VILNIUS UNIVERSITY

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WOMEN'S OF HEALTHY AND REPRODUCTIVE AGE REACTION TO AFFECTIVE IMAGES: RESEARCHES ON SUBJECTIVE EVALUATIONS, CENTRAL AND AUTONOMIC NERVOUS SYSTEM

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VILNIAUS UNIVERSITETAS

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SVEIKŲ REPRODUKTYVAUS AMŽIAUS MOTERŲ REAKCIJA Į EMOCIJAS SUKELIANČIUS VAIZDUS: SUBJEKTYVAUS VERTINIMO, CENTRINĖS IR AUTONOMINĖS NERVŲ SISTEMOS TYRIMAI

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CONTENTS

ABBREVIATIONS

ANOVA - analysis of variance

BHR - baseline heart rate

ECG - electrocardiography

EEG - electroencephalography

ELISA - enzyme immunoassay

ERP - Event Related Potential

GABA - Gamma-Amino-Butyric-Acid

HR - heart rate

IAPS - International Affective Picture System

LH - luteinizing hormone

M - mean

P4 - progesterone

PMDD- Premenstrual dysphoric disorder

PMS - Premenstrual syndrome

PR - progesterone receptors

SAM - Self Assessment Manikin

SD - standard deviation

SE - standard error

η^2 - partial eta squared

INTRODUCTION

Emotions are defined as episodic, relatively short-term, biologically based patterns of perception, experience, physiology, action, and communication that occur in response to specific physical and social challenges and opportunities (Planalp et al., 2009). We experience emotions in a wide range of situations when we feel the pleasure and/or displeasure. Although emotions are subjectively experienced, they can be probed and studied in laboratory conditions (Wilhelm and Grossman, 2010). Emotional state can be evaluated with both discrete or dimensional scales. The basic six emotions - anger, fear, happiness, disgust, sadness and surprise- are described as discrete. International Affective Picture System (IAPS) was devised as a reliable standardized method of eliciting emotions in experimental setting (Lang, et al., 2008). IAPS database consists of 1196 high resolution color photographs, such as buildings, babies, animals, trees or mutilated corpses. This variety of content is similar to a stimulation, which occurs in a real life setting, and covers the entire affective space. The IAPS is based on the dimensional view of emotions. The creators of the instrument emphasize two dimensions of emotional reactions: “pleasure” (valence) and “arousal” (Bradley & Lang, 2007). The former is represented by two separate motivational systems with their own neural pathways. Appetitive system is activated by a stimulus associated with survival and is responsible for the response of approach. Aversive/defensive system starts in the response to a threat stimulus and leads to avoidance response. IAPS is now used for studying emotions, cognition and other areas (Aguilar de Arcos, et al., 2005; Heponiemi et al., 2007; Sharp van Goozen & Goodyer, 2006; Staude-Müller, et al., 2008). Most often IAPS is used together with the Self-Assessment Manikin (SAM) – a self-report measure, in which emotional reactions are studied with a help of figures, accompanying each of the three scales, representing the three main dimensions. It highly correlates with the semantic differential scale based on subjective measures (Bradley & Lang, 1994). However, IAPS is also used in psychophysiological, behavioral and neuropsychological studies (Bradley & Lang, 2007).

A series of emotional events successively occur in temporal context. Emotions involve several physiological and psychological changes. Affective psychophysiology has the potential to contribute, in a significant and unique way, to our understanding of mental health and ilness because emotional disturbances are important features of psychopathology. Moreover, electrophysiological investigation has indicated that passive viewing of affective arousing, compared to nonarousing IAPS pictures, is associated with enhancement of the late positive potential (LPP) (Cuthbert, et al., 2000, Olofsson, et al., 2008). The LPP is a broad positive deflection that reaches maximum amplitude 300–1000 ms after the onset of the motivationally relevant stimulus (Foti, et al., 2009, Moser, et al., 2014) and it can last several seconds. LPP amplitude is more positive in responses to pleasant and unpleasant stimuli than in responses to neutral stimuli, and has a centroparietal maximum topography (Liu, et al., 2012; Cuthbert, et al., 2000). Although different data about reactions to IAPS stimuli exist, the effects of valence and arousal on ERP are not yet completely understood (Rozenkrants and Polich, 2008; Horan, et al., 2010; Olofsson, et al., 2008; Moser, et al., 2010; Liu, et al., 2012; Feng, et al., 2014). Many previous studies have found that the viewing of emotional pictures activates cardiovascular system (Lang et al., 1993; Bradley et al., 1996, 2001; Bernat et al., 2006). Heart rate deceleration is greater under the viewing of unpleasant pictures rather than the viewing of pleasant or neutral pictures. Thus, it is obvious that the fundamental factors of emotion, valence, and arousal elicit physiological and psychological responses. The prefrontal cortex and amygdala, in fact, represent the essence of two specific pathways: affective elicitations longer than 6 seconds allow the prefrontal cortex to encode the stimulus information; briefly presented stimuli access the fast route of emotion recognition via the amygdala (Valenza, et al., 2014).

Recently, an increasing number of studies on emotional processing has been carried out by using neuroimaging techniques and considering gender differences (Lithari, et al., 2010; Andreano and Cahill, 2009; Cahill, 2006; Hamann, 2005). Despite the interest in these issues, most of the studies view these phenomena from psychiatric point of view (Hoeksema, 2012). As a result, there is a lack of studies that focus on the mechanisms related to emotional processing in healthy individuals and take gender differences into account. It is believed that women are more sensitive to emotion-causing situations; besides this, women have a better emotional memory for unpleasant events than men (Rozenkrants and Polich, 2008, Lithari, et al., 2010; Whittle, et al., 2011). Women sex hormones estradiol and progesterone are not only involved in the control of reproductive physiology and behavior, but have been found to more broadly modulate cognitive as well as emotional processes (Ferage et. al., 2008, Lebron-Milad and Milad, 2012, Van Wingen et al., 2011). The latter is, for example, illustrated by symptoms of anxiety and depression being particularly common during periods with extreme changes in ovarian hormone levels, such as during postpartum and perimenopausal periods (Moser-Kolko et al., 2009, Schmidt and Rudinow, 2009). However, effects of ovarian hormones on cognitive-emotional processing have also become evident on the basis of more subtle fluctuations of these hormones as they occur during the menstrual cycle (Sacher et al., 2013). Estradiol levels are low during the early follicular cycle phase, peak before ovulation in the late follicular phase and decrease to a moderate level during the luteal phase, whereas progesterone levels are low during the follicular phase and peak at the mid-luteal phase (Sacher et al., 2013). The growing evidence for menstrual cycle - specific differences suggest a modulating role for sex hormones on the neural networks supporting the integration of emotional and cognitive information (Sacher, et al., 2013; Golstein, et al., 2010; Ferree, et al., 2011; Hoyer, et al., 2013). Morever, the effect of menstrual cycle on cognitive and emotional processing at electrophysiological and behavioral levels is not well established (Kujawa, et al., 2012). Researchers revealed that LPP for different facial expressions during the periovulation phase was larger than during the premenstrual phase (Zang, et al., 2013a). A study carried out by using ERP found that the late positive component (LPC) was increased in women reacting to sexual stimuli compared to neutral in ovulatory phase (Krug, et al., 2000). The other study suggested that although ERP correlates of memory, as measured by the ERP repetition effect, are stable across the menstrual cycle, a correlate of context updating processes is responsive to hormonal fluctuations throughout the menstrual cycle (O'Reilly, et al., 2004). A recent study showed that the LPP to IAPS images of different attractiveness was larger during the late follicular phase than during the luteal phase, but correlations between the LPP amplitudes and the arousal ratings were significant only in the luteal phase (Zhang, et al., 2013b). A new study showed that the N2 amplitude for negative visual stimuli was larger in luteal phase than in follicular (Wu, et al., 2014). Another study showed that while performing classic emotional memory tasks, retention of emotional stimuli in memory was changing depending on the menstrual cycle phase (Ertman, et al., 2011). Despite the fact that these data showed the effect of the menstrual cycle phase on ERP, still the verification of phases by hormone concentration is missing in the so far carried out studies. The level of ovarian hormones was tested in the recent study by Zhang et al. (2015) which revealed that the LPP changes throughout the menstrual cycle phases partly are mediated by progesterone. This study revealed that both neuroticism and menstrual cycle phase, modulated the LPP amplitude (Zhang et. al., 2015). In this study the main effect of the menstrual cycle phase on LPP amplitude was not significant in 300-1000 ms time window. In contrast, the group and phase did not affect the subjective ratings of the affective pictures (Zhang et. al., 2015).

However, at present no study has been carried out which tested the possible effects of menstrual cycle phase on subjective ratings, LPP amplitude and phasic heart rate in response to images of various attractiveness.

The aim of our sudy was to investigate the influence on the ratings of affective images based on the levels of healthy women‘s sex steroid hormone and to compare the responses of central and autonomic nervous system.

Tasks:

1. to verify suitability of IAPS images for the investigation of women's emotions in Lithuania.
2. to test the influence of the sex steroid hormone level on affective images ratings.
3. to investigate the dynamics of central nervous system in response to the images of different affective content with event related potential method, considering the menstrual cycle phase.
4. to investigate the dynamics of autonomic nervous system in response to the images of different affective content with phasic heart rate response method, considering the menstrual cycle phase.

Statements of defend (or hypotheses)

- International affective picture system (IAPS) can be used for women's emotions studies in Lithuania.
- Subjective ratings of different affective images depend on the levels of sex steroid hormone.
- Mean late positive potential amplitude (LPP) depends on the content of affective stimulus, but does not depend on the levels of sex steroid hormone.
- Phasic heart rate response depends on the content of affective stimulus, but does not depend on the levels of sex steroid hormone.

1. THE UNIVERSALITY OF IAPS: EVALUATIONS FROM A SAMPLE OF LITHUANIAN FEMALE

The aim of the first study was to determine the valence, arousal and dominance ratings of IAPS pictures in the sample of Lithuanian female students and compare them with the original United States (US) female norms (n=50) (Lang et al., 2008).

1.1. Methods

1.1.1. Participants

82 psychology female students aged 20 (±2.60), were recruited for the research through class advertisements. For the homogeneity of the sample all the participants were psychology students from Mykolas Romeris University. The study was conducted according to the ethical guidelines of the American Psychological Association’s (APA's) Ethics Code. The participants reported normal or corrected-to-normal vision and good general health.

1.1.2. Materials

The research was performed using the IAPS (Lang et al., 2008), which consists of photographs with a wide range of semantic categories, divided into 20 sets of about 60 photos in each. In this study the 20th set from the IAPS was used with total of 59 images, because at the time of the study the pictures were the latest and consisted to be of the highest resolution. The photographs show food, chess, children, romance, spider, mutilation, car damage, and etc. The specific content of each image is described in IAPS manual (Lang, et al., 2008).

According to the normative data for the US female population (Lang et al., 2008), 20 of the pictures were unpleasant (valence ratings ranged from 1 to 3,99), 20 pictures – neutral (ratings from 4 to 5,99) and 19 pictures were pleasant (ratings from 6 to 9). Mean values (±SD) of ratings in US and LT female sample for the images of each group are presented in Figure 1.1. Pictures were presented randomly, avoiding the sequence of two and more pictures with the same valence.

1.1.3. Evaluation

Emotions of participants were measured by using SAM (Self-Assessment Manikin) method. It is based on the three groups of figures. Each of the group reflects one of the three emotional dimensions: valence (from happy to sad), arousal (from aroused to calm) and dominance (from dominated to dominant). The Participant had to choose three figures, which represented their current emotion (valence, arousal and the aspect of dominance).

1.1.4. Procedure

Each participant was given a consent letter, explaining the features of the experiment. The instructions were based on the standardized guidelines, proposed by Lang and colleagues (2008). The experiment began with three test photographs (4200, 7010, 3100), which helped the participants to understand the standardized presentation format and instructions for the evaluation. Each picture was presented as follows: at first, a slide with the text “Get ready to evaluate the next photo” appeared for 5 seconds, then the photograph was displayed for 6 seconds. After that, the instruction “Write your answer on a sheet x, line x” appeared for 15 seconds.

1.1.5. Data analysis

SAM scales were recorded in the way that higher values indicated more positive valence, higher arousal, and greater feelings of self dominance. Paired Student's t-test and Pearson correlation coefficient were used for analyzing the differences between Lithuanian and US female samples as well as relations between the different dimensions. The alpha level for the significance was set at $p<0.05$.

1.2. Results

The present study used images, which, based on the emotional ratings from US sample, were divided into three main image categories: pleasant, neutral and unpleasant. The average SAM ratings of the each picture were provided by the IAPS manual (Lang, et al., 2008). Such division was used throughout the further analysis of results (it was applied separately for all the images, pleasant, neutral and unpleasant). In all the cases identical images from a specific category were used for the comparison of US and Lithuanian female samples.

Figure 1.1 shows the typical 'boomerang shape' of the distribution of the ratings of IAPS images in the bidimensional (Valence × Arousal) affective space for Lithuanian (n=82) and US (n=50) females.

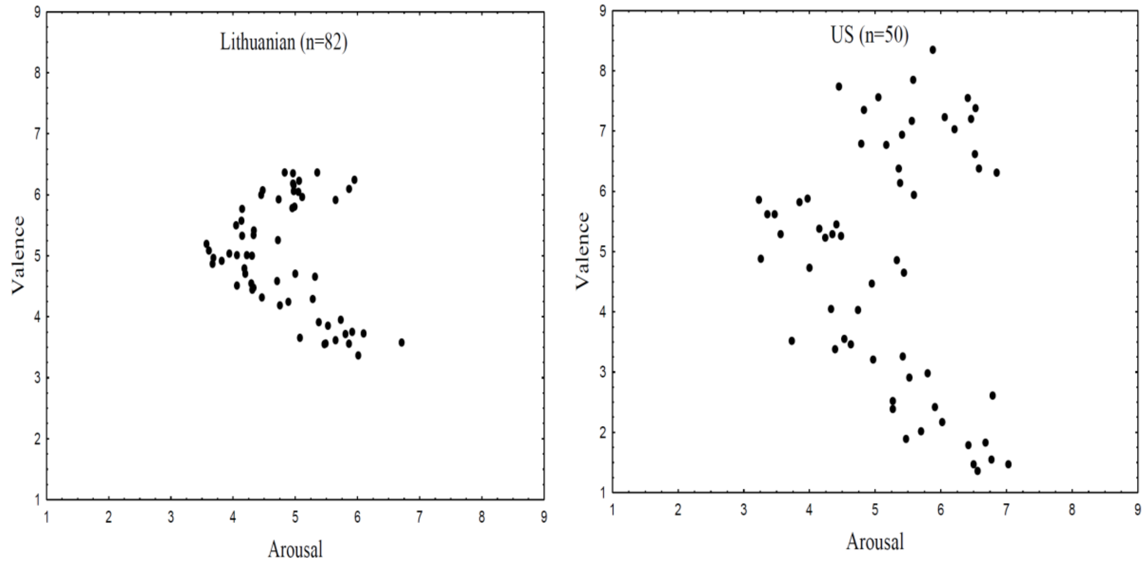

Figure 1.1. Distribution of mean values (for Lithuanian and US females) for 59 IAPS images in the valence and arousal affective dimensions.

Ratings of all the images in the female group revealed no significant differences for valence and dominance ratings ($p>0.05$). Nevertheless, there was a significant difference between samples in arousal ratings: US participants rated arousal of images higher than Lithuanian ($p=0.02$). While comparing the female ratings of pleasant pictures only, there were also significant differences between the two samples. The valence ratings of pleasant pictures were higher in US female sample (6.82±0.47) compared to Lithuanian (5.62±0.24, $p<0.01$). There was a significant difference between the samples for arousal ratings of pleasant pictures ($p<0.01$). In Lithuanian sample arousal (5.14±0.31) was lower than in US sample (5.66±0.72). Valence ratings of unpleasant pictures were lower in US female sample (2.75±0.75) as compared to Lithuanian female sample (4.31±0.29, $p<0.01$). Arousal ratings did not significantly differ between samples ($p=0.11$). There were also no significant differences in ratings of dominance dimension between the pleasant and unpleasant pictures ($p>0.05$). The pleasant pictures were rated as less pleasant and arousing in Lithuanian female group, while unpleasant pictures were less unpleasant compared to US female sample. Ratings of neutral pictures for valence and dominance did not differ significantly between the samples in the female group ($p>0.05$) (Figure 1.2.).

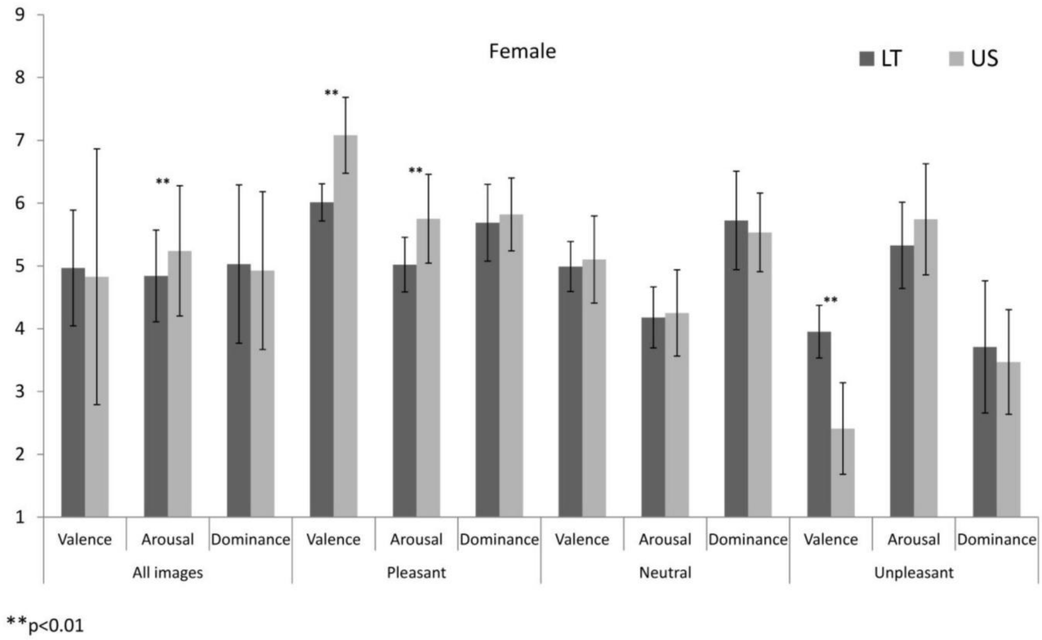

Figure 1.2. Mean values and standard deviations of the ratings of affective pictures for valence, arousal and dominance by Lithuanian and US female participants.

Finally, it must be mentioned that emotional evaluations of images in the Lithuanian female sample positively correlated with US sample in all the three dimensions (Table 1).

Table 1. Pearson correlation between Lithuanian and US ratings for valence, arousal and dominance dimensions for females in all, pleasant, neutral and unpleasant images.

	All images	Pleasant	Neutral	Unpleasant
Valence	0.96	0.60	0.72	0.75
Arousal	0.85	0.66	0.76	0.81
Dominance	0.91	0.77	0.79	0.80

all p<0,01

Although the Lithuanian and US sample's ratings correlated highly, there was a difference in the range of mean ratings. The average valence ratings of different images varied from 3.37 to 6.37 in Lithuanian female sample. In other words, 57.63 % from the 59 selected images were rated from 4 to 6 (i.e. representing neutral emotions), and 22.03

% of them were below 4 (unpleasant), 20.34 % - above 6 (pleasant). The ranges of mean ratings varied much more in the US female sample – from 1.36 to 8.35. 33.89% of them were below 4 (unpleasant), 33.89% – between 4 and 6 (neutral), 32.2% – above 6 (pleasant).

The limited spread of ratings in Lithuanian female sample was also observed in the arousal dimension. 94.92% of the images had an average arousal rating between 3.57 and 6. In comparison, 13.56 % of mean ratings of US female participants were in the range of below 4, 59.32 % in the range from 4 to 6 and 27.12% in the range of above 6.

Nevertheless, the results showed that Lithuanian ratings were dependent on the pleasure category of pictures, which was based on valence ratings according to US norms (that is one third of the images belonged to unpleasant, one third – to neutral, and one third – to pleasant category).

2. THE ESTIMATION OF AFFECTIVE IMAGES IN DIFFERENT PHASES OF MENSTRUAL CYCLE

The aim of the second study was to investigate the influence of levels of sex steroid hormone on evaluating the affective pictures with different attractiveness, regarding the menstrual cycle phase.

2.1. Methods

2.1.1. Subjects

Thirty university students (12 men and 18 women), mean age 23.1 ±2.4 years, participated in this study. Almost all the subjects in the study participated in the four experimental sessions. The female in the early follicular phase (1-4 days of menstrual cycle, n=17), ovulatory phase (24-48 h after positive LH test results, n=16), middle luteal phase (6-8 days after ovulation, n=17) and in the late luteal phase (before menstruation, n=12). One female in the study participated in ovulatory, middle luteal and late lutel phase, but not in follicular phase. All the men in the study participated in the four experimental sessions as a control group.

2.1.2. Materials

Thirty-six images were selected from the International Affective Pictures System (IAPS) and grouped into four sets of nine photographs each: three pleasant, three neutral and three unpleasant. In each experimental session 9 randomly selected IAPS images from the sets 1-4 (9 from set 1 for the first experimental session, 9 from set 2 for the second, 9 from set 3 for the third and 9 from set 4 for the fourth) (Lang et al., 2005). Participants were seated 1 m from a computer monitor as images were presented in a full screen. The images were presented in a random order, and each stimulus was presented for 6 seconds. Subjective emotional reactions to the pictures were assessed using the Self-Assessment Manikin (SAM) (Lang et. Al., 2005). The SAM scale was presented for 15 s. To respond, the participants marked an indicator on any of the five pictographs for each scale. This yielded ratings between 1 and 9 for each dimension, with higher scores indicating greater pleasure or arousal, depending on the scale. The task for the participants was to rate the pictures in valence and arousal scores using the Self-

Assessment Manikin (SAM) instrument. The dominance scale was not included in this study.

2.1.3. Procedure

All the subjects participated in the four experimental sessions. The Intervals between the men's experimental sessions corresponded to the women's inter-session intervals determined by phases. The women were investigated during the follicular, ovulatory, middle luteal and the late luteal phases of menstrual cycle confirmed by salivary 17β-estradiol and progesterone assessment. The mean values of estradiol and progesterone are presented in Figure 2.1.

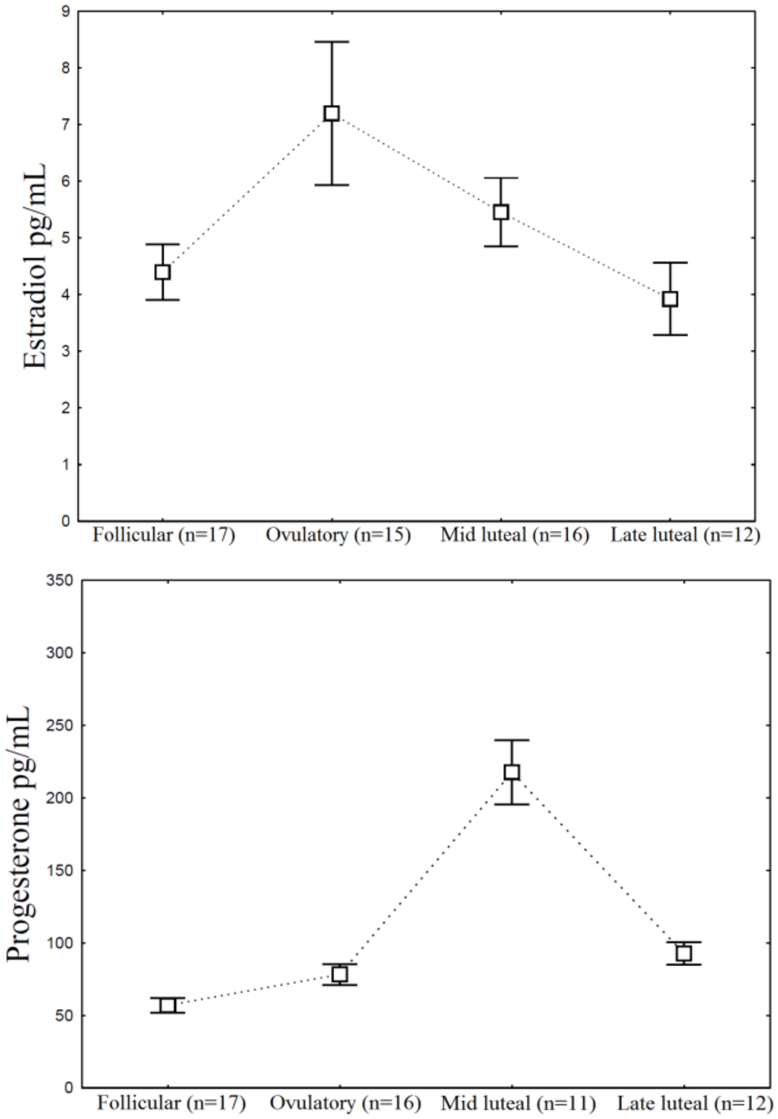

Figure 2.1. Mean value (±SD) estradiol and progesterone levels in women saliva during the follicular , ovulatory, middle luteal and the late luteal phase.

2.2. Results

The mean valence and arousal ratings for different content images during menstrual cycle phases were analyzed by repeated measures 3 (Stimuli Content: pleasant, neutral, unpleasant) x 4 (Menstrual cycle phase: follicular, ovulatory, middle luteal, late luteal) ANOVA. For valence scores the main effect was stimuli content $F(2, 116)=369.06$, $p<0.01$, $\eta^2=0.86$ and interaction between factors (stimuli content x menstrual cycle phase) $F(6, 116)=5.82$, $p<0.01$, $\eta^2=0.23$, but there was no significant effect of phase $F(3, 58)=0.16$, $p=0.92$, $\eta^2=0.008$. Post hoc Fisher LSD test showed that pleasant images were rated with higher valence scores in ovulatory phase than in the middle luteal ($p=0.01$) and the late luteal phase ($p=0.01$). The neutral images were rated with higher valence in the follicular phase rather than in the ovulatory phase ($p=0.02$). The unpleasant images were rated with higher valence scores in the middle luteal phase rather than in the follicular phase ($p<0.01$).

The repeated measures ANOVA analysis showed also, that the main effect for arousal scores were stimuli content $F(2, 116)=79.16$, $p<0.01$, $\eta^2=0.58$ and interaction $F(6, 116)=2.60$, $p=0.02$, $\eta^2=0.12$ between the factors, but there was no significant effect of the menstrual cycle phase $F(3, 58)=1.30$, $p=0.28$, $\eta^2=0.06$. The ratings of pleasant images by arousal scores were higher in ovulatory phase than in the middle luteal phase ($p=0.04$). The ratings of neutral images by arousal were higher in the follicular phase in comparison with the ovulatory ($p=0.02$) and in the late luteal phase ($p=0.03$). All mean valence and arousal values (±SD) are shown in the Figure 2.2.

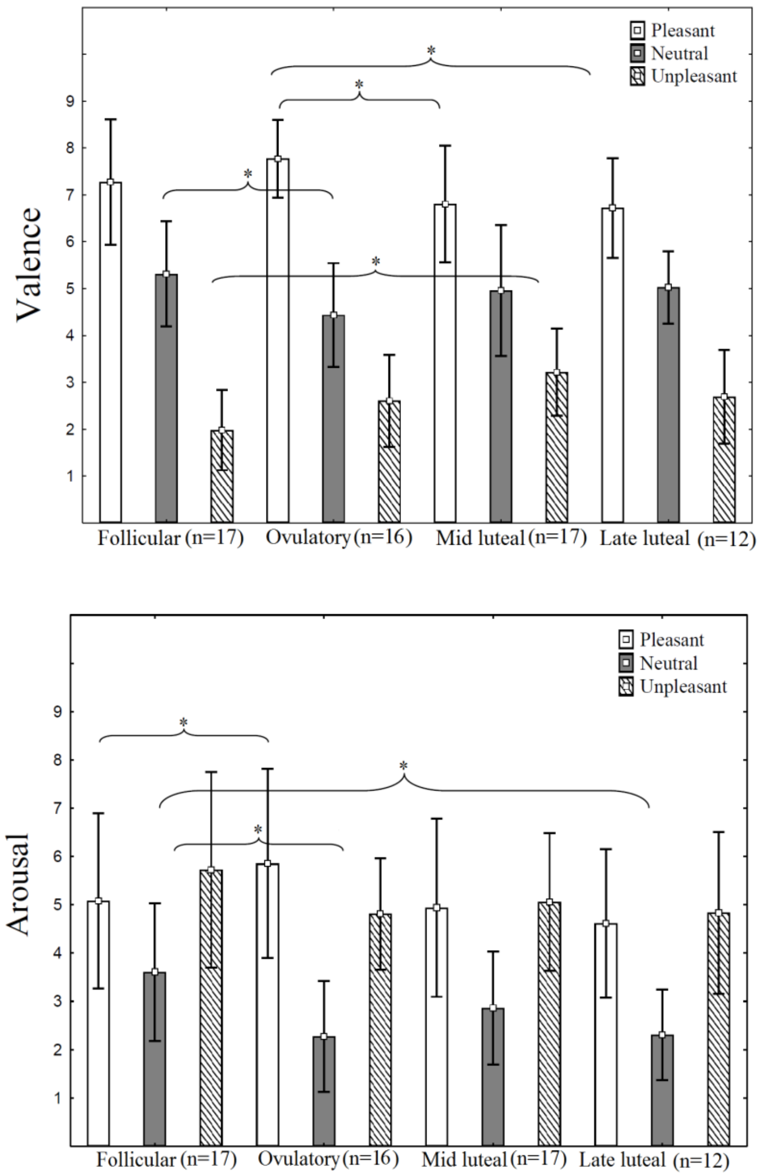

Figure 2.2. Mean valence and arousal rating scores (±SD) during follicular (n=17), ovulatory (n=16), middle luteal (n=17) and late luteal phase (n=12) for pleasant, neutral and unpleasant images.

These results showed that the menstrual cycle phase influence on the image ratings by the valence and arousal scores.

Ratings of the images with different content in ‘male only’ group also were analyzed by repeated measures 3 (Stimuli content: pleasant, neutral, unpleasant) x 4 (Experimental session: first, second, third, fourth) ANOVA. Our results showed that the experimental session (or IAPS stimuli set (from 1 to 4)) was not a significant factor for image evaluation by the valence and arousal scores. For valence ratings the main effect was only stimuli content $F(2, 80)=102.41$, $p<0.01$, $\eta^2=0.72$, but the experimental session $F(3, 44)=0.65$, $p=0.59$, $\eta^2=0.04$ and the interaction between the factors $F(6, 88)=0.74$, $p=0.62$, $\eta^2=0.05$ were of no significance. For images ratings by arousal scores also the main effect was only stimuli content $F(2, 80)=50.56$, $p<0.01$, $\eta^2=0.56$, but not of the the experimental session $F(3, 44)=0.01$, $p=0.99$, $\eta^2<0.01$ and the interaction between them $F(6, 88)=1.09$, $p=0.37$, $\eta^2=0.07$. All the values (±SD) are shown in Figure 2.3.

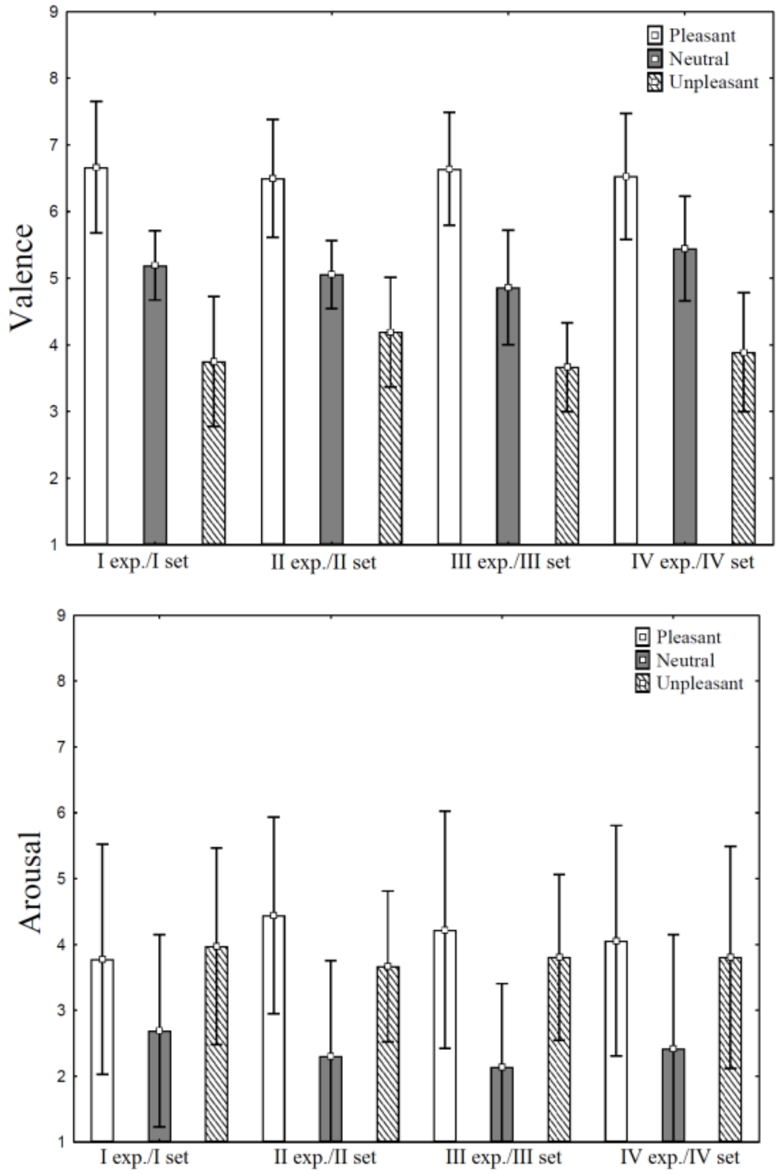

Figure 2.3. Mean valence and arousal rating scores (±SD) during first experimental session (I set), second (II set), third (III set), fourth (IV set) (in all experimental sessions participated 12 male) for pleasant, neutral, unpleasant images.

In the second part of the analysis the ratings of the images with different content by different sex was compared (menstrual cycle phase and experimental session day was not included). The mixed ANOVA 3(Stimuli content; pleasant, neutral, unpleasant) x 2(Gender: female, male) showed that gender $F(1, 108)=5.63$, $p=0.02$, $\eta^2=0.05$, stimuli content $F(2, 216)=396.13$, $p<0.01$, $\eta^2=0,79$ and interaction between them $F(2, 216)=25.65$, $p<0.01$, $\eta^2=0.19$ are the main effects for image rating by the valence scores**.** Post hoc Fisher LSD tests showed that pleasant images are rated with higher valence ($p<0.01$) and arousal ($p<0.01$) scores in female group than in male. Neutral image ratings did not differ between the female and male groups (valence, $p=0.29$; arousal, $p=0.16$). Unpleasant images were rated with lower valence ($p<0.01$) and higher arousal ($p<0.01$) scores in the female group than in male. All values (±SD) are showed in Table 2.1. These results showed that female in general are more aroused by pleasant and unpleasant images than male.

Table 2.1. The mean (±SD) valence and arousal ratings scores for all (n=36), pleasant (n=12), neutral (n=12) and unpleasant (n=12) images in female and male groups.

		All images	Pleasant	Neutral	Unpleasant
Female	Valence	4.91 (2.17)	7.17 (1.19)	4.94 (1.16)	2.62 (1.02)
	Arousal	4.36 (1.93)	5.15 (1.82)	2.81 (1.30)	5.13 (1.61)
Male	Valence	5.20 (1.37)	6.58 (0.89)	5.14 (0.70)	3.88 (0.84)
	Arousal	3.44 (1.67)	4.13 (1.67)	2.39 (1.45)	3.81 (1.37)

To explore the relationships between the sex hormone levels and subjective ratings correlations were calculated between these measures in all the female subjects, excluding the menstrual cycle phase. No significant relationship were found between estradiol levels and image ratings by valence (pleasant $r=0.2$, $p=0.14$; unpleasant $r=0.12$, $p=0.38$) and arousal (pleasant $r=0.25$, $p=0.07$; unpleasant $r=0.03$, $p=0.84$) scores. Neutral image ratings by valence ($r=-0.19$, $p=0.17$) and arousal ($r=-0.12$, $p=0.38$) scores showed no significant correlation with estradiol levels. Whereas progesterone levels significantly positively correlated with unpleasant image ratings by valence scores ($r=0.33$, $p=0.01$) (Figure 2.4). Pleasant ($r=-0.17$, $p=0.20$) and neutral ($r=-0.04$, $p=0.81$) image ratings by valence scores showed no significant correlation with progesterone levels. Moreover, ratings of pleasant (r=-0.04, p=0.77), neutral (r=-0.21, p=0.12) and unpleasant (r=-0.04, p=0.80) images by arousal scores showed no significant correlation with progesterone levels.

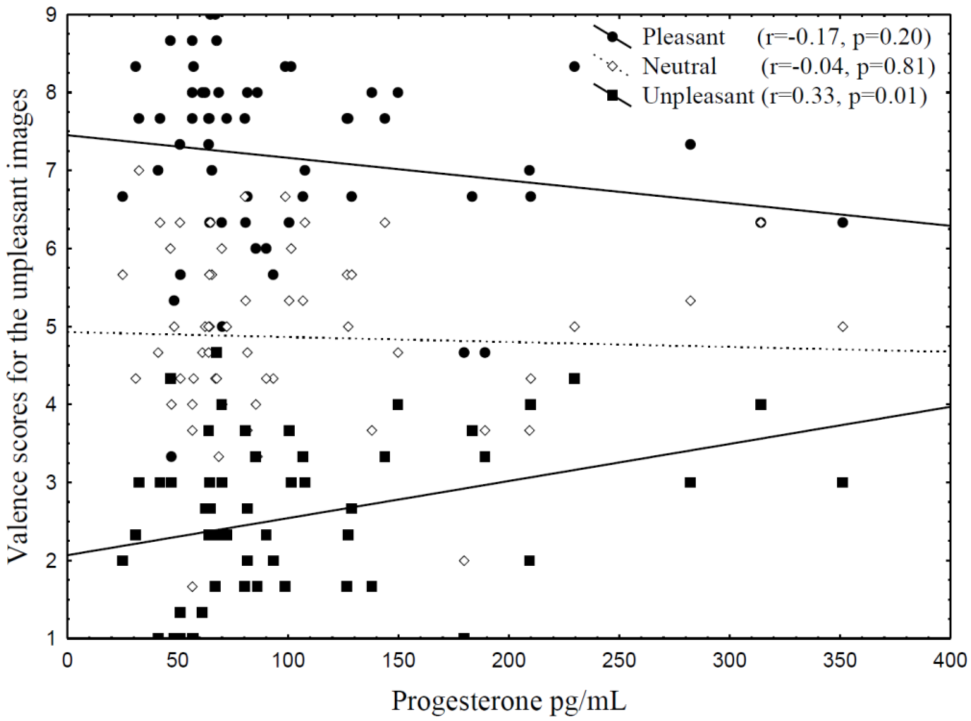

Figure 2.4. Scatterplots of valence scores for the unpleasant, pleasant, neutral images and progesterone pg/mL levels for all the female participants, not considering the menstrual cycle phase.

These results indicated that pleasant, neutral and unpleasant image rating by valence and arousal scores depend on the menstrual cycle phase. The ratings of affective images by men were stable and independent of experimental session day. Pleasant and unpleasant images in general were rated as more arousing by females than by males. The correlation analysis revealed that unpleasant image ratings by valence scores depend on the progesterone level in female saliva, not considering the menstrual cycle phase.

3. THE EFFECTS OF THE MENSTRUAL CYCLE PHASE ON THE EMOTION PROCESSING: A COMBINED ERP, HR CHANGES AND SUBJECTIVE RATINGS STUDY

The third study aimed at examining the differences between the estradiol and progesterone levels in women's salivary and the influences it has on the valence and arousal ratings of the same IAPS images and comparing mean late positive potential (LPP) amplitudes, phasic heart rate responses evoked by pleasant, neutral and unpleasant images.

3.1. Material and methods

3.1.1. Participants

Twenty eight healthy females aged 21-28 (mean age 24.9 years, SD=2.4) participated in the study. Fourteen were in their follicular phase ant the other were in their luteal phase. The Both groups did not differ in their age (p=0.08). The study was approved by the Lithuanian Bioethics Committe and the written consent was obtained from all the subjects prior to the experiment. Only females that reported regular menstrual cycle (24-34 days) and no intake of oral contraceptives or any hormonal threatment during the last 6 months were included to prevent any influences of external hormone administration. All volunteers were asked to refrain from alcohol consumption the night before and coffee intake one hour prior to the experiment. After the screening interview, women were asked to call as soon as they started their menstrual period and the experimental session was booked in one of the two experimental groups: follicular (between 5 and 9 cycle days) or luteal (between 20 - 25 cycle days). The follicular phase was determined by the subject's self report of the first day of her menstrual period. The luteal phase was determined by urine ovulation test strips (*Sure Screen Diagnostics* Ltd, UK), which were used by the study subjects at home according to the instructions by the study investigator. The Experiment was performed 7-10 days after the urinary LH indicated the occurance of ovulation. The actual day of the succeeding menstrual onset was confirmed for each woman by the phone or via e-mail.

3.1.2. Salivary analysis

In order to measure gonadal hormone levels 1 ml of saliva was collected at the test day (5 min after arrival to the laboratory). Saliva was collected in plastic tubes (IBL SaliCap) and kept frozen at -24 °C. Each participant provided a saliva sample at the beginning of each session. Salivary estradiol and progesterone levels were assessed using IBL (IBL International GMBH, Germany) ELISA kits. The sensitivities were 0.4 pg/mL for the determination of estradiol and 3.8 pg/mL for progesterone.

3.1.3. Stimuli

Ninety pictures were selected from the International Affective Picture System (IAPS).

These pictures varying in affective valence and arousal included pleasant, neutral and unpleasant stimuli. IAPS pictures were presented in a random order without repetitions of the same picture category and the particular picture. Participants were seated in an experimental booth facing a computer screen 100 cm in front of them. Each picture was presented for 6 seconds. The experimental design is shown in the Figure 3.1. Stimulating pictures were assessed by valence and arousal immediately after the stimulus offset. Presentation of the collection of stimuli and response were controlled by E-PRIME software (Psychology Software Tools, Inc) running on a PC. The presentation order of the pictures set was randomized for each subject. The pictures were rated using the Self-Assessment Manikin (Lang et. al., 2008). Pleasant and unpleasant images were selected from low to high arousal.

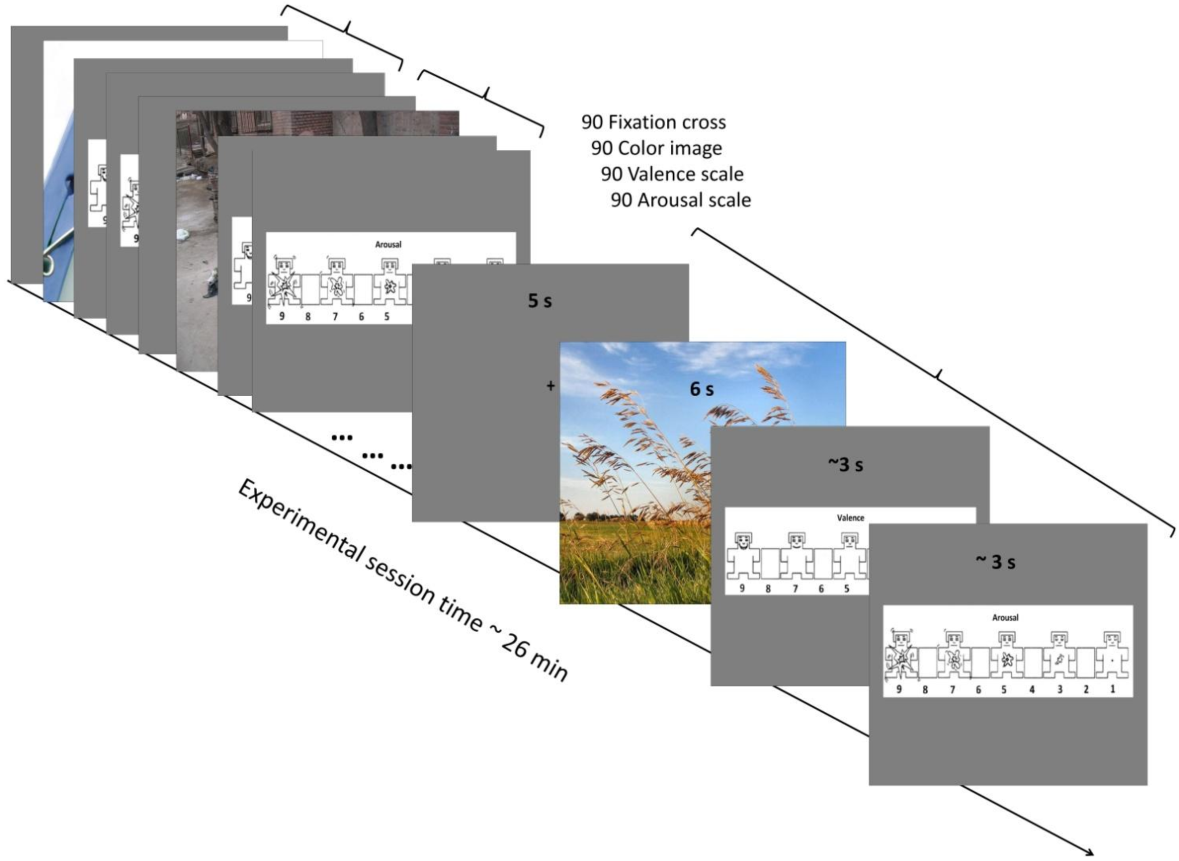

Figure 3.1. Experimental scheme

3.1.4. EEG recording and processing

EEG activity was continuously recorded during the picture viewing task. E-Prime software synchronized presentation of the visual stimuli and triggered EEG recording on each trial. The EEG data were collected using the Advanced Neuro Technology EEG system and a 64-channel Waveguard cap. The EEG data were sampled at 1024 Hz and then down-sampled to 256 Hz. The ground electrode was positioned at AFz and linked mastoids were used as the reference during recording. The average impedance of electrodes was below 10 kOhm.

Artifact removal and ERP analyses was performed using ASA 4.7.3 package (Advanced Source Analysis, ANT Corp.). Thereafter, a bandpass filter of 0.1 - 30 Hz was applied. Continuous EEG data was epoched from -200 ms to 2000 ms surrounding each stimulus presentation. Trials exceeding ±70 μV in magnitude were rejected. The ERP waveforms were analyzed using mean amplitude measures of individual electrodes. Following the previous research, where the strongest LPP amplitudes were reported at the parietal midline sites, we focused our analyses on Pz and CPz electrodes (Liu, et al., 2012; Cuthbert, et al., 2000). The visual inspection of the condition-averaged ERP waveforms across electrode sites revealed that the LPP began on average 450 ms after stimulus onset and continued, on average, till 950 ms after onset. The previous research (Foti et al., 2009; Weinberg and Hajcak, 2011) has demonstrated that important information about the time course of emotional responding may be reflected in the differences between early and late windows of the LPP. Early and late windows of the LPP were defined by splitting the full range of the LPP into two halfs (early: 450-700 ms and late: 700 - 950 ms). These time intervals were used to average signals at each site (Figure 3.2). A 2000 ms window was defined for the ERP epochs, including 200 ms prestimulus baseline.

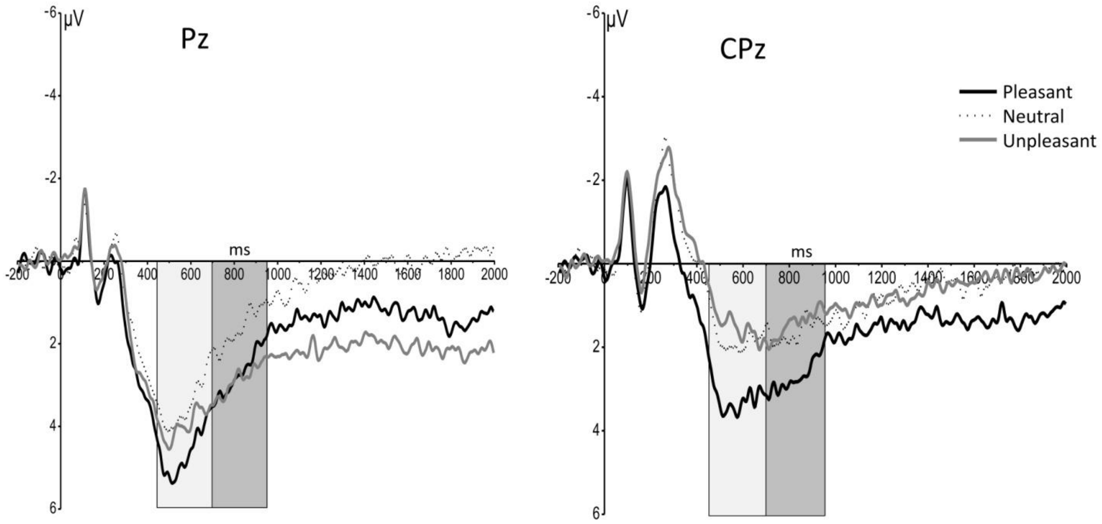

Figure 3.2. Grand average ERP waveforms at electrode sites Pz and CPz evoked by pleasant, neutral and unpleasant images across a 200 ms (pre-stimulus) to 2000 ms (post-stimulus) recording period for all subjects; the early time window (450-700 ms) and the late time window (700-950 ms) used in the analysis are indicated by the light and dark grey color boxes, (n=23).

3.1.5. ECG recording and processing

Electrocardiogram (ECG) was monitored via the Biopac System (www.biopac.com). The E-prime and Biopac systems were synchronized using a marker. The data were sampled at 1000 Hz and stored on a computer running Acqnowledge 3.9.1 MP100 data acquisition software. The ECG signal gain was set at 1000. The low pass (LP) filter for ECG was initially set to 35 Hz and the high pass (HP) filter was set to 0.5 Hz. The time between successive R waves (RR interval) was identified via the MP100 and manually checked and verified.

To quantify phasic stimulus related to heart rate (HR) changes, R-waves were detected from the ECG data and R–R intervals were converted to HR (in beats per minute). The HR in the last second prior to the image onset represented the prestimulus baseline (BHR). Poststimulus difference scores (ΔHR) were derived by subtracting the prestimulus baseline value from the HR-score of each poststimulus 0.5 second. The data were first subjected to visual inspection; only completely artifact-free data were used for estimation of the R-R intervals. Each participant was comfortably seated and fitted with Ag-AgCl adhesive disposable electrodes (lead I, Einthoven). ECG was recorded (sampled at 1kHz) and analyzed by a computer-based data acquisition system and its software Acqnowledge (BIOPAC System Inc., Santa Barbara, CA). The data was first subjected to visual inspection; only completely artifact-free data were used for estimation of the R-R intervals. Then, interbeat intervals were converted to HR as beats per minute (bpm) per real time epoch (500 ms). Each rate was proportionally weighted to the period of time that it occupied. To assess the phasic HR responses, epoch between 2 and 4 seconds after onset of the stimulus were analyzed. Phasic HR responses were scored as task minus baseline change scores in 1/2 second increments.

3.1.6. Statistical analysis

The data was analyzed with the STATISTICA Version 8.0. An independent t-test was performed to compare the levels of hormones between follicular and luteal phase groups. For the subjective ratings, mean valence and mean arousal per picture for three - all subjects, follicular and luteal phase – groups was calculated and results compared by using two-sample t-tests. ERP data was analyzed using 3 (Stimuli content: pleasant, neutral, unpleasant) x 2 (Menstrual cycle phase: follicular, luteal) x 2 (Electrode sites: CPz, Pz) mixed ANOVA in early (450-700 ms) and late (700-950 ms) time windows seperately. Phasic HR changes were analysing using 3 (stimuli content: pleasure, neutral, unpleasant) x 2 (Menstrual cycle phase: follicular, luteal) mixed ANOVA for all the participants. The Data of the four subjects in the follicular and luteal phase groups was excluded because of the noisy EEG signals. Hence, the final subjective ratings and ERP analysis was performed for 10 females in the follicular and 13 in the luteal phase. The Significant main effects and interactions were followed by the post hoc test Fisher LSD. The Values of $p<0.05$ were considered significant. To investigate the relationships between the hormone levels, valence/arousal ratings and mean LPP amplitudes, phasic HR changes Pearson correlation analysis was performed.

3.2. Results

3.2.1. Salivary hormones

Salivary ELISA indicated that mean estradiol and progesterone in follicular phase group was 5.28 ± 2.83 pg/mL and 43.69 ± 14.54 pg/mL, respectively. In the luteal phase group mean estradiol was 5.11 ± 1.95 pg/mL and mean progesterone was 211.23 ± 106.87 pg/mL. The independant t-test indicated higher progesterone in the luteal phase ($p<0.01$), while no significant difference in the estradiol levels ($p=0.88$) was detected.

3.2.2. Subjective ratings by SAM

The mean valence and arousal ratings for each image were analyzed by the independent t-test. Valence scores caused by pleasant images, but not by neutral ($p=0.53$) and unpleasant ($p=0.07$) images were rated significantly higher in follicular phase group rather than in luteal group ($p=0.01$). Arousal ratings for pleasant ($p=0.86$), neutral ($p=0.10$) and unpleasant images ($p=0.16$) did not differ~~ed~~ significantly between menstrual cycle phase groups. The mean (±SD) valence and arousal ratings are shown in Table 3.1.

Based on our hypotheses two separate comparisons in follicular and luteal phase groups of stimuli content on valence and arousal ratings were performed. Valence scores in both phase groups caused by pleasant images were higher than those caused by neutral (Follicular: $t(58)=12.13$, $p<0.01$; Luteal: ($t(58)=12.58$, $p<0.01$) and unpleasant (Follicular: $t(58)=31.75$, $p<0.01$; Luteal: $t(58)=29.97$, $p<0.01$). Also valence ratings caused by neutral images were higher than caused by unpleasant (Follicular: $t(58)=13.97$, $p<0.01$; Luteal: $t(58)=-11.85$, $p<0.01$). Arousal scores in both phase groups caused by the neutral images were lower than by the pleasant (Follicular: $t(58)=9.53$, p<0.01; Luteal: t(58)=10.83, p<0.01) and unpleasant (Follicular: t(58)=-10.54, p<0.01; Luteal: t(58)=11.85, p<0.01). There were no significant differences between arousal scores caused by pleasant and unpleasant images in the follicular phase group (t(58)=-0.88, p=0.38). On the contrary, in the luteal phase group arousal scores caused by unpleasant images were significantly higher than caused by the pleasant images (t(58)=-2.39, p<0.01).

Table 3.1. The mean (±SD) valence and arousal ratings scores in follicular (n=10) and luteal (n=13)phase groups .

	Pleasant	Neutral	Unpleasant
Follicular		*Mean (±SD)*	
Valence	7.20 (0.43)	5.26 (0.76)	2.71 (0.64)
Arousal	5.60 (1.05)	3.49 (0.60)	5.84 (1.06)
Luteal			
Valence	6.81 (0.48)	5.16 (0.76)	2.41 (0.64)
Arousal	5.64 (0.83)	3.73 (0.50)	6.22 (1.04)

3.2.3. ERP data

The effect of visual stimuli of different content, experimental conditions and menstrual phases on LPP mean amplitude was evaluated statistically using mixed ANOVA with stimulus content (pleasant, neutral, unpleasant) as within-subjects factor, phase (follicular and luteal) and recording sites (Pz and CPz) as between-subjects factors in the early and late time windows separately.

3.2.3.1. Early time window (450-700 ms)

The main effect of stimuli content was observed across the both sites (CPz and Pz) for all the participants F(2, 4)=10.22, p<0.01, η^2=0.20 with responses larger to pleasant versus neutral (p<0.01) and unpleasant (p<0.01) images. Responses to the neutral images fell between the two other stimuli content and did not significantly differ from the unpleasant (p>0.05). The main effect of electrode site was also observed F(1, 42)=8.08, p<0;01, η^2=0,16, but menstural cycle phase was no significant F(1, 42)=1.69, p=0.20, η^2=0.04. Responses to all images were higher at Pz site (mean 3.91±0.34) than at CPz (mean 2.01±0.2) site in early time window. The post hoc Fisher LSD test showed that mean LPP at CPz site to pleasant images was higher than to the neutral ($p<0.01$) and unpleasant ($p<0.01$). At Pz site responses to pleasant ($p<0.01$) and unpleasant ($p=0.02$) images were larger than to the neutral (see Figure 3.3). Stimuli content did not interact with electrode site or menstrual cycle phase. All mean LPP values are shown in the Table 2.

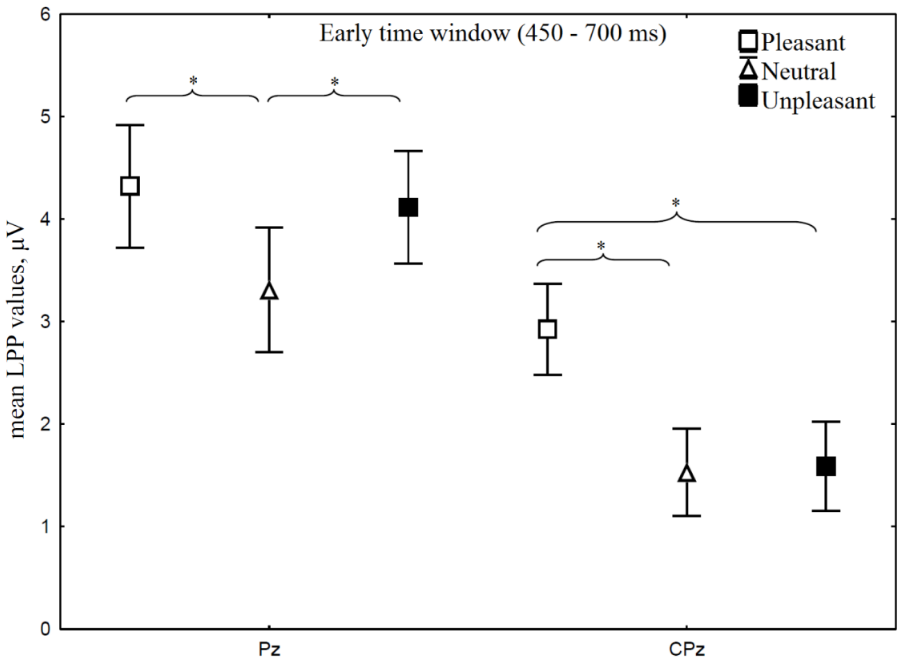

Figure 3.3. Mean LPP (±SE) amplitudes (microvolts) to pleasant (n=30), neutral (n=30) and unpleasant (n=30) images in early time window at Pz and CPz electrodes sites for all the subjects (n=23).

Table 3.2. The mean LPP (±SE) amplitudes (in microvolts) at both, Pz and CPz electrode sites in two time windows for pleasant, neutral and unpleasant images in all the subjects, follicular and luteal phase groups.

	Early (450-700 ms)			Late (700-950 ms)		
	Mean LPP μV (±SE)					
	Pleasant	Neutral	Unpleasant	Pleasant	Neutral	Unpleasant
All subjects						
Both	3.62 (0.38)	2.42 (0.39)	2.85 (0.39)	2.52 (0.35)	1.46 (0.38)	2.37 (0.37)
Pz	4.32 (0.60)	3.31 (0.61)	4.11 (0.55)	2.52 (0.54)	1.48 (0.61)	3.04 (0.59)
CPz	2.93 (0.44)	1.53 (0.43)	1.59 (0.43)	2.52 (0.44)	1.44 (0.48)	1.70 (0.42)
Follicular						
Both	2.86 (0.66)	2.17 (0.60)	2.38 (0.68)	1.97 (0.54)	1.15 (0.57)	2.07 (0.52)
Pz	3.71 (1.06)	3.11 (0.94)	3.70 (0.95)	2.14 (0.79)	1.27 (0.89)	2.80 (0.74)
CPz	2.01 (0.75)	1.23 (0.66)	1.06 (0.80)	1.80 (0.76)	1.03 (0.77)	1.34 (0.69)
Luteal						
Both	4.21 (0.42)	2.61 (0.52)	3.21 (0.46)	2.95 (0.44)	1.70 (0.52)	2.60 (0.52)
Pz	4.79 (0.68)	3.46 (0.83)	4.43 (0.65)	2.81 (0.75)	1.65 (0.86)	3.23 (0.89)
CPz	3.63 (0.46)	1.76 (0.57)	2.00 (0.46)	3.08 (0.49)	1.76 (0.63)	1.97 (0.52)

3.2.3.2. Late time window (700-950 ms)

Analysis at the late time window also revealed the main effect of stimuli content across sites for all participants $F(2,84)=8.33$, $p<0.01$, $\eta^2=0.17$ with responses larger to pleasant and unpleasant versus neutral images (both $p<0.01$) (see Figure 3). Responses to pleasant and unpleasant images did not differ significantly from each other. There was a significant interaction of stimuli content and electrode site $F(2,42)=3.73$, $p=0.03$, $\eta^2=0.08$. The post hoc Fisher LSD test showed that mean LPP at CPz site to pleasant images was higher than to the neutral ($p<0.01$) and unpleasant ($p=0.04$). At Pz site responses to pleasant ($p<0.01$) and unpleasant ($p<0.01$) images were larger than to the neutral (see Figure 3.4.). Responses to pleasant and unpleasant images did not differ significantly from each other. Stimuli content did not interact with the menstrual cycle phase. All mean LPP values are shown in the Table 3.2.

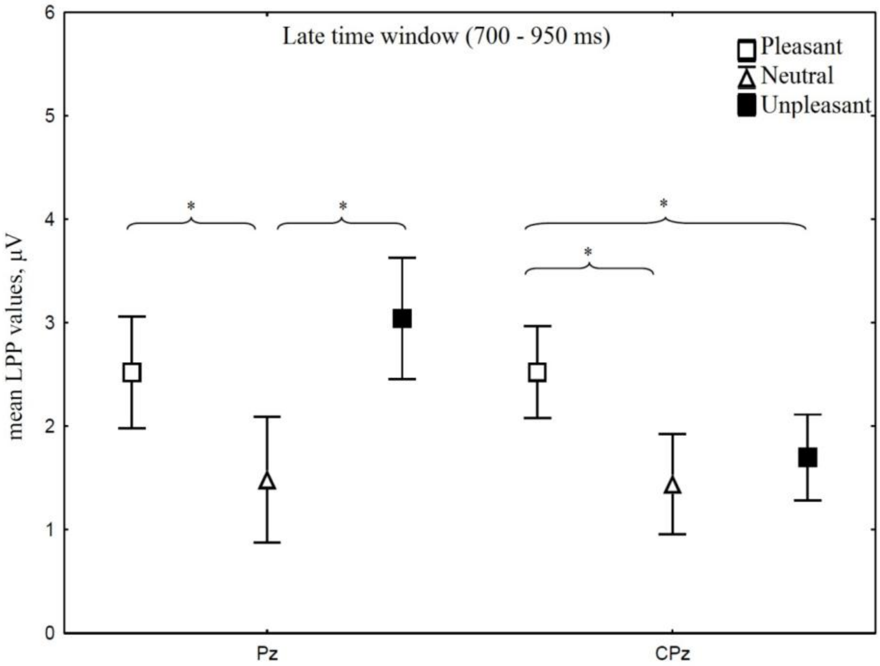

Figure 3.4. Mean LPP (±SE) amplitudes (microvolts) to pleasant (n=30), neutral (n=30) and unpleasant (n=30) images in late time window at Pz and CPz electrodes sites for all the subjects (n=23).

3.2.4. Heart rate responses

Pre-stimulus baseline heart rates did not differ between participants from follicular and luteal phase groups. Figure 3.4 shows the mean HR responses to the pleasant, neutral and unpleasant during the 6 s of stimulus presentation for all the subjects.

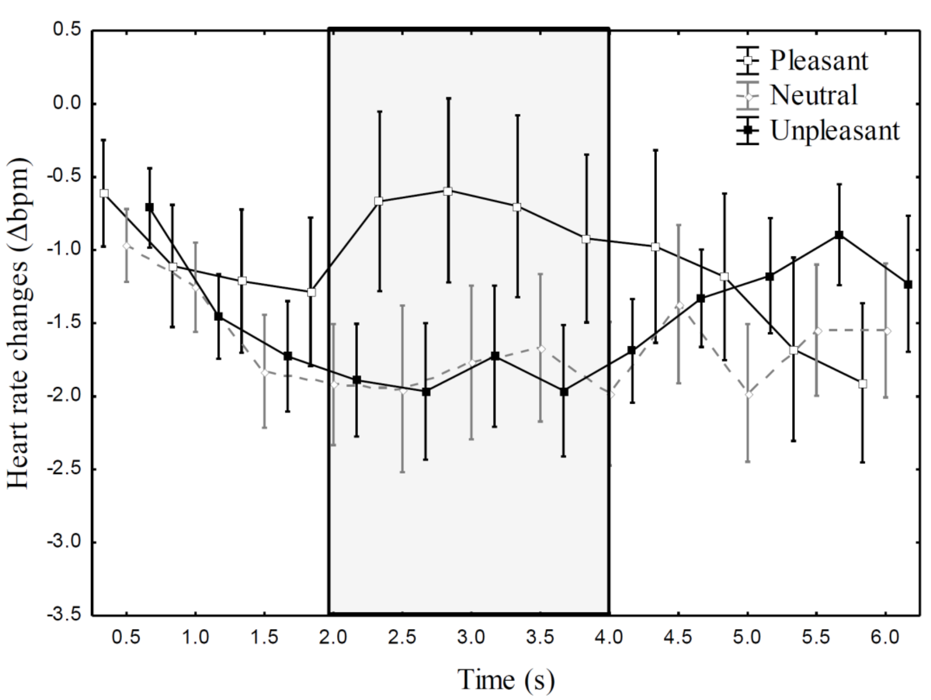

Figure 3.5. Mean heart rate (HR) responses to pleasant, neutral and unpleasant images for all subjects during the 6 s of stimulus presentation, relative to pre-stimulus baseline.

Mixed ANOVA analysis showed that for phasic HR responses during 2-4 seconds after stimulus onset the main effect was stimuli content $F(2, 40)=5.90$, $p<0.01$, $\eta^2=0.23$, but no significant effects on the menstrual cycle phase $F(1, 20)=0.44$, $p=0.52$, $\eta^2=0.02$ and interaction between the factors $F(2, 40)=1.59$, $p=0.22$, $\eta^2=0.07$. The neutral and unpleasant images prompt stronger average heart rate deceleration during 2-4 seconds compared to pleasant ones, as illustrated in Figure 3.6.

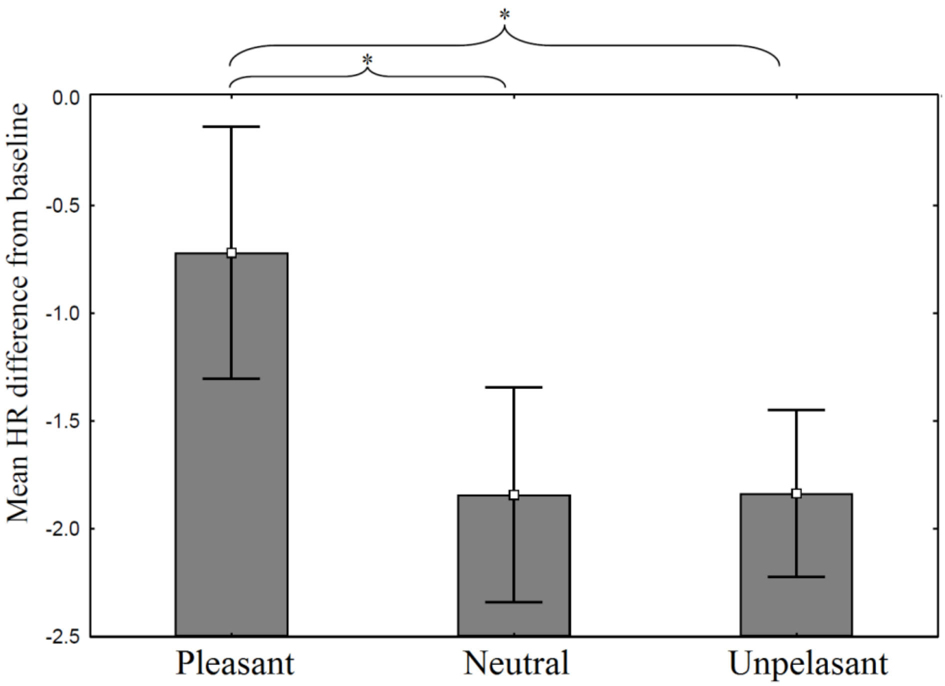

Figure 3.6. Mean HR responses to pleasant, neutral and unpleasant images (n=22).

3.2.4. Correlation analysis

To explore relationships between sex hormone levels, subjective ratings and mean LPP amplitudes, we calculated correlations between these measures in all the subjects, the follicular and the luteal phase groups separately. Significant negative relationships between the progesterone level and arousal ratings of pleasant (r=-0.73, p=0.03) and unpleasant images (r=-0.69, p=0.04) was found only in the luteal phase group (Figure 3.7).

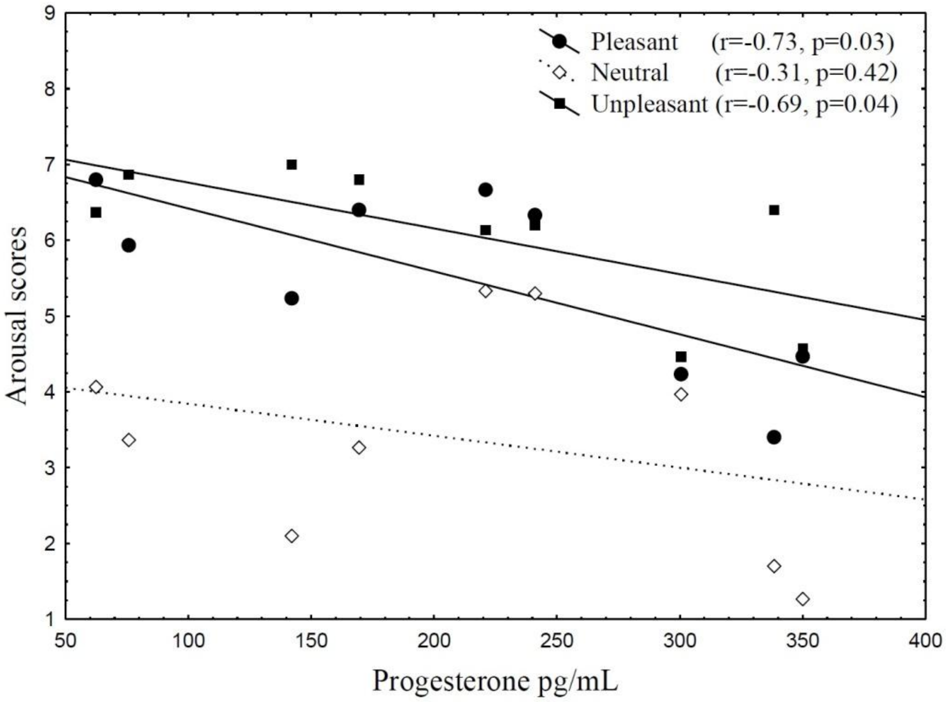

Figure 3.7. Scatterplots of arousal scores for the pleasant, neutral, unpleasant images and progesterone pg/mL levels for the luteal phase group (n=9).

Additionaly, there was a positive correlation between the progesterone level and mean LPP amplitudes at CPz site in the late time window in response to pleasant images (r=0.67, p=0.048) in luteal phase group. There is insufficient evidence to conclude that there is significant linear relationship between mean LPP amplitudes at CPz and progesterone level because the correlation coefficient is not very significant. All correlation values are shown in Table 3.3.

Table 3.3. Pearson correlation between mean LPP apmlitudes in early and late (at CPz and Pz sites) time windows, average phasic HR responses in 2-4 time window, subjective ratings and estradiol/ progesterone levels for all subjects, follicular and luteal phase groups.

		Estradiol pg/mL	Progesterone pg/mL	Estradiol pg/mL	Progesterone pg/mL	Estradiol pg/mL	Progesterone pg/mL
		All (n=18)		Follicular (n=9)		Luteal (n=9)	
Early mean LPP at CPz	Pleasant	0.18 (0.47)	0.35 (0.16)	0.33 (0.39)	0.35 (0.36)	-0.05 (0.91)	- 0.06 (0.88)
	Neutral	0.28 (0.26)	0.23 (0.35)	0.19 (0.36)	0.14 (0.73)	0.48 (0.19)	0.12 (0.76)
	Uneplasant	0.21 (0.42)	0.24 (0.34)	0.18 (0.65)	0.20 (0.61)	0.33 (0.38)	0.11 (0.77)
Late mean LPP at CPz	Pleasant	0.01 (0.96)	0.44 (0.07)	- 0.10 (0.80)	0.07 (0.86)	0.26 (0.49)	0.67 (0.048)*
	Neutral	0.15 (0.55)	0.33 (0.19)	-0.14 (0.72)	0.02 (0.96)	0.65 (0.06)	0.37 (0.33)
	Uneplasant	0.26 (0.31)	0.21 (0.40)	0.07 (0.86)	0.25 (0.51)	0.62 (0.07)	0.46 (0.21)
Early mean LPP at Pz	Pleasant	0.38 (0.13)	0.22 (0.37)	0.45 (0.22)	0.42 (0.26)	0.28 (0.47)	-0.04 (0.92)
	Neutral	0.42 (0.08)	0.12 (0.64)	0.33 (0.39)	0.26 (0.50)	0.60 (0.09)	-0.06 (0.89)
	Uneplasant	0.38 (0.12)	0.27 (0.28)	0.39 (0.30)	0.25 (0.51)	0.43 (0.25)	0.20 (0.61)
Late mean LPP at Pz	Pleasant	0.36 (0.15)	0.30 (0.23)	0.30 (0.44)	0.41 (0.27)	0.48 (0.19)	0.31 (0.41)
	Neutral	0.30 (0.23)	0.22 (0.39)	0.07 (0.85)	0.18 (0.64)	0.64 (0.07)	0.11 (0.78)
	Uneplasant	0.41 (0.31)	0.31 (0.21)	0.34 (0.37)	0.27 (0.48)	0.55 (0.12)	0.38 (0.32)
		All (n=16)		Follicular (n=10)		Luteal (n=6)	
Phasic HR responses	Pleasant	-0.22 (0.42)	0.08 (0.77)	0.01 (0.98)	- 0.11 (0.76)	- 0.66 (0.15)	0.30 (0.57)
	Neutral	-0.23 (0.39)	0.35 (0.18)	- 0.09 (0.82)	0.01 (0.98)	-0.53 (0.28)	0.50 (0.31)
	Uneplasant	- 0.32 (0.23)	0.31 (0.25)	- 0.08 (0.82)	- 0.14 (0.70)	- 0.79 (0.06)	0.64 (0.17)
		All (n=18)		Follicualar (9)		Luteal (n=9)	
Valence	Pleasant	0.14 (0.57)	-0.21 (0.39)	0.07 (0.85)	0.36 (0.34)	0.24 (0.53)	- 0.31 (0.42)
	Neutral	0.28 (0.27)	- 0.19 (0.46)	0.40 (0.28)	0.33 (0.39)	0.23 (0.56)	0.01 (0.98)
	Uneplasant	-0.05 (0.84)	- 0.26 (0.30)	- 0.12 (0.76)	- 0.15 (0.69)	0.16 (0.68)	- 0.41 (0.27)
Arousal	Pleasant	0.03 (0.92)	- 0.37 (0.13)	-0.18 (0.64)	-0.18 (0.65)	0.33 (0.39)	- 0.73 (0.03)*
	Neutral	0.003 (0.99)	- 0.19 (0.46)	- 0.11 (0.77)	- 0.09 (0.81)	0.13 (0.73)	- 0.31 (0.42)
	Uneplasant	- 0.10 (0.69)	- 0.16 (0.53)	- 0.12 (0.76)	- 0.15 (0.69)	- 0.01 (0.99)	-0.69 (0.04)*

These results suggested that subjective ratings of affective images could be related to the level of sex hormones for healthy women with regular menstrual cycle phase without emotional disorders symptoms. There were no significant differences between arousal scores caused by pleasant and unpleasant images in follicular phase group. Contarily, in luteal phase group arousal scores caused by unpleasant images significantly were higher than cased by pleasant images. Also, significant negative relationship between progesterone levels and arousal ratings of pleasant and unpleasant images was found only in the luteal phase group. Progesterone and estradiol levels do not influence on mean late positive potential amplitude and phasic heart rate responses during reaction to pleasant, neutral and unpleasant images.

4. DISCUSSION

The goal of this study was to investigate the influence of menstrual cycle on the subjective ratings of affective images and compare central and autonomic nervous system responses to images from IAPS. First of all, the ratings of IAPS pictures in the dimensions of valence, arousal and dominance in Lithuanian female sample were verified and compared to the original US female sample (Lang et al., 2008). The data provided valence, arousal and dominance ratings for images, which, based on the US norms, could be considered as pleasant, neutral or unpleasant. The results showed that there was a high correlation between Lithuanian and US female samples in all the three emotional dimensions. This supports the usability of IAPS in different cultural backgrounds. The similarity in Lithuanian and US participants' evaluations of images is also supported by the fact that there were no significant differences in the mean ratings of valence and dominance between the two groups. There was, however, a significant difference in arousal ratings. The latter fact was expected, because separate publications showed the inconsistency of arousal ratings in different studies (Drače et al., 2013, Dufey et al., 2011, Grühn & Scheibe, 2008, Lasaitis et al., 2008, Lohani et al., 2013, Moltó et al., 1999, Ribeiro et al., 2005, Vila et al., 2001). Such differences are usually explained by a cultural diversity. Lithuanians are usually considered as reserved people who avoid displaying emotions. Thus, it remains unclear, whether lower mean ratings of arousal in Lithuanian female sample show low arousal evoked by IAPS images or simply unwillingness of participants to report high ones. However, Bradley and Lang (2007) noted that IAPS can be sensitive for intercultural differences in emotional disposition. The reserved nature of Lithuanian people can also be the primary reason of clearly visible differences between Lithuanian and US female participants while comparing the variability of ratings. In the US sample the full range of evaluations was observed, whereas ratings concentrated around middle value (3.37 to 6.37) in Lithuanian female sample. Nevertheless, pictures, which were evaluated as more pleasant by US participants, where also rated higher in valence by Lithuanian female students. This shows that even though specific ratings might differ, the direction of the ratings remains the same. That is, the same pictures are evaluated as evoking positive emotions by both Lithuanian and US female participants. It is important to note some limitations of the study,namely the the stimuli set, which was chosen for the research. The results suggested that valence of emotions evoked by selected pictures were rated as close to neutral. That is why the future studies should consider using more arousing images and lager variety of them. Nevertheless, based on the results of the first study, it can be concluded that IAPS can be used for emotion studies in Lithuanian female, but the tendency of participants to use neutral values should be taken into consideration too.

The results of the second and third studies revealed that IAPS images subjective ratings depend on the menstrual cycle phase. Using the different experimental design the influence of the level of sex steroid hormone on subjective IAPS images ratings was compared. The second study revealed that women estimated pleasant pictures with a higher valence and arousal. Also, women rated unpleasant images with lower valence and higher arousal compared to men. The results of the present study confirm that females are more responsive to emotional stimuli then males (Lithari et al., 2010). These results also suggest that gender is an important factor for rating affective pictures in different cultures (Davis et. al., 2013). Considering the experimental session day the rating of affective images by men was stable and independent of experimental day. Women rated pictures depending on the phase of menstrual cycle. Correlation analysis demonstrated a significant positive relationship between progesterone level and valence ratings of unpleasant images. The third study showed that the rating of pleasant images by valence scores is a significant factor for the menstrual cycle phase groups. Pleasant images are more attractive for female in follicular than in the luteal phase. Whereas unpleasant images are more arousing than pleasant for women in the luteal phase. This confirms that womens sensitivity to pleasant and unpleasant stimuli could be influenced by progesterone levels (Derntl, et al., 2013, Ertman, et al, 2011). Other studies have suggested that the effect of progesterone follows an inversive - U function, rather than a linear correlation (Sakaki & Mather, 2012). Other study demonstrated, when females were tested at two time points with different level of progesterone, for example, they experienced more spontaneous intrusive recollections about unpleasant events when progesterone was high as compared to low progesterone (Ferree & Cahill, 2009). Similarly, compared to the late follicular phase (high estrogen and low progesterone), females in the luteal phase (high estrogen and high progesterone) were more sensitive to facial cues signaling contagion and physical threat (Conway et al., 2007) and increased their heart rates more while watching negative videos (Ossewaarde et al., 2010). Furthermore, a neuroimaging study (Andreano & Cahill, 2010) revealed increased amygdala activity to negative pictures (relative to neutral pictures) when progesterone was high as compared to low progesterone. Human studies employing exogenous progesterone administration also confirmed the facilitative role of progesterone. For example, females in the follicular phase showed increased amygdala activity to angry and fearful faces when they were given progesterone compared with placebo (van Wingen et al., 2008). Progesterone administration also increased physiological reactions to a stress task both in males (Childs et al., 2010) and females (Roca et al., 2003). Taken together, the present and other studies showed that progesterone increases reactions to negative stimuli in female. Furthermore, some studies also observed that progesterone administration weakened subjective reactions to negative stimuli (Childs et al., 2010). One possible reason for this inconsistency is that while progesterone level increases during normal menstrual cycles, enhances reactions to negative stimuli, concentrations of progesterone higher than the normal range might decrease emotional reactions and have calming effects (Andréen et al., 2009). In contrast, both lower and higher concentrations of progesterone produced less negative moods (Andréen et al., 2006). Thus, it appears that the effects of progesterone follow an inverse - U function, rather than a linear correlation. Perhaps, if there is too much progesterone, it no longer has facilitative effects and decreases reactions to negative stimuli (Andréen et al., 2005). Also other authors have suggested amygdala-mediated influences of allopregnanolone – a neuroactive metabolite of progesterone that is thought to influence anxiety-related behavior through its action on the $GABA_A$ receptor – as a possible explanation for finding (van Wingen, et al., 2011). For studies of emotion processing, it is important to consider the menstrual cycle phase, because there are links between sex hormones and female-related mood disorders (Andreano and Cahill, 2010, van Wingen, et al., 2011, Backstrom, et al., 2011, Melcangi, et al., 2011, Schule, et al., 2011, Haimov-Kochman and Berger, 2014, Sacher, et al., 2013). The findings provide new proof that subjective ratings of affective images could be related to the level of sex hormones for healthy women with regular menstrual cycle phase without emotional disorders symptoms.

There are still very few reviews describing the assessment of healthy women’s reactions to affective stimuli depending on the menstrual cycle phase, when anlyzing ERP and phasic heart rate responses. The present study showed that stimuli of different content induced a different mean amplitude of the late positive potentials (LPP), but the menstrual cycle phase did not have effect on this parameter. The results showed that in CPz site mean LPP amplitude to pleasant images was higher than to neutral and unpleasant at both time windows. Whereas at Pz site mean LPP amplitude to pleasant and unpleasant was higher than to neutral images. This result could be explained by the suggestion that for both pleasant and unpleasant pictures, BOLD activity in regions within midline parietal cortex is linearly correlated with the LPP (Liu, et al., 2012). Also this effect is consistent with the results of other studies (Rozenkrants and Polish, 2008, Horan, et al., 2010, Olofsson et al., 2008, Liu, et al., 2012, Feng, et al., 2014). In addition, it has been demonstrated that the LPP amplitudes reflect intensity of subjective emotional experience, as many studies observed that the LPP amplitude predicted the arousal of self-reported emotion evoked by salient stimuli (Moser, et al., 2010, Feng, et al., 2014, Schupp, et al., 2000, Keil, et al., 2002). The results of the present study support observation that the LPP modulation is valence and arousal specific (Rozenkrants and Polish, 2008, Horan, et al., 2010, Olofsson et al., 2008, Liu, et al., 2012, Feng, et al., 2014).

Moreover, the results are consistent with Zang et al. (2015) study results that mean LPP at the time window from 300 to 1000 ms is not influenced by the menstrual cycle phase. However there was a significant effect of menstrual cycle phase on mean LPP at the time window from 2000 to 4000 ms, but this result did not suggest that this time window is associated with emotion processing. Moreover, it is not clear whether this result reflect cognitive or emotion processing. The results also indicate, that the HR changes during affective processing depends on the valence of the stimulus (Kuniecki et al., 2003), but not on the mestrual cycle phase. Heart rate decelerated more during neutral and unpleasant picture viewing compared to pleasant pictures viewing. Larger heart rate deceleration to unpleasant pictures indicates that the defensive motivational system is activated for aversive stimuli, resulting in increased orientation and information intake. The results showed that healthy's women sex steroid hormones do not influence on mean late positive potential amplitude and phasic heart rate responses during the reaction to affective stimuli. Our results showed that progesterone levels influence on IAPS image's subjective ratings.

This is the first study to demonstrate that the same images from IAPS system could be rated by valence and arousal scores differently depending on the women menstrual cycle phase. It is difficult to decide which pattern is more suitable for emotional state research in different menstrual cycle phase. Usage of the same images in different phases for the same participants might cause habituation effects for subjective ratings and mean LPP amplitudes. This effect could be seen in Zang at al. (2015) study, where each female participated in experiment three times (during different menstrual cycle phases) and there were no significant differences between subjective ratings. Possible solution - use of different images from IAPS with the same valence and arousal, but in this case the question remains whether effect is determined by phase, or by stimuli content. One more possible limitation of the present study is that the personality traits were not evaluated. This might be important because it is known that neuroticism could modulate the LPP evoked by emotional pictures (Zang, et al., 2013b, Zang et al., 2015). Further research is important to test the different experimental designs for investigating the effect of menstrual cycle phase on emotional processing. In conclusion, the findings indicate the effect of menstrual cycle phase on subjective affective image ratings in healthy women reporting regular menstrual cycle. The data also point to the need for controlling the phases of the menstrual cycle in the future studies involving emotional processing (Guapo, et al., 2009, Haimov-Kochman and Berger, 2014, Sacher, et al., 2013, Sunstrom Poromaa and Gingell, 2014).

CONCLUSIONS

1. Internationl Affective Pictures System (IAPS) can be used for women's emotion studies in Lithuania.

2. Affective image subjective ratings depend on progesterone levels:

a) unpleasant image ratings by valence scores depend on progesterone levels during the menstrual cycle.

b) higher progesterone levels in the luteal phase are associated with the lower arousal ratings of pleasant and unpleasant images

3. Mean late positive potential amplitude depends on stimuli content, but not on the sex steroid hormone levels.

4. Phasic heart rate responses depend on the stimuli content, but not on the sex steroid hormone levels.

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SANTRAUKA (Summary in Lithuanian)

Reakcija į emocijas sukeliančius vaizdus gali priklausyti nuo sveikų reproduktyvaus amžiau moterų lytinių steroidinių hormonų lygio. Emocijas sukeliančių vaizdų vertinimui valentingumo ir sužadinimo balais gali daryti įtaką individualūs ir tarpkultūriniai skirtumai. Su įvykiu susijusių potencialų ir fazinio širdies susitraukimų dažnio atsakų metodai padeda atskleisti reakciją į emocinio turinio vaizdus. Sveikų moterų reakcija į emocijas sukeliančius vaizdus atsižvelgiant į lytinių steroidinių hormonų lygį yra mažai ištyrinėta. Šio darbo tikslas - nustatyti moteriškų lytinių steroidinių hormonų lygio įtaką reaguojant į emocijas sukeliančius vaizdus subjektyvaus vaizdų vertinimo, su įvykių susijusių potencialų ir fazinio širdies susitraukimų dažnio atsakų metodais. Tuo tikslu buvo atlikti trys tyrimai. Tyrimais įrodyta, kad Tarptautinė emocijas sukeliančių vaizdų sistema (IAPS) yra tinkama lietuvių moterų emocijoms tirti. Gauti rezultatai parodė, kad lytinių steroidinių hormonų lygis gali daryti įtaką vaizdų subjektyviam vertinimui. Padidėjus progesterono koncentracijai moters organizme geltonkūnio fazės metu nemalonūs vaizdai vertinami aukštesniais valentingumo balais. Taip pat gauti rezultatai parodė, kad geltonkūnio fazėje esančios moterys tuos pačius nemalonius vaizdus, kurie buvo rodomi ir folikulinėje fazėje esančioms moterims, vertina aukštesniais sužadinimo balais nei malonius. Taip pat geltonkūnio fazėje esančių moterų progesterono lygis neigiamai koreliavo su malonių ir nemalonių vaizdų vertinimu sužadinimo balais. Atlikus su įvykiu susijusių potencialų ir fazinio širdies susitraukimų dažnio atsakų analizę, reaguojant į emocijas sukeliančius vaizdus, moteriškų lytinių steroidinių hormonų įtaka nebuvo reikšminga. Vidutinė vėlyvojo teigiamo potencialo amplitudė aukštesnė į malonius ir nemalonius vaizdus lyginant su neutraliais, bet nuo lytinių steroidinių hormonų lygio nepriklausė. Širdies susitraukimų dažnis stipriau sulėtėjo reaguojant į nemalonius ir neutralius vaizdus nei į malonius, bet nuo lytinių steroidinių hormonų lygio nepriklausė.

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