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COGNITIVE EFFECTS OF HORMONE BASED CONTRACEPTION IN
WOMEN

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CONTENTS

ABBREVIATIONS	7
1. INTRODUCTION	8
1.1 Aim and objectives.....	10
1.2 Actuality and scientific novelty.....	10
1.3 Defended statements	11
2. METHODS	12
2.1 Participants.....	12
2.2 Tests of cognitive functions	13
2.2.1 Verbal fluency test	13
2.2.2 Mental rotation test	14
2.2.3 Manual speed and accuracy.....	15
2.2.4 Working memory	16
2.2.5 Spatial memory.	17
2.3 Mood	18
2.4 Personality traits.....	18
2.5 Hormones	19
2.6 Design and procedures	19
2.7 Statistical analysis	21
3. RESULTS	22
3.1 Hormones	22
3.2 The effect of experimental session on cognitive performance.....	23
3.3 Verbal fluency	26
3.4 Mental rotation (MRT).....	27
3.5 Manual speed and accuracy.....	30
3.6 Working and spatial memory	32
3.8 Mood	33
4.9 Personality traits.....	35
4. DISCUSSION	36
4.1. Verbal fluency.....	37
4.2 Mental rotation.....	38
4.3 Manual speed and accuracy.....	39
4.4 Memory	40
4.5 Mood.....	40

4.6 Personality traits.....	41
4.7 Limitations	41
CONCLUSIONS.....	42
REFERENCES	43
SANTRAUKA (Summary in Lithuanian)	50
PUBLICATIONS.....	51
ACKNOWLEDGMENTS	53
CURRICULUM VITAE.....	54

ABBREVIATIONS

CA – correct answers

E – extraversion scale (Eysenck questionnaire)

Es – 17 β -estradiol

LIA – luminescence immunoassay

MRT – mental rotation test

N – neuroticism scale (Eysenck questionnaire)

NA – negative affect (PANAS test)

NC – naturally cycling woman.

OC – hormonal (oral) contraceptives user

OC_A – user of hormonal (oral) contraceptives with androgenic properties

OC_{AA} – user of hormonal (oral) contraceptives with anti-androgenic properties

P – psychoticism scale (Eysenck questionnaire)

PA – positive affect (PANAS test)

Pr – progesterone

SD – standard deviation

SE – standard error

T – testosterone

1. INTRODUCTION

Hormonal contraception is one of the most popular methods of reversible contraception, which popularity and the usage duration are increasing worldwide (Schraudenbach and McFall, 2009;Skouby, 2010). Hormonal contraceptives act centrally and peripherally to inhibit follicular growth, reduce overall endogenous sex steroids levels and prevent monthly fluctuations (Gordon and Lee, 1993;van Heusden and Fauser, 1999). However the data how cognitive functions of hormonal contraceptives users are affected by changes in hormonal system induced by synthetic compounds are sparse and contradictory (Gordon and Lee, 1993;Mordecai et al., 2008;Rosenberg and Park, 2002;Rumberg et al., 2010;Wharton et al., 2008).

Action of sex steroid hormones on brain and behavior has been observed 160 years ago, when A. Berthold (1803–1861) performed studies with intact and castrated roosters (Meitzen and Mermelstein, 2011). E. Nicols observation in 1885 that men perceived colors at lower degree of saturation than women, was treated as scientific curiosity (Hampson, 2008). However nowadays there is well established that sex steroid hormones influence the nervous system and behavior. Numerous studies demonstrated that sex steroids acting permanently, temporarily, slowly and rapidly, via changes in gene expression and other cellular processes modulate brain anatomy and physiology (Birzniece et al., 2006;Hampson, 2008;Meitzen and Mermelstein, 2011;Melcangi et al., 2008;Zheng, 2009).

Cognitive abilities in verbal fluency and mental rotation (MRT) are sex-specific and could be the most sensitive to the effects caused by changes in sex steroid levels (Weiss et al., 2003). On a verbal fluency task, women mostly outperform men (Halari et al., 2006;Hausmann et al., 2009;Weiss et al., 2003) (but for critical review see (Wallentin, 2009)), whereas on mental rotation tasks men on average perform better than women (Gouchie and Kimura, 1991;Halari et al., 2006;Jansen and Heil, 2010;Kozaki and Yasukouchi, 2009;Moffat and Hampson, 1996;Peters et al., 2006;Weiss et al., 2003). There are assumptions that androgens are important for better men's performance on spatial tasks (Hooven et al., 2004), and that estrogens play critical role in better women verbal performance (Maki et al., 2002).

The progestins used in the currently available combined hormonal contraceptives could act on androgen receptors as agonists or as antagonists, i.e. they could have androgenic or anti-androgenic properties (Sitruk-Ware and Nath, 2010). Opposite effects of different contraceptives on sex specific and sex steroid sensitive mental rotation and verbal fluency tasks may be observed because of different progestins androgenity. For example, Wharton et al. (Wharton et al., 2008) reported that users of hormonal contraception with more expressed androgenity were better on mental rotation task (MRT) performance as compared with the less androgenic, anti-androgenic hormonal contraception users and non-users. Whereas anti-androgenic hormonal contraception users on the MRT performed poorly in comparison to the androgenic contraceptives users as well as to non-users. As no differences in MRT performance were observed when compared all hormonal contraceptives users and non-users, these authors suggested that the effects were masked by a combination of high and low androgenic progestins in the same analysis.

Data from two studies (Mordecai et al., 2008; Rumberg et al., 2010) demonstrated that hormonal contraceptives users and non-users do not differ in verbal abilities, however there is no evidence that the influence of different progestins in these studies was evaluated and, we did not find published data on effect of androgenic and antiandrogenic contraceptives on other cognitive functions.

Although the influence of hormonal contraceptives on women mood was started to evaluate as soon as it was started to use (Kane, Jr., 1968) and is investigated till now (Caruso et al., 2011), the results are contradictory and the question is still open. In addition mood as well as other factors, like personality traits, could have substantial impact on the performance on general or specific cognitive tasks and should be taken into account.

1.1 Aim and objectives

The aim of the study – to evaluate the effects of hormone-based contraception on young healthy women cognitive functions.

Objectives:

1. To compare naturally cycling women and hormonal contraceptives users with respect to:
 - Sex-specific and sex steroids level sensitive cognitive functions: spatial and verbal abilities;
 - Manual speed and accuracy;
 - Working and spatial memory;
 - Mood;
 - Personality traits.
2. To evaluate the effect of composition of hormonal contraception on the spatial and verbal abilities, memory, manual speed and accuracy, mood.

1.2 Actuality and scientific novelty

For the first time there was demonstrated that:

1. Hormonal contraception with androgenic properties has negative effect on verbal fluency performance;
2. Naturally cycling women are faster on mental rotation task as compared to hormonal contraception users;
3. The number of correct answers decreased significantly with increasing task difficulty in hormonal contraception users, but did not change in non-users group.

Understanding of mechanisms of hormonal contraception action on functions of nervous system is important from both fundamental (understanding how nervous system works is one of the greatest scientific challenges, therefore knowledge about any substance exerting modulatory action on brain activity is important) and applied point of view (knowledge on effects of hormonal contraception on brain activity can be important in diagnosing and treating neuropsychiatric diseases).

1.3 Defended statements

1. Sex-specific cognitive functions could be more sensitive to effect of hormonal contraceptives as compared to other cognitive functions.
2. The cognitive functions could be affected not only by hormonal contraceptives usage/no usage *per se*, but by the composition of contraceptives too.
3. Women who decided to use hormonal contraceptives could have some individual characteristics, for example more or less expressed different personality traits, which could be important for cognitive performance.

2. METHODS

The study was performed at Department of Biochemistry and Biophysics, Faculty of Natural Sciences, Vilnius University. All testing was performed in Lithuanian (native language for all participants).

The design of the study was approved by the Lithuanian Bioethics Committee (License 2007-12-22, Nr. 59). All women gave their written informed consent to participate in the study.

2.1 Participants

The study was performed in several experimental stages (see 2.6 Design and procedures). The information about the number of participants in each stage and some demographic parameters are presented in Table 2.1.

Participants in these experiments were women using (OC group) and not using (NC group) hormonal contraceptives. Subjects were excluded from the study if they were pregnant or used emergency contraception during the last six months, suffered from psychological or endocrine disorders or showed signs of viral/bacterial infections, had a speech impediment or were not native Lithuanian speakers. Only women with a regular menstrual cycle (not shorter than 21 and not longer than 35 days) for at least 3 months or users of monophasic (containing constant dose of synthetic hormones across cycle) combined (estrogen-progestin) hormonal contraception that had been used continuously for at least 3 months took part in experiments.

Table 2.1 The number of subjects and some demographic parameters in the different stages of the study.

	MRT, verbal fluency, spatial memory	Manual speed and accuracy	Working memory	Mood	Personal traits
Participants number (n)	NC=20 OC _A =11 OC _{AA} =12	NC=19 OC _A =10 OC _{AA} =11	NC=29 OC _A =17 OC _{AA} =13	NC=63 OC _A =24 OC _{AA} =22	NC=74 OC=74
Age, years	NC= 21.1±1,5 OC=21.8±2,1	NC= 21.1±1,5 OC=21.8±2,1	NC=21.0±1.3 OC=21.2±1.6	NC=22.3±4.3 OC=23.3±5.1	NC=23.6±3.4 OC=22.2±3.3
Education, years	NC=14.8±1.6 OC=15.0±1.5	NC=15.0±1.7 OC=15.1±1.5	ne	NC=14.8±1.9 OC=14.9±1.8	ne

MRT – mental rotation task, *NC* - naturally cycling, *OC* – oral contraceptive users, *OC_{AA}* – anti-androgenic oral contraceptive users, *OC_A* – androgenic oral contraceptive users, *ne* – not evaluated.

The oral contraceptive users were analyzed as a whole group and as subgroups based on the progestins contained in hormonal contraceptives they used: androgenic progestins containing OCs users – OC_A and anti-androgenic progestins containing OCs users – OC_{AA}.

Subjects from OC_A sub-group used these contraceptives: Logest, Lindynete 20, Lindynete 30, Mercilon and Novynete. In OC_{AA} sub-group were women who used Diane 35, Femina 35, Chloe 35, Belara, Jeanine, Yarina, Yaz and Yasminelle.

The subjects did not differ in age, which could be a significant predictor in mental rotation (Jansen and Heil, 2010) and spatial memory (Lezak, 2004) performance, or in education level, which could affect verbal fluency (Wallentin, 2009) and spatial abilities (Lezak, 2004) (Table 3.1).

2.2 Tests of cognitive functions

All subjects (with exception those who filled questionnaire individually see 2.6 Design and procedures) were tested at three time points (three experimental sessions). During each experimental session they performed the same tests of cognitive functions. The tests of cognitive functions (with the exception working memory and manual performance, see corresponding chapters) were performed by 43 women: 20 naturally cycling, 11 androgenic OC users and 12 anti-androgenic OC users. The data about participant's age, education duration, body mass index are presented at Table 2.

2.2.1 Verbal fluency test

The test was designed to measure the spontaneous production of words beginning with a given letter (letter fluency) or from abstract categories (category fluency) and was performed as described by Spreen and Straus (Spreen and Strauss, 1998). Participants were instructed to generate as many words as possible in one minute excluding proper nouns and variations of the same word. There were three alternate sets from three letters and two categories for the three experimental sessions. The outcome measure was the score, which was the sum of all acceptable words produced in the five (three letters and two categories) one-minute trials.

2.2.2 Mental rotation test

The Shepard and Metzler (Shepard and Metzler J., 1971) two figures paradigm was used for the investigation of mental rotation performance.

Subjects were presented with a pair of stimuli where one figure was rotated with respect to the other (Fig. 2.1). Half of the pairs had identical shapes and the other pairs were composed of different shapes, one figure was the mirror image of the other. Two different 3D figures and their mirror images composed of ten white cubes in black background, rotated by eight different angles around a vertical axis by 45° steps from “Library of Shepard and Metzler type mental rotation stimuli“ (Peters and Battista, 2008) were used. The angular difference between two figures in half of the pairs was 50° and 100° in the other half. Based on figures identity and angular difference there were four types of trials: identical figures with 50° angular difference (Identical 50°), mirror image figures with 50° angular difference (Mirror 50°), identical figures with 100° angular difference (Identical 100°) and mirror image figures with 100° angular difference (Mirror 100°) (Fig. 2.1). The task for subjects was to decide quickly and accurately whether the two figures were the same or different.

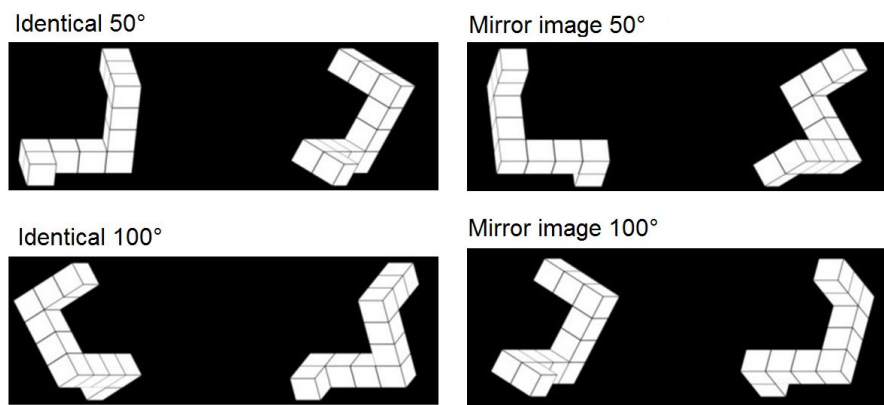


Figure 2.1 The examples of four types of trial used on the mental rotation task. Identical $50^{\circ}/100^{\circ}$ - identical figures with $50^{\circ}/100^{\circ}$ angular difference between two figures in the pair; Mirror image 50° - mirror image figures with $50^{\circ}/100^{\circ}$ angular difference between two figures in the pair.

E-Prime 2.0 software and PST Serial Response Box (PST, Inc.) were used for stimuli presentation and data collection. Trials of identity and angular differences were randomized for each task. The task included six practicing and 64 experimental trials

with a 4-sec time limit for each trial. Participants were instructed to respond with two fingers of the dominant hand, one finger for identical figures=green button and the second for different figures=red button. The outcome measures were the number of correct answers (CA) and response time of correct answers (RT). The number of correct answers was measured in percent. Response time (in ms) was measured from the onset of the test stimulus until the response occurred. Subjects that failed to respond within the time of 4 sec represented just 0.6 % of all presentations. Therefore, these data were simply excluded from analysis. CA and RT values more than two standard deviations from the mean per subject and per type of trial, RT shorter than 100 ms¹ and RT from error trials were excluded.

2.2.3 Manual speed and accuracy.

The modified Target test, measuring manual lateral dominance was used for the assessment of manual speed and accuracy (Borod et al., 1984). The determination of dominant hand was the main reason for the involvement of this test in the study. In addition, there are hypotheses that the differences in women/men performing on some tasks (especially spatial tasks (Rilea, 2008)) could be due to differences in brain lateral activation. Therefore we compared parameters of this task performance between groups/sub-groups and evaluated relationships between MRT and Target test performance.

The paper-and-pencil category Target test was performed by each hand – first by dominant hand, according to subjective participant’s estimates.

The test was composed from 32 targets, arranged in a 4 x 8 matrix (Figure 2.2 A). Each target was composed of four evenly spaced concentric circles, the largest 9 mm in diameter and the center of target - 1 mm in diameter. The task for subjects was to hit the center of each target as quickly and as accurately as they can. Subjects had to begin from the left upper corner and to go through each row from the left side to the right. The score of speed was the number of seconds taken to hit the 32 targets. The accuracy points were allocated by the location of each mark, with a score range for each target from 0 (outside

¹ As representing the effects of anticipation (Di Lollo et al., 2000).

the target) to 8 (the center) (Figure 2.2 B), providing a score range from 0 to 256. In cases where a line occurred instead of a point, the midpoint of the line was scored.

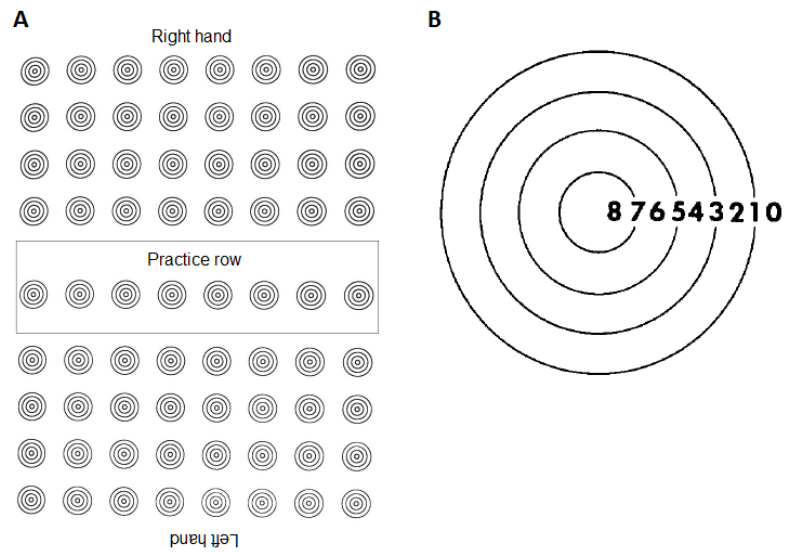


Figure 2.2 A. The target matrix for measuring speed and accuracy. B. Scoring criteria for the accuracy assessing (Borod et al., 1984).

Laterality ratio for each parameter (speed and accuracy) was computed as follows: right hand score (accuracy scores or the time, sec.) minus left hand score divided by right hand plus left hand scores:

$$\text{Laterality ratio} = \frac{\text{right hand} - \text{left hand}}{\text{right hand} + \text{left hand}}$$

Laterality ratio provides a correction for differences in base performance. Positive ratios reflect right-sided dominance and negative ratios left-sided dominance (Borod et al., 1984).

The target Test data from the 40 study participants were used for analysis (19 naturally cycling, 10 androgenic and 11 anti-androgenic hormonal contraceptives users). The data from two subjects were excluded because they demonstrated left-sided dominance and data from one subject were excluded as outliers.

2.2.4 Working memory

Short-term memory was evaluated using computerized visual digit span test based on principles described in digit span tests contained in Wechsler Memory Scale (Lezak, 2004). Sets of digits, consisting of six, seven, eight and nine digits were used in short-term memory test. First, 12 sets consisting of six, and then 12 sets consisting of eight

digits were displayed. Sets of seven and nine digits (12 of each) were displayed after break period. Each set was presented at the centre of monitor for 1000 ms. Time for response was unlimited. Next set was presented when answer to previous task was finished. Task was to remember and immediately type on the computer keyboard digits in correct order. Digits had to be typed forward. The number of correct answers – percentage of correct digits in correct places - was used as a measure of performance.

Working memory test was performed by 59 women: 29 NC, 17 OC_A and 13 OC_{AA}. The age and education duration presented at the Table 2.1.

2.2.5 Spatial memory.

The MCG figures (Fig. 2.3), developed by the Medical College of Georgia (MCG) Neurology Department based on principles described by A. Rey at 1941 (Lezak, 2004; Mitrushina, 2005) were used to investigate the spatial memory.

Three MCG figures (Fig. 2.3), printed on A5 format sheet of paper, in randomized order for the three experimental sessions were used.

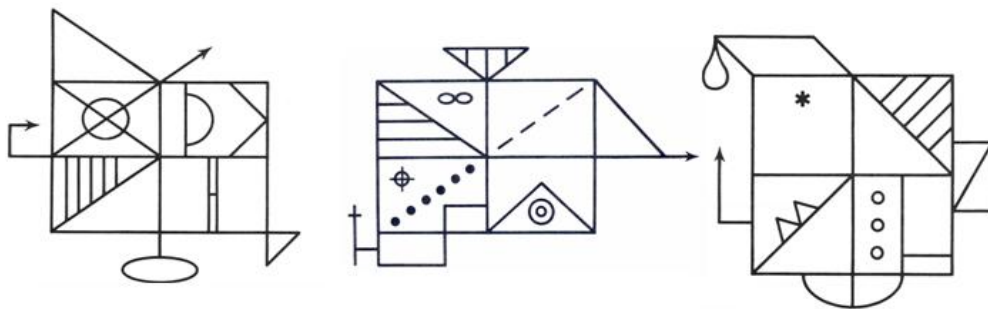


Figure 2.3 MCG complex figures (Lezak, 2004) used for the spatial memory investigation during the three experimental sessions.

The test consisted of two parts – copying and drawing the design from memory (delayed recall after 20-25 minutes). The instruction for copying was simple: “Copy this drawing as well as you can. Make sure you do not leave out anything”. 20-25 minutes later (after other cognitive tests) a reproduction of the drawing from memory was requested. No time limit was used for both parts of the test.

The scoring system described by Lezak (2004) was used. The scoring is based on the accuracy of a subject’s reproduction - each figure is divided into 18 units, which are

considered separately and can earn from 2 (placed properly) to 0 (absent or not recognizable) points. The highest possible score is 36 points.

2.3 Mood

The Lithuanian version (Silinskas G. and Zukauskiene R., 2004) of the Positive and Negative Affect Schedule (PANAS) self-rated scales (Watson et al., 1988) was used for mood evaluation before each experimental session. Participants read a list of 20 descriptions and rated each of them on a 5-point scale at the beginning of the experiment. Ratings for each item ranged from 1 (“very slightly or not at all”) to 5 (“extremely”). Outcome measures included average scores for positive (PA) and negative (NA) affects.

One part of subjects (38,4 %) performed mood tests when coming for the cognitive experimentation (three times). In this case the instruction for the subjects was: "Indicate to what extent you have felt this way right now (that is, after arriving to the experiment)". Other group of subjects filled mood and personality traits (see 2.4) questionnaire individually. These subjects were tested only once and the instruction was: "Indicate to what extent you have felt this way in general, that is, on the average". Due to the difference in instruction and the period evaluated, differently collected data were analyzed separately.

The PANAS test was performed by 112 women: 63 NC, 49 OC: 24 OC_A and 22 OC_{AA}². 43 were tested during cognitive experimentation and 69 – individually. PANAS test subject’s age and education duration are presented at Table 2.1.

2.4 Personality traits

Participants were tested with Lithuanian version of 101-item Eysenck personality questionnaire (EPQ) (Eysenck et al., 1991) including scales for Extraversion (E), Neuroticism (N) and Psychoticism (P).

148 women (74 NC and 74 NC) filled the questionnaire. Subject’s age is presented at Table 2.1.

² Some subjects (from individually tested group) did not indicate the kind of pills used, that is why they were not assigned to none of subgroups.

The test items were rated using a dichotomous yes-no format. E, N and P scales were scored by using key template and summing the item point score for each scale. After summing raw scores were standardized by subtracting raw scores from normative each scale values.

Normative data were compiled during the last five years on 349 young healthy women (Vilnius University students, education 12 years or more, age range 19 - 33 years). The normative data we have used are: Extraversion M=13.3, Neuroticism M=13.0, Psychoticism M=3.5.

2.5 Hormones

Salivary sex steroid (17 β -estradiol, progesterone and testosterone) levels were assayed to quantify free hormone levels, validate self-reported cycle phases and to compare hormone levels between groups. Salivary sampling is a non-invasive, simple, stress-free procedure approved as a useful method for the assessment of ovarian function (Liening et al., 2010; Lu et al., 1999).

The samples of saliva for the determination of free sex steroids were collected from the subjects at the each time point. Participants were asked not to eat, drink, chew gum or brush their teeth for 30 min. before sampling, but to rinse their mouth with cold water 5 min. prior to sample collection. To avoid blood contamination, samples were not collected when oral disease, inflammation or lesions were present. A minimum of 1 ml of saliva was collected into special tubes (IBL SaliCap). Tubes were stored at -24⁰ C until assayed. The concentrations of free 17 β -estradiol, free progesterone and free testosterone in saliva were determined by luminescence immunoassay for in vitro diagnostic quantitative determination in human saliva (IBL-Hamburg, Germany). The analytical sensitivities of the assays varied: the 17 β -estradiol assay was 0.3 pg/ml, the progesterone assay was 2.6 pg/ml, and the testosterone assay was 1.8 pg/ml. All samples were duplicated in the same assay. Fluoroskan Ascent FL from ThermoLabsystems (Finland) was used for luminescence measurement.

2.6 Design and procedures

The study data were collected from three experimental stages – the main cognitive functions testing, the separate study, from which the part of working memory data was

used at our working memory data analysis and the individual testing of mood and personality traits.

To minimize effects of diurnal variations, the main cognitive testing (mental rotation, verbal fluency, manual performance, spatial memory, working memory (some data collected at separate study), mood (some data collected from individual testing) and personality traits (some data collected from individual testing)) was performed in the afternoon from 15.00 h to 20.00 h. Participants were seated in an armchair in a soundproof, light-isolated chamber kept at a constant temperature (20 - 22 ° C) approximately 83 cm from the computer monitor. Each test session was performed in the same sequence: preparation (saliva sampling, Target test (once), PANAS test, Eysenck questionnaire (once)), spatial memory (copying), working memory (six and eight digits sequences), verbal fluency, working memory (seven and nine digits sequences), mental rotation and spatial memory (delayed recall). One session lasted about 60 minutes.

Subjects from both groups were tested at three time points. Naturally cycling women were investigated during three phases of the menstrual cycle: 1) the follicular (Fol) phase (between days 2 and 5 after the onset of menses), 2) the ovulatory (Ov) phase, determined using a commercially available ovulation predictor kits that estimates the LH peak in urine, and 3) the luteal (Lut) phase, determined individually on the basis of the subjects previous three month cycle history and ovulation time (on average, about six days after ovulation). The actual onset of the next bleeding was confirmed by e-mail or a telephone call. Hormonal contraception users were investigated three times during a 28-day cycle at days corresponding to the phases of naturally cycling women.

All three test sessions extended across one or two menstrual cycles depending on which menstrual cycle phase the experiments were started. Phase of menstrual cycle when experiments were started was taken in pseudo randomized order.

The part of personality (70.1 %) and mood (61.6 %) data was collected by individual questioning, not during general cognitive testing. Subjects were presented by questionnaire containing personal questions (age, education duration, family status questions (partnership, children), questions about menstrual cycle (day, regularity and duration), questions about hormonal contraception usage (duration, kind, reason for usage), mood test - PANAS and personality traits questionnaire - EPQ. The questionnaire could be filled on paper or by computer.

Eysenck test questions, outcome measures and data analysis were the same for questionnaires filled during cognitive experiments or during the individual testing, but there were some differences in PANAS testing (see 2.3 Mood).

2.7 Statistical analysis

The statistical analysis was performed with the STATISTICA 8.0 software (StatSoft, Inc., USA). Data are presented as the mean (M) \pm standard error of mean (SEM).

For the data which were normally distributed ANOVAs for repeated measures (RM-ANOVA) were used to evaluate effects of experimental session (practice) and phase of menstrual cycle. Mixed-Model ANOVA was used in cases of random factors (MRT task). Effect sizes were evaluated by partial eta squared (η^2). Post-hoc LSD (Least Square Means Differences) tests were used when appropriate. Pearson correlation analysis was used to calculate relationships between measured parameter.

In cases when data were non parametric (Target test, Eysenck questionnaire) Mann Whitney U test was used to asses differences between groups/subgroups.

A probability value of less than 0.05 was taken as statistically significant.

The data more than two standard deviations from the mean of group/subgroup were removed from analyses.

3. RESULTS

3.1 Hormones

Self-reported cycle phases were validated by the measurement of salivary 17 β -estradiol, progesterone and testosterone. Some samples were eliminated from hormonal assaying and/or data analysis because of possible blood contamination or because hormonal concentrations were not within the range of normal values (concentration was undetectable or more than two standard deviations from the mean in particular cycle phase³). Thus, the hormonal concentration results from 97 of 129 samples (75.2%) were used for calculations. Other studies reported the similar elimination rate (Liening et al., 2010) and the data after outliers elimination were within the normal ranges for salivary hormones obtained in previous studies (Hausmann et al., 2009; Liening et al., 2010; van Anders, 2010).

An independent-sample t-test used for salivary sex steroid analysis showed a significantly higher 17 β -estradiol and testosterone concentrations during the ovulatory menstrual cycle phase and a significantly higher progesterone concentration during the luteal phase in the NC group compared with the OC group (Table 3.1).

Table 3.1 The salivary sex steroids (pg/ml). Mean \pm SE.

	Naturally cycling (48 samples)			Hormonal contraception users (49 samples)		
	Fol. ph. (n=16)	Ov. ph. (n=15)	Lut.ph. (n=17)	Fol. ph. (n=17)	Ov. ph. (n=16)	Lut. ph. (n=16)
Es	2.40 \pm 0.41	5.99 \pm 0.64*	3.45 \pm 0.41	2.29 \pm 0.53	2.26 \pm 0.53	2.32 \pm 0.52
P	36.7 \pm 4.5	52.2 \pm 7.3	153.0 \pm 19.5*	39.3 \pm 4.9	41.8 \pm 4.5	38.8 \pm 5.6
T	19.2 \pm 2.6	20.5 \pm 2.2**	17.6 \pm 2.5	19.9 \pm 2.8	13.9 \pm 1.6	15.5 \pm 2.1

Es - 17 β -estradiol, *P* – progesterone, *T* – testosterone. *Fol. ph.* – follicular phase, *Ov. ph.* – ovulatory phase, *Lut.ph.* – luteal phase.

* - statistically significant difference ($p < 0.01$) compared to with other naturally cycling (NC) subjects phases and all hormonal contraception users (OC) phases.

** - statistically significant difference ($p = 0.02$) compared with ovulatory OC phase.

³ Very high values could be due to blood contamination in saliva, incorrectly determined phase of cycle or other reasons. The data of subjects whom hormonal values did not correspond group or phase, were discarded from the analysis.

3.2 The effect of experimental session on cognitive performance

Each study participant all cognitive tests (mental rotation, verbal fluency and memory) performed three times (three experimental sessions) during the different phases of the menstrual cycle (follicular, ovulatory and luteal). The experimental session added additional – practice effect. The effect of experimental session on all measured cognitive parameters was evaluated using two-way ANOVA for repeated measures (RM-ANOVA). Repeated measurement factor experimental session (1-3) and between-subjects factor subgroup (NC *vs* OC_A *vs* OC_{AA}) were used. The interaction between both these factors (session by subgroup) was also evaluated.

Verbal fluency test. The RM-ANOVA showed significant main effect of experimental session ($F(2,66)=9.78$, $p<0.001$, $\eta^2=0.23$) and subgroup ($F(2,66)=4.67$, $p=0.016$, $\eta^2=0.22$). There was no significant interaction session by subgroup ($F(4, 66)=1.15$, $p=0.34$, $\eta^2=0.07$). All subgroups showed increase in total number of words going from the first session to the second and the third (Fig. 3.1).

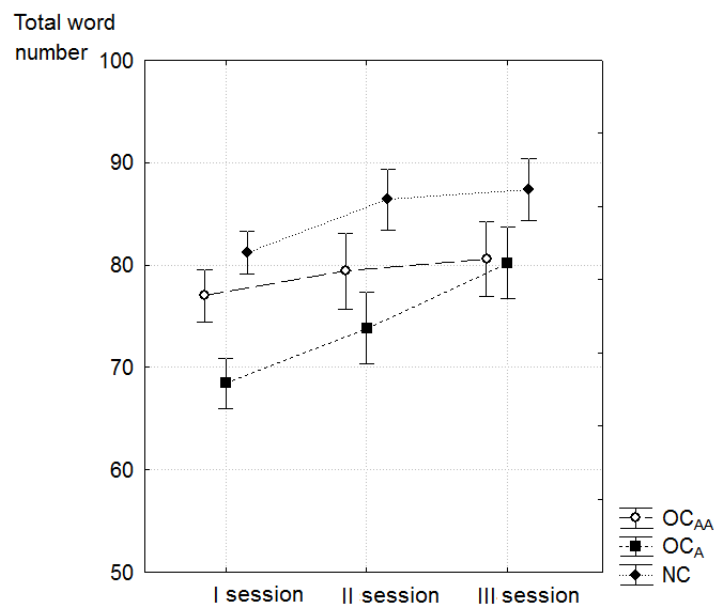


Figure 3.1 The total word number from the verbal fluency test during three experimental sessions in NC (n=19), OC_A (n=11) and OC_{AA} (n=12) subgroups.

Mental rotation test. The RM-ANOVA showed significant main effect of experimental session on both MRT parameters (CA $F(2,306)=23.5$, $p<0.0001$, $\eta^2=0.13$; RT $F(2,306)=50.4$, $p<0.0001$, $\eta^2=0.25$). The subgroup was significant factor for the RT ($F(2,306)=8.37$, $p<0.001$, $\eta^2=0.10$), but not CA ($F(2,306)=0.07$, $p=0.93$, $\eta^2<0.01$). There

were no significant interaction session by subgroup on CA and RT (all $F < 0.52$, $p > 0.72$). All subgroups showed increased CA and decreased RT going from the first to the third session (Fig. 3.2).

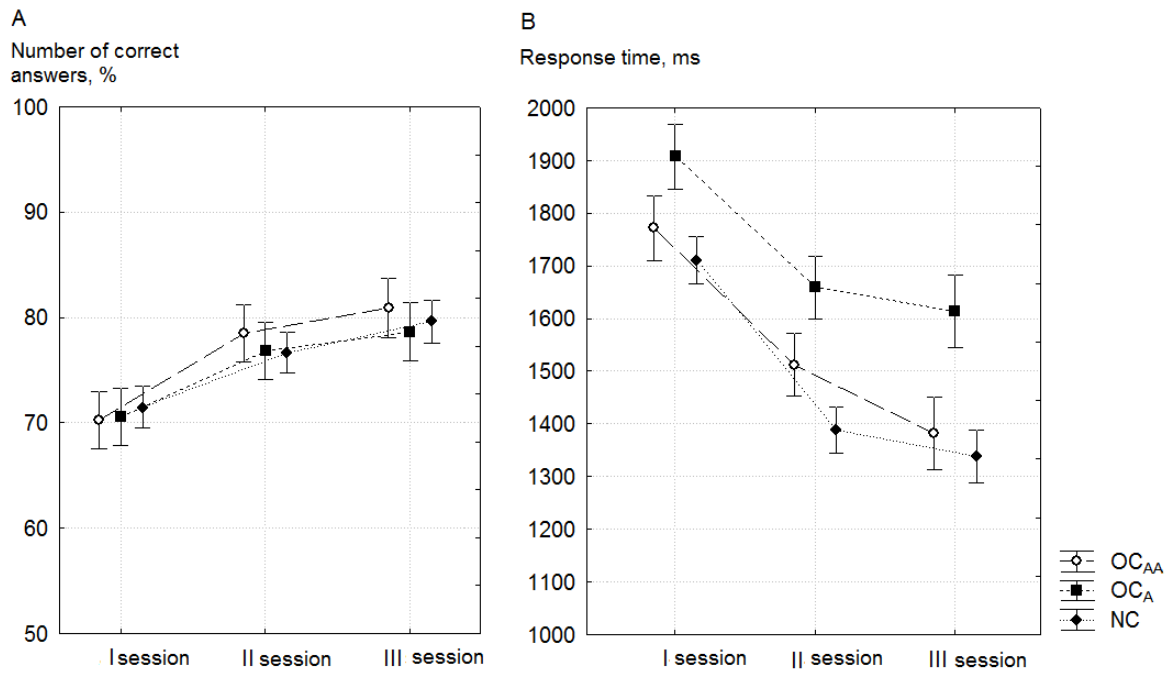


Figure 3.2 The number of correct answers (A) and the response time (B) from the mental rotation test during three experimental sessions in NC (n=20), OC_A (n=11) and OC_{AA} (n=12) subgroups.

Working memory test. The effect of experimental session was significant when seven ($F(2,94)=4.71$, $p=0.01$, $\eta^2=0.09$) and eight ($F(2,94)=9.20$, $p<0.001$, $\eta^2=0.16$), but not nine ($F(2,94)=0.83$, $p=0.44$, $\eta^2=0.02$) digits sets were presented (Fig. 3.3). There was no significant effect of subgroup (all $F < 0.91$, $p > 0.41$), but there was significant interaction session by subgroup when seven and nine digits were presented ($F(4,94) > 2.61$, $p < 0.03$, $\eta^2 > 0.10$). The significant interaction means that the practice was different in different subgroups. However the effect was not regular and interpretation complicated.

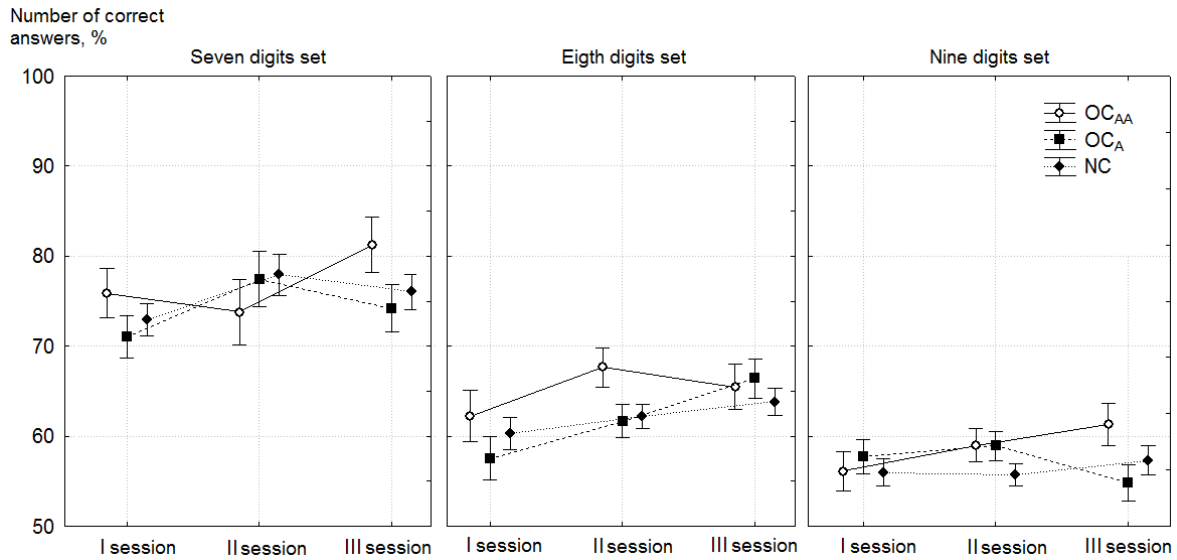


Figure 3.3 The number of correct answers from the working memory test (when seven, eight and nine digit sets were presented) from three experimental sessions in NC (n=29), OC_A (n=17) and OC_{AA} (n=13) subgroups.

Spatial memory test. There was significant effect of experimental session ($F(2,68)=4.45$, $p=0.02$, $\eta^2=0.12$). In NC and OC_A subgroups the number of scores increased going from the first to the third session, whereas subjects from OC_{AA} subgroup most scores collected during the second session (Fig. 3.4). There was no significant effect of subgroup ($F<0.18$, $p>0.83$) as well as interaction session by subgroup ($F<1.7$, $p>0.15$).

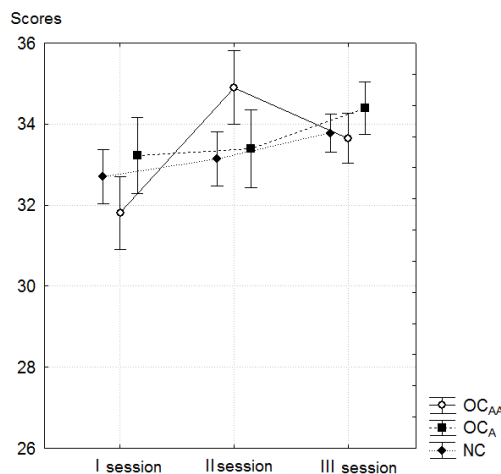


Figure 3.4 The number of scores from the spatial memory test (delayed recall) during three experimental sessions in NC (n=20), OC_A (n=11) and OC_{AA} (n=12) subgroups.

Aiming to avoid effect of practicing, which was not the same for different subgroups on different tests, we decided only data from the first experimental session to use for the next stages of data analysis.

3.3 Verbal fluency

One parameter of the verbal fluency performance, the score, which is the sum of all acceptable words produced during five (three letters and two categories) one-minute trials, was taken for analysis. The scores were removed from analyses if they were more than two standard deviations from the mean of group.

One-way ANOVA, used to verify that the three sets of letters and categories used for the three experimental sessions were equivalent, did not demonstrated significant set effect on the experimental data $F(2, 117)=0.68, p=0.51$.

To avoid influence of practice (see section 3.2) next steps of analysis were performed only on data from the first session excluding data from the second and third sessions.

An additional two-way RM-ANOVA with repeated measurement factor the phase of menstrual cycle (Fol vs. Ov vs. Lut) and between-subjects factor group (NC vs. OC) was computed for the total number of words generated by subjects during the verbal fluency task. No significant main effect of phase or interaction phase by group were found (all $F<1.19, p>0.31$).

The one-way ANOVAs comprising one between-subjects factor group/subgroup were used to evaluate group and subgroup effect on the total number of words generated during the first experimental session (Table 3.2). Naturally-cycling women outperformed OC users by generating more words although the group effect was not significant ($F(1,39)=3.17, p=0.08, \eta^2=0.08$). However there was significant main effect of subgroup ($F(2,38)=4.16, p=0.02, \eta^2=0.18$). The post-hoc analysis revealed that the androgenic OC users generated significantly less words as compared to the naturally cycling women ($p=0.01$) and anti-androgenic OC users ($p=0.03$) (Table 3.2).

A Pearson correlation analysis did not reveal statistically significant relationships between verbal fluency scores (from the first experimental session) and hormones, duration of OC usage or mood (all $r<0.29, p>0.07$).

Table 3.2 Total word number (mean \pm SE) from verbal fluency test in different groups (OC and NC) and subgroups (OC_A and OC_{AA}).

Group/subgroup	Total word number
Naturally cycling, n=19	79.7 \pm 3.0*
Hormonal contraception users, n=22	73.5 \pm 2.0
Androgenic hormonal contraception users, n=11	68.5 \pm 1.7
Anti-androgenic hormonal contraception users, n=11	78.5 \pm 3.0*

* -statistically significant difference ($p \leq 0.03$) compared with the androgenic hormonal contraception users.

Summary: OC_A generated significantly less word as compared to NC and OC_{AA}; there were no other significant differences or relationships.

3.4 Mental rotation (MRT)

The performance of the MRT task was evaluated by correct answers (CA) and response times (RT). The average percents of correct answers and average response times separately for each type of trial (four types of trials: Identical 50°; Mirror 50°; Identical 100°; Mirror 100°), for each subject (43 subjects), from each experimental session (three experimental sessions) were calculated and used for the analysis. CA and RT values more than two standard deviations from the mean per subject and per type of task and RT from error trials were excluded.

To avoid the influence of practice (see section 3.2) the main steps of analysis were performed on the data from the first session. The phase of menstrual cycle as the factor was ignored because the two-way RM-ANOVA with repeated measurement factor cycle phase (Fol vs. Ov vs. Lut) and between-subjects factor group (NC vs. OC) did not demonstrated significant effects of phase or interaction phase by group (all $F < 1.49$, $p > 0.23$).

Mixed-Model ANOVA was used to evaluate how the performance of MRT task depended on the type of trial. The type of trial was taken as random within-subject factor (Identical 50° vs. Mirror 50° vs. Identical 100° vs. Mirror 100°) and subgroup (NC vs. OC_A vs. OC_{AA}) as fixed between-subjects factor. It was showed the significant main effect of type of trial on both MRT parameters (CA ($F(3,152)=7.18$, $p < 0.01$, $\eta^2=0.76$; RT ($F(3,152)=8.42$, $p < 0.01$, $\eta^2=0.75$) and significant main effect of subgroup on RT ($F(2,152)=12.5$, $p < 0.01$, $\eta^2=0.81$), but not on CA ($p=0.88$). The interactions type of trial by subgroup was not significant ($F < 0.75$, $p > 0.62$) for both CA and RT.

Table 3.3 The response time and the number of correct answers (means \pm SE) from mental rotation task in different groups (OC and NC) and subgroups (OC_A and OC_{AA}).

	Response time, ms	Correct answers, %
Naturally cycling, n=20	1730 \pm 44*	69.9 \pm 2.2
Hormonal contraception users, n=23	1845 \pm 41	69.7 \pm 1.7
Androgenic hormonal contraception users, n=11	1908 \pm 57	70.6 \pm 2.7
Anti-androgenic hormonal contraception users, n=12	1799 \pm 63	68.9 \pm 2.5

* - statistically significant difference ($p=0.02$) compared with the androgenic hormonal contraception users.

Post-hoc analyses were performed to analyze differences between subgroups, types of trial and interaction types of trial by subgroups. It was demonstrated that NC subjects responded significantly faster ($p=0.02$) as compared to OC_A (Table 3.3). There were no significant RT differences between OC_{AA} and other subgroups and no significant CA differences between all subgroups (all $p>0.35$) (Table 3.3). Analysis of type of trial effect showed that subjects responded significantly more correctly when angular difference between figures in the pair was 50° as compared to 100° for both identical and mirror image pairs ($p<0.03$). Whereas RT was less affected by angular difference - the RT was significantly shorter for identical figures with 50° as compared to 100° angular difference ($p=0.048$), but there were no RT difference between mirror image figures with 50° and 100° angular difference in the pair ($p>0.38$). The figures identity had no significant effect on CA and RT (all $p>0.22$).

Post-hoc analysis performed with respect to both factors (types of trial by subgroups) did not demonstrated significant between-subgroups differences for CA and RT. However there were some type of trial depended differences within subgroups and tendencies for the type of trial depended differences between subgroups (Fig 3.5). MRT parameters in NC group were the least affected by the type of trial - CA decreased and RT increased in tasks with 100° angular difference as compared to 50°, but changes were insignificant (Fig 3.5). However in both subgroups of OC users CA depended on the type of trial. Subjects from the OC_{AA} group responded the most erroneously when mirror image figures with 100° angular difference were presented. During this type of trial they were significantly ($p<0.05$) less correct as compared to tasks when angular difference was 50° and less correct (not significantly) as compared to NC and OC_A in the same type

of trial. There were significantly less ($p < 0.05$) CA in OC_A subgroup in both types (mirror and identical) of trials with 100° angular difference as compared to trials containing identical figures with 50° angular difference. The RT did not differ between or within subgroups significantly, but NC were faster as compared to OC_{AA} and OC_A in all tasks (with exception of task Identical 50°), as well as OC_A were the slowest in all tasks (Fig 3.5).

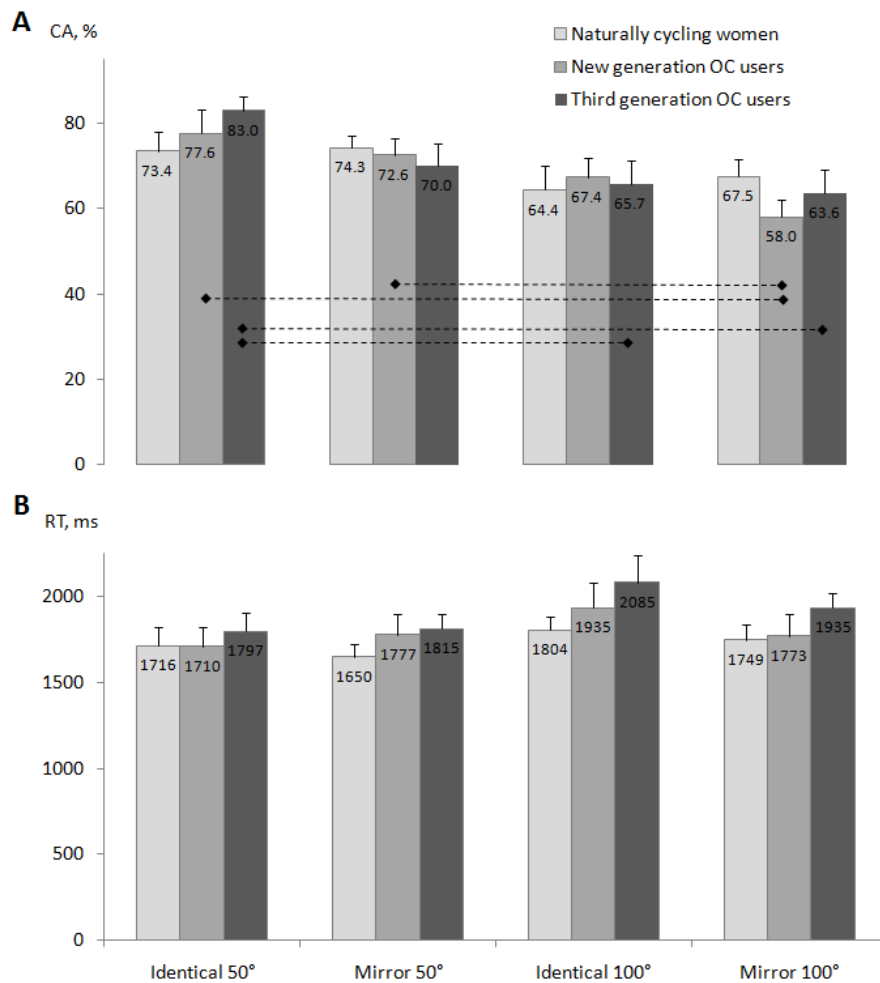


Figure 3.5 Mental rotation parameters of androgenic and anti-androgenic oral contraceptives (OC) users and nonusers by type of trial (figures identity and angular difference in the pair): identical - identical figures, mirror - mirror image figures, 50° and 100° - angular difference between the figures in the pair. A - Correct answers (CA), B - response time (RT). Dashed lines indicate significant differences ($p < 0.05$) between types of trial within subgroups. Error bars are standard errors of the mean (SEM).

A Pearson correlation analysis revealed relationships between EPQ values and MRT performance – OC subjects with higher psychoticism values responded slower ($r=0.50$, $p=0.02$), but more correctly ($r=0.42$, $p=0.06$); in NC group higher extraversion values were related with shorter response time ($r=-0.43$, $p=0.06$), and higher neuroticism values – with longer response time ($r=0.42$, $p=0.06$). There were no stronger and/or significant relationship between MRT parameters and salivary steroids, mood or the duration of OC use (all $r<0.22$, $p>0.06$).

Summary: OC_A group demonstrated the longest and NC group the shortest response time; there were no differences in CA between groups and subgroups; the increasing trial difficulty significantly decreases CA in both OC subgroups, but not in NC; there were several non regular relationships between MRT performance and EPQ parameters; there were no other significant or more expressed differences and relationships.

3.5 Manual speed and accuracy

The speed and accuracy scores and laterality ratios were calculated for evaluation of the performance on the Target test. According to the individual values 41 subjects were classified as right-handed and two as left-handed. Only results of right-handed subjects were used for the following analysis.

The scores of speed and accuracy were compared between groups and subgroups. It was demonstrated that NC subjects were slower, but more accurate as compared to OC users (right hand: the time of performance $t=2.37$, $p=0.02$ (t-test), accuracy $z=1.58$, $p=0.11$ (Mann Whitney test); left hand: the time of performance $t=1.79$, $p=0.08$ (t-test), accuracy $z=2.18$, $p=0.03$ (Mann Whitney test)) (Fig 3.6 A). The comparison between subgroups (NC vs OC_A vs OC_{AA}) revealed that subjects from OC_A subgroup were significantly less correct as compared to NC ($z =1.97$, $p=0.049$) when performed with right hand). There were no significant differences between subgroups in the left hand accuracy and in time of performance by both hands (Fig 3.6 B). There were no laterality ratios differences between groups and subgroups.

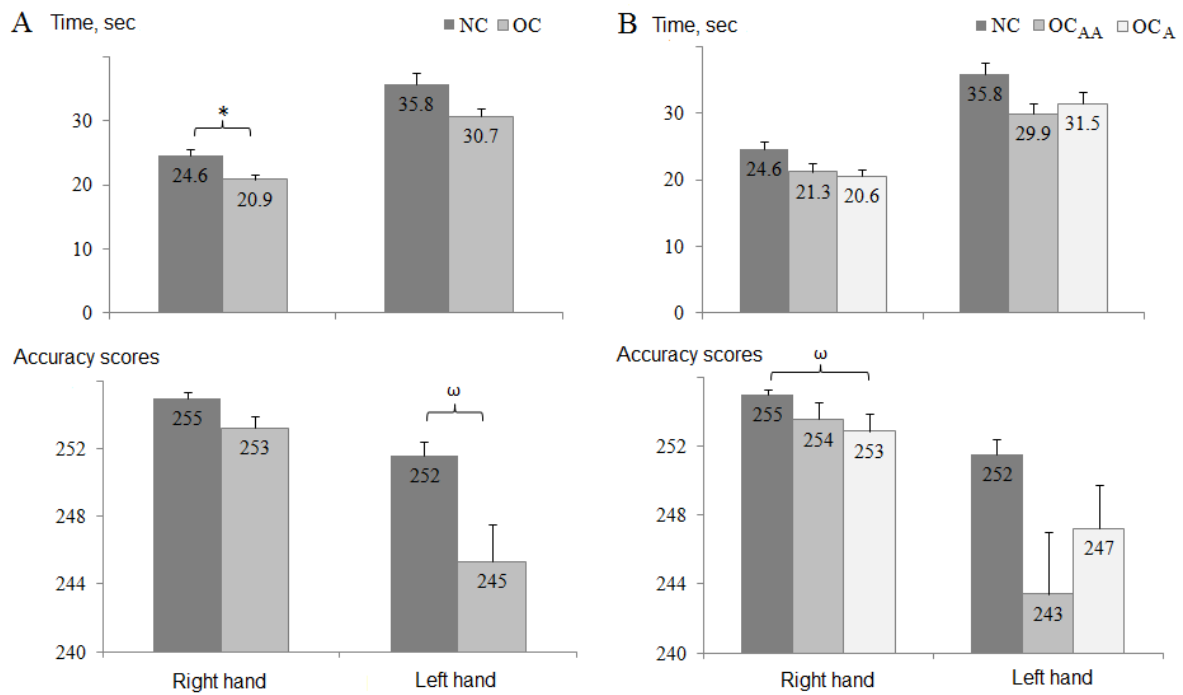


Figure 3.6. Manual speed and accuracy between groups (A) and subgroups (B). * - $p < 0.05$ (t-test), ω - $p < 0.05$ (Mann Whitney test). Error bars are standard errors of the mean (SE). NC $n=19$, OC $n=21$, OC_A $n=10$, OC_{AA} $n=11$.

The correlation analysis demonstrated relationship between neuroticism values and Target test performance when data for all subjects irrespective of group were analyzed. Women with higher values of neuroticism were slower (longer time of performance) (right hand $r=0.47$, $p < 0.01$; left hand $r=0.40$, $p=0.01$), but more accurate (right hand $r=0.40$, $p=0.01$; left hand $r=0.30$, $p=0.07$). Relationships become weaker and /or non significant after dividing subjects into groups (NC group all $r < 0.47$, $p > 0.08$; OC group all $r < 0.38$, $p > 0.01$).

There were no other relationships between performance on Target test and:

- EPQ or PANAS values (all $r < 0.46$ $p > 0.07$).
- the level of salivary steroids (exceptions: in NC group the negative relationship between time of performance with left hand and salivary 17β -estradiol ($r=-0.58$, $p=0.03$); in OC group the negative relationship between time of performance with right hand and salivary testosterone $r=-0.56$, $p=0.03$).
- MRT parameters (all $r < 0.25$, $p > 0.30$).

Summary: NC subjects slower, but more accurate as compared to OC; no speed and accuracy differences between subgroups; no laterality ratios differences between groups/subgroups; accuracy and time of performance positively depended on neuroticism values; no other regular relationships.

3.6 Working and spatial memory

The number of correct digits in correct places in percent was used for the evaluation of performance on working memory test. The scores of accuracy were used for the performance on spatial memory test performance. The results of sequences of six digits at working memory test there were not used because of values being very close to 100 %.

As mentioned in section 3.2 the experimental session was significant factor for working and spatial memory performance, therefore, aiming to avoid the effect of practicing, only the data from the first session were used for the evaluation of differences between groups/subgroups.

There were no working and spatial memory differences between groups/subgroups (Table 3.4) and no effect of the phase of menstrual cycle (all $p > 0.40$).

Table 3.4 Working (the mean of correct answers from seven, eight and nine digits sequences, %) and spatial (scores of accuracy) memory parameters (mean \pm SE) in NC, OC groups and OC_A, OC_{AA} subgroups.

	Working memory,%	Spatial memory, scores
Naturally cycling	64.6 \pm 1.6, n= 27	32.0 \pm 0.7, n= 20
Hormonal contraception users	63.9 \pm 2.4, n=26	31.0 \pm 0.9, n=23
Androgenic hormonal contraception users	63.1 \pm 2.5, n=15	31.7 \pm 1.3, n=11
Anti-androgenic hormonal contraception users	64.7 \pm 2.2, n=11	30.5 \pm 1.3, n=12

There were no significant relationships between performance on memory (working and spatial) tests and salivary steroids, mood, personality traits and OC usage duration (all $r < 1.11$, $p \geq 0.05$).

Summary: no differences between groups/subgroups and no relationships between working and spatial memory performance and other parameters.

3.8 Mood

As mentioned at the methods (see section 2.3), the mood data were collected in two ways, i.e. from two experimental blocks: together with other cognitive tests (20 NC, 23 OC: 12 OC_{AA}, 11 OC_A) and separately, during the individual testing (43 NC, 26 OC: 11 OC_{AA} 13 OC_A).

Mood testing was repeated (the first experimental block) during three experimental sessions and the effect of session on positive (PA) and negative (NA) affect was analyzed first. RM-ANOVAs comprising repeated measurement factor experimental session (1-3) and between-subjects factor group (NC vs. OC) or subgroup (NC vs. OC_A vs. OC_{AA}) were used. The RM-ANOVAs showed a significant main effect of experimental session on positive (all $F > 5.86$, $p < 0.004$, $\eta^2 > 0.13$), but not on negative affect (all $F < 0.23$, $p > 0.82$), no significant effect of group/subgroup on positive and negative affect (all $F < 0.33$, $p > 0.56$) and no significant interaction session by group/subgroup (all $F < 0.23$, $p > 0.92$). All groups/subgroups showed decreased positive affect but no differences in negative affect going from the first session to the second and the third (Fig 3.7).

Similarly designed RM-ANOVA comprising repeated measurement factor the phase of menstrual cycle did not reveal any mood differences during different phases of the menstrual cycle (all $F < 2.00$, $p > 0.14$).

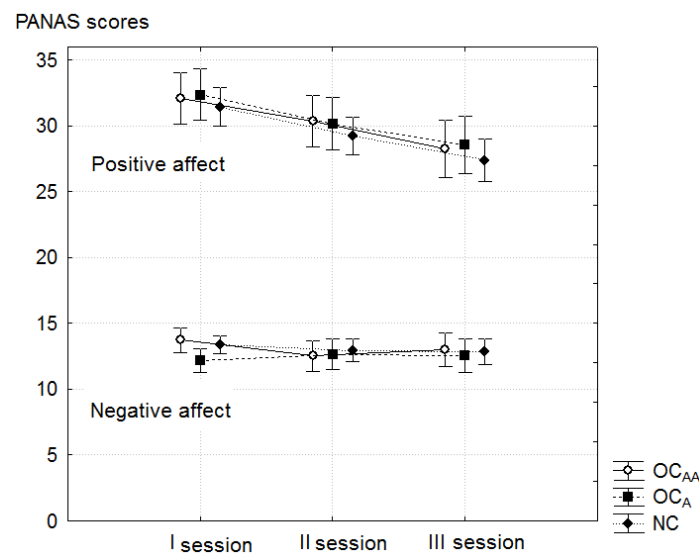


Figure 3.7 Positive and negative affect values from I - III experimental sessions in NC (n=20), OC_A (n=11) and OC_{AA} (n=12) subgroups. Error bars are standard errors of the mean (SE).

The one-way ANOVA did not demonstrated significant group/subgroup effect at the second experimental block (all $F < 1.13$, $p > 0.3$). Student t-test was used for PA and NA comparison between phases of menstrual cycle. NC subjects who filled questionnaire at the follicular phase demonstrated worse mood (lower positive and higher negative affect values) as compared to those who filled questionnaire at the luteal phase: PA ($t = -1.10$, $p = 0.30$), NA ($t = 2.71$, $p = 0.01$) (Fig 3.8). There were no significant differences in OC group, but the tendency was the same as in NC group – lower PA ($t = -1.87$, $p = 0.08$) and higher NA ($t = 1.13$, $p = 0.30$) values at follicular phase.

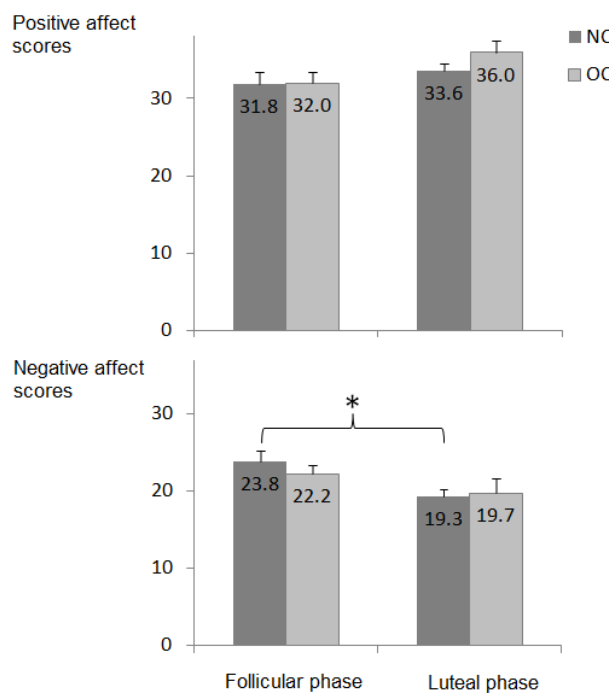


Figure 3.8 The mean values for positive and negative affect during follicular (NC $n = 17$, OC $n = 11$) and luteal (NC $n = 25$, OC $n = 10$) phases of the menstrual cycle in NC and OC groups (the data from the individual testing stage). *- $p = 0.01$. Error bars are standard errors of the mean (SE).

The correlation analysis was performed to analyze PA and NA relationships with salivary steroids (first experimental block), the OC usage duration (both experimental blocks), age and education duration (second experimental block⁴). In NC group there

⁴ At the first experimental block there were almost no variation in subjects age and education duration (Table 2.1).

was tendency of negative relationship between salivary testosterone and PA ($r=-0.48$, $p=0.06$). There were no other stronger or significant relationships between mood and hormones or OC usage duration (all $r<0.23$, $p>0.36$).

The subjects age and education duration was significantly negatively related to negative affect (NC: age $r=-0.47$, $p<0.01$; education $r=-0.45$, $p<0.01$; OC: age $r=-0.40$, $p=0.06$; education $r=-0.41$, $p=0.04$), but not positive affect (all $r<0.29$, $p>0.16$) (Fig. 3.9).

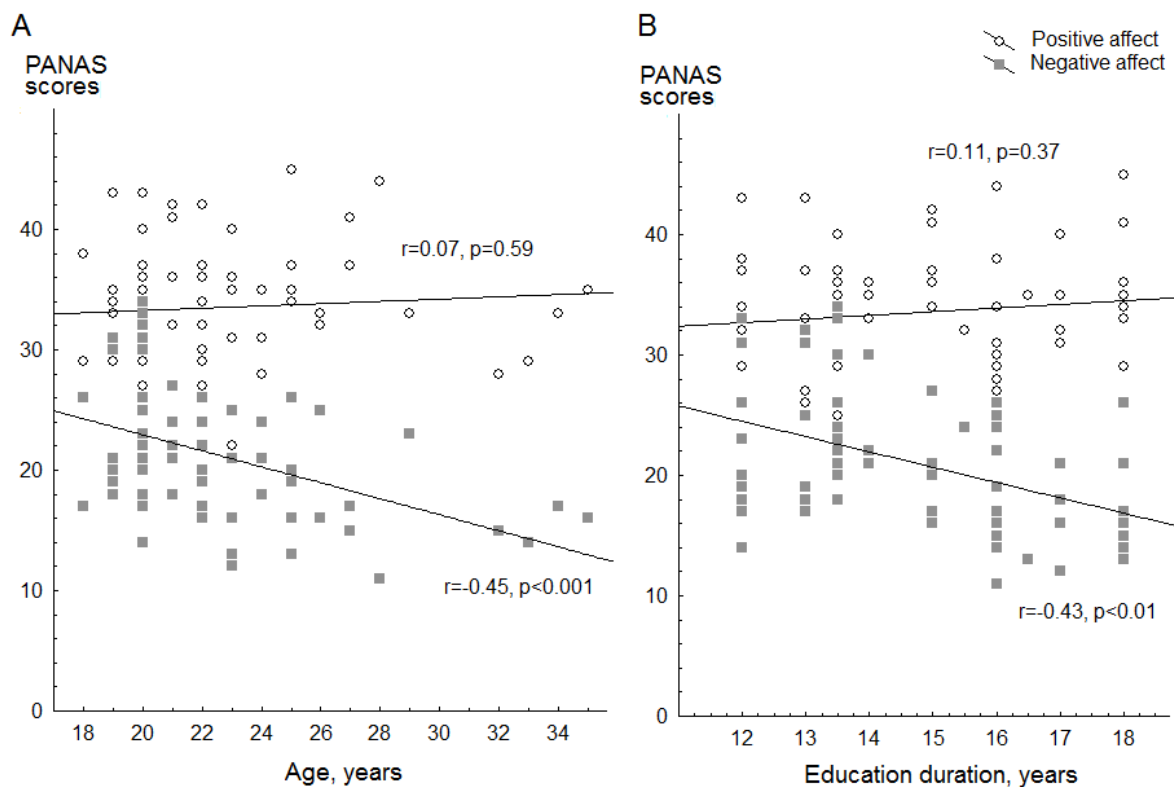


Figure 3.9 The relationships between positive and negative affects with subjects age (A) and education duration (B). The data from the II experimental block and of all subjects, do not divided to groups. N=69.

Summary: there were no PA and NA differences between groups/subgroups; at the second block of experiment there was better mood on the luteal phase; there was medium strength negative relationship between testosterone and PA in NC group; NA, but not PA decreased with age and negatively depended on the education duration.

4.9 Personality traits

The standardized values (see section 2.4) of neuroticism, extraversion and psychoticism were compared (Mann Whitney test) between OC users and nonusers (Fig. 3.10). Neuroticism values were significantly higher in NC group as compared to OC

(NC 0.77, OC -1.24, $z=2.07$, $p=0.04$). There were no significant differences for extraversion (NC -0.04, OC 1.17, $p=0.18$) and psychoticism (NC -0.58, OC -0.20, $p=0.23$) values, however there is tendency that extraversion is more expressed in OC users group (Fig. 3.10).

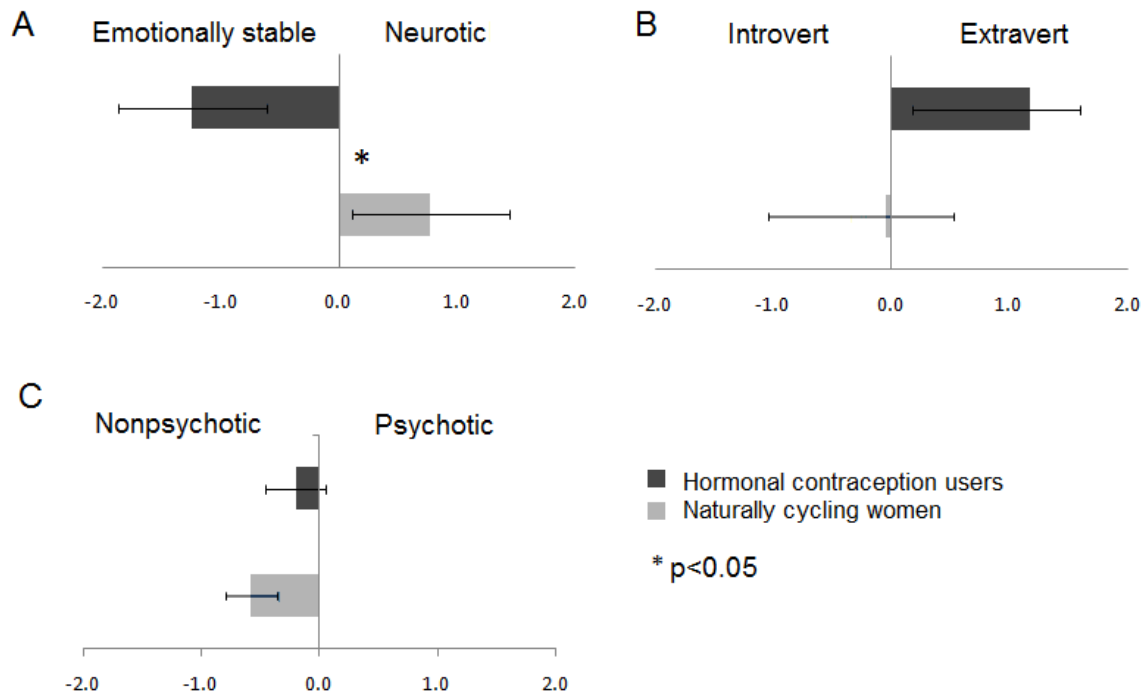


Figure 3.10 The mean values of neuroticism, extraversion and psychoticism in hormonal contraception users ($n=74$) and nonusers ($n=74$) groups.

Summary: in OC group the neuroticism is significantly less and extraversion more (not significantly) expressed as compared to NC group.

4. DISCUSSION

The present study compared the performance on verbal fluency, mental rotation, manual speed and accuracy, working and spatial memory tasks in young, healthy naturally cycling women and women using hormonal contraception with androgenic and anti-androgenic properties. Additionally, mood and personality traits, as factors which could differ between groups and could affect performance on cognitive function tests, were investigated. The levels of salivary 17β -estradiol, progesterone and testosterone were measured.

The salivary 17β -estradiol and progesterone levels were significantly lower ($p=0.001$) in hormonal contraception users. The level of salivary testosterone was slightly lower in the OC users with significant difference ($p=0.02$) only during ovulatory phase. Naturally cycling women performed better on verbal fluency task as compared to hormonal contraceptives users. Subjects who used androgenic contraceptives generated significantly fewer words as compared to users of hormonal contraception with anti-androgenic properties and non-users. The androgenic hormonal contraception users demonstrated significantly longer RT in MRT task as compared to non-users. The increasing MRT task difficulty significantly lowered number of correct answers in hormonal contraception users, but not in nonusers group. NC subjects were slower, but more accurate as compared to OC on manual speed and accuracy task, but there were no differences in laterality ratios and there were no effect of subgroup. The performance on working, spatial memory and the scores of the PANAS scales did not differ between hormonal contraception users and non-users. The MRT, verbal fluency, manual performance, working and spatial memory did not depend on the phase of menstrual cycle. Better mood during the luteal phase as compared to follicular phase of menstrual cycle was observed only in the second block of mood testing (when women only filled questionnaire and did not took part in other testing). Neuroticism was significantly less and extraversion more (not significantly) expressed in hormonal contraception users as compared to nonusers.

4.1. Verbal fluency

We hypothesized that because of lower estradiol concentrations OC group will perform worse in a verbal fluency task. As expected, non-users had higher salivary 17β -estradiol level and demonstrated higher verbal fluency scores. However the correlation analysis did not reveal any significant relationship between 17β -estradiol and the total number of words generated during the verbal fluency task. This result was unexpected, because the positive influence of 17β -estradiol on verbal fluency abilities has been shown in previous studies (Maki et al., 2002; Van Goozen et al., 1995). On the other hand there are critical opinions concerning gender and sex steroids effect on verbal abilities (Wallentin, 2009).

Mordecai et al. (Mordecai et al., 2008) reported that oral contraceptives did not affect verbal fluency, but there are no data about the progestins contained in

contraceptives used by participants in that study. However, the results of our study suggest that the number of words generated depends on the androgenicity of hormonal contraceptives used by the subjects. Based on this result, it is possible to assume that the influence of hormonal contraception in the Mordecai et al. (2008) study was masked by the opposite effects of the different progestins contained in contraceptives.

4.2 Mental rotation

Consistent with previous studies (Mordecai et al., 2008; Rosenberg and Park, 2002; Wharton et al., 2008), no differences in the number of correct responses, i.e. accuracy of mental rotation task performance, between hormonal contraception users and non-users when combined results from all types of task and all OC subjects, were demonstrated. However, analysis of MRT accuracy with respect to the type of task revealed that the increasing MRT task difficulty significantly lowered the number of correct answers in OC, but not in NC group. OC_A subgroup presented more CA as compared to NC when the task was relatively easy - two identical figures with 50° angular difference. OC_{AA} demonstrated less CA as compared to NC when the task was relatively difficult - two mirror image figures with 100° angular difference. It is complicated to compare these results with findings presented by Wharton et al. (2008), where the smallest number of correct responses was demonstrated by subjects using the anti-androgenic hormonal contraceptives, and there were no differences between users of the androgenic contraceptives and non-users. Wharton et al. used Vandenberg and Kuse (Vandenberg and Kuse, 1978) paradigm for the MRT testing, which differs from the Shepard and Metzler (Shepard and Metzler J., 1971) paradigm used in our study. Despite that, our finding that in the more difficult task OC_{AA} users responded worse supports idea presented by Wharton et al. that hormonal contraceptives containing progestins with anti-androgenic properties have a negative influence on MRT performance.

Response time in the mental rotation task was longer ($p=0.06$) in OC group and depended on the progestins contained in hormonal contraceptives – the RTs demonstrated by the OC_A group were significantly longer as compared to non-users ($p=0.02$). Analysis with respect to the type of trial supported differences found between the subgroups - irrespective of trial type the longest RTs demonstrated OC_A users and the

shortest – non-users, although all differences were insignificant. Unfortunately, there are no RT data from studies investigating MRT performance in hormonal contraceptives users and non-users (Mordecai et al., 2008; Rosenberg and Park, 2002; Wharton et al., 2008) to be compared.

Although the salivary testosterone level was slightly higher in the NC group there were no more CA in NC group as well as not significant relationship between MRT performance and testosterone concentration. These findings contradict previous findings demonstrating a positive correlation between testosterone level and spatial performance (Aleman et al., 2004; Gouchie and Kimura, 1991; Moffat and Hampson, 1996; Ostatnikova et al., 2002). However, a recently presented extensive study (160 women and 177 men) demonstrated no relationship between testosterone concentration and spatial performance in young men and women (Puts et al., 2010). This result indicates that the relationship between testosterone and mental rotation performance is rather ambiguous and still requires further clarification.

4.3 Manual speed and accuracy

Naturally cycling women were slower, but more accurate when performing Target test as compared to hormonal contraception users. However, there were no differences in laterality ratio, parameter which provides a correction for differences in base performance between groups. Moreover, the speed and accuracy depended on neuroticism and in NC group neuroticism was more expressed as compared to OC group. Based on this, it is possible to assume that differences in manual speed and accuracy between groups are determined not by hormonal contraception usage/no usage, but by other individual characteristics of subjects, for example personality traits.

Nevertheless, the positive correlation between 17β -estradiol and the performance speed on the subtle motor tasks has been shown in previous studies (Maki et al., 2002; Szekely et al., 1998) and it was observed in our study (significant relationship between the 17β -estradiol and the left hand performance time in NC group ($r=-0.58$, $p=0.03$). Therefore we could not deny the influence of gonadal hormones on hand speed and accuracy.

4.4 Memory

There were no working and spatial memory differences between groups/subgroups. Although there is a lot of data about the positive estrogen effect on memory and hippocampus in animal models, or evidence for the memory decreases after menopause (for review see (Brinton, 2009)), the data about estrogens (or other gonadal steroids) effect on young healthy women memory are scarce. For example, it was shown improvement of spatial, but not other kinds of memory (including digits remembering) during luteal (higher estrogen and progesterone) as compared to menstrual phase of menstrual cycle in one study (Phillips and Sherwin, 1992), but there were no spatial (Mordecai et al., 2008) or working (Konishi et al., 2009) memory relationships with gonadal hormones level in other studies.

4.5 Mood

There were no significant differences in positive and negative affect neither between NC and OC groups nor between OC_A and OC_{AA} subgroups in our study. Relationship between hormonal contraceptives use and mood is rather ambiguous - there are studies showing reduced mood and overall well being in OC group (Brown et al., 2008), as well as showing enhanced positive affect scores by hormonal contraceptives (Almagor and Ben-Porath, 1991).

Our finding that negative affect is significantly related with subjects age (NC $r=-0.47$, $p<0.1$; OC $r=-0.40$, $p=0.06$) and education duration (NC $r=-0.45$, $p<0.01$; OC $r=-0.4$, $p=0.04$) is in contrast to the results presented by Crawford and Henry (Crawford and Henry, 2004), who performed study aimed to evaluate the reliability and validity of the PANAS (Watson et al., 1988) and concluded that the influence education and age (as well as gender and occupation) on PANAS scores can be ignored.

Although positive affect significantly decreased going from the first session to the third ($p<0.004$) and negatively correlated to the testosterone concentration ($r=-0.48$, $p=0.06$) in NC group, there were no significant relationships between PANAS scales scores and cognitive parameters. Based on this we assume that mood had no influence on the main results of our study.

4.6 Personality traits

Consistent with the very early previous studies (Beard et al., 1974; Jacobsson et al., 1981) our data from Eysenck test revealed that in hormonal contraception users the neuroticism is significantly less and extraversion more (non significantly) expressed as compared to non-users. This finding suggests that the personality could be important factor influencing the performance on cognitive tests. Otherwise there were almost no significant relationships between cognitive test results and personality test values (exception Target test) in our study.

4.7 Limitations

There are several limitations of the current study that are noteworthy. First, the methodological limitation in saliva sampling and hormonal analysis has to be mentioned. 25 % of samples were omitted from analysis and consequently data on cognitive parameters has been reduced in some analysis. The main reason for excluding hormonal data was possible blood contamination in saliva. Our experience from subsequent experiments (unpublished data) has proven that the sampling of two saliva samples during each experiment (before and after) results in an enhanced probability to collect good samples for the assay.

Second, the number of subjects in OC subgroups (11 in each) could be a limiting factor, which determined that only tendencies but not significant effects of some factors have been found.

CONCLUSIONS

1. Women using hormonal contraception with androgenic properties performed significantly worse on verbal fluency task and slower on mental rotation task.
2. The accuracy of performing on the mental rotation task significantly negatively depends on the task difficulty in hormonal contraception users, but not in non-users group.
3. The usage of hormonal contraception did not affected:
 - performance on working and spatial memory tests;
 - mood.
4. The neuroticism is significantly less expressed in hormonal contraception users group as compared to non-users.

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SANTRAUKA (Summary in Lithuanian)

Hormoninė kontracepcija - vienas iš populiariausių apsaugos nuo nėštumo metodų. Sintetiniai natūralių hormonų analogai, veikdami taikinius smegenyse ir lytinėje sistemoje, blokuoja folikulų brendimą, panaikina natūralių hormonų lygio svyravimus. Nervų sistemoje lytinių hormonų poveikis neapsiriboja su lytinės sistemos reguliacija susijusiomis sritimis, jis parodytas daugumoje CNS sričių, tame tarpe ir siejamose su kognityvine veikla. Šio tyrimo metu vertinome kognityviųjų funkcijų priklausomybę nuo hormoninės kontracepcijos vartojimo/nevartojimo, bei nuo hormoninės kontracepcijos sudėties. Vienoje grupėje buvo jaunos, sveikos merginos, turinčios natūralų, reguliarių mėnesinių ciklą. Antrąją tyrimo grupę sudarė merginos, vartojančios hormoninę kontracepciją, pusė jų vartojo hormoninę kontracepciją pasižyminčią androgeniniu, antra pusė – anti-androgeniniu poveikiu. Tyrimo rezultatai parodė, kad merginos, vartojančios androgeniniu poveikiu pasižyminčią hormoninę kontracepciją, kalbines užduotis (žodžių vardijimo) atlieka prasčiau, o erdvines (erdvinių figūrų sukimo mintyse) - lėčiau. Be to, erdvinių užduočių sunkinimas reikšmingai blogino hormoninę kontracepciją vartojančių merginų rezultatus ir turėjo mažai įtakos nevartojančiųjų rezultatams. Negavome skirtumų tarp hormoninę kontracepciją vartojančiųjų ir nevartojančiųjų atminties bei nuotaikos. Tačiau, asmenybės bruožų palyginimas parodė, kad tarp merginų, pasirinkusių vartoti hormoninę kontracepciją, mažiau išreikštas neurotiškumas ir labiau išreikšta ekstraversija.

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2. **R. Griksiene**, O. Ruksenas (2009) Cognitive effects of hormone based contraception in young healthy women. // Biologija, 55(3-4), P.115-124.

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2. L. Mačiukaitė, **R. Griškienė**, O. Rukšėnas (2008) “How emotional reactions depend on the phases of menstrual cycle?” (in Lithuanian)// Proc. 5th Conference of junior psychologist, Vilnius, P. 34-40.
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5. L. Maciukaite, **R. Griksiene**, O. Ruksenas. „Evaluation of emotional pictures in different phases of menstrual cycle“// IV International conference ”Psychophysiological and visceral functions in norm and pathology”, Kyiv, 2008, P.32
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