigated the effect of examination stress on the qEEG power spectra in female medical students during the luteal phase of menstrual cycle taking in consideration that examining the same female in

two time periods during the same phase of the cycle (within-subject design) could be more accurate than applying the assessment on two separate female groups.

The fluctuation of female sex hormones during the menstrual cycle has an implication on

her mood and sensitivity to stress (Liu *et al.*, 2017). Observations from previous studies refer to increased physiological responses to stress, including increased adrenocortical activity (Joshi, 2018). Our study results' showed elevated levels of cortisol in examination compared to non-examination sessions. This is in line with the findings of other researchers. Conneely and Hughes (2010) found that increased test anxiety enhanced cortisol reactivity which was liable to be attenuated by social support in highly test anxious college students. Singh *et al.* (2012) found that the stress level (measured by psychological parameters) was correlated with anxiety and salivary cortisol level. These similarities in the results of the previous studies and the current one are logical and reflect that different types of stressors could have the same implications on the cortisol level.

Stress has effects on EEG activities. A previous study documented the effect of premenstrual tension syndrome (that occurs during the luteal phase) on increasing the power of alpha activity of EEG and related that to the effect of stress on the amygdala (Liu *et al.*, 2017). Ossewaarde *et al.* (2013) documented the relationship between the amygdala and stress reactivity as well as the generation or maintenance of alpha rhythm. In the current study, we found insignificant differences regarding the relative power expression in the four studied bands (delta, theta, alpha, and beta) during the non-examination and the examination periods. Khader and Rösler (2011) have reported that alpha oscillation is related to the activation of stored information. Other researchers have found conflicting results regarding the EEG activities in stress compared to non-stress conditions. Hayashi *et al.* (2009) have found that beta activity was higher in the frontal and temporal areas in non-stress than in stress conditions under emotionally unpleasant stimuli. They induced stress by audio-visual stimuli. McNaughton *et al.* (2013) have reported that theta brain rhythmicity is reduced by all anxiolytics. While Gold *et al.* (2013) have

reported that the suggestion that frontal midline theta as a biomarker for anxiety is still unclear. 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 of further studies. Furthermore, the insignificant differences found in our research could be attributed to the basal level of stress that is expressed by the medical students in general even in the non-examination periods. This suggestion can be explained by the hard nature of the study as well as the high concerns of students about their future, academic achievement and future profession (Singh *et al.*, 2012). In addition, females are more liable to be affected by academic stressors, and they have a high level of emotionality (Rana and Mahmood, 2010).

In the present work; no significant change in the expression of frequency band analyzed by the peak power frequency was found. There was no change in the mean relative power of all frequency bands during both time periods. Becker *et al.* (1982) have studied the effect of menstrual cycle phase on the EEG. They found that there are cyclical changes in alpha activity. They have reported slower alpha waves during the follicular phase and faster alpha waves during the luteal phase. Krug *et al.* (1999) studied the EEG oscillation across three phases of the menstrual cycle (ovulatory phase, luteal phase, and menses) during relaxation and mental tasks. They have documented that alpha power didn't change across the cycle during mental tasks while during mental relaxation it was generally decreased in the luteal phase compared to the ovulatory phase and menses. On the other hand, they have found that theta activity was lowered during the luteal phase compared to the ovulatory phase and menses during mental tasks and this lowering was more pronounced during relaxation. The results of the current study are not in line with the results of the previous studies. The reason for this conflict could be due to the difference in methodology and again could be attributed to the emotional

behavior and the basal chronic level of stress expressed by medical students.

Schoofs and Wolf (2009) have declared in their study that they were the first to study the effect of acute stress on memory retrieval with naturally cycling women. They tested 24 hours of delayed free recall under stress and control conditions. They found no effect of acute stress on delayed memory retrieval on women in the luteal phase. While their previous studies with similar study protocols on males showed contrasting results. They have reported that the underlying factor of these conflicting results was the elevated levels of gonadal steroids during the luteal phase which might lead to decreased glucocorticoid sensitivity. They have reported that choosing females in one cycle phase and depending only on the self-reported the identification of the cycle phase were limitations of their study. In the present study, we tried to avoid the limitation of the Schoofs and Wolf, (2009). We identified the phases of the ovarian cycle based on the history and also by the progesterone and estrogen levels.

The current study still has some limitations that should be managed in further studies. We didn't assess the degree of stress, which may have some implications on the results. Another limitation is the current study is the relatively small sample size, although it was out of our hands, as we had to specify the phase of cycle and to apply the memory tasks and EEG recording at the same phase, rather than adjusting these factors to be in non-examination and examination periods of time during the academic year. In conclusion, this study provides an insight into the effect of academic examination stress on brain oscillations during delayed memory retrieval tasks during the luteal phase of the menstrual cycle. To our knowledge, it is one of the few published studies that considered the cyclic changes in qEEG oscillations during examination stress in female medical students. We recommend the application of a similar study on a larger population taking into consideration to grade the

stress (mild, moderate and severe). Future studies should take into consideration the intervening factors that may interact with stress, e.g. depression. Assignments of a female population in the follicular phase of the ovarian cycle, to compare the results with females in the luteal phase to catch the effect of gonadal steroids on electroencephalographic oscillations are encouraged.

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## CONFLICT OF INTEREST

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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