Mood and the pill

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Chapter 5

Mineralocorticoid receptor haplotype, estradiol, progesterone and emotional information processing

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Abstract

Background

Carriers of MR-haplotype 1 and 3 (GA/CG; rs5522 and rs2070951) are more sensitive to the influence of oral contraceptives (OC) and menstrual cycle phase on emotional information processing than MR-haplotype 2 (CA) carriers. We investigated whether this effect is associated with estradiol (E2) and/or progesterone (P4) levels.

Method

Healthy MR-genotyped premenopausal women were tested twice in a counterbalanced design. Naturally cycling (NC) women were tested in the early-follicular and mid-luteal phase and OC-users during OC-intake and in the pill-free week. At both sessions E2 and P4 were assessed in saliva. Tests included implicit and explicit positive and negative affect, attentional blink accuracy, emotional memory, emotion recognition, and risky decision-making (gambling).

Results

MR-haplotype 2 homozygotes had higher implicit happiness scores than MR-haplotype 2 heterozygotes ($p = .031$) and MR-haplotype 1/3 carriers ($p < .001$). MR-haplotype 2 homozygotes also had longer reaction times to happy faces in an emotion recognition test than MR-haplotype 1/3 ($p = .001$). Practice effects were observed for most measures. The pattern of correlations between information processing and P4 or E2 differed between sessions, as well as the moderating effects of the MR genotype. In the first session the MR-genotype moderated the influence of P4 on implicit anxiety ($sr = -.30$; $p = .005$): higher P4 was associated with reduction in implicit anxiety, but only in MR-haplotype 2 homozygotes ($sr = -.61$; $p = .012$). In the second session the MR-genotype moderated the influence of E2 on the recognition of facial expressions of happiness ($sr = -.21$; $p = .035$): only in MR-haplotype 1/3 higher E2 was correlated with happiness recognition ($sr = .29$; $p = .005$). In the second session higher E2 and P4 were negatively correlated with accuracy in lag2 trials of the attentional blink task ($p < .001$). Thus NC women, compared to OC-users, performed worse on lag 2 trials ($p = .041$).

Conclusion

The higher implicit happiness scores of MR-haplotype 2 homozygotes are in line with previous reports. Performance in the attentional blink task may be influenced by OC-use. The MR-genotype moderates the influence of E2 and P4 on emotional information processing. This moderating effect may depend on the novelty of the situation.

Introduction

Female hormones modulate the impact of stress on mood. For instance, high estradiol (E2) concentrations attenuate the negative influence of a psychosocial stressor on mood and promote fear inhibition (Albert et al., 2015; Lebron-Milad & Milad, 2012; Milad et al., 2010). Furthermore, reward-sensitivity and emotional information processing are influenced by the menstrual cycle, probably depending on female hormones like estradiol (E2) and progesterone (P4) (Hamstra et al., 2016; Bayer et al., 2013; Dreher et al., 2007).

Oral contraceptives (OC) contain synthetic estrogens and progestins that also influence human cognition. Better performance on verbal memory, associative learning and spatial attention tasks was observed in OC-users (Gogos et al., 2014; Sundstrom Poromaa & Gingnell, 2014). OC-use may also affect emotion processing, in particular the recognition of negative facial and bodily expressions of emotions as well as decision-making (Suslow et al., 2015; Hamstra et al., 2016, 2015, 2014; Maner & Miller, 2014; Gingnell et al., 2013; Pearson et al., 2009). Cognitive function in post-menopausal women was not improved by hormone replacement therapy with naturally occurring estrogens and a (synthetic) progestin (Lethaby et al., 2008).

The observed effects of OC and female hormones on emotional information processing may be mediated by estrogen and progesterone receptors, which are abundantly expressed in limbic brain structures (Handa & Weiser, 2014). In these limbic areas the sex steroids may modulate the function of mineralocorticoid receptors (MR), that mediate the action of cortisol on vigilance and selective attention (Hermans et al., 2014; Cornelisse et al. 2011) as well as on encoding of spatial (Arp et al, 2014) and emotional memory performance in animal and human studies (Otte et al., 2015; Zhou et al. 2010; Joëls et al., 2008; Otte et al., 2007; de Kloet et al., 2005). Progesterone (P4) binds to the MR with nearly the same affinity as aldosterone and cortisol, and acts as a competitive antagonist (Quinkler et al., 2002; Carey et al., 1995). E2 suppresses the synthesis and transactivation of the MR in brain and vascular endothelial cells (Barrett Mueller et al., 2014; Carey et al., 1995). Consequently, the MR is of relevance in candidate gene studies investigating the influence of female hormones on emotional information processing.

Recent research has identified a common functional MR-haplotype block that is located at the 5'promoter of the gene and is based on two single nucleotide polymorphisms: MR-2G/C (rs2070951) and MR-I180V (A/G, rs5522) (van Leeuwen et al., 2011). Female carriers of MR-haplotype 2 (MR-2C/I180: CA) appeared to have a lower risk of depression during their reproductive years (Klok et al., 2011). Consistent with this, observations in a population-based sample (n = 665) and a clinical cohort from the Netherlands Study of Depression and Anxiety (NESDA; n = 1639) revealed that female carriers of MR-haplotype 2 who reported childhood maltreatment were less likely to develop depression than MR-haplotype 3 carriers who reported

maltreatment (Vinkers et al. 2015). The MR-haplotype also moderates the impact of the menstrual cycle and OCs on emotional information processing. MR-haplotype 1/3 carriers were sensitive to the impact of OC on recall and on the recognition of sad and fearful facial expressions (Hamstra et al., 2015). Within the MR-haplotype 1/3 carriers, OC-users recognized fewer emotions than non-users in the mid-luteal phase of the menstrual cycle (Hamstra et al., 2016). These effects were not observed in MR-haplotype 2 carriers. These observations might explain why some women experience more mood-swings during the menstrual cycle and/or depression-congruent side effects of OC, whereas others do not (Boron & Boulpaep, 2012; Kulkarni, 2007).

The aim of the present study was to investigate the effect of menstrual cycle phases and OC use on emotional information processing in healthy women and the possible moderation of this effect by MR-genotype. Contrary to most previous studies, we used a longitudinal, within-person design. We measured estradiol (E2) and progesterone (P4) concentrations in saliva. We hypothesized that variations in female sex steroid levels affect emotional information processing more strongly in MR-haplotype 1/3 carriers than in MR-haplotype 2 carriers.

Methods

Design

This study had a counterbalanced within-subject design. All data were collected from March till June 2015. OC-users were tested in a counterbalanced entry-order: once in the second week of active OC-use (day 8 - 14) and once during inactive OC-use (day 4–7 of the pill-free week). Naturally cycling (NC) participants were tested at two counterbalanced time-points that are characterized by relatively stable hormone levels of E2 and P4. Once in the early follicular phase (day 2-6), when both hormones are low, and once in the middle of the luteal phase (3–10 days before the onset of the new cycle) when the concentration of P4 is at its maximum and E2 reaches a second peak (Bayer et al., 2014, Boron & Boulpaep, 2012). At intake the average cycle duration of the NC participants was registered. After confirmation of the start of the new cycle, test data were scheduled and adjusted to the individual cycle-duration, reasoning that the luteal phase always lasts two weeks (Boron & Boulpaep, 2012). Participation ended after confirmation of the start of the new cycle. This cycle onset information was used to confirm whether participants had been tested on the right moment.

Participants

Participants were recruited through social media and at Leiden University campus. Eligible participants were healthy, non-smoking female university students (18-35 years) of Northwestern European origin. Naturally cycling (NC) participants had a regular menstrual cycle of between

25-35 days, had not used any hormonal contraceptives for at least three months and did not have premenstrual syndrome (PMS) as determined by the MDQ (Menstrual Distress Questionnaire; Moos, 1968). OC-users took mono-phasic OCs with as compounds ethinylestradiol (EE; 0.03)/ levonorgestrel (LNG; 0.15) for more than three months and applied a pill-free week. Mental health was screened with the General Health Questionnaire 12 item-version, with a cut-off score of X > 2 (Goldberg et al., 1997). Exclusion criteria were self-reported current psychological or psychiatric treatment; pregnancy or lactation; drinking > 14 units alcohol per week; use of cannabis in the past three months; use of MDMA (3,4-methyleendioxymethamfetamin) (> 1 per month during the past three months or any during the past month); any other illegal drug (lifetime); smoking; and current use of prescribed medication likely to interfere with female hormonal levels.

Clinical characteristics

Personality traits (NEO-Five Factor Inventory; McCrae & Costa, 1987) were assessed at the first session. Mood state was assessed by the 20-item state version of the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988), after assessment of the IPANAT. Participants were screened for premenstrual syndrome as determined by the MDQ (Menstrual Distress Questionnaire; Moos, 1968) after completion of the second session.

Procedure

After expressing interest, participants completed an online questionnaire screening for in- and exclusion criteria by an online survey tool (Qualtrics). This was followed up by a screening phone interview. In both sessions tasks were assessed in a fixed order: firstly the IPANAT and the PANAS were assessed and then the first saliva sample was collected. Subsequently, the categorization WCMT, attentional blink task and recall WCMT were assessed. After a short break the second saliva sample was collected and the FERT, RMET and DMT were assessed, after which the third saliva sample was collected. At the end of the first session mucus for MR-genotyping was collected with a buccal swab. All participants provided written informed consent and received € 70 after completion. This study was approved by the Ethics Committee Psychology of Leiden University (CEP 6212909526).

Biological measures

Hormonal assessment

E2 and P4 were assessed in saliva, which was collected at three time-points with 30-minute intervals. In order to control for pregnancy estriol level in saliva was assessed as well. Participants were instructed to avoid eating, drinking, chewing gum 30 min prior to participation. Just before

saliva collection they were asked to rinse the oral cavity with water. Each sample contained approximately 2 ml saliva, collected by polypropylene straws in IBL ultrapure polypropylene tubes (SaliCap Sets; Innovation Beyond Limits, Hamburg, Germany). Samples were immediately stored and kept frozen at −20 °C until the day of assaying. The three samples were pooled and analyzed with highly sensitive luminescence assays of IBL at Ganzimmun Diagnostics AG (D). Reference values of free E2 in saliva were: follicular phase 0.2-10.4 pg/ml; ovulation 5.8 – 21.2 pg/ml; luteal phase 0.8 – 10.8 pg/ml. For free P4 (in saliva): follicular phase 50-100 pg/ml; ovulation 100-150 pg/ml; luteal phase 100-450 pg/ml; post-menopause and OCs: 10-50 pg/ml.

Mineralocorticoid haplotype (MR-haplotype)

Analysis of the rs2070951 and rs5522 polymorphisms.

To determine the rs2070951 and rs5522 polymorphisms, PCR fragments were sequenced using the forward primers (5'-GTTCCYTAGATTCCAGCTCAG-3') respectively (5'-AGAGGAGTTCCCTGGGTGAT-3') and dye terminator chemistry (BigDye v3.1, Applied Biosystems). Sequence reactions were run on an ABI-3730 automated sequencer and sequence data was analysed using SeqScape software (Applied Biosystems).

DNA isolation.

Buccal swabs/saliva from individuals were collected in lysisbuffer (100 mM NaCl, 10 mM EDTA, 10 mM Tris pH 8, 0.1 mg/ml proteinase K and 0.5% w/v SDS) until further processing. Genomic DNA was isolated from the samples using the Chemagic buccal swab kit on a Chemagen Module I workstation (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany).

PCR amplification.

The rs2070951 and rs5522 regions were amplified by PCR using the following primers: a forward primer (5'- GCTGGAAACAGAGCACCTTG -3') and a reverse primer (5'-GCAAGCCACCCACTTCACTA-3'). Typical PCR reactions contained between 10 to 100 ng genomic DNA template, 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 2.5U of Paq5000 DNA polymerase (Agilent Technologies) in a total volume of 30 µl using the following cycling conditions: initial denaturation step of 4 min at 95°C, followed by 40 cycles of 30 sec 94°C, 30 sec 50°C, 120 sec 72°C and a final extension step of 10 min 72°C. After the first PCR 1ul of the amplification product was used directly in a second PCR amplification with nested primers. The following primers were used: a forward primer (5'-GGAGGSCTGGAAATTGAGGA–3') and a reverse primer (5'-CGACAAGCTGTAGTCAATACTC-3'). The PCR reactions contained 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 2.5U of Paq5000

DNA polymerase (Agilent Technologies) in a total volume of 30 µl using the following cycling conditions: initial denaturation step of 4 min at 95°C, followed by 40 cycles of 30 sec 94°C, 30 sec 50°C, 120 sec 72°C and a fin al extension step of 10 min 72°C.

According to the observed frequency in the population (DeRijk et al., 2008), MR-haplotype 1 (GA) is composed by MR-2 (G) and MR-I180V (A), MR-haplotype 2 (CA) by MR-2 (C) and MR-I180V (A), MR-haplotype 3 (CG) by MR-2 (C) and MR-I180V (G) and the in vivo seldom observed MR-haplotype 4 (GG) by MR-2 (G) and MR-I180V (G).

Test battery

Implicit affect

We used the Implicit Positive and Negative Affect test (IPANAT; Quirin et al. 2009), which asks participants to rate the extent to which six artificial words (i.e. SUKOV) in their minds express emotions, using four-point Likert scales. The emotions used were anger (angry, irritated, annoyed), sadness (sad, down, unhappy), anxiety (anxious, afraid, fearful) and happiness (happy, lucky, good-humored). Since the stimuli have no meaning, ratings are thought to reflect (latent) affect states (Brosschot et al., 2014).

Word categorization and memory task (WCMT)

The WCMT holds sixty personality characteristics that are generally rated as either disagreeable (e.g. domineering, untidy, hostile) or agreeable (e.g. cheerful, honest, optimistic) (Anderson, 1968). All characteristics were presented on a computer screen for 500 ms. Disagreeable and agreeable words were matched in terms of word length, ratings of frequency used and familiarity ('meaningfulness'). Participants were asked to indicate as quickly as possible whether they would like or dislike being associated with the characteristic. Before the start of the task, they were informed that they would be given a recall test later (but were not told when). Delayed recall was tested after completion of the attentional blink task. We analyzed the number of correctly recalled positive and negative characteristics and positive and negative false recognitions (traits not previously presented).

Attentional blink

Attentional blink (AB) is an impairment in the detectability of the second of two similar targets (T1 and T2; both randomized non-identical single-digits) that appear within a rapidly presented sequence of stimuli (separately presented letters). Each trial started with a fixation cross, presented for 1,000 ms at the center of the computer screen. Next, a rapid serial visual presentation stream of 20 letters and two single-digits (T1 and T2) was presented. All items were displayed for 26 ms, separated by 80-ms blanks. The task contained 80 randomized trials, preceded by 12 practice

trials with feedback on performance. T2 appeared either as the second (lag2) or seventh stimulus (lag7) after T1. Analyses were performed on mean T1 and T2 accuracy, where T2 accuracy was computed as the percentage of correctly identified stimuli if T1 was correct. A response was considered correct if both the identity and the temporal order were reported correctly (Akyurek et al., 2012).

Facial expression recognition task (FERT)

The FERT displays five basic emotions (happiness, sadness, fear, anger and disgust) taken from the Pictures of Facial Affect Series (Ekman & Friesen, 1976). A male and a female face were morphed between each prototype and the neutral expression in 10% steps. Each face was also presented in a 100% neutral expression. Four trials of each emotion were presented at each intensity level, leading to a total of 204 stimuli. These were presented in a randomized order for 500 ms and replaced by a blank screen. Participants were asked to respond as quickly and accurately as possible by pressing the corresponding buttons of a response box. Total accuracy and mean reaction times (of the correctly recognized trials) were calculated per emotion.

Reading the mind in the eye task (RMET)

The RMET is an advanced emotion recognition test, a measure of adult social intelligence or cognitive empathy. The task consists of 36 photographs of the eye region that express more complex emotional states (e.g., contempt). It is a multiple-choice test: four adjectives are presented with each trial. The photographs display 11 negative, 7 positive and 16 neutral states and 2 mixed states ["preoccupied" (negative or neutral) and "interested" (positive or neutral)]. Accuracy was calculated in proportion correctly recognized expressions per valence (positive, negative, neutral) (Harkness et al., 2005).

Risky decision-making task (DMT)

The DMT assesses risky decision-making by presenting trials with low or high probabilities to win a low or high number of credits, in the context of a low or high (probability of) potential losses (Rogers et al., 2003). This adaptation of the Iowa Gambling Test consists of 80 trials in which participants had to choose between a relatively safe gamble (50% chance of winning or losing 10 cents) and a riskier gamble (e.g., 60% chance of winning 30 cents but 40% chance of losing 70 cents) (see also Hamstra et al., 2015). The order of trials was randomized within four blocks of 20 trials, yielding each trial type twice per block. The aim of the test was to gain as many cents as possible and participants were to keep the amount gained at the end of the test (most often between €1 and €3).

Statistical analyses

Mean scores on personality dimensions (NEO-FFI) and age were compared with t-tests and committed relationship status by chi-square tests. Outcomes are presented in table 1.

We analyzed the outcomes of the test battery with repeated measures multivariate analyses of variance (rm MANOVAs) with time (session 1, 2) as within-subject factor and hormonal status (OC, NC), order (high/low hormones) and MR-haplotype (amount MR-haplotype 2 alleles: 0, 1, 2) as between-subject factors. (Interaction) effects on time ('learning-effects') are reported but not interpreted in the discussion (Tops & Weijers, 2011). Follow-up (M)ANOVAs with split on OC/NC and MR-haplotype were performed if interaction effects in MANOVA were significant. Effects on MR-haplotype ($p<.05$) were investigated with contrasts (simple last). If assumptions of sphericity were violated, a Greenhouse-Geisser correction was reported. Multivariate influential cases (Cook's distance ≥ 1) were excluded from analyses. Estimates of effect size were partial eta squared (η_p^2) and power. We corrected for multiple comparisons by interpreting only effects with $p \leq .005$.

Moderated regression analyses were applied to explore the correlations between sex steroids, MR-haplotype and the test battery outcomes of the first and second session. For reasons of multicollinearity, analyses were performed for E2 and P4 separately. Moderated regression analyses included three terms: MR-haplotype, E2 or P4 concentrations and the corresponding interaction. All variables were entered in a single step and independent variables (E2 and P4) were mean-centered. Log-transformed P4 values were applied in the rm MANOVAs and depicted in the scatterplots. For reasons of multicollinearity however, untransformed P4-values were used in the regression analyses. The regression analyses were done with and without outliers (standardized residuals ≤ -3 and ≥ 3) and influential cases (Cook's distance ≥ 1). Significant interaction effects between MR-haplotype and hormonal levels were followed-up by separate regression analyses ($p < .05$). Semi-partial correlations (sr) were reported, because they reflect the unique contribution of the variable to the model (Tabachnik & Fidell, 2007).

Results

Participant characteristics

A total of 368 women showed interest in our study, 147 of whom fulfilled the inclusion criteria and 118 signed informed consent. During participation 15 women were excluded due to irregular menstrual cycles (< 25 days or > 35 days), another two fell ill and five withdrew, leaving 96 participants. OC-users were more often in a committed relationship, but no other significant differences existed between OC and NC groups. MR-haplotype 2 homozygotes scored lower on NEO-FFI Agreeableness than MR-haplotype 2 heterozygotes ($p < .05$). None of the participants screened positive for premenstrual dysphoric disorder.

Table 1. Sample characteristics

	Pill status		OC			NC		
	OC	**NC**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**
N (% total sample)	57 (59)	39 (41)	25 (26)	22 (23)	10 (10)	20 (21)	13 (14)	6 (6)
Age	20.7 (.2)	20.9 (.33)	20.7 (.3)	20.7 (.4)	20.8 (.5)	20.5 (.5)	21.2 (.5)	20.5 (1.0)
Committed Relationship	36* (63)	12* (31)	15 (60)	13 (59)	8 (80)	7 (35)	2 (15)	3 (50)
NEO-FFI Agreeableness	35.8 (.6)	34.8 (.7)	36.0 (.9)	35.5 (.9)	35.8 (1.2)	34.9 (1.0)	36.2 (1.0)*	31.3 (1.0)*
NEO-FFI Conscientiousn.	41.0 (.4)	40.8 (.4)	41.1 (.6)	40.7 (.8)	41.4 (.4)	40.7 (.6)	41.2 (.9)	40.2 (1.2)
NEO-FFI Extraversion	40.5 (.3)	39.5 (.5)	40.7 (.5)	40.5 (.6)	39.9 (.7)	39.0 (.7)	39.9 (.9)	40.5 (.7)
NEO-FFI Neuroticism	33.1 (.5)	33.6 (.7)	33.6 (.9)	32.5 (.7)	33.9 (1.4)	33.8 (.9)	34.5 (1.0)	31.0 (1.9)
NEO-FFI Openness	34.9 (.5)	34.2 (.7)	34.5 (.7)	35.7 (.7)	34.1 (1.1)	34.3 (.9)	34.9 (1.3)	32.5 (1.0)

Notes: N (%) or Means (SE). *p < .05 (t-tests). *Abbreviations:* OC = oral contraceptives users; NC = naturally cycling women; MRHT 1/3 = mineralocorticoid receptor haplotype 1/3; MRHT 2 het = mineralocorticoid receptor haplotype heterozygotes; MRHT 2 hom = mineralocorticoid receptor haplotype homozygotes; NEO-FFI = NEO Five-Factor Inventory.

Table 2. Mean (SE) hormonal levels per subcategory.

	E2 pg/ml	**P4 pg/ml**
Early follicular phase (EF)	2.6 (.2)	72.9 (17.6)
Inactive OC-use (IU)	2.3 (.2)	43.6 (2.9)
Mid-luteal phase (ML)	3.4 (.2)	148.2 (16.1)
Active OC-use (AU)	2.1 (.2)	53.7 (5.7)

Abbreviations: E2=estradiol; P4=progesterone.

Genotype frequencies of the rs 5522 SNP were in Hardy-Weinberg equilibrium ($\chi^2(1) = .1$; $p = .750$), however those of the rs 2070951 SNP were not ($\chi^2(1) = 4.0$; $p = .045$). Information on demographic variables and MR-genotype outcomes are given in table 1.

Hormonal measures

Analyses failed of four samples from the first session and one from the second session. P4 values of four participants were significant outliers (± 3 SDs) or exceeded the corresponding reference values: one of these samples was collected in the first and three in the second session. We included these outliers in our analyses, since their estriol levels did not indicate pregnancy and large interpersonal differences in P4 exist (Boron and Boulpaep, 2012). The findings showed the expected pattern (see table 2).

Test battery

Implicit positive and negative affect

Tests of within-subject effects showed that session 2 scores were higher for IPANAT anxiety [($F(1,85) = 10.9$; $p = .001$; $\eta_p^2 = .11$; power = .90] and IPANAT sadness [($F(1,85) = 5.4$; $p = .023$; $\eta_p^2 = .06$; power = .63]. Between-subjects effects showed an interaction effect of MR haplotype and OC/NC status on IPANAT anxiety, which was statistically significant but did not survive correction for multiple comparisons [$F(2,85) = 3.5$; $p = .035$; $\eta_p^2 = .08$; power = .64]: IPANAT anxiety scores differed between MR-haplotype carriers only in OC-users [$F(2,51) = 3.5$; $p = .037$; $\eta_p^2 = .12$; power = .63]. MR-haplotype groups scored significantly different on IPANAT happiness [between-subject effects; $F(2,85) = 7.3$; $p = .001$; $\eta_p^2 = .15$; power = .93]: MR-haplotype 2 homozygotes scored higher on IPANAT happiness than MR-haplotype 2 heterozygotes ($p = .031$) and MR-haplotype 1/3 carriers ($p < .001$) (see figure 1). This effect remained significant after correction for multiple comparisons. All scores are listed in table 3.

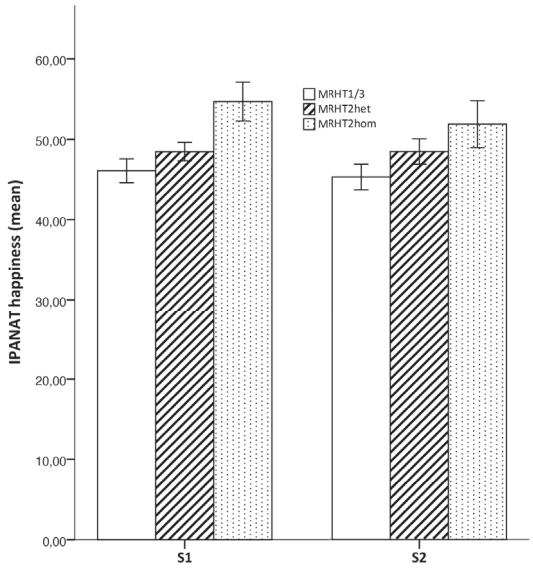

Figure 1. Implicit happiness scores by MR-haplotype.
Notes: Error bars: +/- 1 SE. *Abbreviations:* MRHT = mineralocorticoid receptor haplotype; S1 = session 1; S2 = session 2; IPANAT = implicit positive and negative affect.

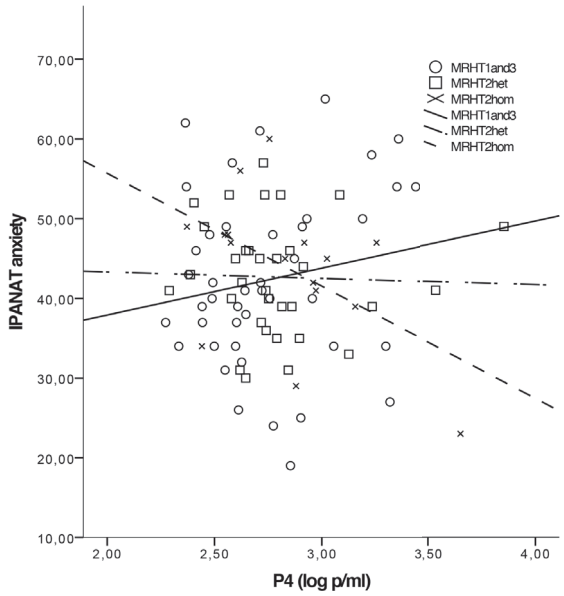

Figure 2. Progesterone (P4) correlations with implicit anxiety scores (session 1).
Abbreviations: MRHT = mineralocorticoid receptor haplotype; IPANAT = implicit positive and negative affect.

Word categorization and memory task

Accuracy. Within-subject effects revealed significant time-effects: participants recalled more positive [(F(1,85) = 64.5; p < .001; η_p^2 = .43; power = 1.0] and negative [(F(1,85) = 48.5; p < .001; η_p^2 = .36; power = 1.0] characteristics in the second than in the first session.

Semi-partial correlations: Memory performance was not associated with E2 or P4 (p > .063).

Attentional blink

Accuracy. Within-subject effects revealed that accuracy improved between sessions [F(1,85)=162.6; p < .001; η_p^2 = .66; power = 1.0]. As expected, the second target was recognized less frequently in lag2 than lag7 trials, confirming the AB phenomenon [F(1,85) = 45.1; p < .001; η_p^2 = .35; power = 1.0]. Between-subjects effects showed that NC women, compared to OC-users, performed worse on lag 2 trials [F(1,85) = 4.3; p = .041; η_p^2 = .05; power = .54] and lag 7 trials [F(1,85) = 4.2; p = .044; η_p^2 = .05; power = .52]. This effect was no longer significant after correction for multiple comparisons. All scores are listed in table 3.

Semi-partial correlations: In the first session performance on lag 7 trials was negatively correlated with MR-haplotype (sr = -.23; p = .030). In the second session performance on lag 2 trials was negatively correlated with E2 (sr = -.35; p < .001) and P4 (sr = -.35; p < .001). See figure 3.

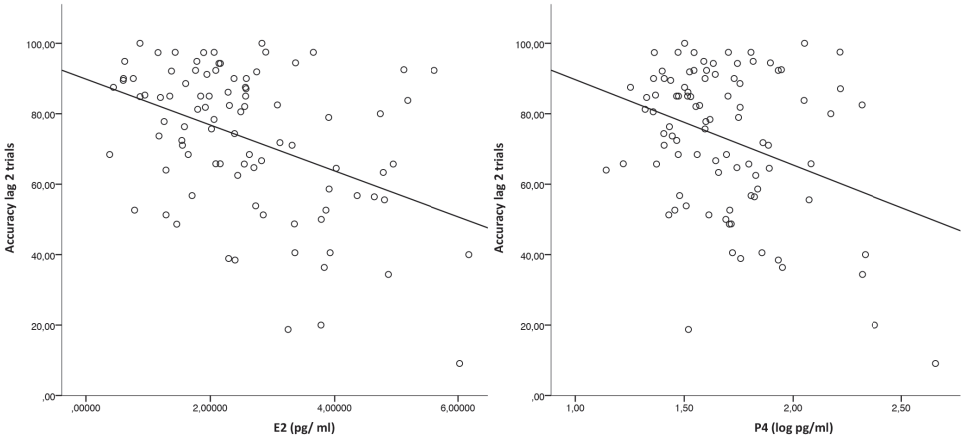

Figure 3. Estradiol (E2) and progesterone (P4) correlations with performance in lag 2 trials (session 2)

Facial expression recognition test

Accuracy. Within-subject effects revealed a significant time-effect on accuracy in the recognition of anger, disgust, fear, happy and sad (all p < .002), that improved from session 1 to session 2. Between-subject effects revealed that OC-users recognized fewer expressions of happiness

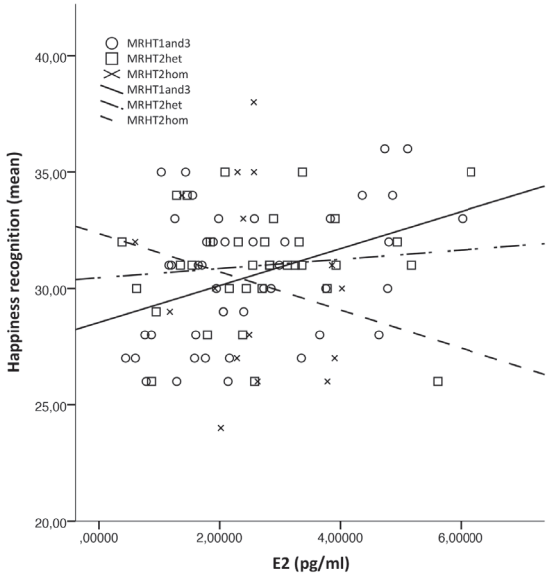

Figure 4. Estradiol (E2) correlates with happiness recognition accuracy (session 2)
Abbreviations: MRHT = mineralocorticoid receptor haplotype

[F(1,85) = 4.0; p = .047; η_p^2 = .05; power = .51] and sadness [F(1,85) = 4.0 p = .048; η_p^2 = .05; power = .51]. This effect was no longer significant after correction for multiple comparisons. All scores are listed in table 3.

Semi-partial correlations. In the first session E2 was positively correlated with the recognition of sadness (sr = .20; p = .048) and an interaction effect was observed between P4 and MR-haplotype in disgust (sr = .21; p = .046) and happiness recognition (sr = .21; p = .047): in follow-up analyses these effects were not significant anymore (p > .081). In the second session MR-haplotype correlated with disgust recognition (sr = .30; p = .005). Happiness recognition was correlated with E2 (sr = .29; p = .005) and an interaction effect between E2 and MR-haplotype was observed (sr = -.21; p = .035): follow-up regression revealed that only in MR-haplotype 1/3 E2 was positively correlated with happiness recognition (sr = .39; p = .008). See figure 4.

Reaction times. Reaction times (RTs) were positively skewed so were log transformed. Three participants were excluded from the analyses since they misclassified all sadness expressions, so no reaction times for correct trials were registered. One participant was excluded from the analyses, because her RTs were extremely long in the second session (> 5 SDs). Univariate tests of within-subject effects revealed that reaction times of correctly recognised expressions of anger, disgust, fear and sad were shorter in the second session (all p≤.002). Between-subject effects showed that OC-users were quicker to recognize anger [F(1,81) = 5.9; p = .017; η_p^2 = .07; power = .67] and

happiness [F(1,81) = 8.2; p = .005; η_p^2 = .09; power = .81] than NC participants. MR-haplotypes had different RTs on fear [(F(2,81) = 3.8; p = .026; η_p^2 = .09; power = .68] and happiness [(F(2,81) = 8.7; p < .001; η_p^2 = .18; power = .96]: MR-haplotype 2 homozygotes reacted slower to happy faces than MR-haplotype 1/3 (p = .001). After correction for multiple comparisons the effects on happiness remained. See table 3 for all means.

Semi-partial correlations: In the second session P4 was positively correlated with disgust RTs (sr = .25; p = .011) and MR-haplotype was positively correlated with the RTs in happiness trials (sr = .27; p = .01).

Reading the mind in the eye test

Between-subject effects revealed that the recognition of positive characteristics differed between MR- haplotype carriers [F(2,85) = 4.4; p = .015; η_p^2 = .09; power = .74]: MR-haplotype 2 homozygotes performed worse than MR-haplotype 1/3 (p = .017). An interaction-effect between OC/NC status and MR-haplotype was also observed F(2.85) = 3.9; p = .024; η_p^2 = .08; power = .69], follow-up analyses did not reveal any significant effects. Finally, OC-users recognized more positive characteristics than NC women [F(1,85) = 11.0; p = .001; η_p^2 = .12; power = .91], which remained significant after correction for multiple comparisons. All scores are listed in table 3.

Semi-partial correlations: In the first session MR-genotype was correlated with the recognition of positive characteristics (sr = -.25; p=.019) and an interaction-effect was observed between MR-genotype, E2 (sr = .23; p = .029) and P4 (sr = -.25; p = .013): only in MR-haplotype 2 homozygotes P4 was negatively correlated with positive characteristics recognition (sr = -.54; p = .038).

Risky decision-making task

As expected, participants gambled more in trials with a high probability of winning than in trials with a low probability [F(6,508) = 59,1; p < .001; η_p^2 = .41; power = 1.0], in trials with large vs small expected gains [F(6,508) = 88,7; p < .001; η_p^2 = .51; power = 1.0] and in trials with small vs large expected losses [F(6,526) = 94,6; p < .001; η_p^2 = .53; power = 1.0]. A significant effect of time on the number of trials in which participants chose to gamble was also observed [F(2,84) = 8.5; p < .001; η_p^2 = .17; power = .96]. In trials with low losses risky decision-making differed between MR-haplotypes [between-subject effects; F(2,85) = 3.8; p = .026; η_p^2 = .08; power = .68]: MR-haplotype 2 homozygotes gambled more than MR-haplotype 1/3 carriers (p < .009). This effect did not survive correction for multiple comparisons, however. Mean scores are listed in table 3. See figure 5.

Table 3. Mean outcomes (SE)

Task	Session 1					Session 2				
	Pill status		MR-haplotype			Pill status		MR-haplotype		
	OC	NC	MRHT 1/3	MRHT 2 het	MRHT 2 hom	OC	NC	MRHT 1/3	MRHT 2 het	MRHT 2 hom
PANAS PA	28.7 (.6)	28.4 (.9)	29.2 (.8)	28.0 (.9)	28.1 (.9)	27.1 (.9)	25.5 (1.1)	26.9 (1.0)	26.1 (1.1)	26.0 (1.4)
PANAS NA	12.3 (.3)	13.1 (.6)	12.0 (.4)	13.3 (.6)	13.0 (.9)	13.0 (.5)	13.8 (1.0)	12.5 (.7)	13.3 (.6)	15.6 (1.8)
IPANAT happin.	48.3 (1.1)	48.5 (1.7)	46.1 (1.5)**	48.5 (1.1)*	54.7 (2.4)**,*	47.5 (1.4)	47.5 (1.7)	45.2 (1.6)*	48.4 (1.6)	51.8 (2.9)*
IPANAT sadness	44.5 (1.1)	44.3 (1.2)	43.3 (1.2)	45.1 (1.4)	45.9 (2.0)	45.6 (1.2)	46.8 (1.4)	45.0 (1.4)	46.4 (1.4)	48.6 (1.9)
IPANAT anxiety	41.5 (1.2)	44.1 (1.5)	41.9 (1.7)	42.8 (1.2)	43.8 (2.3)	43.8 (1.4)	46.5 (1.6)	44.2 (1.6)*a	42.8 (1.6)*b	51.3 (2.7)*a,*b
IPANAT anger	46.7 (1.3)	49.8 (1.4)	48.4 (1.7)	47.9 (1.2)	46.9 (2.3)	48.4 (1.3)	50.2 (1.4)	49.3 (1.6)	49.1 (1.4)	48.6 (2.1)
AB lag 2 (%)	67.9 (2.5)	60.3 (4.3)	67.0 (3.5)	63.4 (3.9)	61.6 (5.2)	75.4 (2.1)	69.8 (4.0)	74.7 (3.2)	70.9 (3.5)	73.7 (3.8)
AB lag 7 (%)	94.5 (.7)	93.1 (.9)	95.2 (.5)	93.3 (1.1)	91.7 (1.4)	96.5 (.5)*	94.2 (.9)*	95.7 (.7)	95.2 (.9)	95.9 (.9)
WCMT positive	4.1 (.3)	4.6 (.3)	4.4 (.3)	4.3 (.4)	4.2 (.5)	6.0 (.4)	6.2 (.3)	5.9 (.3)	5.8 (.4)	7.0 (.7)
WCMT negative	3.7 (.3)	4.0 (.4)	3.8 (.3)	3.7 (.4)	4.1 (.5)	5.0 (.3)	5.2 (.4)	5.4 (.3)	4.5 (.3)	5.6 (.6)
FERT happy	28.8 (.4)	29.9 (.4)	29.4 (.4)	28.8 (.5)	29.8 (.9)	30.4 (.4)	31.0 (.5)	30.5 (.4)	31.0 (.4)	30.3 (1.0)
FERT sad	15.4 (1.1)*	19.0 (1.1)*	16.0 (1.2)	17.3 (1.4)	18.4 (1.4)	20.2 (1.1)	23.4 (1.1)	20.9 (1.2)	21.4 (1.4)	23.4 (1.5)
FERT fear	25.7 (.4)	25.6 (.5)	25.5 (.4)	26.0 (.5)	25.5 (.8)	25.8 (.3)	26.7 (.4)	26.0 (.4)	26.7 (.5)	25.6 (.6)
FERT anger	18.4 (.7)	19.0 (.8)	18.1 (.8)	18.9 (.8)	19.6 (1.2)	20.6 (.8)	21.4 (.9)	20.6 (.8)	21.0 (1.0)	21.6 (1.3)
FERT disgust	26.9 (.6)	27.6 (.8)	26.4 (.8)	27.4 (.7)	28.7 (.9)	28.8 (.4)	28.9 (.8)	28.3 (.7)	29.2 (.5)	29.4 (.9)
FERT happy RT	693 (23)	739 (28)	669 (25)*	764 (33)*	722 (32)	623 (21)*	753 (73)*	586 (22)**	713 (48)**	855 (150)
FERT sad RT	1033 (40)	1115 (66)	1041 (54)	1076 (60)	1122 (90)	931 (44)	972 (44)	866 (39)*,**	1069 (60)**	926 (62)*
FERT fear RT	996 (41)	1034 (52)	970 (49)	1097 (57)	952 (48)	874 (37)	958 (51)	833 (32)*a	1042 (65)*a,*b	844 (49)*b
FERT anger RT	1034 (41)*	1181 (57)*	1068 (52)	1148 (61)	1062 (64)	1036 (96)	1041 (48)	1029 (115)	1063 (63)	1011 (57)
FERT disgust RT	903 (40)	912 (47)	871 (47)	947 (50)	922 (58)	775 (30)	847 (51)	761 (34)	875 (56)	782 (58)
DMT low loss	18,3 (.7)	18,1 (,8)	17,1 (.6)*	18,7 (1,0)	20,6 (1,1)*	17.6 (.7)	17,3 (,8)	16,6 (,6)	17.8 (1.0)	19.3 (1.3)
RMET neutral	11.8 (.3)	12.5 (.3)	11.9 (.4)	12.6 (.3)	11.6 (.5)	12.2 (.3)	12.2 (.3)	11.9 (.4)**a	11.9 (.4)**b	13.2 (.3)**a,**b
RMET positive	7.2 (.1)*	6.7 (.2)*	7.1 (.2)	7.1 (.2)	6.6 (.3)	6.8 (.2)	6.8 (.2)	7.0 (.2)	7.3 (.2)*	6.5 (.3)*
RMET negative	9.1 (.3)	9.1 (.3)	8.9 (.3)	9.0 (.3)	9.5 (.4)	8.9 (.3)	8.9 (.3)	8.7 (.3)	8.8 (.3)	8.9 (.4)

Notes: N (%) or means (SE); *p < .05; **p < .01 (t-tests); [a,b] Different superscripts within rows indicate significant group differences.
Abbreviations: OC = oral contraceptives users; NC = naturally cycling women; MRHT 1/3 = mineralocorticoid receptor haplotype 1 and 3; MRHT 2 het = mineralocorticoid receptor haplotype 2 heterozygotes; MRHT hom = mineralocorticoid receptor haplotype 2 homozygotes; PANAS = positive and negative affect scale; PA = positive affect; NA = negative affect; IPANAT = implicit positive and negative affect test; AB lag 2 = attentional blink lag 2 trials; AB lag 7 = attentional blink lag 7 trials; WCMT = word categorization and memory task; FERT = facial expression recognition task; RT = reaction times; DMT low loss = decision making task in trials with low losses; RMET = reading the mind in the eye task.

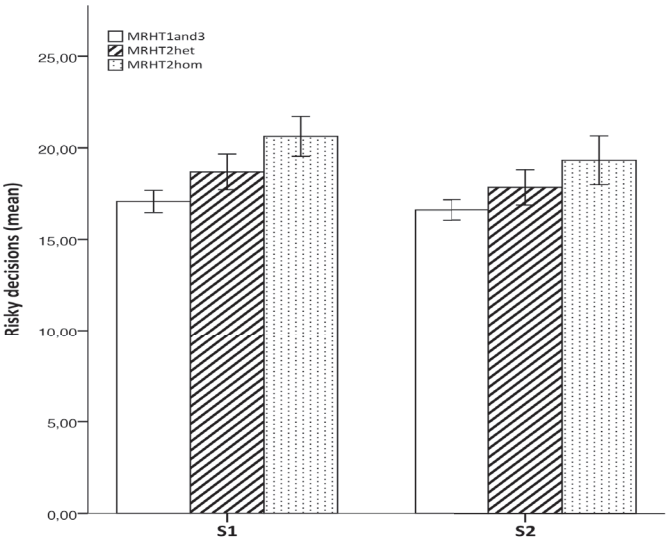

Figure 5. Risky decision-making in trials with low losses by MR-haplotype.
Notes: Error bars: +/- 1 SE. *Abbreviations:* MRHT = mineralocorticoid receptor haplotype; S1=session 1; S2 =session 2;

Semi-partial correlations: In the first session E2 (sr = .21; p = .039) and MR-haplotype (sr = .24; p = .017) were positively correlated with risky decision-making in trials with low losses. In the second session a correlation with MR-haplotype (sr = .22; p = .031) was also observed.

Discussion

The aim of the present study was twofold. Firstly, we investigated the effects of cycle phase and OC use on emotional information processing. We used a counterbalanced within-subject design, whereas our previous studies were cross-sectional. Secondly, we tested the hypothesis that carriers of MR-haplotype 1/3 are more sensitive to the impact of (natural and synthetic) sex steroids on emotional information processing than MR-haplotype 2 carriers. We also examined whether MR-haplotype 2 homozygotes and heterozygotes score differently.

We observed main effects of MR-haplotype, regardless of hormonal status. MR-haplotype 2 homozygotes had higher scores on implicit happiness (p = .001) than MR-haplotype 1/3 carriers. This is in line with a previous observation of higher explicit optimism scores in MR-haplotype homozygotes in a cohort study (Klok et al., 2011). They had also longer reaction times in a facial emotion recognition test, in particular at the trials with happy expressions (p < .001). In prior studies, MR-haplotype 2 carriers showed more efficacious cortisol and ACTH responses

to a psychological stressor, suggestive of a relatively resilient phenotype (van Leeuwen et al., 2011). Our MR-haplotype 2 homozygotes tended to gamble more in trials with low expected losses. This might be interpreted as signaling a more rational or an optimistic attitude, but this finding was no longer significant after correction for multiple comparisons, so must be regarded as preliminary. Previous studies reported comparable risky decision-making patterns in MR-haplotype 2 carriers (Bogdan et al., 2010; Hamstra et al., 2016).

The modulation of MR-functioning by E2 and P4 (Stancycz et al., 2014; Barrett-Mueller et al., 2014; Quinkler et al, 2002; Carey et al., 1995) depending on MR haplotype may be related to stress resilience and vulnerability to depression (Vinkers et al., 2015; TerHeegde et al., 2015; Klok et al., 2011). In our second session, so after habituation, high E2 levels were associated with higher happiness recognition scores. This effect was moderated by MR-genotype: in MR-haplotype 1/3 carriers, E2 was positively correlated with the recognition of happy expressions (sr = .39; p = .008).

Better happiness recognition may reflect a subtle shift towards a more positive information processing bias, which has also been observed in healthy volunteers after single doses of an antidepressant (Harmer et al., 2003; Harmer et al., 2009). We previously found effects of OC-use on information processing in the opposite direction in MR-haplotype 1/3 carriers (Hamstra et al. 2015, 2016). Although we did not measure hormone levels in these studies, the observed effects may have been caused by OC's suppressing E2 or by an intrinsic effect of the synthetic estrogens.

P4 was associated with an increase in implicit anxiety (sr = .25; p = .015). The role of P4 in anxiety is controversial. Most studies report anxiolytic effects of P4 because of metabolization to neurosteroids (Bitran et al. 1995). However, fMRI research observed anxiogenic effects of P4 as reflected by an increased activity of the amygdala (van Wingen et al. 2008). Of interest is in this respect that the positive correlation of P4 with anxiety was only observed in the first session, when the test situation is still novel. In MR-haplotype 2 homozygotes P4 was negatively correlated with implicit anxiety (sr = -.61; p = .012). MR function in the limbic brain is linked to coping with fear and anxiety (Rozeboom et al., 2007; de Kloet et al., 2016), which may be affected by P4 as a competitive antagonist of cortisol at the receptor level (Carey et al. 1995; de Quinkler et al. 2005).

The strengths of our study were that we assessed emotional information processing in a counterbalanced, mixed between-subject (OC/NC) and within-subject design (phase). We tested a homogenous sample of OC-users during (in)active use and NC women on personalized time-points in their menstrual cycle. Additionally, we verified hormonal status with the saliva concentration of E2 and P4. However, our attempt to measure across a broad range of information processing phases resulted in a large number of tests, raising the risk of chance findings. Consequently, some main differences between OC-users and NC women were no longer significant after correction for multiple comparisons, such as the effect of OC-use

on facial expression recognition. In line with our earlier studies, we observed that OC-users recognized fewer facial expressions of sadness than NC women (Hamstra et al., 2014, 2015). OC-users performed better in lag 2 and lag 7 trials of the attentional blink task, but this effect was also no longer significant after correction. In line with Hollander et al. (2005) we observed negative correlations between attentional blink performance and E2 and P4 (both sr = -.35; $p < .001$), however. These correlations remained significant and are in line with the OC-effect on attentional blink performance, since OCs suppress endogenous levels on E2 and P4. Finally, we observed that OC-users, compared to NC women, recognized more positive characteristics in the RMET ($p = .001$). As far as we know, this effect has not been reported earlier.

The pattern of correlations differed between sessions. It is unclear why some correlations were not observed in the first session, but only in the second session. Personal differences at baseline and habituation to a novel setting may play a role. Furthermore, P4 (van Wingen et al., 2008; Bitran et al., 1995) and E2 (McEwen, 2014; McEwen, 2002; McEwen and Alves, 1999) are involved in responses to stress and novelty and may have different effects at both sessions. Oxytocin, a hormone interacting with E2 and also implicated in emotion recognition, was also associated with opposite states (trust vs. anxiety) in a first compared to a second session (Tops et al., 2013). The pattern of the correlations of E2 and P4 with emotional information processing is also quite complex, dependent on genotype and test familiarity, thus awaits further analysis.

Interpretation of the current results should be considered within the context of study limitations. The rs 2070951 distribution deviated significantly from the Hardy-Weinberg equilibrium. Our sample was small for a genetic association study as several subgroups had a low number of participants, making our estimated effect imprecise. Furthermore, practice effects were found in all tasks, except for the RMET. Depending on the test, an effect of repeated testing may reflect simple learning or practice effects (e.g., in the memory test), a change of strategy (e.g., in the gambling task) and increased familiarity (less tension). Reports about practice-effects in emotional test batteries are mixed, with studies reporting that repeated testing did (Thomas et al., 2015) and did not (Adams et al., 2015) affect performance on memory and emotion recognition tests. An extra training session may solve a part of this problem, since performance seems to stabilize after the second session (Thomas et al, 2015). If the research question involves the response to novelty, a between-subjects design may be preferred, despite the obvious limitations.

Finally, future studies in NC women should take care to verify cycle-phase by confirmation of the next cycle onset. We had to exclude 15 cases because their current cycle was shorter or longer than expected. These women had a reported regular cycle of 25-35 days, but they were also young (mean age = 20.7) and menstrual irregularities are more frequently observed in young women (Boron and Boulpaep, 2012).

In conclusion, MR-haplotype 2 homozygotes scored higher on implicit happiness. This

might reflect an optimistic attitude, suggestive of a stress-resilient phenotype. Furthermore, the MR-haplotype may moderate the influence of estradiol and progesterone on emotional information processing, which is in line with our previous findings. We also observed that the pattern of correlations between sex hormones and cognitive performance differs between sessions, which may be related to novelty.