ABSTRACT

Aim To investigate the role of sex hormones in the modulation of specific cognitive functions across the menstrual cycle of young healthy women, and to apply improved study design by addressing limitations recognized in previous studies.

Methods A homogenous group of 16 young healthy women, with no history of health problems related to menstrual cycle, major psychiatric and neurological disorders or addictions was included in study. All participants were medical students of similar age (21.56±0.15 year). They were subjected to various cognitive tasks at three different phases of the menstrual cycle: early follicular phase, proven ovulatory phase and mid-luteal phase. Special concern was taken to validate blood hormone levels and to determine preovulatory luteinizing hormone (LH)-peak.

Results Analysis of blood hormone levels confirmed that the test sessions were performed at appropriate time points. Most women were presented with the above average results on utilized cognitive tasks, with no significant changes in immediate memory, working memory, delayed recall, verbal learning, delayed verbal learning or verbal fluency in any phase of the menstrual cycle. In addition, test results did not correlate to measured hormone levels.

Conclusions The results suggest that changes in estrogen and progesterone levels during each menstrual cycle did not affect women’s everyday functioning to any significant extent.

Keywords: menstruation, estrogen, progesterone, cognition
INTRODUCTION

It has been observed that women’s cognitive functioning changes during their lifetime. This change in cognitive functioning has been attributed to fluctuations of estrogen (E2) and progesterone (PROG) (1). Investigations in the field of neurobiology have given evidence that sex hormones could govern some cognitive processes and are important for preservation and protection of normal brain structures (2). In contrary, latest studies on the effects of hormone replacement therapy (HRT) on the cognitive well-being in postmenopausal women have demonstrated disappointing results (3-6).

Estrogenic influence on the brain starts very early in prenatal life and continues throughout the postnatal period, when it seems to take part in sex specific organization of the brain (2,7). Its diverse effects can be explained by a diffuse distribution of E2 receptors (ER) in the brain and the influence of E2 on neurotransmitter systems (8-10). E2 receptor beta (ERβ) is thought to mediate cognitive rather than reproductive processes and appears to be the main ER subtype (11-13). Functional neuroimaging studies of women while undergoing specific cognitive tasks have demonstrated an association between tested cognitive functions and specific activated brain regions (14). It has also been demonstrated that E2 modulates the structure and function of the dorsal hippocampal formation, an area of the brain that governs memory and learning (15).

The effects of PROG on the brain are less known. PROG receptors (PR) have been found in the frontal cortex where its metabolites pregnanolone and allo-pregnanolone bind to the GABA-A receptor complex, potentiating GABAergic inhibitory mechanisms (16-19). Studies suggest that PROG could play an important role in facilitating cognition (20-22).

In contrast, recent meta-analyses on high number of randomized control trials on the effects of HRT and E2 replacement therapy (ERT) have yielded disappointing results (3-6). These studies showed that ERT and HRT do not prevent cognitive decline in older postmenopausal women when given as short term or longer term (up to five years) therapy (5). Some positive effects were mainly seen in short term studies (<4 months) (4). In addition, long term (HRT) application increased the risk of cancer (23,24).

EXAMINEES AND METHODS

Participants

In order to get more convincing results we applied a controlled design to our study, with strict and well defined rules (as described bellow).

Thirty six healthy young women aged 20-23 (M 21.56±0.15 year), all students at the School of Medicine Josip Juraj Strossmayer, University of Osijek (Croatia), volunteered to participate in this study. Participants were selected based on
the results of a survey carried out among second to fourth year medical students who were interested in participating in this study. The selected young women (a) had regular menstrual cycles (i.e. 28 ± 2 days) with no history of skipping cycles, (b) were not using oral contraceptives (OC) six months prior to engagement in this study, (c) had negative history of major neurological or psychiatric disease, (d) had no evidence of alcohol abuse or any kind of addictions and (f) were not using medications that are known to influence the central nervous system. All 36 young women who participated in this study met the inclusion criteria (assessed by a questionnaire prior to inclusion in the study). However, 17 women did not finish all three test sessions, either because they could not confirm ovulatory cycle (by luteinizing hormone (LH)-urinary strips, n=11) or because they did not show up on one or more test sessions (n=6). The study was done in collaboration with the Clinical Hospital Center Osijek. All participants gave their written informed consents and did not receive any compensation for taking part in this study. The study conformed to the standards set by the latest revision of the Declaration of Helsinki and it was approved by the Ethical Committee of the School of Medicine Osijek, Croatia.

Test sessions

Cognitive tasks were performed in three different phases of the menstrual cycle: (a) from the 2nd to the 4th day of the menstrual cycle in the EF phase (menstruation, early follicular phase), (b) in the confirmed O phase, and (c) 7 to 9 days post ovulation in midluteal phase (ML) phase. Most of the tested women (11/16.69%) completed the test sessions in the following order: EF, O, ML phase. Of these women, six were tested during a single menstrual cycle, whereas others were tested throughout at least two separate cycles. Five out of 16 women (31%) were tested in one of the different orders: O, ML, EF; ML, EF, O; EF, ML, O or O, EF, ML.

To confirm the date of ovulation, ELISA-based LH urinary strips were used (Biostrip LH Ovulation Test, Innovatek Medical inc., Vancouver, B.C., Canada). Hormone levels in the assigned phases of the menstrual cycle were assessed from venous blood to confirm menstrual cycle phases. Their mood was not assessed in this study, but the earliest (day 1) and last (day 25-28) cycle days were excluded to minimize the effect of cycle-related variations in the mood (27). In addition, the women were questioned for known symptoms of premenstrual syndrome.

Each test session was performed at the same part of the day to avoid circadian rhythm changes. One complete test session took 40 minutes.

Hormone levels

Serum levels of estrogen (E2), progesterone (PROG), luteinizing hormone (LH), follicular stimulating hormone (FSH) and testosterone (TESTO) were assessed from venous blood. The blood was taken at the beginning of each test session and immediately delivered to the Department of Nuclear Medicine and Radiation Protection at Clinical Hospital Centre Osijek; standard radioimmunoassay (RIA) kits were used (BioSource Europe S.A., Belgium). To confirm the preovulatory increase of LH, before the results of hormone levels measured from venous blood were available, commercial ELISA-based LH urinary stripes (Biostrip LH Ovulation Test, Innovatek Medical inc., Vancouver, B.C., Canada) were used.

Cognitive tasks

To examine global cognitive functions, the alternate form of Rey’s Auditory verbal Learning Test (AVLT) published by Lezak in 1995 was utilized (28). This cognitive task has shown to reflect not only specific verbal learning and memory, but also global cognitive functions (29-31). At each test session two lists (A and B) of 15 words were used, for repeated tests parallel lists of words were available. Five presentations of the list A were given, each followed by attempted recall. The first trial of the AVLT is a measure of immediate memory. After five trials a second 15-word list (list B, distraction list) was read and followed by a recall trial of this list and then another recall trial of the list A (measure of delayed recall). After a delay interval of 30 minutes and no further presentations of the lists, delayed verbal memory was assessed by recall trial of the list A.

Each participant underwent three types of verbal fluency tests: phonemic, semantic and ideational (28). Each test consisted of trials (90 second duration) directing participants to generate as many
words as possible (a) that began with a particular letter (‘F,’ ‘A’ or ‘S’) excluding proper names and variations of the same word, (b) that were exemplars of an unprimed semantic category (e.g. animals) or (c) that were exemplars of an unprimed ideational category (e.g. metal objects). The final score was given as a number of correct words generated during the first 60 seconds.

The Auditory Digit Span Task (ADST) is one of the Wechsler’s group of intelligence tests used to access the immediate and working memory function (32,33). A list of random numbers was used and read out loud at the rate of one per second. The participants were asked to repeat an increasing array of numbers in backwards (working memory) or forward order (immediate memory) until they committed an error.

The d2 Attention Loading Test was constructed by German psychologist Rolf Brickenkamp in 1962. We used an updated and revised form published in 1994. The d2 attention loading test is one of the general competence tests that has been used for measuring attention and ability to concentrate – sustained attention. In the present study, an official form of the attention loading test was used (Copyright by Hogrefe –Verlag GmbH & Co, KG Göttingen, 1994). It is a timed test composed of the letters „d” and „p” with one, two, three or four dashes arranged either individually and/or in pairs above and below the letter. There are 14 lines of 47 characters each. Subjects were given 20 seconds to scan each line and cross all the „d” marked with two dashes.

The test analysis appoints quality and quantity of processed data, in general and during the time of the test. Several variables were used for the test analysis, as follows: TN – total number of processed data, E – total errors (OE – omitted errors + RE – replacing errors), TN-E – quantitative measure defined by total number of processed data minus number of total errors, MC – mean concentration, qualitative measure calculated as a number of correctly processed data reduced for number of omitted errors. MC is a variable that protects from possible cheating on the test (random data processing cannot increase MC).

The Digit Symbol Substitution Test (DSST) is also a part of Wechsler’s Adult Intelligence Test, which has been used to evaluate attention, perceptual speed, motor speed, visual scanning and memory. The test consists of 9 digit-symbol pairs (e.g. 1/-, 2/┴ ... 7/Λ, 8/X, 9/=) followed by a list of digits. During the examination subjects were asked to substitute numbers on the list with corresponding symbols within a limited time period (120 seconds). The success on the test is reported as the number of correctly substituted symbols. Nevertheless, many of our participants were able to finish the task in less than 120 seconds preventing us from measuring the fine differences between the test sessions. Therefore, the results of the tests could not be presented here.

Statistical analysis

The sample size required for significant effect on studied variables was calculated on preliminary data in 9 subjects with alpha=0.05 and statistical power of 80% for paired t-test and ANOVA, showing a needed sample size of 16 participants. All data values are presented as means ± SEM and were analyzed by one-way ANOVA repeated measures or Friedman repeated measures analysis of variance on Ranks followed by post hoc Holm-Sidak or Tukey tests, when appropriate. Paired t-test was used to compare differences between the two groups. Correlation between hormone levels and scores achieved on certain cognitive tasks were analyzed by Spearman rank of order. Data distribution was tested by Shapiro-Wilk normality test. The level of statistical significance was determined at p<0.05.

RESULTS

Three of the 19 (3/19) participants that completed all three test sessions were excluded from the data analysis, because their hormone levels did not show the expected fluctuations over the menstrual cycle. Therefore, the results are based on data from sixteen healthy, normally cycling young women (M 23±0.25 year; range 22-24). The serum concentrations of hormones (taken at the time of testing) showed that the participants underwent cognitive test sessions at appropriate points of the menstrual cycle. All measured hormone levels were congruent with referent values set by the routine diagnostic laboratory where the blood was analyzed. The LH and E2 were the highest in the O phase which, together with the positive result of the ovulation detection kit, showed that all participants had ovulatory cycles.
and were tested on time. The E2 level during ML phase was at least twice as high as in EF phase (p<0.001). The E2 level in the O phase was significantly higher than in the ML or EF phase in all tested women (p<0.001). The highest PROG level was in the ML phase of the cycle (p<0.001). All participants had TESTO concentrations equal to referent values during the entire menstrual cycle (referral values: 0.38-2.74 nmol/L) (Figure 1 A-D).

All tested women showed above average results in most of the performed tasks for evaluation of cognitive function (Table 1 and 2). In addition, the results on the Digit Symbol Substitution Task (DSST) test could not be analyzed as most of the participants were able to finish their test before the testing time (120 sec) expired.

There was no significant difference found among phases in immediate memory measured as a number of correctly recalled words after the first read-

<table>
<thead>
<tr>
<th>Table 1. Scores achieved on tested cognitive tasks</th>
</tr>
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<tbody>
<tr>
<td>Cognitive function</td>
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<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Immediate memory</td>
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<td></td>
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<tr>
<td>Verbal learning</td>
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<td></td>
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<tr>
<td></td>
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<tr>
<td>Verbal fluency</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Attention - all</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Attention – randomized</td>
</tr>
<tr>
<td>(by the order of test sessions)</td>
</tr>
</tbody>
</table>

Figure 1. Levels of A) estrogen (E2), B) progesterone (PROG), C) luteinizing hormone (LH) and D) follicle-stimulating hormone (FSH) at the time of test sessions EF, early follicular phase, O, ovulatory phase, ML, mid-luteal phase of the menstrual cycle. *statistically significant difference p=0.001
In AVLT and as a number of correctly repeated numbers in forward order in ADST, respectively (Table 1). Having compared the phases, there was no significant difference in delayed recall or delayed verbal memory measured by AVLT. The 3rd recall in AVLT test was significantly reduced in O phase compared to EF phase, but not ML phase (p=0.037). This change could not be connected to hormone variations and its impact remains negligible as no similar tendency could be observed in other four recalls. In addition, the learning curve was of normal shape in O phase (Figure 2). There was no difference among any of the other recalls between the phases of the menstrual cycle. The learning curve in EF phase was slightly altered, showing decrease in 5th recall (Figure 2). This decrease was not significant when compared to scores achieved on 4th or 3rd recall. However, the score achieved on 5th recall was more frequently lower than the one achieved on 4th recall in EF phase when compared to O and ML phase (5/16 vs 2/16).

Verbal fluency (phonemic, semantic or ideational) did not vary among the menstrual cycle in tested subjects. Working memory was tested by ADST backwards and did not change among the cycle. First analysis of d2 attention loading task, including all 16 participants showed a significantly better performance (as measured by TN-E and MC) in the ML phase as compared to the EF phase of the cycle (p<0.001) (Table 1). However, further analysis of five participants that underwent test sessions in one of the randomized orders revealed that the test was susceptible to negative effects of anxiety caused by the limited time interval for the given task. Reduced level of anxiety by repeated test sessions resulted in significantly improved results achieved on the d2 test. The results of our study revealed that the d2 attention loading task can be learned by repeated testing, even when there was a period of a few weeks between the test sessions. Therefore, the results of d2 test had to be discharged. (TN-E and MC were p=0.008 and p=0.002, respectively) (Table 1). Further evidence was found when their results on d2 task were compared ordered by phase of the menstrual cycle, where we found no significant difference (TN-E and MC were p=0.270 and p=0.379, respectively) (Table 1). Each of five of these women had an increasing TN-E and MC score on repeated test sessions. In addition, there was no significant correlation found between test results and measured hormone levels for any of the used tasks (Table 3).

**DISCUSSION**

Although several studies have demonstrated changes in the working memory and verbal fluency during the menstrual cycle, the results are controversial.

<table>
<thead>
<tr>
<th>Cognitive function</th>
<th>Task</th>
<th>E2</th>
<th>PROG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate memory</td>
<td>AVLT (1) - first trial</td>
<td>0.114</td>
<td>0.442</td>
</tr>
<tr>
<td></td>
<td>ADST - forward</td>
<td>0.136</td>
<td>0.356</td>
</tr>
<tr>
<td></td>
<td>AVLT (2) - second trial</td>
<td>-0.087</td>
<td>0.558</td>
</tr>
<tr>
<td></td>
<td>AVLT (3) - third trial</td>
<td>-0.104</td>
<td>0.483</td>
</tr>
<tr>
<td></td>
<td>AVLT (4) - fourth trial</td>
<td>0.042</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td>AVLT (5) - fifth trial</td>
<td>0.305</td>
<td>0.037</td>
</tr>
<tr>
<td>Delayed recall</td>
<td>AVLT - delayed recall</td>
<td>0.248</td>
<td>0.092</td>
</tr>
<tr>
<td>Working memory</td>
<td>ADST - backwards</td>
<td>0.211</td>
<td>0.150</td>
</tr>
<tr>
<td>Delayed verbal memory</td>
<td>AVLT - late recall</td>
<td>0.193</td>
<td>0.193</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>Semantic task</td>
<td>-0.001</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>Ideational task</td>
<td>-0.062</td>
<td>0.679</td>
</tr>
<tr>
<td></td>
<td>Phonemic task</td>
<td>0.161</td>
<td>0.448</td>
</tr>
</tbody>
</table>

*Level of statistical significance was determined at p<0.05; results are presented as means± SEM E2, estrogen; PROG, progesterone;
ring the menstrual cycle, the results of this study did not confirm those findings (34-36). In most previously conducted studies women were tested only twice, in the EF and ML phase and observed improvements in tested cognitive functions during ML phase have been attributed to E2 (27,35,37). Even though participants in this study were tested at three different time points, under more strict and controlled conditions than many other studies, no change in working memory and verbal fluency was observed. Previous investigations discovered that women perform better in “women favoured” skills (i.e. verbal speed and fluency, fine motor skills) ML phase when the E2 and PROG levels are high, compared to the EF phase (34-36,38,39). Furthermore, when the hormone levels are low (menstruation, EF phase) they also show better performance in “men favoured” skills (i.e. mental rotations, target directed motor task, mathematical reasoning) (40,41). However, several studies have failed to show any kind of change in performance of cognitive tasks across the menstrual cycle (42,27). Mordecai et al. reported no cyclic changes in verbal memory, but the studies did reveal that oral contraceptive users performed better on the same task during the active pill phase (containing reproductive hormones) as compared to inactive pill phase (placebo). Studies on acute ovarian hormone withdrawal, caused by Gonadotropin Hormone Releasing Hormone agonists (GnRHa), demonstrated a sex-hormone influence on visual working memory (43,44). Conversely, Owens et al. did not find any effect of gonadotropine releasing hormone (GnRHa) mediated suppression of ovarian hormones (45). Studies outside the laboratory, in complex, everyday situations (e.g., flying for women pilots) failed to show any correlations between variations in women’s performance on a flight simulator and hormonal changes during the cycle (46).

The d2 attention loading task had to be discharged and no conclusions could be made about the influence of sex hormones on attention in this study. However, Solis-Ortiz and Corsi-Cabrera have found that sustained attention in the early luteal phase is favored by PROG and the visual-spatial memory by E2 in O phase (47). They used a principal component analysis to show that the PROG level and the success on attention task co-varied together. Hatta and Nagaya failed to show changes in memory but found enhanced attention measured as achievement on the Stroop task in ML phase (37). In the functional magnetic resonance imaging (fMRI) and positron emission tomograph (PET) studies, the Stroop task has shown to activate the same part of the brain known to possess a significant number of PROG receptor. In our study the learning curve (AVLT test) was slightly altered in EF phase, showing decrease in the 5th recall. This decrease was not significant when compared to scores achieved on the 4th or 3rd recall in the EF phase. However, score achieved on 5th recall was more frequently lower than the one achieved on the 4th recall in EF phase when compared to O and ML phase (5/16 vs. 2/16). Although not significant, this observation suggests reduced ability to maintain attention during the AVLT test in EF phase when hormone levels are low.

This study used a very strict design in terms of validation of cycle phases and a homogenous group of young women, but at cost of increasing the number of repeated test sessions for one subject. In addition, a lack of systematic randomisation of subjects further increased the risk of learning. Being aware of this, it utilized different forms of tests where it was appropriate (i.e. three lists of 15 words in AVLT, different forms of semantic and ideational verbal fluency). The limitation of this study could be that our tests were not sensitive enough to detect differences in cognitive function in intellectually over average women. However, this is unlikely, because used tests are standardized and used in clinical practice.

In conclusion, the lack of any measured effect of sex hormones on the tested cognitive functions, even though the experimental methodology was improved, raises once more the question of the significance of the influence of sex-hormones (E2 in particular) on everyday cognitive functioning of a young woman. Sex-hormone levels throughout the menstrual cycle and reproductive period of life seem to be sufficient for maintenance of cognitive wellbeing, and changes that happen during each menstrual cycle, normally, do not affect women’s everyday functioning to any significant extent.

ACKNOWLEDGEMENTS

Authors thank Mrs Pašezada Kosović from the Department of Nuclear Medicine, Clinical Hospital Centre Osijek, who performed measurements of sex hormone levels.
FUNDING

No specific funding was received for this study.

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Utjecaj menstruacijskog ciklusa na osnovne kognitivne funkcije u kontroliranoj skupini žena

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SAŽETAK

Cilj Istražiti utjecaj ženskih spolnih hormona na modulaciju specifičnih kognitivnih funkcija tijekom menstruacijskog ciklusa u mladih zdravih žena, te primjeniti poboljšani ustroj studije koji se temelji na opisanim ograničenjima prethodnih studija.

Metode U studiji je sudjelovalo 16 mladih zdravih žena, bez prijašnjih zdravstvenih problema vezanih uz menstruacijski ciklus, te neuroloških i psihijatrijskih poremećaja ili ovisnosti. Sve sudionice bile su studentice medicine, približno iste životne dobi (21.56±0.15 godina). Ispitanice su bile podvrgnute različitim testovima kognitivnih sposobnosti u tri faze menstruacijskog ciklusa: ranoj folikularnoj fazi, dokazanoj ovulaciji i srednjoj luteinskoj fazi. Tijekom studije posebna je pozornost posvećena određivanju koncentracije ženskih spolnih hormona u krvi, kao i određivanju preovulacijskog vala luteiniziranog hormona (LH).

Rezultati Analize koncentracije ženskih spolnih hormona u krvi potvrdile su kako su testiranja izvršena u odgovarajućim fazama menstruacijskog ciklusa. Većina ispitanica je imala iznadprosječne rezultate u korištenim testovima kognitivnih sposobnosti uz menstruacijski ciklus, kao ni psihiatrijskim i neurološkim poremećajima ili ovisnostima. Sve sudionice bile su studentice medicine, približno iste životne dobi (21.56±0.15 godina). Ispitanice su bile podvrgnute različitim testovima kognitivnih sposobnosti u tri faze menstruacijskog ciklusa: ranoj folikularnoj fazi, dokazanoj ovulaciji i srednjoj luteinskoj fazi. Tijekom studije posebna je pozornost posvećena određivanju koncentracije ženskih spolnih hormona u krvi, kao i određivanju preovulacijskog vala luteiniziranog hormona (LH).

Zaključak Promjene u fazama menstruacijskog ciklusa nisu bile značajne, te su se rezultati ispitivanja istovremeno sa korištenjem koncentracija hormona u krvi.

Ključne riječi: menstruacija, estrogen, progestron, kognitivne funkcije