Mood and the pill

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Chapter 6

Oral contraceptives positively affect mood in healthy PMS-free women: a longitudinal study

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Abstract

Objective

Menstrual cycle phase and oral contraceptives (OC) use influence mood and cognition and these effects may be moderated by the mineralocorticoid receptor (MR) genotype. The effect of menstrual cycle phase on mood may be increased if participants know that this is the focus of study. We assessed aspects associated with reproductive depression such as mood, interpersonal sensitivity, affect lability and depressive cognitions in MR-genotyped OC-users and naturally cycling (NC) women in a carefully masked design.

Methods

A homogenous sample of healthy, PMS-free, pre-menopausal MR-genotyped women (n=92) completed online questionnaires eight times during two consecutive cycles.

Results

The masking of the research question was successful. OC-users did not differ significantly from NC women in positive and negative affect at the time of assessment, personality characteristics (e.g. neuroticism) or mental and physical health. Both groups reported more shifts in anger in the first cycle week ($p < .001$; $\eta_p^2 = .08$). Compared to NC women, OC-users reported fewer mood-shifts between depression and elation in the mid-luteal phase of the menstrual cycle ($p = .002$; $\eta_p^2 = .10$) and had fewer ruminating thoughts at all phases ($p = .003$; $\eta_p^2 = .11$). Effects of MR-genotype were not significant after correction for multiple comparisons.

Conclusion

OC users scored more favorably on measures associated with reproductive depression. OC users also showed a decreased affect variability possibly indicating an emotional blunting effect, which is in line with previous reports on affect-stabilizing effects of OC. Limitations were loss of cases due to irregularities in the menstrual cycle length and possible confounding by the 'survivor effect', since almost all OC-users took OC for more than a year.

Introduction

A beneficial effect of oral contraceptives (OC) on mood swings occurring during the menstrual cycle has frequently been reported (e.g. Keyes et al., 2013; Cheslack-postava et al., 2014; Yonkers et al., 2016). On the contrary, negative mood effects such as irritation and alexithymia have been observed since the introduction of OC. These are still one of the main reasons to discontinue OC-use (Kulkarni 2007; Hamstra et al., 2015; Zethraeus et al., 2017). Apparently, inter-individual differences exist in the sensitivity to the negative mood effects due to the synthetic hormones in OC (Poromaa & Segebladh, 2012). Due to the lack of consistent research methods it is difficult to pinpoint which OC users in particular may be at risk for these adverse effects (Schaffir, Worly & Gur, 2016).

The hypothalamic-pituitary-ovarian axis drives the menstrual cycle: neurons in the hypothalamus release gonadotropin releasing hormone (GnRH), which results in the release of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the gonadotrophs. FSH and LH at their turn stimulate the ovary to secrete estrogens and progestins. Together with peptides that are also produced by the ovary, FSH and LH provide both positive and negative feedback on the hypothalamus and anterior pituitary resulting in a monthly pattern of hormonal fluctuations (Jones, 2012; Rivera et al., 1999).

Like the natural ovarian steroids, OC feed back directly to the hypothalamus and the gonadotrophs in the anterior pituitary, accruing in a suppressed release of gonadotropins, FSH and LH. This mechanism prevents follicular development, thus ovulation, and suppresses the monthly hormonal fluctuation. Monophasic or fixed-combination OC are taken during a period of 21 days (active use), followed by 7 pill-free days in order to mimic a menstrual cycle of 28 days. During active OC use the levels of LH, FSH, progesterone and estradiol are lower in OC users than in NC women (Jones, 2012; Rivera et al., 1999).

The mood effects of OC may be induced by an interaction of sex steroids with the serotonergic and noradrenergic pathways. Interestingly, the hypothalamic-pituitary-adrenal (HPA) axis may also be involved in the effects of female hormones on mood and depression (Handa & Weiser, 2014; Jones, 2012). The HPA axis represents a complex neuroendocrine feedback loop, which is activated by a stressful event. The HPA end-product cortisol is secreted by the adrenals and promotes coping with a stressor. This action exerted by cortisol is mediated by mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). The MR is involved in the appraisal of a stressful event, the regulation of the initial psychological stress reactions like vigilance, selective attention, selection of an appropriate coping style and encoding of emotional memory (Cornelisse et al., 2011; de Kloet et al., 2005; Henckens et al., 2012). Endogenous estrogen may suppress the expression of the MR in the limbic brain (Carey et al., 1995) and in vascular endothelial cells (Barrett Mueller et al., 2014). Progesterone binds to the MR with nearly the same affinity as its ligands cortisol and aldosterone, but acts as an antagonist (Carey

et al., 1995; Quinkler et al., 2002). Consequently, the MR gene has been studied within the context of the influence of the female hormonal status on affective processes associated with mood and cognition.

Genetic variation in the MR was found to predict individual differences in emotion and mood disorders. The single nucleotide polymorphisms (SNP) MR-2G/C (rs2070951) and MR-I180V (A/G, rs5522) have been studied with a haplotype approach (van Leeuwen et al., 2011). A sex-dependent role for these MR-haplotypes was found in the Netherlands Study of Depression and Anxiety (NESDA): in survivors of childhood maltreatment (n=1639), female carriers of MR-haplotype 2 (C/A) were less likely to develop depression than MR-haplotype 1 (G/A) carriers (Vinkers et al., 2015). Female carriers of MR-haplotype 2 (C/A) also showed higher optimism scores (Klok et al., 2011; Hamstra et al., 2017), fewer thoughts of hopelessness during sad mood and a lower risk of depression (Klok et al., 2011). These effects were restricted to premenopausal women supporting the effect of female gonadal steroids on the function of the MR in this context.

The MR-genotype also moderates the effect of female hormones on cognition, particularly with respect to the appraisal and processing of emotionally relevant information. Healthy pre-menopausal volunteers with MR-haplotype 1/3 who used OC showed an information processing bias that is consistent with an increased vulnerability to depression (Hamstra et al., 2015). A follow up study revealed that the MR-genotype not only moderated the influence of second-generation OC, but also the impact of menstrual cycle phase on emotional information processing: OC-users with MR-haplotype 1/3 performed worse on the facial expression recognition task than naturally cycling (NC) MR-haplotype 1/3 carriers in the mid-luteal phase of the menstrual cycle. This effect was not observed in carriers of MR-haplotype 2 (Hamstra et al., 2016). Our most recent study revealed that the influence of the MR-haplotype on emotional information processes depended on circulating estrogen and progesterone (Hamstra et al., 2017). These differences in emotional sensitivity to hormonal change were all observed in healthy pre-menopausal volunteers.

The term ‘reproductive depression’ refers to a heightened sensitivity to hormonal shifts, which may be characterized by increased irritability and interpersonal sensitivity, depressed mood and cognitive dysfunction (Deecher et al., 2008; Harald & Gordon, 2012; Soares & Zitek, 2008). Scores on these domains also varied between the late follicular and late luteal phase in a healthy, PMS-free sample (Gonda et al., 2008). However, it has also been shown that reports of psychological and somatic symptoms related to the menstrual cycle may be increased if participants are aware that the menstrual cycle is the focus of study (Aubuchon & Calhoun, 1985). Taking the current evidence together, it seems reasonable to postulate that hormonal changes during the cycle can trigger mood changes in a MR-genotype dependent manner.

In this study we tested the hypothesis that naturally cycling women had higher scores on measures associated with reproductive depression than OC-users. We further hypothesized that naturally cycling women with MR-haplotype 1/3 had higher scores on these measures than MR haplotype 2 carriers. We carefully masked our research objective to prevent artefacts caused by expectations concerning menstrual cycle symptoms.

Experimental procedures

Participants

All participants were university students, who were recruited via social media and posters distributed at the campus of Leiden University. Eligible participants were healthy, non-smoking women (18-35 years) of Northwestern European origin. Naturally cycling (NC) participants had a regular menstrual cycle (25-35 days) and had not used any hormonal contraceptives for at least 3 months. OC-users took mono-phasic pills 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 determined by the General Health Questionnaire 12 item-version. Participants with a score X > 2 were excluded from participation (Goldberg et al., 1997; Aalto et al., 2012). Further exclusion criteria were self-reported current psychological or psychiatric treatment; pregnancy or lactation; use of abortion-pills in the past three months; alcohol abuse; use of nicotine or cannabis in the past three months; history of any hard drug use; use of XTC (> 1 per month during three months or any use during the past month) and current use of any prescribed medication.

Procedure

Design

This study had a prospective longitudinal design. All data were collected from March till July 2015 using an online survey tool (Qualtrics, Provo, UT). We assessed all participants at four time-points during two consecutive months, so eight times. After completion of the eighth assessment, participants were screened on premenstrual syndrome (PMS) by the Menstrual Distress Questionnaire (MDQ; Moos, 1968).

Masking procedure

We masked our research objective by informing participants that we investigated the impact of chronotype and sleep on mood during two months in healthy MR-genotyped women. Questionnaires measuring chronotype and sleep quality were also administered but are not

reported. After completion of the eighth assessment and the MDQ (see 2.2.1.), we asked participants to guess the underlying research question (open-ended question), to check whether the masking was successful. Subsequently they completed a screening for premenstrual syndrome (MDQ; Moos, 1968) after which they were debriefed.

Biological measures

Cycle phase assessment

Naturally cycling (NC) participants filled out questionnaires on four time-points (T) of two consecutive menstrual cycles: the early-follicular (EF; T1; day 4), late-follicular (LF; T2; day 13), mid-luteal (ML; T3; day 21) and late-luteal phase (LL; T4; day 27). Care was taken to adjust the time-points to the individual cycle-duration, reasoning that the luteal phase always lasts two weeks (Jones, 2012). At intake the average cycle duration of the NC participants was registered or – if the duration was unknown – a cycle was awaited before inclusion. The actual participation started with the confirmation by the participant that a new menstrual cycle had started. The study was completed after receiving confirmation of the third cycle onset: this information was used to recalculate if the NC participants had been tested in the pre-calculated phase. Only participants with at least one valid session per time-point were included in the analyses.

Oral contraceptive (OC) users were assessed at four equivalent time-points during two consecutive months: on day 4 (T1), day 13 (T2) and day 21 (T3) of active OC-use and on day 6 of the pill-free week (T4). Their actual participation started with a new pill strip.

Assessment of estradiol and progesterone

We controlled for cycle phase and pregnancy by levels of estrogen, progesterone and estriol in saliva at two sessions in our lab. In OC-users, samples were collected 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). In naturally cycling (NC) participants, samples were collected 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 progesterone is at its maximum and estrogen reaches a second peak (Jones, 2012).

Procedure: Saliva samples were collected in our laboratory at two sessions. At the first meeting buccal swaps for MR-genotyping were taken and personality traits were assessed (NEO-Five Factor Inventory; McCrae & Costa, 1987) also. During each laboratory session saliva was collected at three time-points with an interval of 30 minutes each. 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 estradiol (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 progesterone (in saliva): follicular phase 50 - 100 pg/ml; ovulation 100 - 150 pg/ml; luteal phase 100 - 450 pg/ml; post-menopause and OC-users: 10 -50 pg/ml.

Mineralocorticoid (MR) haplotype

BaseClear Ltd. (Leiden, The Netherlands) realized DNA isolation, PCR amplification and analyses of the rs2070951 and rs5522 polymorphisms. According to the observed frequency in the population, we composed MR-haplotype 1 (GA) 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) (DeRijk et al., 2008).

Questionnaires

Interpersonal Sensitivity

The Interpersonal Sensitivity Measure (IPSM; (Wilhelm et al., 2004; Boyce & Parker, 1989; Boyce et al., 1991) is a 28-item questionnaire that assesses four dimensions of interpersonal sensitivity. The subscale 'interpersonal awareness' (IPSM IA) reflects vigilance against the behavior and feelings of others (''I care about what other people feel about me"). 'Separation anxiety' (IPSM SA), refers to the fear of becoming separated from others. 'Timidity' (IPSM T) measures lack of assertiveness in fear of irritating others. 'Fragile inner self' (IPSM FI) assesses the extent to which a person has difficulty disclosing his feelings out of fear for being rejected by others ("My value as a person depends enormously on what others think of me"). Participants rated the applicability of each item during the past week on a 4-point Likert scale, ranging from 1 (not applicable at all) to 4 (yes, completely).

Affect Lability

The tendency to experience strong and variable emotions was measured by the Affect Lability Scale (ALS; Oliver & Simons, 2004; Aas et al., 2015). We used three items to assess mood shifts from anxiety to depression (ALS AD; "One minute I can be feeling OK and then the next minute I'm tense, jittery, and nervous"), three items for shifts from depression to elation (ALS

DE; "There are times when I feel absolutely wonderful about myself but soon afterwards I often feel that I am just about the same as everyone else") and three items for shifts in anger (ALS A; "There are times when I'm so mad that my heart starts pounding and/or I start shaking and then shortly afterwards I feel quite relaxed"). Participants rated to what extent a given statement corresponded with their mood during the past week. Ratings were on a 4-point Likert scale from 0 (not at all) to 3 (very much).

Cognitive Reactivity

A shortened version of the Leiden Index of Depression Sensitivity-Revised (LEIDS-R; Van der Does, 2002; Solis et al., 2016) was used to measure depression vulnerability. Before answering the items, participants were instructed to take a moment to imagine or recall a mildly sad mood state. Subsequently, cognitive reactivity to sad mood was assessed by three subscales: rumination (LEIDS RUM; 4 items; "When I feel down, I feel more overwhelmed by things"), aggression (LEIDS AGG; 6 items;"In a sad mood, I am bothered more by aggressive thoughts") and risk avoidance (LEIDS RAV; 4 items; "When I feel sad, I am less inclined to express disagreement with someone else"). Participants rated the extent to which each statement applied to them on a 5-point Likert scale from 0 (not at all) to 4 (very strongly).

Positive And Negative Affect States

Positive and negative affect states were assessed by the 20-item state version of the PANAS (Watson et al., 1988), using a 5-point Likert scale from 1 (not at all) to 5 (extremely). Participants were asked to rate how they felt 'right now'.

Statistical analyses

Statistical analyses were performed in SPSS 22. Mean scores on personality dimensions between both groups (OC-users and NC women) were compared with analyses of variance and chi-square tests. In case of significant group differences, we added these scores as a covariate to our model in further analyses. In order to control for confound by historical effects, we calculated a mean score over two months for each time-point. A mean score was calculated per item and over the subscales of the questionnaires consecutively.

We analyzed the outcomes of the questionnaires with repeated measures multivariate analyses of variance (rm MANOVAs) with time (time-points 1-4) as a within-subject factor and hormonal status (OC, NC) as a between-subject factor. In the explorative analyses MR haplotype (amount MR-haplotype 2 alleles: 0, 1, 2) was added as a between-subject factor to the model. Effects on MR-haplotype ($p < .05$) were investigated with simple last contrasts, thus comparing MR-haplotype 1/3 and MR-haplotype 2 heterozygotes with MR-haplotype 2 homozygotes.

Follow-up (M)ANOVAs were performed if interaction effects in MANOVAs were significant ($p < .05$). Means (SE) on outcomes (IPSM, ALS, LEIDS-R, PANAS) are presented in table 3.

Within-subject univariate tests and between-subject effects were reported, but only if effects were significant ($p < .05$). Estimates of effect size were partial eta squared (η_p^2) and power. If assumptions of sphericity were violated, a Greenhouse-Geisser correction was reported. Multivariate influential cases (Cook's distance ≥ 1) were excluded from analyses. We corrected for multiple comparisons by reporting only effects with $p < .005$.

Results

Participant characteristics

A total of 368 women showed interest in our study, of whom 147 seemed to fulfill in- and exclusion criteria after a brief screening and were included. Twenty-nine women withdraw before partaking for reasons of non-compliance (i.e. not willing to apply a pill-free week; not being able to complete questionnaires during two months) and 118 women signed informed consent. During participation 15 women were excluded due to irregular menstrual cycles (< 25 days or > 35 days), illness (2) or low compliance (5). Data of 4 participants were excluded from the analyses because of too many missing data, leaving a final sample of 92 participants. Almost all OC-users (95%) were on the pill (EE 0.03; LNG 0.15) for more than one year and 75% for more than two years. Of all NC women, 16 (46%) had been using OC in the past, of whom 7 (20%) discontinued OC-use for reasons of negative side-effects or feared long-term consequences (physical complaints, negative mood, increased risk of cancer). None of the participants had premenstrual syndrome according to the MDQ (Moos, 1968). There were no significant differences on personality traits (NEO-Five Factor Inventory; Costa & McCrae, 1992) between both groups except that more OC users were in a committed relationship, which we added as covariate to our model. Information on demographic variables and personality scores are given in Table 1.

The masking of the research question had worked well: 83 (90%) participants thought the study investigated the influence of sleep, emotions (n = 78; 85%), female hormones (n = 47; 51%) and /or cycle phase (n = 27; 28%). None of the participants guessed that we investigated sensitivity to menstrual cycle related mood-swings.

Table 1. Sample characteristics

	NC	OC
N (%)	35 (38)	57 (62)
MRHT 2 (0, 1, 2 alleles) (%)	17 (49); 13 (37); 5 (14)	25 (44); 22 (39); 10 (18)
Age	20.63 (.33)	20.74 (.22)
Committed relationship (%)	10 (29)*	36 (63)*
NEO-FFI Agreeableness	35.03 (.71)	35.77 (.56)
NEO-FFI Conscientiousness	40.60 (.48)	41.02 (.41)
NEO-FFI Extraversion	39.51 (.56)	40.47 (.33)
NEO-FFI Neuroticism	34.06 (.67)	33.19 (.54)
NEO-FFI Openness	34.23 (.69)	34.89 (.45)

Notes: N (%) or Means (standard errors). *$P < .05$. *Abbreviations*: OC = oral contraceptive users; NC = naturally cycling women; MRHT 2 = amount mineralocorticoid receptor haplotype 2 alleles; NEO-FFI = NEO Five-Factor Inventory.

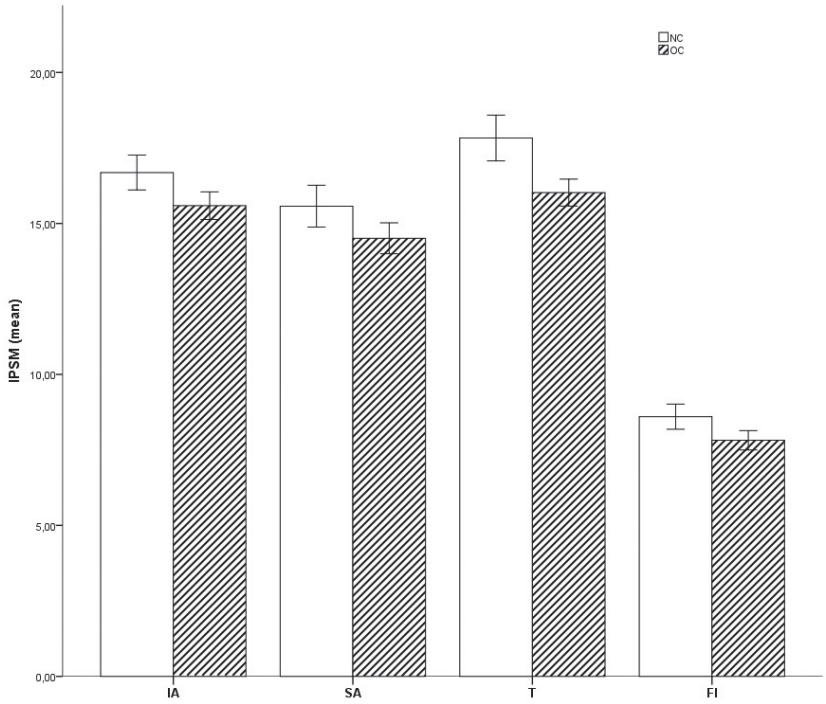

Figure 1. Overall mean scores on interpersonal sensitivity per subscale of NC women compared with OC-users. *Notes.* Error bars represent ± 1 SE. *Abbreviations.* NC = naturally cycling women; OC = oral contraceptives users; IPSM = interpersonal sensitivity measure; IA = interpersonal awareness; SA = separation anxiety; T= Timidity; FI = fragile inner-self.

Hormonal measures

Analyses failed of four samples from the first session and one from the second session. Progesterone 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 progesterone exist (Jones, 2012). The findings showed the expected pattern (see table 2).

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

	Estradiol (pg/ml)	**Progesterone (pg/ml)**
Early follicular phase (EF)	2.6 (.2)	75.2 (19.5)
Inactive OC-use (IU)	2.3 (.2)	43.6 (2.9)
Mid-luteal phase (ML)	3.4 (.2)	136.4 (15.5)
Active OC-use (AU)	2.1 (.2)	53.7 (5.7)

Questionnaires

Interpersonal Sensitivity

OC versus NC. Figure 1 shows that OC-users had lower interpersonal sensitivity scores, although these differences were not significant after correction for multiple comparisons.

Moderating effect MR genotype. No significant (interaction) effects for MR-haplotype were observed.

Covariate. Between-subject effects on IPSM Timidity revealed a main effect of hormonal status (OCNC; [$F(1,89) = 8.0$; $p = .006$; $\eta_p^2 = .08$; power = .80]), with NC women scoring higher. Consistently, women who were in a committed relationship had lower IPSM T scores [$F(1,87) = 4.5$; $p = .037$; $\eta_p^2 = .05$; power = .55) as 78% them were OC-users.

Affect Lability

OC versus NC. Regardless of hormonal status, scores per time-point differed significantly for ALS anger [$F(2,214) = 7.5$; $p < .001$; $\eta_p^2 = .08$; power = .97]. Paired t-tests revealed that ALS anger scores at T1 (EF) were higher than at T2 (LF) ($p = .004$; df 91; $t = 2.9$), T3 (ML) ($p < .001$; df 91; $t = 3.7$) and T4 (LL) ($p = .016$; df 91; $t = 2.5$). Univariate tests revealed a significant interaction effect of group (OCNC) and time on ALS DE scores [$F(3,255) = 2.8$; $p = .040$; $\eta_p^2 = .03$; power = .67]. The follow-up MANOVA showed that OC-users reported fewer moodswings between depression and elation than NC women at T2 [$F(1,90) = 4.7$; $p = .032$; $\eta_p^2 = .05$; power

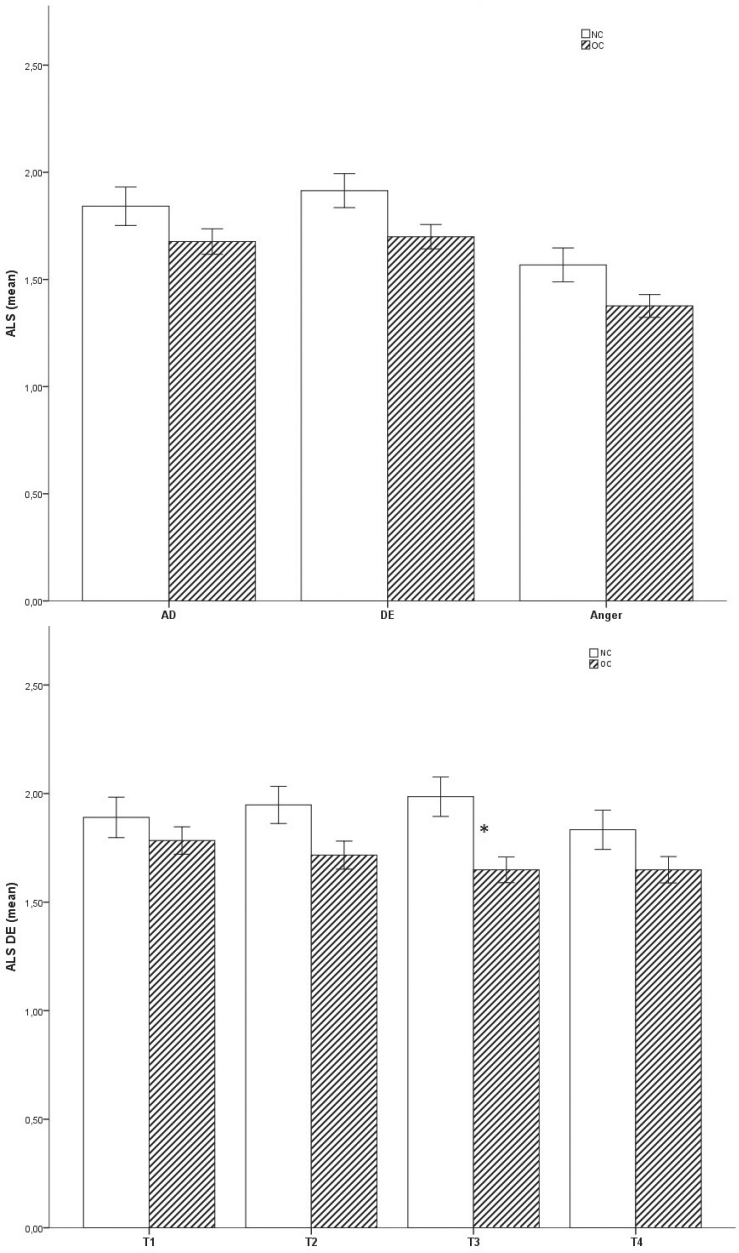

Figure 2. Affect lability scores. *First panel*: overall mean affect lability scores of NC women compared to OC-users. *Second panel:* Scores on mood shifts between depression and elation per time point of NC women compared to OC-users. *Notes.* * p < .05; Error bars represent ± 1 SE. *Abbreviations.* NC = naturally cycling women; OC = oral contraceptives users; T = time point;
ALS = affect lability scale; AD = mood shifts between anxiety and depression; DE = mood shifts between depression and elation.

= .58] and at T3 [F(1,90) = 10,5; p =.002; η_p^2 = .11; power = .89], which remained significant after correction for multiple comparisons. See figure 2.

Moderating effect MR genotype. After correction for multiple comparisons no effects remained.

Covariate. No within- and between-subject effects (interaction) effects involving committed relationship were found. Outcomes as reported by OC versus NC remained.

Cognitive Reactivity

OC versus NC. OC users scored lower than NC on LEIDS RUM [F(1,90)=9.5; p=.003; η_p^2=.10; power=.86]. See figure 3.

Moderating effect MR genotype. Reported effects on OC vs NC remained. No (interaction) effects involving MR-genotype were found.

Covariate. No (interaction) effects involving committed relationship was found. Between-subject effects in OC/NC on LEIDS RUM remained significant after correction for multiple comparisons [F(1,87) = 12.5; p = .001; η_p^2 = .13; power = .94].

Positive And Negative Affect States

Neither significant (interaction) effects were observed of group, cycle phase, or time * groups on negative or positive mood states, nor any significant (interaction) effects involving MR-haplotype.

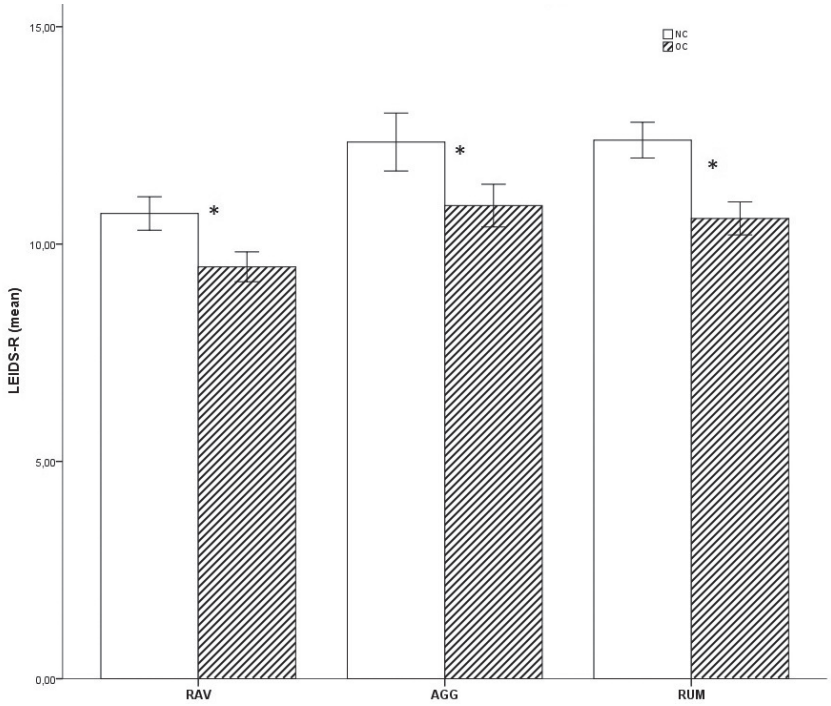

Figure 3. Overall mean scores on depression-congruent cognitions.

Notes. * $p < .05$; Error bars represent ± 1 SE. *Abbreviations:* OC = oral contraceptives users; NC = naturally cycling women. LEIDS-R = Leiden index of depression sensitivity-revised; RAV = risk avoidance; AGG = aggression; RUM = rumination.

Table 3. Mean outcomes (*SE*) of OC-users compared to NC women

		Time point 1		Time point 2		Time point 3		Time point 4	
Measure	**Subscale**	**NC**	**OC**	**NC**	**OC**	**NC**	**OC**	**NC**	**OC**
IPSM	IA	17.10 (.67)	15.78 (.48)	16.47 (.61)	15.58 (.48)	16.56 (.60)	15.26 (.47)	16.61 (.58)	15.74 (.47)
	SA	15.73 (.80)	14.52 (.51)	15.94 (.76)	14.53 (.54)	15.29 (.64)	14.28 (.55)	15.33 (.74)	14.71 (.54)
	T	17.89 (.77)	16.33 (.49)	17.43 (.75)	16.22 (.45)	18.17 (.80)*	15.93 (.48)*	17.83 (.84)*	15.60 (.45)*
	FI	8.54 (.45)	7.58 (.29)	8.64 (.44)	7.92 (.34)	8.57 (.42)	7.86 (.35)	8.64 (.43)	7.91 (.35)
ALS	AD	1.81 (.11)	1.71 (.07)	1.85 (.11)	1.65 (.07)	1.90 (.10)	1.67 (.07)	1.81 (.10)	1.68 (.07)
	DE	1.89 (.09)	1.78 (.06)	1.95 (.09)*	1.72 (.06)*	1.99 (.09)*	1.65 (.06)*	1.83 (.09)	1.65 (.06)
	Anger	1.73 (.10)*	1.42 (.06)*	1.50 (.08)	1.36 (.05)	1.46 (.08)	1.36 (.05)	1.58 (.10)	1.36 (.06)
LEIDS-R	RAV	10.64 (.38)*	9.59 (.36)*	10.47 (.42)*	9.62 (.40)*	11.01 (.43)*	9.35 (.36)*	10.69 (.50)*	9.34 (.38)*
	AGG	12.31 (.67)	10.86 (.46)	12.81 (.77)	11.18 (.52)	11.94 (.73)	10.78 (.54)	12.31 (.68)	10.71 (.54)
	RUM	12.63 (.45)**	10.63 (.37)**	12.37 (.46)*	10.56 (.42)*	12.27 (.45)*	10.61 (.45)*	12.29 (.46)*	10.56 (.41)*
PANAS	PA	22.23 (1.09)	24.53 (.83)	23.49 (1.12)	24.12 (.89)	21.87 (1.18)	23.93 (.85)	22.20 (1.17)	23.21 (.85)
	NA	14.49 (.68)	12.96 (.45)	14.34 (.72)	13.27 (.52)	15.17 (.79)	13.64 (.61)	14.21 (.61)	13.53 (.52)

Notes. * $P < .05$; ** $P < .001$; *Abbreviations*: SE = standard errors of the mean; NC= naturally cycling women; OC = oral contraceptives users; IPSM Interpersonal Sensitivity Measure; IA= isolation anxiety; SA = separation anxiety; T = timidity; FI = fragile innerself; ALS = Affect Lability Scale; AD = anxiety – depression; DE = depression – elation; LEIDS-R = Leiden Index of Depression Sensitivity- Revised; RUM = rumination; RAV = risk avoidance; AGG = aggression; PANAS = Positive and Negative Affect Scale;
PA = positive affect; NA = negative affect.

Discussion

We investigated mood and cognition across the menstrual cycle in healthy MR-genotyped women in a longitudinal design. We compared mood scores of OC-users during active use and in the pill-free week with those of NC women on personalized time-points in their menstrual cycle. Additionally, we verified the assessment of cycle phase and phase of oral contraceptives use with the concentration of estradiol and progesterone in saliva.

OC-users were significantly less likely to ruminate ($p = .003$), an effect that remained after controlling for committed relationship status. In general, women report or are observed to use more emotion-focused strategies such as rumination (Thayer et al., 1994; Nolen-Hoeksema, 2012; Brody et al., 2016). Rumination is defined as 'the process of thinking persistently about one's feelings and emotions' (Nolen-Hoeksema, 1991) and is the strongest predictor of the development and relapse of depression of all cognitive reactivity subscales (Kuehner & Weber, 1999; Kruijt et al., 2013; Figueroa et al., 2015).

Not only rumination, but also interpersonal sensitivity, affect lability and negative cognitions associated with anger and risk avoidance tended to be reduced in OC-users. And they did not differ significantly from NC women in positive and negative affect at the time of assessment, personality characteristics (e.g. neuroticism) or mental and physical health. Thus, OC-users tended to score lower on outcomes associated with reproductive depression than NC women. This effect has been observed previously in both healthy and clinical samples (e.g. Svendal et al., 2012; Toffol et al., 2012; Teatero et al., 2014; Robakis et al., 2015). Although women below the age of 20 may be more susceptible to negative mood effects following OC use (Lisofsky et al., 2016; Oinonen & Mazmanian, 2002; Skovlund et al., 2016), we did not observe this in our sample (mean age 20,6).

Only affect lability scores varied significantly across the menstrual cycle. NC women and OC-users reported an increase in mood shifts towards anger at time point 1, thus at the beginning of the new cycle ($p <. 001$). As participants rated mood-shifts during the past week, NC women reported increased irritability before and at the menses onset and OC-users during their pill-free week. Both are periods in which natural or synthetic hormonal levels decline, which may contribute to increased irritability (Dougherty et al., 1997; Ritter, 2003; Pearlstein et al., 2005). Reports on increased irritability in OC-users in the pill-free week are mixed, however. This might be because the mood effects of OC especially developed to alleviate PMS and PMDD, for instance drospirenone-containing OC, have been studied in a shortened (24-4) or absent pill-free week. In the mid-luteal phase, OC-users reported fewer mood-swings between depression and elation than NC women ($p = .002$). This reduction in mood-swings may be interpreted as a beneficial, stabilizing effect of OC on mood (Ott et al., 2008; Keyes et al., 2013; Cheslack-Postava et al., 2014; Yonkers et al., 2016), but may also be experienced as a condition of reduced emotional

reactivity ('emotional blunting'). For instance, a positive mood induction exerted weaker effects in OC-users than in NC women (Jarva & Oinonen, 2007). The slightly worse recognition of bodily and facial expressions of emotions by OC-users (Suslow et al., 2015; Hamstra et al. 2014, 2015, 2016, 2017) may also reflect emotional blunting.

Genetic variation in the MR was found to predict individual differences in emotion and mood disorders. These effects were restricted to premenopausal women supporting the effect of female gonadal steroids on the function of the MR (Klok et al., 2011; Hamstra et al., 2015, 2016, 2017). Insight in biological determinants of emotional lability due to hormonal changes may be of importance in the prevention, detection and treatment of psychopathology in women. In general, mood instability is an important aspect of psychopathology and can occur in the prodrome of attention-deficit disorder, depressive disorder and bipolar disorder (Broome et al., 2015). About 50% of all women with bipolar depression experience significant mood changes during the menstrual cycle (Robakis et al., 2015; Teatero et al., 2014). Furthermore, increased affect lability during the menstrual cycle and hormonal contraceptives use may predict depressive episodes post-partum and at peri-menopause (Teatero et al., 2014; Stahl, 2013; Payne et al., 2009; Kornstein & Clayton, 2002). Although we observed moderating effects of the MR-genotype on the influence of the hormonal status on mood, these effects were not significant anymore after correction for multiple comparisons. We applied a stringent correction in order to avoid over-interpretation of possibly spurious findings, but given the exploratory nature of our study, future studies might consider a less stringent approach as well. Furthermore, future studies should investigate homozygous and heterozygous MR-haplotype 2 carriers separately in bigger samples, considering dropouts due to menstrual cycle irregularities.

Some issues need to be addressed in future studies. Our favorable findings with regard to OC use in healthy premenopausal women could be confounded by the so-called "survivor effect". Novel OC-users experiencing adverse effects will often discontinue OC use, and usually within one year (Trussell & Kost, 1987; Kay, 1984). They are also more likely to become part of the NC group and to volunteer for OC–related research (Wiebe, 2013; Kutner & Brown, 1972). Furthermore, we did not assess estradiol and progesterone levels on all stages of the menstrual cycle and had 15 dropouts due to irregularities in the menstrual cycle duration, even though we included only women with self-reported regular cycle duration. As menstrual irregularities are more frequently observed in young women (Jones, 2012), the high dropout rate was possibly due to the relatively young age of our sample.

Strengths of our study include that we collected all data in a single season (Spring) and that we verified the hormonal status by assessment of levels on estradiol and progesterone in saliva. We investigated a healthy PMS-free sample homogenous on age and education level. All OC-users were on the first-choice combined oral contraceptive (EE, 0.03; LNG, 0.15).

We corrected for multiple comparisons and applied contrasts on menstrual cycle phase scores only if the main effect for phase in follow up analyses was significant. Confound by historical effects was reduced by averaging the outcomes per time point over two months. We adjusted the timing of assessments in NC women to the actual cycle-duration and checked correct timing of assessment with third cycle onset information. Finally, OC-users and NC women were compared on personality traits and committed relationship status.

In our study participants were 'blinded' successfully to the underlying research question, as none of the participants guessed that we were investigating sensitivity to menstrual mood-swings. Consistently, OC-users did not differ significantly from NC women in positive and negative affect, but had lower scores on scales associated with depression. Although the effects of OC-use and menstrual cycle phase on (depressed) mood have been studied extensively, concealing the research topic has only been done in very few of these studies. Consequently, reported findings on psychological and physical symptoms related to OC-use and/ or the menstrual cycle phase may have been increased (Aubuchon & Calhoun, 1985). Future studies may investigate in a masked design if the stabilizing effect of OC on mood is perceived as a pleasant or negative side effect by assessing mood during active OC-use, in the pill-free week and in different menstrual-cycle phases in a larger cohort. Not only questionnaires and diaries on PMS symptomology should be applied but also ecological momentary assessment (EMA; Shiffman et al., 2008), in order to detect and explain perceived differences in daily life experiences (Bosman et al., 2016).