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# Mood and the pill

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# Chapter 8

## General discussion

## 8.1 Aim of this thesis

In this PhD project the mood-effects of oral contraceptives (OC) and the moderating effect of the mineralocorticoid receptor (MR) gene were examined in four research projects. The results were reported in six publications (see chapter 2 to 7) and are compiled in chapter 9 (Summary).

All outcomes in this PhD project on the effects of female hormonal status are summarized and interpreted in paragraph 8.2. Paragraph 8.3 involves main effects of MR-gene variation using a haplotype-approach, followed by paragraph 8.4 on interaction effects between the female hormonal status and MR-gene variation. In paragraph 8.5 some methodological aspects concerning this research topic will be discussed.

## 8.2 Main effects of the female hormonal status

In six chapters we investigated effects of the menstrual cycle phase and oral contraceptive (OC) use – summarized by the term 'the female hormonal status' – on emotional processing, some of which could be consistently interpreted. We observed no differences between OC-users and naturally cycling (NC) women in mental and physical health, personality traits and affective state, with the exception of our second study (chapter 3) in which OC-users showed significantly fewer negative cognitions in response to sad mood.

### 8.2.1 Conclusion behavioral lab studies (chapter 2, 3, 4 and 5)

Female hormonal status may influence the attentional blink, facial expression recognition and risky decision-making.

***Attentional blink***

Attentional blink is an impairment in the detectability of the second of two neutral stimuli (single digits): the larger the proportion of failures in the detection of the second target, the bigger the attentional blink. Our research (chapter 5) revealed that OC-users had fewer attentional blinks than NC women. Consistently, lower levels of E2 and P4 were significantly correlated (sr =. 35; p <.001) with fewer attentional blinks, which is in line with Hollander et al. (2005).[6]

***Facial expression recognition task***

The facial expression recognition task (FERT) assesses accuracy and speed in the recognition of five facial expressions of emotions (anger, disgust, sadness, anxiety and happiness; Ekman & Friesen, 1976) in 204 trials and has been used in both healthy and clinical populations (Bone et

[6] Consistently, NC women performed worse on the attentional blink task than OC-users. This effect was no longer significant after correction for multiple comparisons, however (see chapter 5).

al., 2019; Harmer et al., 2003; 2004; 2009). Importantly, we never observed ceiling effects in this task, enabling us to detect subtle differences between OC-users and NC women whereas other variants of this task might have failed (Osório et al., 2018).

This PhD project started with the coincidental finding that OC-users recognized fewer expressions of the negative emotions anger, sadness and disgust than NC women and had shorter reaction times in this task (see chapter 2). Although the three consecutive studies had different designs, shorter RTs were observed in OC-users in each study (chapter 3[7] and 5). Furthermore, an association of OC use with impaired recognition of emotional expressions was observed in all studies, albeit the patterns and magnitude of effects on RTs and accuracy differed importantly (chapter 2, 3, 4 and 5).

***Reading the mind in the eye task***

The reading the mind in the eye task (RMET) was originally designed to measure social sensitivity or cognitive empathy. It requires assessment of 36 complex mental states from the eye area only. In our studies participants were instructed to take as much time as they need to answer (Harkness et al., 2005), resulting in a more cognitively oriented paradigm on emotion processing than assessed with the FERT. We observed that OC-users recognized positive mental states better than NC women (chapter 5). However, we did not observe equivalent effects in our previous study (see chapter 4) and outcomes on RMET performance of OC-users in other studies diverged considerably (see e.g., Radke et al., 2016; Pahnke et al., 2019, Shirazi et al., 2020), suggestive of a spurious finding.

***Emotional working memory***

In a verbal working memory paradigm, validated pictures served as positively, neutrally or negatively rated distracters. OC-users and NC women did not perform differently (chapter 4).

***Word categorization and memory task***

We observed inconsistent effects on the recall of characteristics (chapters 3 and 5). Other studies reported various results too: from improved or altered verbal (emotional) memory performance in OC-users to null-findings (see e.g., Gogos, 2013; Mordecai et al., 2008; Bayer et al., 2014; Warren et al., 2014).

***Decision-making task***

The decision-making task is an adaptation of the Iowa Gambling Test. The goal is to win as much money as possible. In each trial, a decision has to be made between a safer and a riskier option.

[7] Only in OC-users carrying MR-haplotype 2 (chapter 3). In chapter 4 no outcomes on FERT reaction times are reported.

The riskier option results in a gain (that can be high or low) or a loss (that can be high or low). The probability of winning or losing also varies. In each trial the participant may choose between a riskier or safer option. Our outcomes suggest that the female hormonal status may influence risky decision making. Women in the mid-luteal phase (ML) gambled more than women in the early follicular phase (EF), if the risk of losing was not too high. This indicates that MF women had more confidence in the possibility of winning and/ or were less sensitive to possible loss (chapter 4). Consistently, OC-users gambled less than NC women in our second study (chapter 3), as levels of endogenous estradiol and progesterone in OC-users are low (see chapters 5, 6 and 7). However, we observed no effects of female hormonal status in our within-subject study (chapter 5), which is possibly due to learning effects (see 8.5.3).

### 8.2.2 Conclusion longitudinal study (chapter 6)

***Depressive cognitions during the menstrual cycle***

Our longitudinal study (chapter 6) revealed that OC-users reported fewer sub-clinical symptoms of depression. Especially rumination scores were significantly lower in OC-users. Other negative cognitions during sad mood, interpersonal sensitivity and affect lability were reduced as well. These patterns remained when we corrected for committed relationship status. Participants were successfully 'blinded' to the research question – making confounding effects of experimenters' or participants' expectancy unlikely – and did not differ significantly in positive and negative affect at the time of assessment, personality characteristics (e.g. neuroticism) or mental and physical health. Hence, OC-users tended to score lower on outcomes associated with reproductive depression than NC women.

### 8.2.3 Conclusion resting state EEG study (chapter 7)

The theta/ beta ratio (TBR), a biomarker of cognitive control, did not differ between healthy PMS-free OC-users and NC women. TBR was also not found to be associated with levels of estradiol (E2) and progesterone (P4) (chapter 7). Hence, our data suggest that the female hormonal status does not affect cognitive control.

### 8.2.4 Discussion: OC influence emotional information processing, for better and for worse

***OC and reproductive depression***

In 1970 Herzberg and co-workers reported not only increased rates of depression in first generation OC-users, but also more variability of menstrual-cycle-related depressive feelings in NC women. So, this early paper hinted at both a positive (mood-stabilizing) and a negative

(depression-inducing) effect of OC. Since then, OC-use has repeatedly been associated with increased risk of depression, especially in young women (e.g. Kulkarni, 2007; Skovlund et al., 2016). However, it has also been reported that OC protect against developing depression in healthy premenopausal women (e.g. Toffol et al. 2012; Svendal et al. 2012). OC may also decrease premenstrual dysphoric feelings – both in clinical samples (e.g. Teatero et al., 2014; Robakis et al., 2015) and in healthy females (e.g. Ott et al., 2008; Cheslack-Postava et al., 2015). Consistently, we observed that women using OC for more than one year had more favorable scores on cognitions associated with depression than NC women. Especially the scores of OC-users on ruminative cognitions following sad mood were decreased (chapter 6). Of all depressive cognitions, rumination is the strongest predictor of the onset and relapse of depression (Kuehner & Weber, 1999; Kruijt et al., 2013; Figueroa et al., 2015). Lower rumination scores in OC-users might be explained by the suppressive effect of OC on cortisol as measured in saliva. OC-users showed lower levels of "free" cortisol than NC women after exposure to psychosocial stress (Kirschbaum et al., 1993), and after administration of 0.25 mg ACTH1–24 (Kirschbaum et al., 1999). Lower levels of salivary cortisol have been linked to decreased rumination (Zoccola et al., 2010; Shull et al, 2016).

***OC[8] reduce affect variability***

Little (experimental) research has been conducted on the influence of OC on positive mood states. Hence, subtle effects of OC in satisfied OC-users – approximately 75% of all users– may be under-reported. In our study OC-users reported not only lower scores on depressive cognitions, but also experienced reduced mood shifts between depression and elation (chapter 6), which may be perceived as either a stabilizing or a blunting effect, depending on the appraisal by the OC-user. This effect of OC has also been observed in experimental paradigms: compared with naturally cycling women, OC-users did not respond to positive mood induction (Jarva & Oinionen, 2007) and recognized fewer facial expressions of emotions (chapter 2, 3, 4 and 5).

***OC may influence sensitivity to facial expressions of emotions***

Several studies were undertaken to investigate the effects of the menstrual cycle on emotion recognition, revealing an effect of natural variation in estrogens and progesterone. For instance, in the luteal phase, when levels of progesterone and estrogen are elevated, increased affective responsiveness to negative emotions was found. The identification of the negative emotions anger, sadness and fear was impaired in the early follicular phase, when endogenous gonadal hormonal levels are low (Derntl et al., 2008; Gasbarri et al., 2008). At a neural level, decreased

[8] It is important to note that we investigated only users of second-generation monophasic OC. OC which contain the same amount of estradiol and progestins across the cycle contributed to improved mood stability (Oinonen and Mazmanian, 2002; Mitchell & Welling, 2020).

responsiveness of different brain structures during facial emotion processing in OC-users has been reported (see for a narrative review on this topic Osorio et al., 2018). In line with this, we observed that OC-users – whose decreased levels of natural hormones (estradiol and progesterone) mirror the early follicular phase – reacted quicker but recognized fewer facial expressions of emotions (chapter 2, 3, 4 and 5)[9,10].

A decreased sensitivity to facial expressions of emotions may have its implications. According to Ochsner & Gross (2014) emotions arise via the appraisal of internal and external stimuli and may therefore affect the evaluation of the situation itself. Hence, the perception of these stimuli is not only the result of the prior action, but also serves as input for the next emotion. So, if we perceive fewer social cues – as reflected by (facial expressions of) emotions – this may affect how we feel about and react to the world around us. Blunted emotion recognition may therefore contribute to impaired emotional and social learning, resulting in an increased risk for negative peer interactions (Rudolph et al., 1994). In line, non-human primates displayed more aggression and reduced anxiety after hormonal contraception administration. They spent more time sitting close to another animal and spent less time fearfully scanning (see for a review on this topic Welling, 2013). Importantly, this effect may become pronounced in younger women, as adolescents – compared with adults – showed decreased sensitivity to subtle changes of facial emotion expressions, suggestive of an ongoing social learning process until adulthood (Thomas et al., 2007).

***Female hormonal status modulates attentional blink and reward reactivity***

Reactivity to a possible reward is moderated not only by the menstrual cycle phase (Bayer et al., 2013; Ossewaarde et al., 2010; Dreher et al., 2007; chapter 4), but possibly also by OC use (chapter 3). Sex hormones modulate different processes during the anticipation of gain and loss magnitude, which might be explained by modulatory mechanisms as OC reduce total and free testosterone levels of an adrenal and ovarian source (see for a systematic review and meta-analysis on this topic Zimmermann et al., 2014). Reduced amygdala reactivity to high rewards was also reported in a placebo-controlled blinded study in healthy pre-menopausal women in which administration of a gonadotropin-releasing hormone (GnRH) agonist resulted in a significant reduction in testosterone and estradiol (Macoveanu et al., 2016).

Attentional blink and reward reactivity cover early versus late stages of information processing, but both functions may be affected by the central dopaminergic system, which seems to be modulated by levels of progesterone and in particular estradiol (Colzato & Hommel, 2014;

[9] Although the patterns and magnitude of effects differed importantly (see also 8.5.3).

[10] As far as we know, the influence of OC on RTs in facial emotion recognition studies has not been reported previously. However, lower progesterone levels have been associated with decreased RTs (Kamboj et al., 2015; Osorio et al, 2018), which is in line with our observations on shorter RTs in OC-users.

Gillies et al., 2014). Mesocortical projections of dopamine neurons influence frontal cortical activities underlying cognitive functions involved in planning, decision-making and attention mechanisms (Cools, 2006; Gillies et al., 2014; Felten et al., 2015). Decreased dopamine release was associated with a smaller attentional blink, reflecting a better performance on the attentional blink task (Slagter et al., 2012). OC-users had fewer attentional blinks, so performed better on the non-emotional attentional blink task (chapter 5). Clinical implications of this finding remain unclear, as only a few studies have investigated the non-emotional attentional blink effect in mood disorders. Morrison et al. (2016) observed that worse performance on the attentional blink task among individuals with social anxiety disorder was limited to those suffering from current comorbid depression, which suggests we observed a beneficial effect of OC on the attentional blink.

Processes like motivation, reward reactivity and risky decision making are also influenced by dopamine. Dopamine neurons in the ventral tegmental area send mesolimbic projections to the nucleus accumbens, amygdala and hippocampus (Miller, 2000; Felten et al., 2015). Activity during reward processing in dopamine-rich brain areas covaried with fluctuations of estrogen and progesterone levels. Furthermore, during the premenstrual phase increased activity was observed in the ventral striatum, which is a target region of the mesolimbic dopaminergic system (Dreher et al., 2007; Ossewaarde et al., 2010; Bayer et al., 2013). The decline in gonadal hormone levels during the premenstrual phase and OC use may lead to a down-regulation of dopaminergic activity, causing a withdrawal like state and increased craving for drugs and food (Ossewaarde et al., 2010). Indeed, increased appetite and food-craving are common side effects associated with OC-use (Bancroft & Rennie, 1993; McVay et al., 2011).



***Premenstrual irritative mood in OC-users may be alleviated by intake regimen***

Already in 1970 premenstrual depressive mood was reported not only by NC women, but also by OC-users (Herzberg et al., 1970). In our study we observed significantly ($p < 0.001$) more mood shifts towards anger in NC women around menses and in OC-users during their pill-free week (chapter 6). 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). Anger regulation problems are main symptoms of depression in children and adolescents (disruptive mood dysregulation disorder; American Psychiatric Association, 2014), and increased irritability is observed in 30 - 40% of all depressed adults (Fava et al., 2009; Verhoeven, 2014). According to the so-called serotonin hypothesis, anger in depression has been linked to serotonergic impairment, which may be related to an intensified receptiveness for annoying occurrences and decreased interpersonal sensitivity (Van Praag, 1996). Consistently, estrogen levels suppressed by chronic administration of a gonadotropin-releasing hormone

(GnRH) agonist were accompanied by lower serotonergic neurotransmission and depressive symptoms in a double-blind placebo-controlled study in healthy euthymic volunteers (Frokyaer, 2015; Macoveanu et al., 2016). Furthermore, another argument favoring the serotonergic hypothesis is that selective serotonin reuptake inhibitors are effective in reducing the symptoms of premenstrual syndrome, whether taken continuously or even only in the luteal – in other words the premenstrual – phase (Marjoribanks et al., 2013).

Premenstrual irritability in OC-users may be reduced by taking OC which are especially developed to alleviate premenstrual syndrome and premenstrual dysphoric disorder, f.i. drospirenone-containing 4th generation OC (Sundström-Poromaa & Segebladh, 2012). An extended OC regimen may also be considered. According to the recently updated clinical guidance of the FSRH[11] on combined hormonal contraception the recommendation to take OC on a 21 – 7 regimen is outdated: OC-users who still would have a regular 'period' could consider taking OC on an 84 – 4 schedule (FSRH, 2019). With regard to this regimen, the guideline from the Dutch College of General Practitioners (DCGP, 2012, 2020) is more liberal and leaves the choice to the OC-user in consultation with her GP (Kurver et al., 2012).

## BOX-1. Summary Female Hormonal Status

- Lower affect variability and reduced sensitivity to facial expressions of emotions may be appraised as a stabilizing or blunting effect of OC.
- Blunted emotion recognition in OC-users may influence their social interactions, this effect may become pronounced in adolescent OC-users.
- Female hormonal status modulates attentional blink and reward reactivity.
- Premenstrual irritative mood in OC-users may be alleviated by an alteration in the OC intake-regimen.
- Reduced ruminative cognitions in OC-users might be linked to decreased cortisol levels.

[11] Faculty of Sexual & Reproductive Healthcare of the Royal College of Obstetricians & Gynecologists

## 8.3 Main effects of MR gene variation

In chapter 3 and 4 carriers of MR-haplotype 2 (homo- and heterozygotes combined) were compared with MR-haplotype 1/3. Following feedback of researchers in this field of research, we decided to compare three groups: MR-haplotype 1/3, MR-haplotype 2 heterozygotes and MR-haplotype 2 homozygotes, thus counting the number of MR-haplotype 2 alleles (see chapters 5, 6 and 7). In general, the MR-haplotypes did not differ significantly in mental and physical health, personality traits and self-reported affective state, but differed in performance on some tests and EEG measures.

### 8.3.1 Conclusion behavioral lab studies (chapter 2, 3, 4 and 5)

MR-haplotype 2 is associated with positive implicit affect and more risky decision-making.

***Implicit affect***

Implicit affect is assessed by non-existing but pronounceable words: participants are asked to rate the extent to which these words express different emotions to them (anger, sadness, anxiety and happiness). Since the words have no meaning, this is hypothesized to reflect a subliminal affective state (Brosschot et al., 2014). MR-haplotype 1/3 carriers scored higher on implicit anger and sadness (chapter 4). Consistent results were found in the next study (chapter 5), with MR-haplotype 1/3 carriers having lower implicit happiness scores than MR-haplotype 2 heterozygotes and MR-haplotype 2 homozygotes. This effect was linear: the more MR-haplotype 2 alleles, the higher the scores on implicit happiness (see figure 1; chapter 5). Importantly, these implicit affect scores were not correlated with self-reported positive or negative affect, thus suggest a subliminal state of heightened dispositional optimism.

***Attentional blink***

MR haplotypes did not differ in attentional blink performance[12] (chapters 4 and 5).

***Facial expression recognition task***

MR-haplotypes had different reaction times on happy faces: MR-haplotype 2 homozygotes reacted slower than MR-haplotype 1/3 carriers (chapter 5).

***Reading the mind in the eye task***

MR-haplotype 2 homozygotes performed worse on positive cognitive empathy, as they recognized fewer positive characteristics than MR-haplotype 1/3 carriers (chapter 5).

[12] Lag 2 trials

***Emotional working memory***

No difference in memory performance was observed between the MR-haplotypes.

***Word categorization and memory task***

We found no association between the MR-haplotypes and emotional memory bias (chapters 3 and 5).

***Decision-making task***

MR-haplotype 1/3 carriers gambled more when their risk to lose was relatively small, whereas MR-haplotype 2 carriers – hetero- and homozygotes together – gambled more, regardless of their odds on winning or losing (chapter 4). This effect became more pronounced in the next study: figure 5 in chapter 5 shows a linear relationship between MR-haplotype 2 and risky decision-making: the more MR-haplotype 2 alleles, the larger the proportion of risky decisions in the decision-making task.

### 8.3.2 Conclusion longitudinal study (chapter 6)

We observed no significant association between MR-gene variation and self-reported symptoms of depression in our longitudinal study.

### 8.3.3 Conclusion resting state EEG study (chapter 7)

According to our observations, MR-haplotypes differ in neural marks of attentional control. A low rs EEG theta/ beta ratio (TBR) is associated with better attentional control and a more adaptive appraisal of stressors, which may decrease odds on developing stress-related psychopathology (Mogg & Bradley, 2016; Joormann & Stanton, 2016). MR-haplotype 2 homozygotes displayed the lowest TBR – implying better cognitive control – compared with the other MR-haplotype carriers. An explorative follow-up analysis revealed that theta power declined with the amount of MR-haplotype 2 alleles, with significantly lower theta power in MR-haplotype 2 homozygotes than in MR-haplotype 1/3 carriers (Franke, 2017; $p = .044$).

### 8.3.4 Discussion: MR haplotypes differ in neural state, traits and behavior associated with stress susceptibility

***Methodological comments***

We observed several main effects of the MR haplotypes, although not initially hypothesized. Furthermore, we observed important differences between MR-haplotype 2 homo- and heterozygotes. This became explicit in our studies, showing linear relationships between the amount MR-haplotype 2 alleles and optimism, reduced theta power, and increased risky-decision-making. Consequently, merging homo- and heterozygotes into one group ('MR-haplotype 2 carriers') may obscure findings or result in spurious findings.

***Linear relationship between MR-haplotype 2 alleles and dispositional optimism***

In this project with healthy volunteers the MR-haplotypes did not differ in self-reported affective state and personality traits. Still, most of our findings are in line with earlier studies revealing that MR-haplotype 2 carriers showed more signs of optimism, either in (implicit) affect or in riskier decision-making in a financially rewarding context (chapters 4 and 5) (Solberg Nes, 2016). Consistently, MR-stimulation resulted in riskier decisions (Deuter et al., 2017) and female carriers of MR-haplotype 2 scored higher on self-reported optimism and were less susceptible to depression (Klok et al., 2011). In addition to earlier findings, we observed a linear relationship: the more MR-haplotype 2 alleles, the higher dispositional optimism as reflected by subliminal happiness and increased risky-decision making in a rewarding context.

***MR-haplotype 2 homozygotes show signs of stress-resilience in paradigms without deliberate stress induction***

Previous studies showed an improved ability to cope with stressors in MR-haplotype 2 carriers. For instance, MR-haplotype 2 carriers had lower scores on self-reported chronic stress (van Leeuwen et al., 2011) and showed resilience to childhood trauma (Vinkers et al., 2015). In our study MR-haplotype 2 homozygotes had the lowest parietal rsEEG theta/ beta ratio (TBR) reflecting improved regulatory cortical control over the affective system. In contrast with previous studies, this was observed at rest (chapter 7). TBR has a very high test-retest reliability over weeks, suggesting that TBR reflects attentional control as a trait instead of a state (Angelides et al., 2016; van Son, 2019). Lower TBR in MR-haplotype 2 homozygotes may reflect a lower baseline reactivity to affective stimuli (theta) and an improved ability to down-regulate amygdala reactivity (beta), suggesting a more rational and less empathic way of coping. Consistently, we observed that MR-haplotype 2 carriers reacted slower to facial expressions of happiness and performed less on positive cognitive empathy (chapter 5). Other studies revealed equivalent patterns, although it was originally hypothesized that MR-haplotype 2 carriers should be more social and more sensitive to interpersonal cues. MR stimulation did not lead to greater cognitive empathy compared to placebo (Wingenfeld & Otte, 2018). In support, adolescent carriers of MR-haplotype 2 who reported lower parenting related stress had lower levels of prosocial behavior (Endedijk et al., 2019). Carriers of the MR-gene variant rs5522 AA with low levels of childhood emotional neglect showed reduced amygdala reactivity to threatening stimuli (Bogdan et al., 2012) and had higher scores on social poise, fearlessness, and stress immunity (Hall & Benning, 2006; Durand, 2018a, 2018b). These observations may reflect not only a decreased sensitivity to interpersonal cues, but also a more rational attitude. This rational approach together with increased dispositional optimism might evoke a less flexible behavioral coping style which will make MR-haplotype 2 carriers more likely to persist rather than giving up (Segerstrom 2001; Solberg Nes et al., 2016).

In sum, MR-haplotype 2 carriers – especially homozygotes – appear to be less sensitive to interpersonal emotional cues, show in general a more favorable behavioral response after and under stress[13], and are more likely to expect positive outcomes. Hence, the genetic make-up of MR-haplotype 2 carriers may be beneficial especially when they encounter challenges.

***MR-haplotype 1/3: sensitivity may also be adaptive***

The term distress stands for a range of negative emotional states and is comprised of a plethora of cognitive, biological and social processes establishing an interaction between a person and his/ her environment, with a negative impact on the individual (Matthews et al., 2003; Matthews, 2016). MR-haplotype 1/3 carriers may be more prone to distress as they showed increased subliminal negative affect in two consecutive studies with newly-recruited healthy volunteers (chapter 4, 5). Higher rsEEG TBR and theta power are in line with the distress-proneness hypothesis in MR-haplotype 1/3 carriers (chapter 7). In general, this may contribute to increased odds on developing stress-related psychopathology and depression in MR-haplotype 1/3 carriers (Klok et al., 2011; Vinkers et al., 2015; Endedijk et al., 2020).

Hence, MR-haplotype 1/3 carriers may be more sensitive to stress and negative mood. This increased sensitivity may also apply to interventions targeting their susceptibility[14]. Carriers of MR-gene variant rs 5522 G showed more effective reward learning in a no stress condition (Bogdan et al., 2010) and were more sensitive to interpersonal cues as they reacted quicker to facial expressions of happiness (chapter 5). At a neural level, MR-haplotype 1/3 carriers showed increased theta power (chapter 7). Increased theta power has not only been associated with trait anxiety, but also with behavioral adaptation (Cavanagh & Shackman, 2015).

Furthermore, in our RCT confounded by OC effects (chapter 2) exploratory analyses in the NC group revealed that a single dose of fludrocortisone (0.5 mg) was associated with better and quicker recognition of facial expressions of fear and happiness and an improved recall of positive characteristics, but only in carriers of MR-haplotype 1/3 (Hamstra, 2013). Similar effects were observed in a placebo controlled double-blind study in healthy female volunteers: a single dose of citalopram improved the recognition of fear, happiness (Harmer et al., 2003) and the recall of positive characteristics (Harmer et al., 2011; Harmer et al., 2004). Equivalent patterns were also observed in MR-genotyped patients suffering from cardiac failure receiving spironolactone treatment. Carriers of the MR-gene variant rs2070951 G[15] displayed such a high response to standardized spironolactone treatment that they were at increased risk for pathological K+ levels, suggesting that MR-genotyping (SNP rs2070951 (C/G) may be useful in predicting

[13] … and physiological response (see chapter 1)

[14] See also 'One pill may not fit all' under 8.4.4.

[15] The single SNP shows lower MR gene expression in its G allele and consistently higher circulating aldosterone levels and higher systolic blood pressure (van Leeuwen et al., 2010) suggestive of a less efficient, maladaptive stress response.

the potassium response to spironolactone. According to Cavallari and co-workers (2015) it is possible that the MR-genotype may also influence cardiac response to spironolactone. Yet, it may be postulated that these effects might reflect an (over)adaptive mechanism by which MR-haplotype 1/3 carriers compensate for their susceptibility[16].

These observations might also explain why these common genetic variants have not been eliminated from the genetic pool through general selection (Homberg & Lesch, 2011). Further insights in these adaptive mechanisms in distress-prone, but healthy populations may be of help in the prevention and treatment of stress-related psychopathology.

***MR-haplotype 2 heterozygotes***

MR-haplotype 2 heterozygotes may have the best of both worlds, as they carry one pair of alleles representing the sensitivity of MR-haplotype 1/3 and another pair representing the stability and resilience of MR-haplotype 2 homozygotes. From an evolutionary point of view, this might provide an explanation in the overrepresentation of MR-haplotype 2 heterozygotes (Hamstra, 2013; chapter 3, 4, 5, 6, 7).

***No differences on attentional and memory performance among MR-haplotypes***

In this project, we observed no differences among MR-haplotypes on attentional and memory performance (chapter 2, 3, 4 and 5). Whereas many studies reported cognitive effects associated with MR functioning (see for a review on this topic Wingenfeld & Otte, 2018), others failed to report differences among MR-haplotypes in attention and memory (Vogel et al., 2014; Keller et al., 2016). However, variation in other SNPs in the MR gene NR3C2 (not studied in this PhD project) predicted verbal memory performance (i.e *rs5525, rs4835488, rs10213471, and rs17484245*) and the *rs5534* SNP was associated with a negative memory bias after childhood trauma (Vogel et al., 2014). The MR-haplotype in this PhD project is based on different SNPs *rs2070951* and *rs5522*.

***Clinical implications***

Apparently, the MR-haplotype is associated with neural states related to attentional control and appraisal of stressors, traits like stress immunity, interpersonal sensitivity and optimism, and behaviors like risky decision-making. This is in line with other research revealing that the function of the MR is important for the sensitivity or threshold of the stress response and the subsequent choice of coping style. Insights in such resilience or susceptibility to stress might be of clinical relevance not only in pharmacotherapy, but also in the psychological treatment of mood disorders. From a clinical

[16] A comparable line of reasoning was followed with regard to observations on increased social conformity in carriers of the distress-prone short allele of the serotonin transporter gene (5-HTTPLR) (e.g. Antypa, 2011; Homberg, 2012; Homberg & Lesch, 2011).

perspective, whereas MR-haplotype 2 carriers may be less sensitive to stress-related psychopathology, affected MR-haplotype 1/3 carriers may be more likely to benefit from psychological treatment as they showed more effective reward learning and heightened interpersonal sensitivity.

## BOX-2. Summary Main Effects of MR-gene Variation

- The MR-gene is associated with neural cognitive control over the affective system, and with traits like optimism and risky decision-making.
- We observed a linear relationship between MR-haplotype 2 alleles and dispositional optimism.
- The genetic make-up of MR-haplotype 2 carriers may be beneficial especially when they encounter challenges.
- Healthy MR-haplotype 1/3 carriers seemed not only more susceptible to distress, but may also be more responsive to interventions.
- Although MR-haplotype 2 homo- and heterozygotes share one MR-haplotype 2 allele, important differences between both genotypes may exist.
- MR-haplotypes did not differ in attentional and memory performance.

## 8.4 Main outcomes of the interaction between MR-gene variation and the female hormonal status

In general, the subsamples did not differ significantly in mental and physical health, personality traits and affective state.

### 8.4.1 Conclusion behavioral lab studies (chapter 2, 3, 4 and 5)

MR-haplotype may influence the impact of the female hormonal status on implicit affect and facial expression recognition.

***Implicit affect***

In chapter 5 we observed an interaction effect between MR-haplotype and hormonal status, but only in the first of two sessions. In the novel situation, MR-haplotype 2 homozygotes had lower scores on implicit anxiety when their levels of progesterone were higher. This may reflect reduced subliminal anxiety because self-reported negative affect did not differ between groups.

***Attentional blink***

Our hypothesis that the MR-haplotype would influence the impact of the female hormonal status on attentional blink performance on lag 2 trials was rejected.

***Facial expression recognition task***

In chapter 3 OC-users with MR-haplotype 1/3 recognized not only more facial expressions of fear and sadness, but also at a lower intensity and with slower reaction times, reflecting an increased sensitivity to negative stimuli. Although these effects were not exactly replicated in our studies, consistent patterns were observed. In chapter 4 MR-haplotype 1/3 carriers, compared with MR-haplotype 2 homo- and heterozygotes, were more sensitive to the influence of the female hormonal status on emotion recognition. In the next project – a within-subject study – this effect became more specific: in the second session, so after habituation to the experimental setting, estradiol (E2) levels were positively correlated with higher happiness recognition scores, but only in MR-haplotype 1/3 carriers (chapter 5).

***Word categorization and memory task***

In chapter 3 OC-users with MR-haplotype 1/3 recalled more negative characteristics, showing a negative memory bias consistent with their performance on the facial expression recognition task. It seems unlikely that the negative memory bias observed in OC-users with MR-haplotype 1/3 is due to a selection bias, as both experimenter and participants were blind to their MR-haplotype. However, this effect was not replicated in our within-subject study, possibly due to learning effects (chapter 5).

***Decision-making task***

No interaction effects were observed, only main effects on female hormonal status (see 8.2) and MR-haplotype (see 8.3).

### 8.4.2 Conclusion longitudinal study (chapter 6)

MR-haplotypes did not influence the impact of the female hormonal status on self-reported symptoms of depression. Although we observed moderating effects of the MR-haplotype on the influence of the female hormonal status on mood in our longitudinal study (chapter 6), effects were no longer significant after correction for multiple comparisons. We applied a stringent correction in order to avoid over-interpretation of possibly spurious findings, as we lost many cases due to menstrual cycle irregularities, resulting in small samples.

### 8.4.3 Conclusion resting state EEG study (chapter 7)

MR-haplotype did not moderate the influence of the female hormonal status on neural marks of attentional control.

### 8.4.4 Discussion: MR-haplotype moderates the influence of the female hormonal status on important aspects of emotion processing

Our research was among the first to show that the genetic makeup of healthy women may play a role in the influence of the female hormonal status on emotional information processing (Hamstra et al., 2015; Sundström-Poromaa, 2018; Wingenfeld & Otte, 2019). We observed moderating effects of the MR-haplotype on the influence of the female hormonal status on (implicit) mood and the recognition of facial expressions of emotions (chapter 3, 4 and 5). Remarkably, this moderating effect was only observed in tasks in which subtle cues related to the early processing of emotions were assessed.

***One pill may not fit all***

OC-users with MR-haplotype 1/3 showed biases in perception and emotional memory (chapter 3) which have been observed earlier in (formerly) depressed and dysphoric patients and their offspring (Joormann & Gotlib, 2007; Fritzsche et al., 2010). Our next study revealed that emotion recognition performance was influenced by the female hormonal status only in MR-haplotype 1/3 carriers: OC-users recognized significantly fewer facial expressions of emotions than NC women. This effect became pronounced when we compared OC-users with women in the mid-luteal phase of the menstrual cycle, thus when levels on natural hormones peak (chapter 4).

Importantly, these effects were not observed in OC-users with MR-haplotype 2 (homo- and heterozygotes). The question remains if this effect is induced by the synthetic hormones in

OC or the suppressive effect of OC on natural hormones. Our observations with regard to a possible anti-depressant effect of estrogen in MR-haplotype 1/3 carriers (see below) point into the direction of the latter.

***Estrogen***[17]

Estrogens were shown to downregulate the transcription of MRs in the rat hippocampus (Carey et al. 1995), in line with this we hypothesized that the MR-gene might moderate the impact of estrogen on emotional information processing. We observed that only in MR-haplotype 1/3 carriers increased levels of estrogen were associated with improved happiness recognition (chapter 5). This suggests an antidepressant effect of estrogens as the administration of antidepressants – compared with placebo – resulted in comparable positive biases on the same task in healthy volunteers (Warren et al., 2019; Harmer et al., 2009). This antidepressant effect of estrogen was also observed in two double blind RCTs in healthy euthymic volunteers (Frokyaer, 2015; Macoveanu et al., 2016).

Although we did not measure levels of estradiol in chapters 3 and 4, the observed depressogenic effects in OC women carrying MR-haplotype 1/3 are in line, as OC suppress natural estrogen. So, we were the first who found indications that MR haplotype 2 carriers (homo- and heterozygotes) may be protected against the depressogenic effects of oral contraceptives (Hamstra et al., 2015; Wingenfeld & Otte, 2018).



***Progesterone***

Progesterone[18] exhibits almost the same affinity for the MR as aldosterone and cortisol (Quinkler et al., 2002). Consequently, MR functioning may be affected by progesterone as a competitive antagonist of these steroids at the receptor level (Quinkler & Diederich, 2002). In chapter 5 was reported that the MR-gene might moderate the influence of progesterone on implicit anxiety in novel – emotional – situations: only in MR-haplotype 2 homozygotes levels of progesterone (P4) were negatively correlated with anxiety. Consistently, studies have shown that the MR in the limbic brain is linked with choice of coping with fear and anxiety (see a.o. Rozeboom et al., 2007; Vogel et al., 2016). Sex-dependent effects are observed in an experiment investigating behavioral flexibility in a fear conditioning paradigm (Ter Horst, 2012). In line, behavioral adaptation to novelty depended on the cycle phase of female mice (Ter Horst et al., 2013).

[17] Our findings on MR-haplotype moderated effects of estrogen and progesterone should be considered with caution as they are based on findings in a study in which we observed time and order (interaction) effects, which could not be explained (chapter 5; see for methodological considerations chapter 8.5.3).

[18] We investigated women using OC containing levonorgestrel (LNG). LNG exerts a stronger effect on the progesterone receptor than progesterone. In contrast with progesterone, LNG has no anti-mineralocorticoid effect (Stanczyk, 2003; Mitchell & Welling, 2020).

Human studies revealed that an increase in progesterone may be an adequate endocrinological response to social stressors (Wirth, 2011). Women with lower social anxiety scores displayed an increase in progesterone after social exclusion, whereas socially anxious women showed a drop (Maner et al., 2010). So, progesterone may be of help if individuals seek social contact with others to reduce the negative effects of stress and manage feelings of sadness and anxiety (Schultheiss et al., 2003; Wirth & Schultheiss, 2006; Maner & Miller, 2014; Mitchell & Welling, 2020).

***Genomic versus nongenomic effects***

It is difficult to disentangle whether we observed either genomic or nongenomic effects in our experiments[19]. Genomic MR signaling is difficult to detect because even with low basal circulating cortisol, this receptor is largely occupied even under rest as a result of its high affinity for cortisol (Lu et al., 2006; De Kloet et al., 2018). Blockade of the MR caused increased basal and stress-induced cortisol levels, for instance. In addition to research reporting that nuclear GR and MR mediate slow genomic effects of cortisol, animal and human studies revealed rapid non-genomic effects (Losel & Wehling, 2008; Karst et al. 2005, 2010). The non-genomic MR-actions are implicated in the rapid appraisal and selection of coping responses, and have also been observed in the amygdala (Karst et al., 2010). The high affinity of the MR allows sensing the rising corticosterone levels in response to a stressor. As a result, the MR is also involved in behavioral reactivity to latent stressful situations (Holsboer, 2000; De Kloet, 2016).

***Further clinical implications***

While it is commonly accepted that progesterone is the symptom-provoking hormone in premenstrual dysphoric disorder, research has shown that estrogen plays an important role as well (Frokyaer, 2015; Macoveanu et al., 2016; Sundström-Poromaa, 2018). Besides, – as estrogen upregulates progesterone receptors – an increased availability of estrogen results in more progesterone receptors and enhanced progesterone reactivity subsequently (Schmidt et al., 1998; Boron & Boulpaep, 2012; Sundström-Poromaa, 2018).

A cohort study following women across natural pregnancy and postpartum revealed that women with an increased sensitivity to shifts in estrogen levels were more likely to develop a post-partum depression, while plasma estrogen levels did not differ across groups (Mehta et al., 2014, 2019). Hence, our outcomes suggest that female MR-haplotype 1/3 carriers are more likely to experience (sub)clinical symptoms of depression when their estrogen levels are low, which might increase their odds on developing reproductive mood disorders like premenstrual

[19] 'Genomic' mechanisms affect gene transcription, physiological responses occur with a delay of at least 15 min (Haller et al., 2008). 'Nongenomic' mechanisms are not sensitive to inhibitors of gene transcription or translation and are observed within a rapid time frame (see for a review on this topic Groeneweg et al., 2012).

dysphoric disorder and postpartum depression. In line with this, Klok and co-workers (2011) observed that MR-haplotype 2 carriers were less susceptible to depression during their fertile years, which might reflect the finding that MR-haplotype 2 carriers are not as sensitive to shifts in estrogen levels as MR-haplotype 1/3 carriers.

## BOX-3. Summary Influence of MR-Gene Variation on the Impact of the Female Hormonal Status

- MR-haplotype 2 carriers may be protected against the depressogenic effects of oral contraceptives.
- MR-haplotype 1/3 carriers recognized more expressions of happiness when their levels on estrogen were high, suggestive of a MR-haplotype dependent anti-depressant effect of estrogen.
- Higher levels of progesterone may be associated with lower anxiety in a novel situation, but only in MR-haplotype 2 homozygotes.
- The MR-gene may moderate the influence of the female hormonal status on cognitive-emotional processes associated with depression. Fundamental insights underlying this hypothesis should be obtained by studies designed to examine the action mechanism of estrogens and progesterone and their synthetic analogs on the function of the brain MR.

## 8.5 Methodological considerations and suggestions

This PhD project had its limitations. Firstly, the careful selection and screening of our participants resulted in a sample with limited generalizability. Secondly, we investigated the moderating role of MR-gene variation, using haplotypes constructed from two functional SNP's, resulting in limited – though promising – insights in how the MR-gene may influence the impact of the female hormonal status on cognitive processes related to depression. Hereafter some methodological considerations will be discussed concerning this research topic.

### 8.5.1 The Hawthorne effect in OC research

The term Hawthorne effect was conceived in 1958 by H.A. Landsberger when he was analyzing earlier experiments from 1924 to 1932 at the Hawthorne Works. The workers' productivity seemed to improve with higher levels of light at the factory, being the aim of the study, but collapsed as soon as the observations ended, suggestive of a motivational effect of the study itself on the performance of the workers (Adair, 1984). Although the interpretation by Landsberger has been criticized for being distorted by its historical context and some methodological aspects (Wickstrom & Bendix, 2000), the Hawthorne effect may influence the practical value of clinical research importantly. A systemic review confirmed that consequences of research participation for behaviors being investigated do exist; not only if participants are directly observed, but also if they are otherwise made aware of being studied (McCambridge et al., 2014).

We recruited the participants for our experimental studies by communicating that we were investigating the influence of stress vulnerability and the female hormonal status on emotional processing. It is certainly possible that the mere fact of being a participant in research may have had consequences for our participants' behaviors (in the broadest sense). Another difficulty with this design is that OC-users and NC women are self-selected populations who may change their contraceptive status frequently (Warren et al., 2014). We did control carefully for the latter potential confound, however. One of the strengths of our studies was that neither the participants, nor the researchers knew the participants' MR-haplotype as we genotyped later. Furthermore, in our longitudinal study, which appeared to be the most vulnerable to the Hawthorne effect, we managed to mask the research topic successfully (chapter 6). Concealing the research topic has only been done in very few of these studies (Warren et al., 2014).

### 8.5.2 The survivor effect in OC research

The survivor effect encompasses the following[20]. Approximately 25% of all novel OC-users experience adverse effects and depressed mood is actually one of the most reported adverse effects

[20] See for an extensive review on this phenomenon Oinonen & Mazmanian, 2002.

(Kay 1984). These users will often discontinue OC-use, and usually within one year (Trussell & Kost, 1987). Consequently, a group of OC-users who have not experienced these negative effects remains, and women who experienced negative effects are more likely becoming part of the NC group (Kutner & Brown, 1972). The 'survivor effect' may therefore result in an OC group which is less sensitive to depression, rather than being 'protected' against depression or mood swings by OC-use. The survivor effect may account for some of the variability in our studies. Although we did not distinguish between first-time and brand-switchers in the OC group and did not screen for history of OC-use in the NC group – the latter due to feasibility reasons –, we included only women using monophasic second-generation OC for more than three months and controlled for duration of OC use (see chapters 3 to 7). Furthermore, consistent with the recommendations of Oinonen and Mazmanian (2002), we controlled for factors predisposing certain women to OC-related negative mood-effects. We measured explicit and implicit positive and negative affect in OC users and NC women at the beginning of each assessment. We also screened for a history of mood disorders, dysmenorrhea, premenstrual mood symptoms and (history of) pregnancy. Additionally, we controlled for committed relationship status as this may protect against depression. Last but not least, even in spite of the possible presence of a survivor effect in our samples, we were able to detect subtle and consistent differences in the mood-effects of OC among carriers of different MR-haplotypes.

In sum, we compared self-reported symptoms on reproductive mood in a relatively small sample of 'surviving' OC-users with NC women in a longitudinal study, and obtained insights in the potentially mood-stabilizing effects of OC. These effects of OC-use may be perceived not only as stabilizing thus beneficial, but also as blunting (chapter 6). In contrast with Oinonen & Mazmanian (2002), we observed increased irritability during menses, not only in NC women but also in OC-users. In order to optimally manage the surviving effect, future studies should not only investigate new users of OC in larger cohorts. Women who use OC to their full satisfaction should also be included, and women using other generations of OC. By including the last group, we will gain more insight into a group of OC users, who are probably more prone to experiencing negative mood effects of OC[21].



### 8.5.3 Concerns about replicability in psychological studies also affect OC research

***Did we contribute to the replicability crisis in OC research?***

The smaller the sample sizes and effect sizes of a study conducted in a scientific field, the less likely the outcomes are valid and replicable (Ioannidis, 2005). Indeed, high-quality research examining the impact of OC on emotional information processing is scant, with inconsistent study designs

[21] With regard to the Dutch situation as second-generation monophasic OC are the first choice (Kurver et al., 2012).

assessing various cognitive testing batteries on a large variety on OC-users (Warren et al., 2014). Furthermore, the greater the variety in designs, definitions, results and statistical analyses, the less likely the research findings can be replicated (Ioannidis, 2005). Especially the limited sample size in this area of research is of concern, resulting in limited statistical power, in particular in studies with a cross-sectional design (Warren et al., 2014; Sundström-Poromaa, 2018). In retrospect, the studies of this PhD project may have contributed to 'the reproducibility crisis' as well, with relatively small sample and effect sizes. Furthermore, we not always applied corrections for multiple comparisons and unexpectedly encountered important learning effects, resulting in unexplainable time and order (interaction) effects (chapter 5). However, we observed a rather consistent pattern in our consecutive studies and avoided over-interpretation as much as possible.

***Or not (1), as we controlled for many confounds***

Starting from a coincidental finding we undertook a series of studies in which we tried to control for confounds following insights obtained in the previous study[22]. Furthermore, we used validated cognitive tasks which had shown to be sensitive enough to detect subtle differences in depression status, depression vulnerability or to antidepressant treatment. We also controlled for age, education, circadian phase and physical or psychiatric comorbidities[23], all of which may affect cognitive performance. Although assessors were not blind to the participants' OC status, the likelihood of investigator bias significantly influencing performance-based cognitive test results is not high (Warren et al., 2014).

***Or not (2), as we controlled rigorously for the female hormonal status***

In our consecutive studies we applied an increasingly strict screening for menstrual cycle phase and types of OC used. For instance, we started in our first study with the registration of OC use. In our second project (chapter 3) we assessed type of OC (chapter 4). Whereas we controlled in our last projects (chapter 5, 6 and 7) not only for menstrual cycle phase with confirmation of the third cycle onset, but also for levels of estradiol and progesterone in saliva. With regard to types of OC used, we included in the chapters 4, 5, 6 and 7 only second-generation OC containing ethynyl estradiol and levonorgestrel, which has low binding affinity with MR, while progesterone has a high binding affinity (Krattenmacher, 2000).

***In sum***

We observed that future studies examining the influence of the menstrual cycle should include

[22] After completion of our studies we discovered that this approach should not be necessary, as effects should be large enough to remain, even in studies including a large variety in participants (see f.i. Goodstein, 2010; Harris, 2017).

[23] In the first studies (chapter 2, 3 and 4) we relied on self-reports, whereas in later projects (chapter 5, 6 and 7) validated screening methods were applied.

OC-users as well[24]. Inclusion of OC-users may contribute to more explained variance, making the effects of the female hormonal status in multivariate factorial statistical models more pronounced. Furthermore, studies examining the influence of the menstrual cycle phase should take care to verify cycle-phase by confirmation of the next cycle onset.

### 8.5.4 Limitations of the assessed experimental tasks

The information processing approach provides an empirical methodology to investigate emotional cognitive processes and offers opportunities to assess mediating and moderating cognitive variables, without relying exclusively on self-reports. Another advantage of this approach is that the results of several paradigms can be presented and interpreted within a coherent and meaningful context (Ingram, 1986). Nevertheless, we have some considerations with regard to the selected paradigms and interventions. Firstly, we did not select one ecologically valid model of emotional information processing at the beginning of this research project. As a result, we covered only a few stages and aspects of emotional information processing with our test battery. Furthermore, we did not assess the influence of hormonal intrauterine devices – as these are reported to exert a systemic effect as well (see f.i. Aleknaviciute et al., 2017).

Furthermore, we observed some task-related caveats. We observed a ceiling effect in accuracy in emotional working memory performance, so this version may have been too easy to detect subtle effects (Warren, 2014). Furthermore, some negative IAPS pictures may have blunted the memory performance – as some of the pictures were considered too aversive (chapters 4 and 5) – therein overriding subtler interpersonal differences. Assessment of reaction times could have been a subtle behavioral measure of working memory performance instead. Finally, although the reading the mind in the eye task (RMET) has been assessed effectively in many paradigms, the RMET has also been reviewed critically as some items can be interpreted ambiguously (Wilhelm et al., 2014).

### 8.5.5 Methodological aspects genotyping

***Limitations MR-haplotype approach***

Single nucleotide polymorphisms (SNPs) and haplotypes are categorical variables as they represent pairs of nucleotides. Therefore, SNPs and haplotypes are not suited for multivariate statistics which are designed for quantitative analyses. In a way we solved this by dichotomizing and later counting the amount of MR-haplotype 2 alleles, although the variability in the MR-haplotype 1/3 alleles remained ignored[25]. Furthermore, there are many genetic contributions

[24] Considering intake-regimen and compounds.

[25] Increasing odds for MR-haplotype 1/3 on not being in HWE

to stress susceptibility, making high dimensional - low sample size data such as genetics and genomics more sensitive to spurious effects (Beaton, 2017). Our results may have been more convincing if we reported outcomes in self-collected data and in an external dataset for validation. Another – though costlier – approach is applied by Vogel et al. (2014), who firstly performed a gene-wide statistical analysis, considering all detected variation in NR3C2 concurrently, followed by post-hoc SNP and haplotype analyses.

***Alternative SNP***

The functionality of the MR is influenced by common genetic variants (see chapter 1, figure 4). In this PhD project we focused on two SNPs (rs5522, rs2070951), which are located in the promoter region of the receptor. The two SNP's lead to altered transcription of the MR gene. The three haplotypes constructed from these SNP's – the 4$^{th}$ haplotype has not been detected – also showed profound function changes in receptor regulation. Another investigated individual SNP is rs5534 G/A (minor allele frequency: 44.2%), which may be associated with negative memory bias in the self-referent encoding/evaluation task. This SNP is located in the 3' untranslated region of the MR gene which appeared to be responsive in two cell lines to microRNA383 repression (Vogel et al., 2014; De Kloet et al., 2016).

***Contemporary genomics***

Multiple pathways lead to stress-susceptibility and depression, and proteins from multiple genes are likely to interact in susceptibility to the stress-prone phenotype (Charney & Manji, 2004; Raymer et al., 2005). Hence, future studies should make a shift toward 'contemporary genomics', which include the association of genes with stress related psychopathology and their interactions, genome-wide analyses and epigenetic individual variations, if we aim to establish the role of gene markers as the MR-haplotype in models examining the relation between the female hormonal status and mood disorders (Opmeer, 2013).

***Conclusion***

We have investigated small samples for a genetic study. The size of the MR-haplotype groups differed importantly also, as we did not a-priori select on MR-haplotype. In particular, we had small subsamples with MR-haplotype 2 homozygotes due to the relatively infrequent MR SNP rs5522 (minor allele frequency 11.8%) (De Kloet et al., 2016). However, SNPs rs5522 and rs2070951 have been investigated separately and with a haplotype approach earlier in many studies and different paradigms, revealing results which were mostly in line with our observations.

### 8.5.6 Summary of strengths and weaknesses of this PhD project

## BOX-4. Summary of Strengths of this PhD Project

- Studies were hypothesis driven[26].
- Project concerns a topic with substantial societal and clinical relevance.
- This was the first pharmacogenetic research project investigating mood-related side effects OC.
- A new topic involving the mineralocorticoid receptor and sex steroids interaction was investigated.
- Investigated OC were controlled for compounds, intake regimen, duration use.
- Focus on second generation OC containing levonorgestrel, which are the most widely used in the Netherlands and are first choice according to the Contraception Guidelines of The Dutch College of General Practitioners[27].
- Meticulous handling menstrual cycle phase assessment including saliva E2 and P4 determinations[28].
- OC-users and NC women were all college students. They were also carefully screened on mental and physical health and NC women were screened for PMDD with validated questionnaire, resulting in homogeneous subsamples.
- Participants were recruited based on self-reported Northwestern European ancestry.
- We applied computerized tests that had previously shown to be sensitive to depression status (nondepressed, remitted, depressed) or sensitive to neurotransmitter manipulations (e.g., sensitive to tryptophan loading or depletion or to a single dose of antidepressants in healthy volunteers).
- Tests were covering different aspects of emotional information processing.

## BOX-5. Summary of Weaknesses of this PhD Project

[26] Except chapter 7.

[27] https://www.nhg.org/standaarden/samenvatting/anticonceptie.

[28] Especially in chapters 5, 6 and 7.

- We assessed healthy women who were college students of Northwestern European ancestry. Hence, our study population is not representative for all Dutch OC-users.
- We did not assess behavior around ovulation.
- We did not control for PMS or PMDD by daily diary method, which is considered the golden standard.
- We investigated OC-users taking OC > 3 months, while most side-effects are observed within the first months following initiation of OC-use.
- We did not control for previous OC-use in NC participants.
- We did not assess testosterone levels and the stress hormone cortisol.
- Hormonal levels in saliva were measured with luminescence assays[29].
- In our consecutive studies there was an inconsistence in statistics (i.e. differences in p-value thresholds, corrections for multiple comparisons and follow-up on interaction effects).
- Our results may have been more convincing if we reported outcomes in self-collected data and in an external dataset for validation.
- We relied on self-reported Northwestern European ancestry and did not apply ancestry informative principal components.
- We focused solely on one – although evidence based – MR-haplotype and ignored the variability in the MR-haplotype 1/3 alleles.
- We investigated small samples for a genetic study. We did not calculate if the samples were in Hardy-Weinberg Equilibrium and Linkage Equilibrium.
- Computerized psychological test battery was not complemented with neuroscience methods (ERPs, fMRI, rTMS, DTI, rsfMRI and PET), resulting in less sensitive measures.

[29] Although luminescence assays are valid and widely used in studies on naturally cycling women, the present gold standard for E2 analysis is mass spectrometry, which gives reliable results even when E2 levels are extremely low (e.g., as in postmenopausal women) (Sommer et al., 2018). Thus, luminescence assay outcomes are less reliable than analyses performed with mass spectrometry.

## 8.6 Final conclusions

This PhD project started with my own curiosity in interpersonal differences in the psychological effects of OC. It revealed that the female hormonal status – including OC-use – and stress vulnerability – as defined by the MR-gene variation – have practical implications in experimental psychological researchg. Furthermore, incorporation of these variables in models of emotional information processing may be of help in understanding and treating mood disorders in women. Of course, the information processing approach is abstract in nature and cannot directly be applied in the clinical field. The insights in this PhD project can be applied in both experimental and clinical studies, however. For instance, longitudinal studies investigating mood in pre-menopausal women should control for the female hormonal status. Furthermore, the female hormonal status and stress vulnerability are clinically relevant variables, which are rooted in real-world adaptive and mal-adaptive functioning.

Our findings regarding the MR-haplotypes are generally consistent with previous observations (Klok et al, 2011; Vinkers et al., 2015). MR-haplotype 2 carriers are the more optimistic individuals, also in 'unstressed' conditions. Healthy MR-haplotype 1/3 carriers may be more prone to distress, but might also be more likely to respond to interventions aiming at their susceptibility. These effects were subtle, sometimes only detectable with implicit measures, but even small biases in a positive or negative direction may affect information processing and may contribute to the development of negative mood states, eventually depression or other stress-related psychopathology. These associations should be interpreted with caution however, as genes code for proteins and not for traits or behavior.

Following our observations, it may be hypothesized that endogenous estrogen and progesterone as well as oral contraceptives use are factors that contribute to the effects of MR-gene variation. Namely, MR-haplotype 1/3 carriers seemed more sensitive to mood-effects of their menstrual cycle phase and depressogenic side-effects of OC. Fundamental insights with regard to this hypothesis could be obtained by studies in vivo and in vitro examining the reactivity of MR-haplotype carriers to the synthetic hormones in second generation OC – ethynyl estradiol and levonorgestrel – in relation to the concentration of endogenous female hormones. In addition, it should be noted that the influence of the female hormonal status on the neuroendocrinological mechanism of stress coping is currently under-investigated and urgently needs further research.

The idea of a 'resilience or vulnerability gene' has fascinated many scientists. Genotyping may be a clinically helpful and economically promising instrument in 'social engineering' by selecting the more vulnerable to psychiatric disease and developing personalized pharmacological treatments (Mukherjee, 2016). However, stress-related psychopathology is very heterogeneous by nature and proteins from multiple genes are likely to interact in the stress-susceptibility phenotype. Last but not least, we should not ignore that the increased vulnerability of women

to mood disorders is the result of a plethora of biological, psychological and sociological factors.

Finally, one may wonder what my own opinion is about OC after completing this PhD project. My family background may be exemplary for the impact of OC on the position of women all over the world. My grandmother raised eight children and could not pursue a personal career. My mother[30] decided to take the pill as soon as it became available in the Netherlands and – like so many women at that time – took some side-effects for granted (Van den Broeck, 2014). The pill enabled her to complete her education, to give birth to her first child (me) at the age of 30, to pursue her career and – as a result – to become and stay financially independent. Furthermore, the introduction of OC did not only change the role of women in the economy, but also contributed to positive health outcomes for women, such as reduced rates of maternal death and fewer cases of endometrial and cervical cancer (Welling, 2013). Compared with the physical effects of OC, which have been reported extensively although not without controversy (Liao & Dollin, 2012), the psychological and behavioral effects of OC have only become recently topic of scientific interest (see f.i. Pletzer & Kerschbaum, 2014; Montoya & Bos, 2017; Mitchell & Welling, 2020). Fully justified, much media attention has been paid to these psychological side-effects. As a result, women apply more 'natural' ways of birth control which are often not as reliable as hormonal contraceptives, increasing odds on unintended pregnancies. Hence, more attention should be paid to the psychological side-effects of synthetic hormones during birth-control consultation. According to the recently published revised guidelines (NHP; 2021), the general practitioner should ask for a history of depression before subscribing OC[31]. According to our observations, the GP should also screen for a history of depressive symptoms following OC-use, not only in the client, but also in her (grand)mother.

The lower affect variability and reduced sensitivity to interpersonal emotional cues that we observed in OC-users may be experienced as either a stabilizing or a blunting effect of OC, perhaps depending on the individual's appraisal. The lower depression scores of OC-users in the longitudinal study suggests a protective effect of monophasic OC on symptoms of reproductive depression. Future studies should investigate (former) OC-users in larger cohorts including novel users, satisfied users and 'brand-switchers' in order to control for the survivor effect.

[30] This may erroneously suggest that only women are responsible for birth control

[31] Richtlijnen.nhg.org/standaarden/anticonceptie#volledige-tekst-anamnese.