Mood and the pill

Hamstra, D.A.

Chapter 4

Mineralocorticoid receptor haplotype moderates the influence of oral contraceptives and menstrual cycle on emotional information processing

Published as:

Hamstra, D. A., de Kloet, E. R., Tollenaar, M., Verkuil, B., Manai, M., Putman, P., & Van der Does, W. (2016). Mineralocorticoid receptor haplotype moderates the effects of oral contraceptives and menstrual cycle on emotional information processing. *Journal of psychopharmacology,* 30(10), 1054-1061.

Abstract

Rationale

The processing of emotional information is affected by menstrual cycle phase and by the use of oral contraceptives (OC). The stress hormone cortisol is known to affect emotional information processing via the limbic mineralocorticoid receptor (MR).

Objectives

We investigated in an exploratory study whether the MR-genotype moderates the effect of both OC-use and menstrual cycle phase on emotional cognition.

Methods

Healthy premenopausal volunteers (n = 93) of West-European descent completed a battery of (emotional) cognition tests. Forty-nine participants were OC-users and 44 naturally cycling, 21 of whom were tested in the early follicular (EF) and 23 in the mid luteal (ML) phase of the menstrual cycle.

Results

In MR-haplotype 1/3 carriers, ML women gambled more than EF women when their risk to lose was relatively small. In MR-haplotype 2, ML women gambled more than EF women, regardless of their odds of winning. OC-users with MR-haplotype 1/3 recognized fewer facial expressions than ML women with MR-haplotype 1/3.

Conclusion

MR-haplotype 1/3 carriers may be more sensitive to the influence of the female hormonal status. MR-haplotype 2 carriers showed more risky decision-making. As this may reflect optimistic expectations, this finding may support previous observations in female carriers of MR-haplotype 2 in a naturalistic cohort study.

Introduction

Emotional information processing is influenced by oral contraceptives (OC) and menstrual cycle phase. Furthermore, increased levels of natural or synthetic sex steroids had a positive effect on the reactivity to a potential reward (Bayer at al., 2013; Dreher et al., 2007), memory and attention (Gogos et al., 2014; Warren et al., 2014). Facial expression recognition may be affected as well. For instance, women performed better in emotion recognition when levels on natural sex steroids were increased (Sundstrom Poromaa & Gingnell, 2014; Maner & Miller, 2014; Roos et al., 2012; Pearson et al., 2009). In contrast, OC-users made more errors in a facial expression recognition test in comparison to naturally cycling women (Hamstra et al., 2014, 2015).

The OC-effects on emotion cognition might be due to the suppression of cyclical changes in ovarian hormones (Boron & Boulpaep, 2012; Kornstein & Clayton, 2002). This is because OC decrease the secretion of gonadotropin releasing hormone (GnRH) which results in too low levels of FSH and LH to support ovulation (Boron & Boulpaep, 2012; Rivera et al., 1999). Circulating estrogen and progesterone concentrations are also significantly lower in OC-users than in the mid-luteal phase (day 18 - 25) of naturally cycling women (DeBondt et al., 2013). Furthermore, OC decrease the hypothalamic – pituitary – adrenal (HPA) axis response to stress in women. Moreover, the increase in cortisol-binding globulin caused by ethinylestradiol in OC may blunt salivary-free cortisol responses to physical and psychosocial stress (Nielsen et al., 2013; Kirschbaum et al., 1999; Carr et al., 1978).

The mineralocorticoid receptor (MR) mediates the effect of cortisol on the regulation of initial stress reactions like vigilance and selective attention (Hermans et al., 2014; Cornelisse et al. 2011), appraisal processes (Vogel et al. 2014; Schwabe et al, 2013; Oitzl & de Kloet, 1992), encoding of spatial (Arp et al, 2014) and emotional memory in animal and human studies (Otte et al., 2015; Zhou et al. 2010; Joëls et al., 2008; Otte et al., 2007). These rapid effects are mediated by the MR in the subgenual anterior cingulate cortex, amygdala and hippocampus, which are limbic structures with a crucial function in the processing of stressful information (Joëls et al. 2012; De Kloet et al., 2005). In these regions early life adversity, chronic stress and depression were found to downregulate MR expression (ter Heegde et al., 2015; Bogdan et al., 2012; Klok et al., 2011b; Champagne et al., 2008).

The sensitivity of the human MR is genetically determined. The MR-haplotypes are constituted by two single nucleotide polymorphisms MR-2G/C (rs2070951) and MR-I180V (A/G, rs5522) (van Leeuwen et al., 2010, 2011). Female carriers of MR-haplotype 2 (MR-2C/I180A: CA) showed higher dispositional optimism scores, fewer thoughts of hopelessness during sad mood and a lower risk of depression. These effects were restricted to premenopausal women suggesting an effect of female gonadal steroids on the function of the MR (Klok et al., 2011a). The MR-haplotype 2 sex-dependently moderated the relation between childhood maltreatment

and depressive symptoms in a population-based cohort and an independent clinical cohort (Vinkers et al. 2015). MR-haplotype status is also associated with the effect of hormones on cognition, particularly the processing of emotionally relevant information: OC-users with MR-haplotype 1 or 3 recognized more sad and fearful faces and performed better in the recall of negative characteristics, whereas MR-haplotype 2 carriers did not (Hamstra et al., 2015). The observed differences in sensitivity between different MR-haplotypes may also explain why some women experience depression-like side effects of OC whereas others do not (Gingnell et al., 2013; Poromaa & Segebladh, 2012; Boron & Boulpaep, 2012; Kulkarni, 2007).

In the present study we investigated the influence of OC and menstrual cycle phase on emotion cognition in MR-genotyped healthy female volunteers. We administered a test battery covering multiple stages of emotional information processing associated with depression (Beck & Clark, 1997). The battery existed of tasks that already have shown to be sensitive to the influence of OC (facial expression recognition and risk decision-making; Hamstra et al., 2014, 2015) and 'new' tasks (implicit negative and positive affect, emotional working memory, reading the mind in the eye). We hypothesized that MR-haplotype modulates the impact of OC-use and menstrual cycle phase on all stages of emotional information processing.

Experimental procedures

Study population

Eligible participants were 18 – 35 year old women of Northwestern European origin with a regular menstrual cycle (between 25 and 35 days). Users of hormonal contraceptives other than hormonal pills were excluded. Further exclusion criteria were self-reported current or past psychological or psychiatric treatment; pregnancy or lactation; dyslexia; alcoholism; use of nicotine or cannaboids in the past three months; a history of regular use of (hard) drugs including MDMA (3,4-methyleendioxymethamfetamin); use of prescription medication; (self-) medication likely to interfere with the study (e.g. antidepressants, St John's Wort, benzodiazepines, stimulants). Participants were recruited at various sites at Leiden University. All participants provided written informed consent before the start of the study and received course credit or € 15. The study was approved by the Ethics Committee Psychology of Leiden University (CEP: 6940117280).

Design and procedure

This study had a cross-sectional, parallel-group design. After the intake, DNA was collected with a buccal swab. Subsequently participants completed a test battery in a psychology laboratory. Tasks were assessed in a fixed order; the duration of each task was between 5-15 min. Naturally cycling (NC) women with a regular menstrual cycle (25 – 35 days) were tested in the early

follicular (EF; day 2 – 5) or mid-luteal phase (ML; day 18 – 25) of the menstrual cycle. The mid-luteal phase was calculated after confirmation of the onset of the cycle. In case women had a cycle of more than 28 days, time of assessment in the mid-luteal phase was scheduled after day 20. Only women using monophasic OC containing ethinylestradiol (0.03 mg) and levenorgestrel (0.15 mg) were included. OC-users were tested outside their pill-free week and had been using an OC for at least three months.

Instruments

Clinical characteristics

Mood state was assessed by the 20-item state version of the Positive and Negative Affect Scales (PANAS) prior to testing (Watson et al., 1988). Personality traits were assessed with a short form of the NEO-Five Factor Inventory (NEO-FFI; McCrae & Costa, 1987). Depression vulnerability was measured with the Leiden Index of Depression Sensitivity - Revised (LEIDS-R; Van der Does, 2002).

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 a 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 final 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).

Emotional cognition

Implicit affect

We used the Implicit Positive and Negative Affect test (IPANAT) to measure implicit affect. Participants were asked to rate the extent to which six artificial words (i.e. SUKOV) expressed different emotions, on four-point Likert scales (Quirin et al. 2009). The main emotions used were anger (assessed by the adjectives: angry, irritated, annoyed), sadness (sad, down, unhappy), fear (anxious, afraid, fearful) and joy (lucky, happy, good-humored). Since the stimuli have no meaning, the ratings are thought to reflect (latent) affect states (Brosschot et al., 2014).

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 given in a 100% neutral expression. Since all trials were presented twice, four trials of each emotion were presented at each intensity level. A total of 204 stimuli 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. Accuracy was calculated for the total correctly recognized expressions and per emotion.

Emotional working memory

In a verbal working memory paradigm involving the recall of letters (Sternberg), International Affective Pictures System (IAPS; Lang et al., 2005) pictures served as positively, neutrally or

negatively rated distractors (Krause-Utz et al., 2012). Each trial started with a fixation-cross, followed by the presentation of three letters on a computer screen (the memorandum). After a delay three target letters were shown. During the delay interval the IAPS pictures were presented. Participants had to press a 'yes' button, when they recognized a target letter previously presented (present-target trials), or a 'no' button, when they did not perceive the target letter (absent target trials) in the recognition display after a short delay (750 ms). Only one target letter was present in the present-target trials. The task consisted of 96 randomly presented trials and four practice trials with feedback (showing neutral distractors). The task delivered 16 trials per each of six conditions (e.g. present-target trials with a negative distractor, absent-target trial with a positive distractor).

Decision-making task (DMT)

The decision-making task (Rogers et al., 2003) is an adaptation of the Iowa Gambling Test and consisted of 80 trials in which participants have to choose between a relatively safe gamble (50% chance of winning or losing 10 cents) and a riskier gamble (see also Hamstra et al., 2015). The task assesses risk decision making over different probabilities to win or lose varying points and differentiates between risk-seeking and risk-aversive behavior. The aim of the test was to gain as many points as possible and participants were to keep the amount gained at the end of the test (mostly between €1 and €3).

Reading the mind in the eye task (RMET)

This is an advanced emotion recognition test, a measure of adult social intelligence. The task consisted of 36 photographs of the eye region. Together with each photograph, four adjectives describing possible emotional states were presented. The photographs together expressed 12 negative, 8 positive and 18 neutral states, since the state "preoccupied"' can be interpreted as negative or neutral and "interested" as a positive or neutral (Harkness et al. 2005). Participants were asked to make a forced choice between four words (the standardized correct response and three distracters) that illustrated what the person in the photograph might be thinking or feeling. Accuracy was calculated in proportion correct per valence (positive, negative, neutral) and total correctly recognized expressions (Harkness et al., 2005).

Statistical analyses

Depending on the distribution of the data, square root, log, reciprocal and/ or Poisson distribution transformation was applied on variables that were not normally distributed. If transformations were unsuccessful, the findings were followed-up with equivalent non-parametric tests (Mann-Whitney U; Kruskal-Wallis H). Multivariate influential cases (Cook's distance > 1) were excluded from analyses.

Mean scores on personality dimensions (NEO-FFI; LEIDS-R; PANAS) were compared

with t-tests. Distribution over groups was analyzed by chi-square tests. Multivariate analyses of variance (MANOVAs) were applied to analyze the test scores, with hormonal status (EF: early follicular; ML: mid luteal; OC: oral contraceptives) and MR-haplotype (homozygote or heterozygote MR-haplotype 2 vs MR-haplotype 1/3) as between subject factors. Explorative analyses on separate subsamples were performed only if interaction effects in MANOVA were (nearly) significant ($p < .10$), in order to avoid erroneous analyses of interactions. If assumptions of sphericity were violated, a Greenhouse-Geisser correction was reported. Partial eta squared (η_p^2) and power were reported as estimates of effect size. Pairwise comparisons between OC, EF and ML were performed as well, using a Scheffé-correction for multiple testing.

Results

Participants

Two hundred and forty-four volunteers expressed interest in the study, 50 of whom withdrew before inclusion, and 100 did not meet inclusion criteria. All participants were students. One participant did not show up on the testing day. Table 1 shows the personality characteristics of the 93 participants, divided per subsample [OC-users, early follicular (EF) and mid luteal (ML) menstrual phase]. Since there were few homozygous MR-haplotype 2 carriers in both groups, we compared homo- and heterozygous MR-haplotype 2 carriers with carriers of MR-haplotypes 1 or 3. MR-haplotypes (2 vs. 1/3) were equally distributed across groups ($\chi^2 = 3.0$; df(2); $p = .22$). Table 1 reveals that early follicular women (EF) carrying MR-haplotype 1/3 scored higher on NEO FFI openness than EF women with MR-haplotype 2 ($t(19) = 2.4$; $p = .03$). ML-women carrying MR-haplotype 2 scored higher on PANAS negative affect than ML women with MR-haplotype 1/3 ($t(20) = -2.5$; $p = .02$). OC-users with MR-haplotype 1/3 scored higher on the aggression ($t(47) = 2.4$; $p = .02$) and rumination ($t(47) = -2.6$; $p = .04$) subscales of cognitive vulnerability to depression.

Emotional cognition

Implicit positive and negative affect

ANOVA on the separate adjectives showed a main effect of MR-haplotype on 'angry' [$F(1,3) = 26.6$; $p = .014$; $\eta_p^2 = .90$; power= .91] and 'down' [$F(1,5) = 23.2$; $p = .006$; $\eta_p^2 = .83$; power = .96]. See table 2. The outcomes on the IPANAT did not correlate with explicit positive and negative affect (PANAS).

Table 2. Participants' characteristics divided by MR-haplotype and hormonal status

	Early follicular day 2 – 5		**Mid luteal day 18 – 25**		**OC EE (0.03)/ LNG (0.15)**	
	MR HT 1/3	**MR HT 2**	**MR HT 1/3**	**MR HT 2**	**MR HT 1/3**	**MR HT 2**
N	11	10	8	15	15	34
Age	20.3 (.4)	20.5 (.5)	20.8 (1.0)	21.2 (.6)	20.6 (.4)	20.2 (.3)
Comm. Rel. n (%)	1 (17)*	5 (50)*	5 (63)*	3 (38)*	12 (80)	24 (71)
NEO-FFI Agreeableness	35.1 (1.3)	32.7 (1.2)	36.6 (1.2)	35.4 (1.2)	34,1 (1.0)	34.8 (.7)
NEO-FFI Conscientiousness	42.9 (.8)	40.7 (.9)	40.0 (.8)	41.7 (.7)	41.0 (1.0)	40.1 (.6)
NEO-FFI Extraversion	43.2 (2.0)	45.6 (1.6)	39.9 (3.1)	43.7 (2.1)	46.4 (1.4)	43.4 (.9)
NEO-FFI Neuroticism	34.5 (2.4)	34.2 (1.6)	33.9 (2.4)	32.3 (1.9)	34.3 (2.3)	33.4 (1.2)
NEO-FFI Openness	35.5 (1.2)*	31.5 (1.0)*	34.3 (.8)	35.3 (.9)	33.9 (1.0)	33.8 (.6)
LEIDS-R Acceptance	3.0 (1.1)	.6 (.2)	.9 (.4)	1.5 (.4)	1.6 (.5)	1.2 (.3)
LEIDS-R Aggression	6.9 (1.5)	6.8 (.9)	6.6 (1.7)	8.3 (1.2)	9.5 (1.0)*	6.6 (.7)*
LEIDS-R Perfectionism	7.7 (1.8)	10.6 1.6)	7.8 (1.1)	8.1 (1.2)	9.1 (1.4)	8.0 (.6)
LEIDS-R Hopelessness	6.1 (1.6)	5.1 (.9)	5.1(1.2)	4.7 (.5)	6.1 (1.2)	5.2 (.7)
LEIDS-R Risk Avoidance	11.5 (1.6)	8.4 (1.0)	10.0 (1.3)	10.5 1.0)	10.7 (1.2)	8.7 (.8)
LEIDS-R Rumination	9.9 (1.5)	10.4 (.7)	11.1 (1.9)	13.1 1.1)	13.4 (1.1)*	10.3 (.8)*
LEIDS-R Total	45.09 (6.6)	41.9 (2.4)	41.5 (5.1)	46.2 (2.9)	50.4 (4.5)	40 (2.5)
PANAS Negative Affect	12.1 (.6)	12.3 (.5)	11.0 (.4)*	13.0 (.7)*	12.9 (.6)	12.7 (.4)
PANAS Positive Affect	29.5 (1.4)	27.4 (1.0)	26.1 (2.3)	26.8 (1.9)	28.7 (1.3)	28.0 (.8)

Notes: N (%) or means (SE); *p < .05; *Abbreviations*: OC = OC-users; EE = ethinyl-estradiol; LNG = levonorgestrel; Comm. Rel. = committed relationship; NEO-FFI = NEO – Five Factor Inventory; LEIDS-R = Leiden Index of Depression Sensitivity – Revised; PANAS = Positive Negative Affect Scale (state); MR HT = mineralocorticoid receptor haplotype

Facial expression recognition

ANOVA on total accuracy revealed a trend-level main effect of hormonal status [F (2,87) = 3.0; p = 0.053; η_p^2= .07; power = .57] and a trend-level interaction effect between hormonal status and MR-haplotype [F (2,87) = 2.5; p = .086; η_p^2=. 06; power = .49]. Hormonal status influenced total accuracy only in MR-haplotype 1/3 carriers [F (2,31) = 4.7; p = .016; η_p^2=. 23; power = .75], with a significant difference between OC and ML ($p_{Scheffe}$ = .016). See figure 1 and table 2.

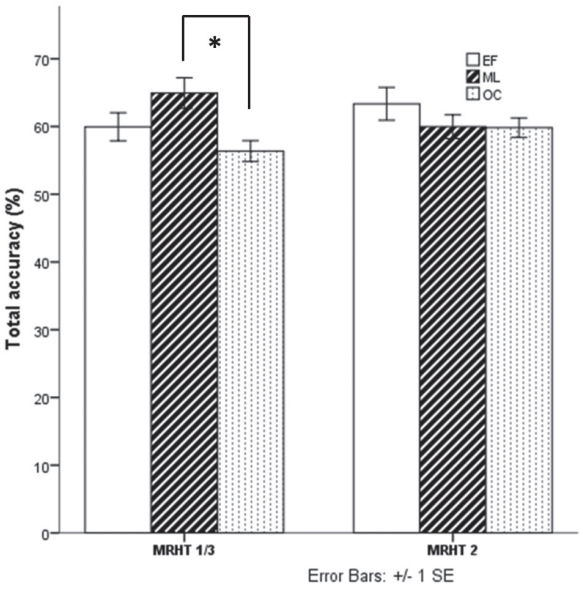

Figure 1. MR-haplotype, hormonal status and facial expression recognition scores.
Error bars represent ± 1 SE; MRHT = Mineralocorticoid receptor haplotype; * = $p < .05$

Emotional working memory

Two participants did not complete the task because they found some of the pictures too aversive. Accuracy was analyzed with a repeated measures MANOVA with condition (target yes/no) and valence of the distractor (positive, neutral, negative) as within-subject factors and hormonal status and MR-haplotype as between-subject factors. Significant main effects were found for condition [$F (1, 85) = 100.3$; $p < .001$; $\eta_p^2 = .54$; power = 1.0] and valence [$F (2,169) = 36.2$; $p < .001$; $\eta_p^2 = .30$; power = 1.0]. An interaction effect was found between condition and valence [$F (2,159) = 6.8$; $p = 0.002$; $\eta_p^2 = .07$; power = .9]. No (interaction) effects for hormonal status and MR-haplotype were observed.

Decision-making task

Analyses of variance revealed no main effect of hormonal status and MR-haplotype. Since the DMT scores remained significantly different from normal distribution after log-, arcsine- and Poisson-transformation, we applied non-parametric tests (Mann-Whitney U tests) subsequently. Regardless their odds of winning, MR-haplotype 2 carriers in the mid-luteal phase gambled significantly more than those in the early follicular phase ($W = 88$; $Z = -2.34$; $p = .019$). In trials with a high probability to win high gains in the context of low losses, decision-making was influenced by hormonal status ($\chi^2(2) = 9.1$; $p = .010$). This effect was only present in MR-

haplotype 1/3 ($\chi^2(2) = 7.5$; p = .024) with differences between EF and ML (W = 81; Z = -2.6; p = .009) (see figure 2 and table 2).

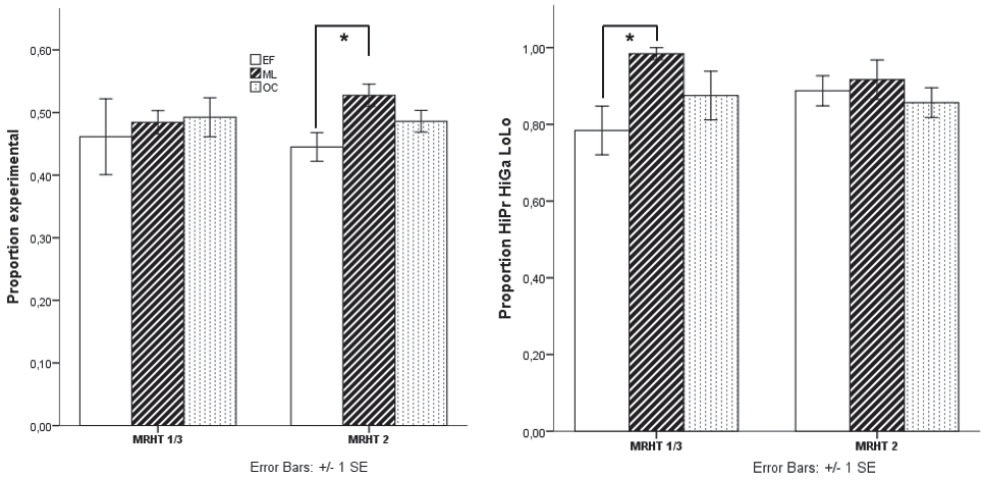

Figure 2. MR-haplotype, hormonal status and gambling behavior in all trials (left panel) and in the most favorable gambling conditions (high probability to win high gains risking low losses; right panel). *Notes*: Error bars represent ± 1 SE. *Abbreviations*: MRHT = Mineralocorticoid receptor haplotype; * = p < .05.

Reading the mind in the eye task

Analyses of variance revealed no main effect of hormonal status and MR-haplotype. Since the scores remained significantly different from normal distribution after transformation, we applied non-parametric tests (Mann-Whitney U tests) subsequently. No significant effects for MR-haplotype or hormonal status were found on total accuracy rates. Analyses of the accuracy per valence revealed that the (proportion) of correctly recognized positive emotions may be moderated by hormonal status (trend; χ^2 (2) = 5.4; p = .066). This effect was carried by MR-haplotype 2 ($\chi^2(2) = 6.0$; p = .051), with differences between ML and OC (W = 749.5; Z = -2.3; p = 0.023). See table 2.

Table 2. Information processing scores per MR-haplotype and hormonal status

	Early follicular day 2 – 5		**Mid luteal day 18 – 25**		**OC EE (0.03)/ LNG (0.15)**	
	MR HT 1/3	**MR HT 2**	**MR HT 1/3**	**MR HT 2**	**MR HT 1/3**	**MR HT 2**
N	11	10	8	15	15	34
IPANAT anger	17.3 (1.2)	14.6 (1.3)	15.4 (1.7)	14.1 (.9)	18.2 (1.0)	16.0 (.6)
IPANAT down	16.7 (.8)	15.1 (1.2)	15.4 (1.8)	13.9 (.9)	16.2 (.7)	15.3 (.6)
FERT total	119.9 (4.1)	126.7 (4.8)	129.9(4.5)*	119.9 (3.5)	112.7 (3.1)*	119.7 (2.9)
DMT total gambles	36.9 (4.8)	35.6 (1.8)*	38.8 (1.5)	42.2 (1.4)*	39.4 (2.5)	38.9 (1.4)
DMT HiP HiG LoL	6.3 (.5)*	7.1 (.3)	7.9 (1.3)*	7.3 (.4)	7.0 (.5)	6.9 (.3)
RMET positive	6.2 (.3)	5.7 (.5)	6.5 (.6)	6.9 (.3)*	6.8 (.2)	6.1 (.2)*

Notes: N or means (SE); *p < .05 (t-tests); *Abbreviations*: EE = ethinylestradiol; LNG = levenorgestrel; MR HT = mineralocorticoid receptor haplotype; IPANAT = implicit positive and negative affect; FERT = facial expression recognition task; EWM = emotional working memory; DMT = decision making task; HiP HiG LoL = high probability to win high gains risking low losses; RMET = reading the mind in the eye task

Discussion

This exploratory study revealed a possibly increased sensitivity to the female hormonal status in MR-haplotype 1/3 carriers. OC-users with MR-haplotype 1/3 performed worse in the facial expression recognition task than MR-haplotype 1/3 carriers in the mid-luteal phase of the menstrual cycle. Furthermore, in line with previous research (Bayer et al., 2013), naturally cycling women gambled more in the ML phase than in the EF phase. In the MR-haplotype 1/3 carriers ML women gambled more than EF women, but only when their risk to lose was relatively small. In contrast, MR-haplotype 2 carriers in the ML phase took more risky decisions, regardless their odds to win. These scores may reflect an optimistic expectation about the outcomes of one's decisions (Murphy et al., 2001; Carver & Scheier, 2014). Signs of dispositional optimism were previously found in MR-haplotype 2 carriers in a naturalistic cohort study. The same study revealed that female carriers of MR-haplotype 2 were protected against depression, but only during their fertile years, which suggests a moderating effect of female hormones on the MR as well (Klok et al., 2011a).

A number of issues need to be addressed in future studies. Due to our cross-sectional design, causal effects of hormonal phase or OC use cannot be inferred. Our sample size was small and too small to investigate homozygous and heterozygous MR-haplotype 2 carriers separately. The hormonal status of the participants could not be controlled, since we did not measure circulating sex hormone concentrations to confirm menstrual cycle phase and OC use and we did not register the onset of the next cycle. Finally, we did not apply a standardized clinical interview, but screened our participants by self-reported medical and psychiatric disorders before inclusion

and on the day of assessment itself. Since we did not exclude former OC-users, the naturally cycling (NC) group may have included women who previously have experienced side effects of OC, which is known as the 'survivor effect' (Warren et al., 2014; Oinonen & Mazmanian, 2002; Kutner & Brown, 1972). However, effects of OC-use were observed exclusively in MR-haplotype 1/3 carriers and both researchers and participants were blind to MR genotype, which makes confounding less likely.

For future studies we suggest to compare effects of OC with different MR properties on indices of emotional information processing. Compounds containing androgenic (levenorgestrel) and anti-androgenic progestins (drospirenone) (Sitruk-Ware, 2005) exert differential effects on fusiform and frontal gray matter volume and face recognition performance (Pletzer et al, 2014). Drospirenone, a derivative of spironolactone, acts as an MR-antagonist (Oelkers, 2003). Drospirenone therefore counteracts sodium retention and lowers blood pressure. It also attenuates depressogenic side-effects of ethinylestradiol (Mallareddy et al., 2007). Given the different effects on mainly the MR, however, a comparison of these OC would be both interesting and important, as it would provide more information about the role of the MR in the effects of oral contraceptives on emotional information processing.

It is remarkable that there are so few studies that consider OC and menstrual cycle phase as a critical variable of interest. In line with our previous studies we showed that not only OC-type, but also menstrual cycle phase should be registered, when fertile women are investigated. Our study may also provide in further insights into experienced mood changes during the menstrual cycle and OC use. These alterations in mood may occur not only during the menstrual cycle, but also during reproductive events as puberty, post-partum and the peri-menopause (Teatero et al., 2014; Stahl, 2013; Boron & Boulpaep, 2012; Kornstein & Clayton, 2002). In order to investigate this more closely, future studies may assess mood and behavior in MR genotyped women during their menstrual cycle or during inactive and active OC-use in a within-person design.