Mood and the pill

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Chapter 3

Mineralocorticoid receptor haplotype, oral contraceptives and emotional information processing

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Abstract

Background

Oral contraceptives (OC) affect mood in some women and may have more subtle effects on emotional information processing in many more users. Female carriers of mineralocorticoid receptor (MR) haplotype 2 have been shown to be more optimistic and less vulnerable to depression.

Aim

To investigate the effects of OC on emotional information processing and a possible moderating effect of MR haplotype.

Methods

Cross-sectional study in 85 healthy premenopausal women of West-European descent.

Results

We found significant main effects of OC on facial expression recognition, emotional memory and decision-making. Furthermore, carriers of MR haplotype 1 or 3 were sensitive to the impact of OC on the recognition of sad and fearful faces and on emotional memory, whereas MR haplotype 2 carriers were not.

Limitations

Different compounds of OC were included. No hormonal measures were taken. Most naturally cycling participants were assessed in the luteal phase of their menstrual cycle.

Conclusions

Carriers of MR haplotype 2 may be less sensitive to depressogenic side-effects of OC.

Introduction

Oral contraceptives (OC) have been on the market for over 50 years now and are used by approximately 100 million women worldwide (Hather et al., 2007). Early studies investigating the association between OC use and depression revealed inconsistent findings (Oinonen & Mazmanian, 2002): varying from increased to decreased rates of depressed mood in OC-users (Cullberg, 1972; Deijen et al., 1992). Some studies found no association (e.g., Vessay et al., 1985). More recent studies have shown that in most women OC reduce the variability of affect across the entire menstrual cycle. This is due to the suppression of cyclical changes in ovarian hormones (Kornstein & Clayton, 2002). OC also prevent negative affect during menstruation (Oinonen & Mazmanian, 2002). Recent cohort studies have also shown that OC may protect against depression (Svendal et al., 2012; Toffol et al., 2012). However, depressogenic side effects remain one of the major reasons for discontinuation of OC (Oinonen & Mazmanian, 2002; Boron & Boulpaep, 2012). In experimental research, OC-users showed a blunted response in positive affect (i.e., reduced positive affect variability) when exposed to emotional stimuli (Jarva & Oinonen, 2007).

Recently, subtle psychological consequences of OC use have been discovered that may also affect users who do not experience subjective effects on mood (Pletzer et al., 2010; Cobey & Buunk, 2012). For example, OC induce a preference for less masculine faces (Bobst et al., 2014; Little et al, 2013). Partnered women using OC reported significantly higher levels of jealousy than naturally cycling partnered women in their non-fertile cycle phase (Cobey et al., 2012). Furthermore, endogenous and/ or exogenous female hormones affect not only psychological processes and the associated brain activity patterns, but also brain structure (Pletzer et al., 2014; DeBondt et al., 2013; Pletzer et al., 2010). Taken together, psychological effects of OC may have implications on both societal and individual levels.

Previously we have detected that OC-users make more errors in the recognition of facial expressions of sadness, anger and disgust (Hamstra et al., 2014). Natural variations in estrogens and progesterone also affect emotional information processing. For instance, stress vulnerability fluctuates during the menstrual cycle (Ossewaarde et al., 2010). Sex hormones influence also facial emotion recognition: specifically, estrogen seems to be implicated in the recognition of negative emotions such as sadness, anger and fear (Guapo et al., 2009). Furthermore, recognition errors for facial expressions of sadness and disgust were significantly higher in the follicular phase than in the luteal phase, when progestins dominate the female cycle (Gasbarri et al., 2008). Not only emotion recognition, but also the reactivity of the reward system is moderated by the menstrual cycle phase (Ossewaarde et al., 2011). In the mid-follicular phase of the menstrual cycle, when estrogen is unopposed by progesterone, more reward anticipation in the monetary incentive delay task was observed (Dreher et al., 2007). Since the most frequently used OC

contain a synthetic progestin and estrogen (Hather et al., 2007), these findings may also be relevant for research into the influence of OC on information processing.

OC suppress the release of endogenous female hormones by the hypothalamic-pituary-gonadal axis. OC feed back at the hypothalamus, decreasing the secretion of gonadotropin releasing hormone (GnRH), and at the gonadotrophs in the anterior pituary, resulting in low levels of Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH), disabling normal folliculogenesis and ovulation (Boron & Boulpaep, 2012; Rivera et al., 1999). Hormonal contraceptives suppress endogenous hormonal levels: concentrations of estrogen and progesterone are significantly lower in OC-users than in naturally cycling (NC) women in the mid-luteal phase of the menstrual cycle (day 18 - 25) (DeBondt et al., 2013). Furthermore, regardless of the estrogen or progestin type, combined OC reduce total and free testosterone levels (Zimmermann et al., 2013; Alexander et al., 1990).

Exogenous gonadal hormones also influence the responsiveness of the hypothalamic-pituary-adrenal (HPA) axis. OC decrease HPA axis activity and cortisol response to a stressor in women (Nielsen et al., 2013; Kirschbaum et al., 1999). OC may also suppress cortisol secretion by affecting the feedback action of cortisol in the brain, which is mediated by mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). Previous studies in rats revealed that endogenous estrogen and particularly progesterone affect the expression and binding characteristics of the MR in the brain, which may explain the effects of the MR on HPA axis activity (Quinkler et al., 2002; Carey et al. 1995). It is not known to what extent the synthetic estrogens and progestins may interact with the MR and GR. The MR in particular was found to be associated with the processing of emotional information (Joëls et al., 2012) and stress reactions (Otte et al., 2007).

More specifically, the MR seems to be involved in the regulation of initial psychological stress reactions like vigilance, selective attention, emotional expressions and formation of emotional memory (Otte et al., 2007). This is consistent with the fact that the MR is located especially in limbic structures such as the subgenual anterior cingulate cortex, amygdala and hippocampus, which are crucial for processing of stressful information (De Kloet, 2005). In depressed individuals, MR expression is approximately 30% lower in the hippocampus, inferior frontal gyrus and cingulate gyrus than in non-depressed controls (Klok et al., 2011b). Recent studies have revealed that MR haplotype 2 is associated with higher dispositional optimism, fewer thoughts of hopelessness during sad moods and lower risk of depression. These effects are restricted to premenopausal women suggesting a moderating effect of female gonadal steroids on the function of the MR (Klok et al., 2011b). 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 (Boron & Boulpaep, 2012; Oinonen & Mazmanian, 2002).

The goal of the current study was to further investigate the effects of OC use on information processing (Hamstra et al., 2014), considering potential moderating effects of MR-haplotypes. Firstly, OC- users were compared with NC women on indices of emotional information processing. Secondly, we hypothesized that performance on these tasks would be moderated by MR-haplotype. Specifically, we hypothesized that the effects of OC on emotional information processes would be larger in carriers of MR haplotype 1/3 than in MR haplotype 2.

Experimental procedures

Participants

Eligible participants were women of North-Western European origin with a regular menstrual cycle (between 25 and 35 days). Age limits were between 18 and 35 years. Users of hormonal contraceptives other than hormonal pills were excluded. Further exclusion criteria were pregnancy or lactation, dyslexia, alcoholism, habitual smoking, a history of regular use of (hard) drugs including XTC and cannabis and use of medication likely to interfere with the study (e.g. antidepressants, St John's Wort, benzodiazepines, ADHD medication). 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 euro. The study was approved by the Ethics Committee Psychology of Leiden University (CEP 7099926055).

Design and procedure

This study had a cross-sectional, parallel-group design. Participants completed a test battery in a psychology laboratory. NC women were tested between day 6 and 26 of their menstrual cycle. OC-users were tested outside their pill-free week. OC brand name and chemical compound, duration of OC use and in case of NC women first day of last menses were registered.

Instruments

Clinical characteristics

Mood state was assessed by the 20-item state version ('today') of the Positive and Negative Affectivity Scales (PANAS) prior to testing (Watson et al., 1988). Personality traits were assessed by the NEO Five Factor Inventory (NEO-FFI) (Hoekstra et al, 1996). Depression vulnerability was measured with the Leiden Index of Depression Sensitivity - Revised (LEIDS-R; Van der Does, 2002).

Information processing tests

Facial expression recognition task (FERT)

The FERT displays five basic emotions taken from the Pictures of Facial Affect Series (Ekman and Friesen, 1976; see figure 1). Male and female examples of these pictures expressing happiness, sadness, fear, anger and disgust were morphed at ten intensity levels (10–100%). This morphing procedure involved taking a percentage of the shape and texture differences between the two standard images 0% (neutral) and 100% (full emotion) in 10% steps (Young et al., 2007). Each face was also given in a neutral expression, resulting in a total of 204 stimuli that 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 one of the buttons of a response box corresponding with the perceived facial expression: anger, sad, fear, happy, disgust or neutral.

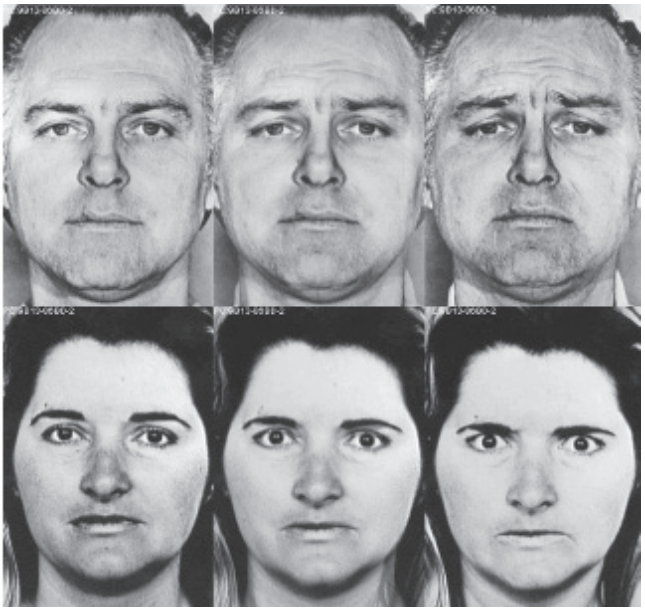

Figure 1. Sample stimuli form Ekman's facial expression dataset

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 classify as quickly as possible whether they would

like or dislike being associated with the characteristic. Twelve minutes later, after completion of the FERT, participants were asked to recall as many of the characteristics from the categorization task within 2 minutes. We analyzed the number of correctly recalled positive and negative characteristics and positive and negative false recognitions (traits not previously presented).

Decision-making task (DMT)

The DMT (Rogers et al., 2003) is an adaptation of the Iowa Gambling Test and consists of 80 trials. Each participant started with 100 points (eurocents) and in each trial she was asked to choose between two simultaneously presented options: a control and an experimental gamble. In the control gamble, participants had a 0.5 probability of winning or losing ten points (the 'safe' option). Experimental gambles came in eight types, that vary in the probability of winning (.4 vs .6), the magnitude of the expected gains (30 vs 70 points) and the magnitude of the expected losses (30 vs 70 points). Additionally, eight 'wins or losses only' trials were presented: in these conditions the participant chose between a certain loss (or win) of 30 points and a gamble with a .5 probability of losing (or winning) nothing and .5 probability of losing (or winning) 60 points. 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 points as possible and participants were to keep the amount gained at the end of the test (on average between €1 and €3).

Genotyping

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 95oC, followed by 40 cycles of 30 sec 94oC, 30 sec 50oC, 120 sec 72oC 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.

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). According to the observed frequency in the population (DeRijk et al., 2008), MR-haplotype 1 (GA) was constituted by MR-2 G and MRI180V A, MR-haplotype 2 (CA) by MR-2 C and MRI180V A, MR-haplotype 3 (CG) by MR-2 C and MRI180V G and the in vivo seldom observed MR-haplotype 4 (GG) by MR-2 G and MRI180V G.

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, we followed-up the findings with equivalent non-parametric tests. Multivariate influential cases (Cook's distance and Leverage) were excluded from analyses. Mean scores on personality dimensions between both groups (OC-users and non-users) were compared with t-tests. Multivariate analyses of variance (MANOVAs) were used to compare the test scores, with OC use (yes/no) and MR-haplotype (homozygote or heterozygote MR-Haplotype 2 vs remaining) as between subject factors. Partial eta squared (η_p^2) and power are reported as estimates of effect size. When assumptions of sphericity were violated, a Greenhouse-Geisser correction on p-values was applied.

For each emotion in the FERT (anger, disgust, fear, happy and sad) the total accuracy rate was calculated separately. Furthermore, we summed all correctly recognized trials per intensity in expression of each emotion. This resulted in an accuracy score ranging from 0 to 4 per intensity (10-100%) for each emotion. These accuracy scores were analyzed using rm-ANOVAs with intensity (10 to 100%) as within-subject factors and emotion as measures. Mean reaction times (RTs) were calculated for all correct trials and analyzed with a MANOVA.

Outcomes on the EMT were analysed with valence (positive vs. negative characteristics) as within- subject factors and correct and intrusive recall as measures. The dependent variables of the DMT were the number of trials in which participants chose the experimental (risky) gamble. We applied analyses of variance to control for the effects of OC use and (interaction with) 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.

Results

Participant characteristics

Hundred and thirty-three volunteers signed up, 26 of whom withdrew before inclusion and 19 of whom did not meet inclusion criteria. Eighty-five women took part, 44 of whom were using OC. Forty-one NC volunteers were included, of whom eleven were tested in the follicular phase (day 6 – 14) and 29 in the luteal phase (day 15 - 26) of the menstrual cycle. Since there were few homozygous MR-haplotype 2 carriers in both groups (see table 1), we compared all 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 users and non-users of OC (χ^2 = 1.872; df = 1; p =.171).

Users and non-users did not differ significantly on age and personality scores, except for a trend on Extraversion (p = .055). OC-users scored lower on depression vulnerability (LEIDS-R total) (p = .03). Ninety-one percent (n = 40) of our participants used OC with estrogen and/or progestin as compounds (see table 2). When we excluded OC with other compounds (n = 4) from our analyses, the main outcome of this study remained. Furthermore, we did not control for a possible confounding influence of the circadian secretion pattern on steroid hormones since we tested. Participants were tested according to their own availability at different times of the day (see table 4). The distribution of timeslots (morning, early and late afternoon) over the subsamples (haplotypes, OC and NC) was not significantly different (χ^2 = 3.0; df = 2; p = .227).

Table 1. Participants' characteristics

	OC	**NC**
N	44 (52)	41 (48)
Age	20.4 (0.3)	20.2 (0.4)
OC use > 1 yr (%)	38 (86)	-
Pilltype (monophasic, estr. and prog.)	39 (89)	-
Menstrual cycle phase: luteal (%)	-	29 (73)
In a committed relationship (%)	20 (45)	12 (24)
NEO-FFI Neuroticism	31.6 (1.2)	34.12 (1.2)
NEO-FFI Extraversion	45.3 (0.8)*	42.78 (1.1)*
NEO-FFI Openness	33.7 (0.4)	34.44 (0.6)
NEO-FFI Agreeableness	34.3 (0.6)	34.88 (0.5)
NEO-FFI Conscientiousness	40.6 (0.4)	40.29 (0.6)
LEIDS-R Total	35.2 (2.3)**	41.61 (1.7)**
PANAS Positive Affect	26.1 (0.9)	25.83 (0.9)
MR Haplotype 1 or 3 (%)	18 (41)	11 (27)
MR Haplotype 2 homozygotes (%)	7 (16)	6 (15)
MR Haplotype 2 all (%)	26 (59)	30 (73)

Notes: N (%) or Means (SE); ** $p < .05$; * $p = .055$; *Abbreviations*: OC = oral contraceptives; NC = naturally cycling; NEO-FFI = NEO Five Factor Inventory; LEIDS-R = Leiden Index of Depression Sensitivity; PANAS = Positive and Negative Affect Schedule (state); MR = mineralocorticoid receptor.

Table 2. Type of OC used

Compounds (mg)	**N**
EE (0.02)/ LNG (0.10)	1
EE (0.03)/ LNG (0.15)	32
EE (0.05)/ LNG (0.125)	1
EE / LNG[1]	4
EE (0.03, 0.04, 0.03)/ LNG (0.05, 0.075, 0.125)	1
EE (0.35)/ CPA	2
EE (0,03)/ DRSP	3
Total	44

[1] Doses EE and LNG unknown. *Abbreviations*: EE = Ethinylestradiol; LNG = Levonorgestrel; CPA = Cyproteronacetate; DRSP = Drospirenone

Facial expression recognition task

Accuracy

The accuracy rates in the FERT are shown in Figures 1 and 2. Due to a technical failure, FERT data of one participant are missing. Tests for between-subject effects of the MANOVA revealed a significant main effect of OC use on anger [F (1,80) = 5.51; p = .021; η_p^2 =. 064; power =. 64] and sadness [F (1,80) = 6,58; p = .012; η_p^2 =. 076; power =.72] and an interaction effect between OC use and MR haplotype for fear [F(1,80) = 5.45; p = .022; η_p^2 = . 064; power =.64] and sadness [trend; F(1,80) = 3.00; p = .087; η_p^2 = . 036; power =.40]. Non-parametric Mann - Whitney U tests revealed that OC-users performed worse at the recognition of anger than NC participants in the haplotype 2 group [W= 606; z = -2.070; p = 0.038], although haplotype 1/3 showed the same pattern (p = 0.215). MR haplotype 1/3 carriers who were OC-users were better at identifying fear [W=113; z = -2. 360; p = 0.018] and sadness [W = 113; z = -2. 367; p = 0.018] than non-users. This pattern was not found in haplotype 2 carriers (see figure 2, top panels). When we added scores on depression vulnerability as covariates to our rm-ANOVA in order to correct for significant group differences, effects remained the same.

Subsequently we applied a repeated measure MANOVA on all correctly recognized trials per intensity for anger, fear and sadness. Multivariate tests revealed the expected significant main effect of intensity for these three emotions [F (9,72) = 64.1; p < .001; h_p^2 = .889; power = 1.00]. An interaction effect between OC use and intensity was found for anger [F (9,72) = 4.0; p = .001; h_p^2 = .332; power = 1.0] and fear [F (9,72) = 2.9; p = .006; h_p^2 = .263; power =. 94]. Outcomes on fear revealed also a trend-level interaction effect between intensity and MR haplotype [F (9,72) = 1.9; p = .068; h_p^2 = .192; power = .78]. Within subject effects showed an additional interaction effect between OC use and intensity for sadness [Greenhouse-Geisser correction; F (7,532) = 2.5; p = .017; h_p^2 = .031; power =. 87]. Fear and sadness were better recognized at lower intensity levels by OC-users with MR haplotype 1 and 3. Figure 3 shows the score patterns of MR-haplotype 2 versus MR-haplotype1/3 on the recognition of sadness and fear across intensity levels.

Reaction times

Two participants were identified as multivariate influential cases (indicated by Cook's distance > 1.3) and were excluded from analyses involving RTs. The pattern of results for RTs mirrored the pattern for accuracy scores (see figure 1, bottom panels). Multivariate tests of the rm-ANOVA revealed similar effects as observed for the accuracy scores.

The interaction between intensity * OC use * MR haplotype for happiness was a trend [F(6,480) = 1.89; p = .080; η_p^2 = .024; power=.71]. Furthermore, tests of between-subject effects showed an additional interaction effect for OC use * MR haplotype for disgust [F(1,78) = 6.58; p = .012; η_p^2 = . 078; power = .72].

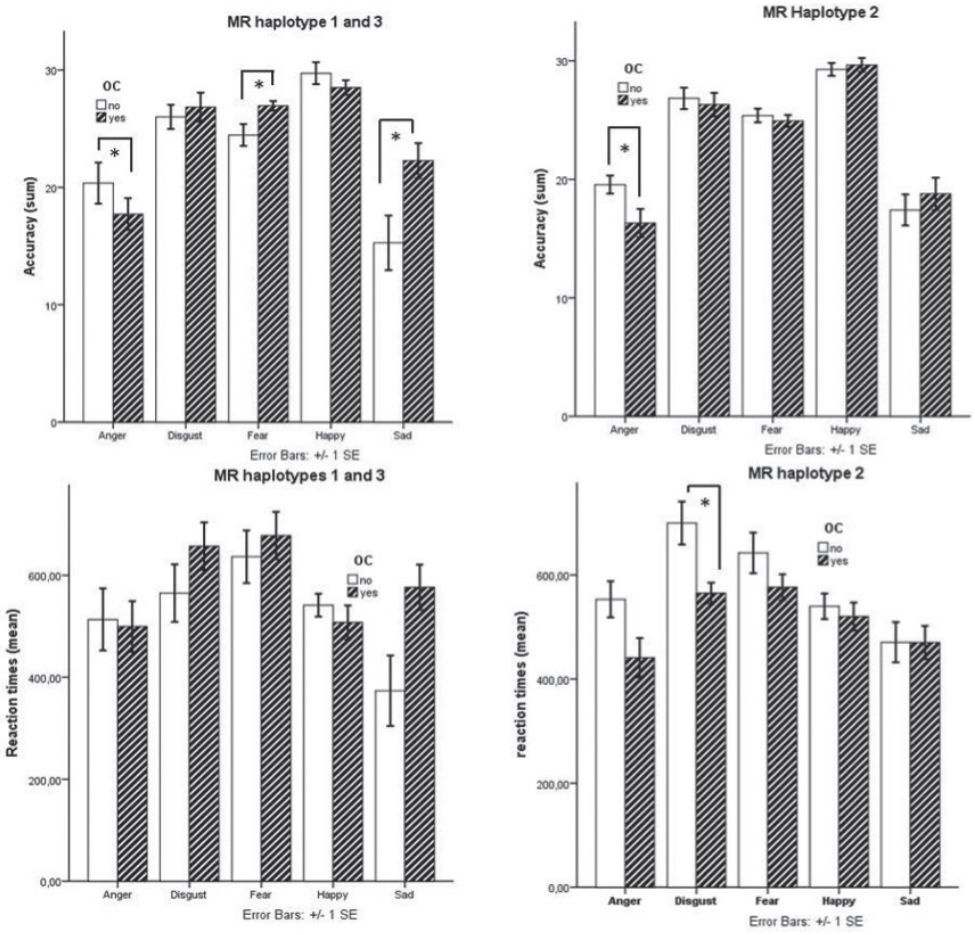

Figure 2. The impact of OC use on emotion recognition (top panels) and reaction times (bottom panels) in MR-haplotypes 1 and 3 (left panels) and haplotype 2 (right panels); * $p < .05$.

Word categorization and memory task

The memory scores are presented in table 3. Multivariate tests of the rm-ANOVA (Wilks' Lambda) revealed a significant within-subjects effect for valence of correctly recalled characteristics [$F(2, 80) = 25.4$; $p < .001$; $h_p^2 = .39$; power = 1.0], valence*OC use [$F(2, 80) = 4.5$; $p = .015$; $h_p^2 = .10$; power = .75] and valence*OC use*MR haplotype [trend; $F(2, 79) = 2.4$; $p = .067$; $h_p^2 = .07$; power = .54]. With correction for violation of sphericity (Greenhouse-Geisser), significant interaction effects were found between valence and OC use for intrusive characteristics [$F(1,81) = 9.02$; $p = .004$; $h_p^2 = .10$; power = .84]. Furthermore, a significant interaction effect between

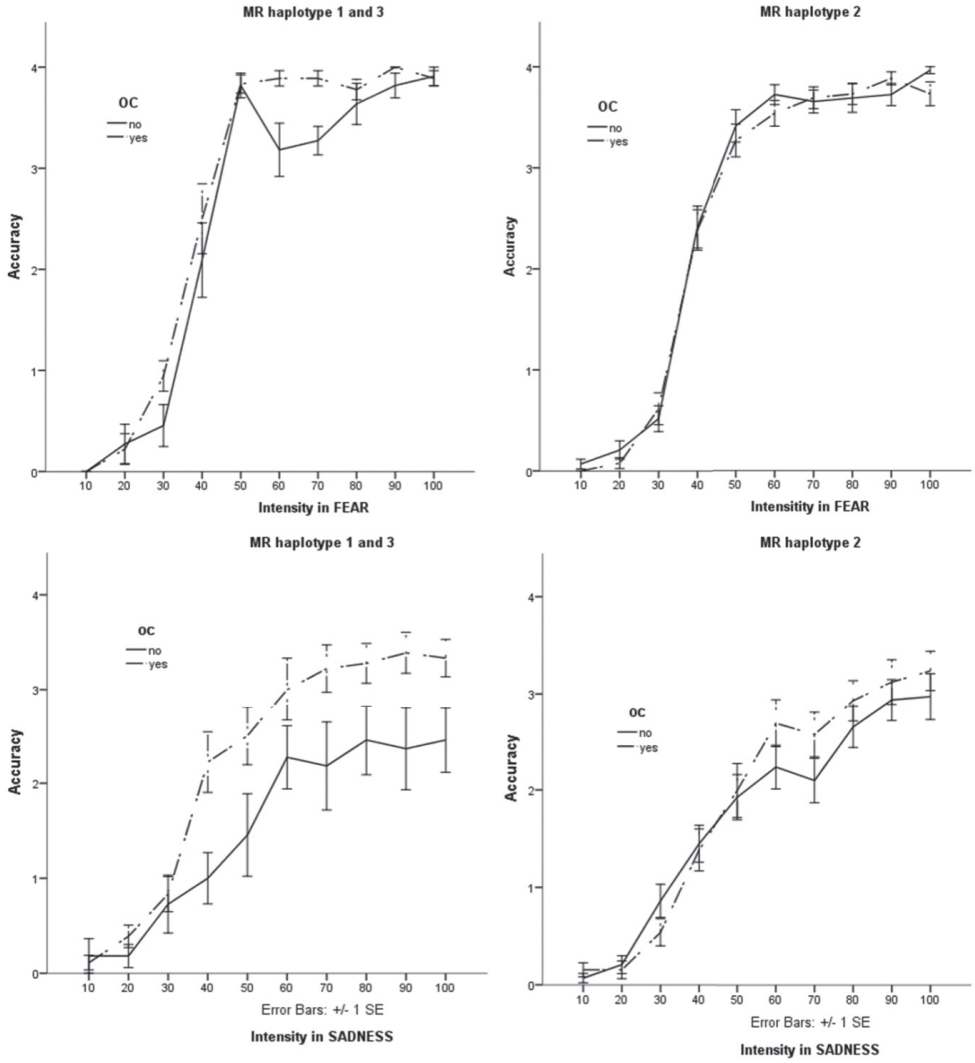

Figure 3. Recognition of fear and sadness by carriers of haplotype 1/3 (left panel) and haplotype 2 (right panel); * $p < .05$.

valence, OC use and MR haplotype was found for intrusive characteristics [$F(1,81) = 4.3$; $p = .040$; $h_p^2 = .051$; power = .54]. The patterns were unchanged after addition of extraversion and LEIDS-R total scores as covariates. Nonparametric tests confirmed an effect of OC use on correctly recalled negative characteristics [trend; W= 1549; z = -1.915; p = .055] and intrusive characteristics [W = 1680; z = -1.987; p = .047]. When analyzing both groups of MR haplotype carriers separately, we found that OC use was associated with a better recall of negative stimuli in

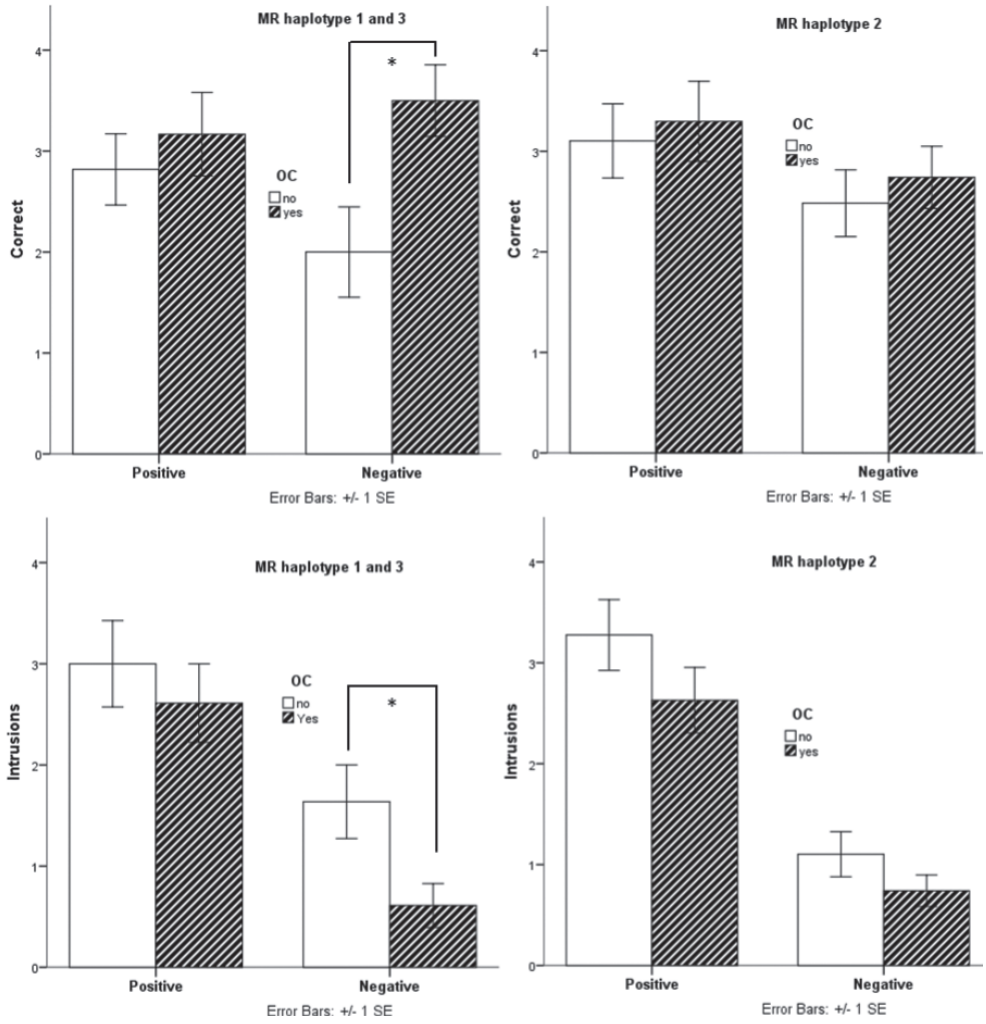

Figure 4. Influence of OC use on correctly recalled (top panels) and intrusive characteristics (bottom panels) in carriers of MR haplotype 1 or 3 (left panel) and haplotype 2 (right panel); * $p < .05$.

the haplotype 1/3 group [W = 111; z = -2. 469; p = 0.014] (see figure 4). OC-users in this group also had more negative intrusive memories [W = 220; z = -2. 409; p = 0.016]. This pattern was not observed in MR haplotype 2 carriers.

Decision-making task (DMT)

As expected, participants gambled more in trials with a low probability of losing than in trials with a high probability [$F(6,76) = 27.4$; $p = .001$; $\eta_p^2 = .68$; power = 1.0] and in trials with large vs small expected gains [$F(6,76) = 82.3$; $p = .001$; $\eta_p^2 = .87$; power = 1.0]. These effects did not interact with OC use or MR haplotype. In trials with a low probability to win a large amount, OC-users tended to be more reluctant to gamble. This occurred both when expected losses were low [$W = 1649$; $z = -2.160$; $p = 0.031$] and high [trend; $W = 1700$; $z = -1.769$; $p = 0.077$] (see figure 5, right panel).

As expected, participants were much more likely to gamble in the losses-only condition than in the wins-only condition [$F(1,81) = 58.3$; $p = .001$; $\eta_p^2 = .419$; power = 1.0]. This risk-seeking behavior may have been influenced by OC use [trend; $F(1,81) = 3.00$; $p = .087$; $\eta_p^2 = .036$; power =.40] (see figure 4, left panel). Non-parametric tests also showed that OC-users tended to gamble more than non-users in losses-only trials [trend; $W = 1545$; $z = -1.940$; $p = 0.052$] and in gains-only trials [trend; $W = 1714$; $z = -1.733$; $p = 0.083$].

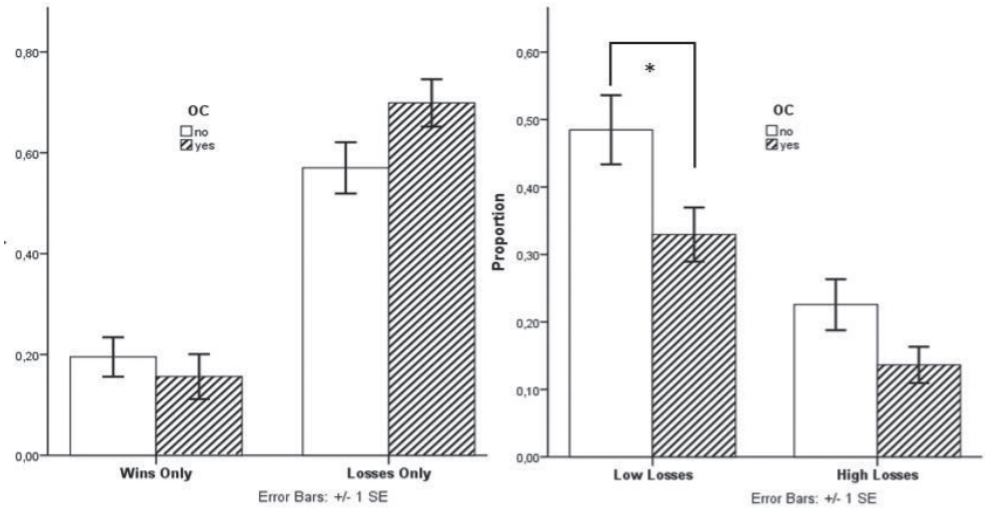

Figure 5. Proportion of choice for the experimental gamble in the wins- and losses-only conditions (left panel) and in conditions with a small chance to gain a large amount in the context of low or high potential losses (right panel); * $p < .05$.

Table 3. Means (standard errors) FERT, CT and DMT

	OC		NC		Pill status		MR-haplotype	
	MR HT 1/3	MR HT 2	MR HT 1/3	MR HT 2	yes	no	MR HT 1/3	MR HT 2
N	18	26	11	30	44	41	29	56
FERT anger	17.7 (1.3)	16.3 (1.2)	20.4 (1.7)	19.6 (.8)	16.9 (.9)**	19.8 (.7) **	18.7 (1.1)	18.0 (.7)
FERT disgust	26.8 (1.2)	26.3 (1.0)	26.0 (1.0)	26.8 (.9)	26.5 (.8)	26.6 (.7)	26.5 (.8)	26.6 (.7)
FERT fear	26.9 (.4)**	24.9 (.5)**	24.5 (.9)	25.3 (.6)	25.8 (.4)	25.1 (.5)	26.0 (.5)	25.2 (.4)
FERT happy	28.5 (.6)	29.7 (.6)	29.7 (.9)	29.3 (.5)	29.2 (.5)	29.4 (.4)	29.0 (.5)	29.5 (.4)
FERT sad	22.3 (1.5)*	18.8 (1.4*)	15.3 (2.3)	17.4 (1.3)	16.8 (1.1) **	20.2 (1.0)**	19.6 (1.4)	18.1 (.9)
WCMT pos. correct	3.2 (.4)	3.4 (.4)	2.8 (.4)	3.1 (.4)	3.3 (.3)	3.0 (.3)	3.0 (.3)	3.2 (.3)
WCMT neg. correct	3.5 (.4)*	2.7 (.3)*	2.0 (.4)	2.5 (.3)	3.0 (.2)	2.4 (.3) *	2.9 (.3)	2.6 (.2)
WCMT pos. intrusions	2.6 (.4)	2.7 (.3)	3.0 (.4)	3.2 (.4)	2.6 (.3)	3.2 (.3)	2.8 (.3)	3.0 (.2)
WCMT neg. intrusions	.6 (.2)	.7 (.2)	1.6 (.4)	1.1 (.2)	.7 (1.1)**	1.2 (.2) **	1.0 (.2)	.9 (.1)
DMT losses only	5.9 (.6)	5.4 (.5)	4.5 (.8)	4.6 (.5)	5.6 (.4) *	4.6 (.4) *	5.3 (.5)	5.0 (.4)
DMT LoPr HiG LoLo	2.6 (.5)	2.7 (.4)	3.2 (.8)	4.1 (.5)	2.6 (.3) **	3.9 (.4) **	2.8 (.4)	3.5 (.3)
DMT LoPr HiG HiLo	1.4 (.4)	.9 (.2)	1.8 (.7)	1.8 (.3)	1.1 (.2) *	1.8 (.3) *	1.6 (.4)	1.4 (.2)

Notes: N (%) or means (SE); t-tests: * = .05 < p < .10; ** = p < .05; *Abbreviations*: OC = oral contraceptives; NC = naturally cycling; MR HT = mineralocorticoid receptor haplotype; FERT = facial expression recognition task; WCMT = word categorization and memory task; DMT = decision making task; LoPr HiG LoLo = low probability high gain low loss; LoPr HiG HiLo = low probability high gain high loss.

Discussion

We observed that users of OC perform differently than non-users on tests of facial expression recognition, emotional memory and decision-making. Furthermore, a moderating effect of MR- haplotype was observed on some of these outcomes: carriers of MR haplotype 1 or 3 were sensitive to the impact of OC on the recognition of sad and fearful faces and on emotional memory. The effects of OC on recognition of anger and on risky decision-making were not moderated by MR-haplotype.

The association of OC use with a worse recognition of anger is in line with our previous study (Hamstra et al., 2014). Furthermore, in accordance with our hypothesis, genotype-dependent effects of OC use were found especially in carriers of MR-haplotype 1 or 3. OC use seems to be associated with biases in the perception of negative information in MR haplotype 1 and 3 carriers. In this group, OC use was associated with better recall of negative information and with a better recognition of sadness and fear in facial expressions. The reaction times of correctly recognized expressions were also longer for these emotions. Apparently, the confrontation with sadness and - although less explicitly - fear slowed down reaction times. These effects are subtle and may not always be noticed, but are detectable in the laboratory. Comparable biases in perception and memory have also been observed in (remitted) depressed and dysphoric patients and in daughters of depressed mothers (Joormann & Gotlib, 2007; Joormann et al., 2007). Memory biases in depressed vs. non-depressed samples are found most consistently in free recall tasks: for example, currently and formerly depressed participants had a better recall of negative words than positive words (Fritzsche et al., 2009). Depressed and formerly depressed individuals also have an attentional bias away from happy faces towards sad faces (Fritzsche et al., 2009; Joormann et al., 2007). Furthermore, these groups have greater accuracy and response bias for sad expressions only, especially at low intensities (Ellis et al., 2014; Milders et al., 2010).

We observed that some but not all of these effects of OC were moderated by MR haplotype. OC reduced the recognition of anger and influenced risky decision-making in all OC-users. A recent meta-analysis confirmed that combined OC (containing estrogens and progestins) reduce levels of androgen, especially testosterone, by inhibiting ovarian and adrenal androgen synthesis and increasing levels of sex hormone-binding globulin. Regardless of the estrogen or progestin type, such combined OC reduce total and free testosterone levels (Zimmermann et al., 2013; Alexander et al., 1990). Possibly, the less accurate but faster recognition of facial expressions of anger in OC-users in our previous (Hamstra et al., 2014) and current study can be explained by this endocrine effect. These outcomes agree with other evidence on the relationship between testosterone and response to facial expressions of anger (Terburg et al., 2012; Wirth et al., 2007; Hampson et al., 2006).

The observed reduction of risky-decisions in trials with a low probability to win high gains

may be the consequence of the testosterone-suppressing effects of OC as well (Zimmermann et al., 2013; Alexander et al., 1990). Recent research revealed that testosterone-treated rats preferred a large risky reward over a smaller safe reward at low and moderate shock intensity risks (Cooper et al., 2014). Testosterone-treated rats also choose a large delayed reward more frequently than a small immediate reward (Wood et al., 2013). Both women and men with high levels of endogenous testosterone made riskier choices in the Iowa Gambling Task than their low-testosterone counterparts. This effect was especially pronounced in women (Stanton et al., 2010).

Our findings may also have implications for mood disorders. The increased vulnerability of women to depression is the result of a complex interplay between sex-specific hormonal and environmental factors with non-sex-specific genes on other chromosomes. The rise and fall of estrogen and progesterone in women related to reproductive events such as puberty, pre-menses, post-partum and peri-menopause affect neurotransmitter, neuroendocrine, and circadian systems involved in depression. Women have the same frequency of depression as men before puberty and after menopause, but during their fertile years the female-to-male prevalence ratio for current, remitted, first-episode, and lifetime depression remains nearly constant at 2:1 (Kornstein & Clayton, 2002). Considering the present and earlier findings (Klok et al., 2011a) future studies may assess mood in MR genotyped women during their menstrual cycle and before and after the onset of OC use in a within-person design.

Previous studies indicated that sex hormones moderate facial emotion recognition. The early follicular phase, which is characterized by lower levels of progesterone and estrogen, was found to be associated with a decrease in the identification of the negative emotions anger, sadness and fear. Women in their luteal phase, when endogenous estrogen and progesterone levels are elevated, showed increased affective responsiveness to negative emotions (Derntl et al., 2008; Gasbarri et al., 2008). Since neural reactivity to possible reward is moderated by the menstrual cycle phase (Ossewaarde et al., 2011; Dreher et al., 2007), risk decision-making may alter during the menstrual cycle as well. However, we were not able to compare outcomes of NC volunteers in the luteal and follicular phase separately, since 73% of our NC participants were assessed in the luteal phase of the menstrual cycle.

A number of issues need to be addressed in future studies. Since we did not exclude former OC-users, the NC group may have included women who previously have experienced side-effects of OC. This is known as the 'survivor effect' (Kutner & Brown, 1972). Depressogenic side effects of OC use were observed at a significantly higher level in MR haplotype 1 and 3 carriers, however. This is consistent with the finding that MR haplotype 2 carriers are less vulnerable to depression during their child-bearing years (Klok et al., 2011).

Due to our cross-sectional design, causal relations between MR-haplotypes and OC use cannot be inferred. Neither investigators nor participants were blind to their condition (OC

use or not), but participants did not know the hypotheses and both participants and researchers were blind to the MR- genotype. Furthermore, the sample size was too small to investigate homozygous and heterozygous MR-haplotype 2 carriers separately. NC volunteers were not tested on pre-determined days in the follicular respectively luteal phase of the menstrual cycle. We did not validate the estimated menstrual cycle phase by measures of estrogen and progesterone, nor did we assess levels of testosterone. We did not assess cycle days of OC-users. We did not control for a possible confounding influence of the circadian secretion pattern on steroid hormones since we tested participants according to their own availability during the day. However, no significant differences were observed between the subsamples with regard to their time of assessment, making confounding as such unlikely. Although we did not apply a standardized clinical interview, we screened our participants on medical and psychiatric current and past disorders before inclusion and on the day of assessment itself. Finally, we did not select participants on predetermined compounds of OC, resulting in an inclusion of OC with various compounds and different doses of synthetic hormones (see table 2). Nonetheless, when we excluded OC with other compounds than estrogen and/ or progestin from our analyses, the main outcome of this study remained.

In sum, our study revealed that OC use is associated with lower accuracy in the recognition of anger. OC use also influenced risky decision making. OC use may also be associated with changes in information processing that are consistent with a depressogenic side-effect. Carriers of MR-haplotype 2, however, seem to be protected against these negative effects of OC on perception and memory. Our findings may provide further insights into why some but not all women experience mood swings due to the synthetic hormones in OC (Oinonen & Mazmanian, 2002; Boron & Boulpaep, 2012; Gingnell et al., 2012).