Mood and the pill

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Content

Chapter 1

General introduction

"Depression is the aloneness within us made manifest.
It destroys not only the connection to others,
but also the ability to be peacefully alone with oneself
(Solomon, 2001)"

1.1 Depression in women

Depression is the leading cause of disability worldwide, as in 2017 more than 264 million people of all ages suffered from depression (James et al., 2018). One of the main symptoms of depression is to see no hope and no future, it may be perceived as if you are locked in a dark tunnel which is blocked at both ends (Haig, 2015). Depression is characterized by feelings of persistent sadness and detachment, as if you are living under a bell jar that prevents you from connecting with others (Plath, 1963). Depressed people lose their interest in activities which were enjoyable earlier and report disturbed patterns on sleep and appetite (e.g. Solomon, 2001; Haig, 2015; Wax, 2015). Depression also affects one's cognitive abilities, it induces concentration difficulties and hampers cognitive performance. As described by R. Wax (2013): "If it was an acute episode, I wouldn't be able to type these words; I wouldn't be able to connect one thought with the next or even comprehend the meaning of words". Finally, depression induces cognitive biases, it makes you think things that are wrong (Solomon, 2001; Haig, 2015). Each depressive episode may be experienced and expressed differently, not only between but also within persons (Kirmayer et al., 2001; Milak et al., 2005). Nevertheless, specialists search for insights in factors that contribute to the onset, maintenance and recurrence of depressive episodes.

One fundamental question is why more women are affected by depression than men. This phenomenon may be partially attributable to socio-economic and cultural differences. The world is still dominated by men, and women are more likely to remain poor and to become a victim of violence (Nolen-Hoeksema, 1995; Jack, 2019). Biological differences between women and men may also play a role[1]. For instance, women have the same risk of depression as men before puberty and after menopause, but during their fertile years the female-to-male prevalence ratio for first-episode and recurrent depression remains nearly constant at 2:1 (Weisman & Klerman, 1977; Kornstein & Clayton, 2002). This observation has been replicated in many countries and cultures (Kornstein & Clayton, 2002; Fauser et al., 2013).

In general, the symptom profile during depressive episodes is comparable between men and women. However, symptoms of 'atypical' depression – characterized by increased appetite, weight and sleep – are 1.5 times more often observed in women than in men, and those of seasonal affective disorder even three times more. Comorbidity in depression is 2-3 times more common in women than in men: depressive women suffer more often from various anxiety disorders, eating disorders and medically unexplained symptoms. Substance abuse is seen more often in men suffering from depression, however (Nolen-Hoeksema, 1995; Marcus et al., 2008). The heritability of depression vulnerability is slightly higher in women than men (approximately 40% vs. 31% Kendler 2008). Sex-dependent genetic patterns exist, suggesting that different genes

[1] Although we should keep in mind that "it is dangerous to imply through the choice of the label that an aspect of women's reproductive biology is central to psychiatric illness" (S. Nolen-Hoeksema).

contribute to the risk for major depression in men and women (Wray et al., 2018). Furthermore, in women this inheritable susceptibility may be influenced by hormonal shifts occurring in the menstrual cycle, during and after pregnancy (Kornstein & Clayton, 2002; Payne et al., 2009; Harald & Gordon, 2012; Stahl, 2013).

1.2 The female hormonal status and its implications for the onset and course of mood disorders

Some specific forms of female depression are related to reproductive events, suggestive of a biological basis. Postpartum depression, premenstrual depression and peri-menopausal depression are all associated with important shifts in female hormones. These hormonal shifts contribute to physical and psychological symptoms. The impact of these shifts may vary importantly among persons. Previous studies revealed that sensitivity to alterations in the female hormonal status may be linked to vulnerability to depression. Women suffering from premenstrual syndrome and menstrual mood swings were more likely to develop depressive episodes. Increased affect lability during the menstrual cycle and hormonal contraceptives use may also predict depressive episodes post-partum and in the peri-menopause (Kornstein & Clayton, 2002; Payne et al., 2009; Harald & Gordon, 2012). Moreover, oral contraceptives pills (OC) contain synthetic analogs of estrogens and progestins which also may cause changes in mood and affect (Oinonen & Mazmanian, 2002; Sundström-Poromaa & Segebladh, 2012; Stahl, 2013; Teatero et al., 2014).

Clinical populations

Women respond better to SSRIs (60% positive response) than to TCAs (40 % positive response), while in men the opposite has been observed (Fauser et al., 2013). Interestingly, women respond better to TCAs after they have entered menopause (Stahl, 2013; Fauser et al., 2013). Fifty percent of women with bipolar depression experience large mood shifts during their menstrual cycle. These women respond better to mood stabilizing medication (lamotrigine) if they use OC as well (Teatero et al., 2014). Depressed women using OC containing a synthetic estrogen and progestin had less severe depression, had fewer comorbid anxiety disorders, and a better physical functioning than depressed women not using OC (Young et al., 2007). However, the use of OC induces more mood swings in women with severe borderline personality disorder (DeSoto et al., 2003). Finally, depressive symptoms in women with a life-time diagnosis of depression tend to change significantly around menopause (Kornstein & Clayton, 2002). In summary, the female hormonal status may have consequences not only for the onset, but also for the successful treatment of mood disorders.

1.3 The menstrual cycle

The hypothalamus-pituitary-gonadal (HPG) 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 anterior pituitary gonadotrophs in the blood. FSH and LH at their turn stimulate the ovaries to secrete estrogens and progestins. These two steroid hormones regulate in complex feedforward and feedback interactions with GnRH, FSH, LH and other peptides (e.g. inhibin and activin) in a monthly pattern of hormonal fluctuations the menstrual cycle as depicted in figure 1 (Boron & Boulpaep, 2012; Rivera et al., 1999).

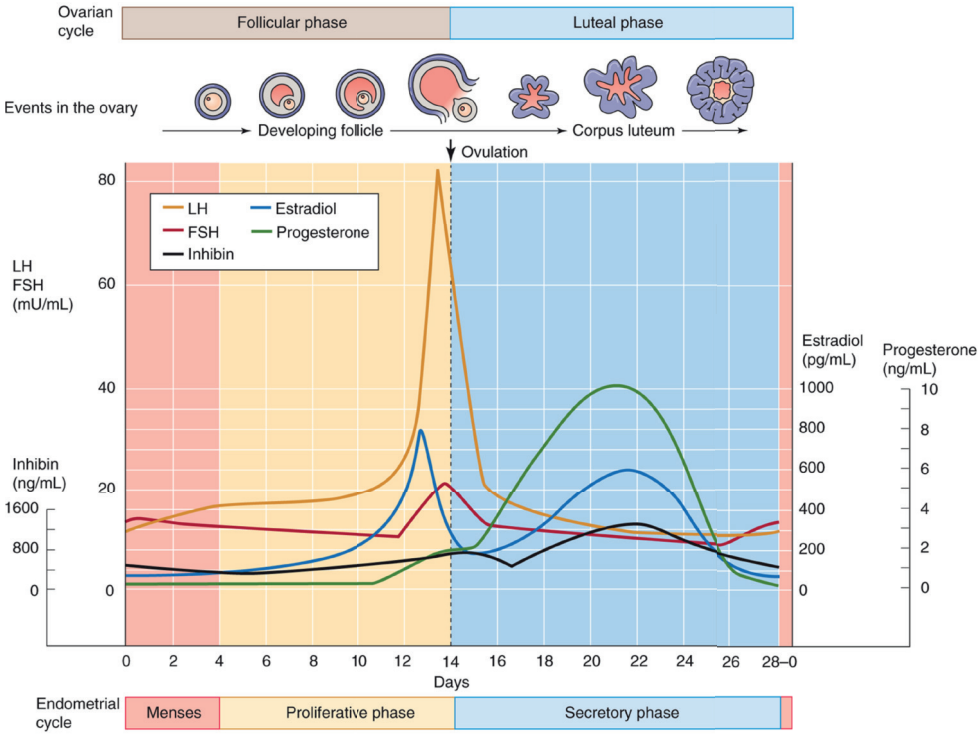

Figure 1. Hormonal changes during the menstrual cycle (Boron & Boulpaep, 2012).
FSH = follicle-stimulating hormone; LH = luteinizing hormone

The menstrual cycle actually involves cyclic changes in two organs: the ovary and the uterus. The ovarian cycle includes the follicular and the luteal phase, separated by the ovulation. The endometrial cycle includes the menstrual, proliferative and the secretory phases (see figure 1).

The first phase of the ovarian cycle is the follicular phase during which FSH stimulates the growth of follicles (folliculogenesis). The follicular phase begins with the onset of the menstruation (menses) and averages 14 days in length, but is also the most variable phase of the menstrual cycle. In the early follicular phase, the gonadotrophs in the anterior pituitary contain little LH and FSH. Estrogen and progesterone levels are also low. During folliculogenesis the granulosa cells of the follicles increase the production of the estrogen estradiol (E2), which stimulates LH and FSH synthesis, but inhibits their secretion. During the late follicular phase, the follicle selected for ovulation matures and FSH levels decrease but LH levels remain, as E2 inhibits FSH secretion more than LH secretion. In the ovulatory phase E2 levels peak and progesterone levels increase as well. The luteal phase starts when the dominant follicle has become a corpus luteum after releasing the ovum and usually lasts 14 days. The corpus luteum secretes primarily progesterone in increasing quantities, peaking 6 to 8 days after ovulation. Progesterone stimulates the development of the secretory endometrium, which is necessary for embryonic implantation. As levels of circulating estradiol and progesterone are high during the luteal phase, LH and FSH levels decrease. If no pregnancy occurs, estradiol and progesterone levels decrease in the late luteal phase as well, following which the corpus luteum dissipates and menses begins (Boron & Boulpaep, 2012; Rivera et al., 1999).

Menopause

In the Western world most women enter menopause between 45 and 55 years of age. Even before the onset of menopause, significant hormonal changes occur very early during reproductive life. As the follicles decline little by little, the ovarian production of estrogen decreases gradually. This reduces the negative feedback to the anterior pituitary and induces increased levels of FSH. Increased levels of FSH are seen as early as 35 years of age, even though menstrual cycles continue. Accordingly, older — but premenopausal — women have diminished estradiol production and decreased luteal function during natural cycles compared with younger women (Boron & Boulpaep, 2012; Fauser et al., 2013). This is why we investigated only women younger than 36 years of age in this PhD project.

1.4 Oral contraceptives

History

In 1950 Margaret Sanger, a then 71-year-old woman who had been lobbying for 50 years for the right of women to control their own fertility, provided a small grant to the famous endocrinologist Gregory Pincus which enabled him to discover oral contraceptives (Eig, 2014). In 1960 the FDA approved Enovid as an oral contraceptive, but women were not allowed to use it for longer

than two years because of its serious side-effects (Junod & Marks, 2002). A German company asked dr. Fernand Peeters – a Belgian gynaecologist – to improve the formula of Enovid and to test his inventions on his clientele. In 1962 Anovlar (meaning 'without ovulation') came on the market in Europe: a pill with two main compounds, a synthetic estrogen and progestin, making it safe and effective and the true precursor of today's contraceptive pill (van den Broeck, 2014). Lyndiol® was the first contraceptive pill produced by Organon in The Netherlands. Since 2012 OC use is declining, especially in younger women (21 – 30 years). Still, in 2017 1.7 mln women were using hormonal contraceptives of whom 1.2 mln women were taking second generation OC, which is the topic of this thesis (SFK, 2018)[2].

Working mechanism

The effectiveness of OC is the result of several actions. Like the natural ovarian steroids, OC exert a negative feedback action in the hypothalamus and the gonadotrophs in the anterior pituitary, resulting in a suppressed release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). This suppression prevents follicular development and ovulation. The progestin in OC induces thickening of the cervical mucus and makes it viscid and scant, inhibiting the sperm to enter the uterus. It also makes the endometrium less suitable for implantation of the embryo (Rivera et al., 1999; Jones, 2012).

Generations OC

Contemporary OC always contain ethynylestradiol (EE), but the progestins have evolved over time, resulting in improved 'generations' of OC (Lawrie et al., 2004):

a. First generation: norethisterone and norethindrone acetate

b. Second generation: levonorgestrel (Microgynon, morning after pill, etc.)

c. Third generation: desogestrel, gestodene and norgestimate (Arianna, Careza, Marvelon, etc.)

d. Fourth generation: drospirenone or other new progestin containing pills (Yasmin, Yaz, etc.)

These different synthetic progestins have similar effects on the endometrium, but differ in effects on other tissues. This is because they have similar effects on progesterone receptors, but act differently on other receptors such as androgen receptors (AR), glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) (Lawrie et al., 2014). Second generation OC containing levonorgestrel are the most widely used in the Netherlands and they are first choice according

[2] Stichting Farmaceutische kengetallen (2018).
https://www.sfk.nl/publicaties/PW/2018/minder-vrouwen-aan-anticonceptie

to the Contraception Guidelines of The Dutch College of General Practitioners[3]. Therefore, we focused in this PhD project on OC users taking second generation monophasic OC[4].

Regimen

Monophasic or fixed-combination OC are taken daily 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 naturally cycling (NC) women (Rivera et al., 1999; Boron & Boulpaep, 2012). During the pill-free week a withdrawal bleeding is likely to occur. The HPG axis 'recovers' resulting in increasing levels of FSH and estrogen, making this period comparable with the early follicular phase of the natural menstrual cycle (van Heusden & Fauser, 1999, 2002). Besides the HPG-axis, OC influence also the physiological stress response system (see section 1.7).

1.5 Stress response system: HPA axis and clinical consequences of chronic stress

The HPA axis and the stress response

The hypothalamus-pituitary-adrenal (HPA) axis represents a complex neuroendocrine feedback loop, which is involved in the regulation of behavioral adaptation in response to a potential stressor. When the brain detects a threat, freeze or fight-or-flight behavior may follow. This is an integrated acute defense reaction which originates within the central nervous system (CNS), and is mediated by the autonomic nervous system (adrenaline). This immediate reaction is the result of processing of sensory information in the cortex and amygdala, activating noradrenergic neurons in the locus coeruleus (LC) which signal to almost every part of the central neural system. Activation of the hypothalamic paraventricular nucleus (PVN) induces an autonomic nervous system, immune-, behavioral and endocrine response. The adrenal medulla releases catecholamines (adrenaline and noradrenaline). The slower endocrine response of the PVN involves release of corticotropin-releasing hormone (CRH) from the parvocellular neurons of the paraventricular nucleus in the portal vessel blood. Co-localized vasopressin is also released, which potentiates the CRH signal on the anterior pituitary corticotrophs. This triggers the subsequent release of adrenocorticotropic hormone (ACTH) from the pituitary gland, leading to the production and secretion of glucocorticoids by the adrenal cortex (see figure 2). Hence,

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

[4] Monophasic second-generation OC contain the same amount of estradiol and levonorgestrel across the cycle.

adrenaline facilitates immediate resources for the onset of the stress-response and fight-or-flight defense reactions. Subsequently, glucocorticoids promote in a slower and more lasting mode the allocation of metabolic energy in order to support stress coping.

The nature of the stressor is appraised by the medial prefrontal cortex (mPFC). At the same time the memory function of the hippocampus is activated in order to retrieve similar experiences from the past. Next, the mPFC promotes appropriate behavioral adaptation to the stressor and starts – simultaneously – restraining the neuroendocrinological, autonomic and behavioral components of the stress response. These processes are facilitated by glucocorticoids which exert for this purpose a negative feedback action on the brain and the pituitary (de Kloet et al., 2018). Consistently, this neuroendocrine system is also implicated in the pathophysiological changes which occur in response to – chronic – stress, from early experiences into adult life (Lupien et al., 2009).

Consequences of (chronic) stress

Important consequences of (early life) stress have been observed in the famous Adverse Childhood Experiences study (also known as the ACE Study) conducted by Felitti and co-workers (1998). The ACE study revealed that traumatic childhood experiences were far more common than expected. It also showed that exposure to childhood adversity was associated with increased adult psychopathology with a cumulative effect: the more adverse childhood experiences, the higher the adult risk for stress-related (psycho)pathology such as depression, suicide, substance abuse, lung cancer, ischemic heart disease, chronic pulmonary disease, diabetes, and liver disease (Felitti et al., 1998; Van der Kolk, 2015).

The relationship between early life stress and psychopathology became pronounced in a follow-up study of Putnam and co-workers (2014). People with more childhood adversities had more separate DSM-IV diagnoses over various DSM-IV disorder categories (mood, anxiety, substance abuse, and impulse control disorders), again with a cumulative effect. Hence, these results show convincingly that increased childhood adversity is associated with adult psychopathology such as depression. Besides sociological, environmental and psychological factors, (chronic) dysregulation of the HPA-system may play a role in this increased susceptibility on developing depression following childhood trauma. In the majority of depressive patients altered regulation of corticotropin (ACTH) and cortisol secretory activity is observed, reflecting changes in the setpoint of the HPA-axis. Closer analyses of the HPA-system in patients suffering from major depression revealed that corticosteroid receptor signaling is impaired, resulting in among others increased production and secretion of corticotropin-releasing hormone (CRH) in various brain regions involved in depression (Raadsheer et al., 1994; Plotsky et al., 1998; Holsboer, 2000; De

Kloet et al., 2005, 2007, 2016). Whether these biological changes underlie depression or even may provide prospective evidence on the onset, relapse or recurrence of depression is still topic of debate (Kennis et al., 2019).

1.6 The female reproductive system and the stress response system interact

As shown in figure 2, the response of the hypothalamic-pituitary-adrenal (HPA) axis is affected by estradiol and progesterone. Corticotropin releasing hormone (CRH), produced by the parvocellular neurons of the paraventricular nucleus (PVN) of the hypothalamus, expresses inter alia estrogen receptors (ER) and receives input from brain regions that are themselves target for sex- and stress hormones (Handa et al., 1994; Kirschbaum et al., 1999; Handa & Weiser, 2014; Heck & Handa, 2019). In its turn, the HPA axis influences levels of these hormones (Kudielka & Kirschbaum, 2005).

Reports on direct inhibition of neuroendocrine stress responses by estradiol are mixed, possibly due to inconsistent experimental conditions. However, estradiol may indirectly influence HPA axis activation. The PVN receives serotonergic projections from the median and dorsal raphei nuclei of the brainstem which activate the HPA axis in general. This effect is partially decreased by estradiol as it desensitizes serotonin signaling in the PVN (Van de Kar & Blair, 1999; Raap et al., 2000; Heck & Handa, 2019). This process is hypothesized to play an important role in reproductive depression (Kornstein & Clayton, 2002; Fauser et al., 2013).

Administration of progesterone alone suppresses HPA axis activity in rodents (Patchev et al., 1994; Carey et al., 1995). Furthermore, progesterone may reduce the influence of estradiol on the activity of the HPA axis. The extent by which estradiol increases the output of the HPA axis depends on the background level of progesterone (Viau & Meaney, 1991; Patchev et al., 1994). Consistently, the salivary cortisol response to psychosocial stress was present in women in the early and mid-follicular phase, but not in the luteal phase (Rohleder et al., 2001). Hence, estrogen and progesterone may play a role in regulating stress responsiveness across the menstrual cycle (Heck & Handa, 2019).

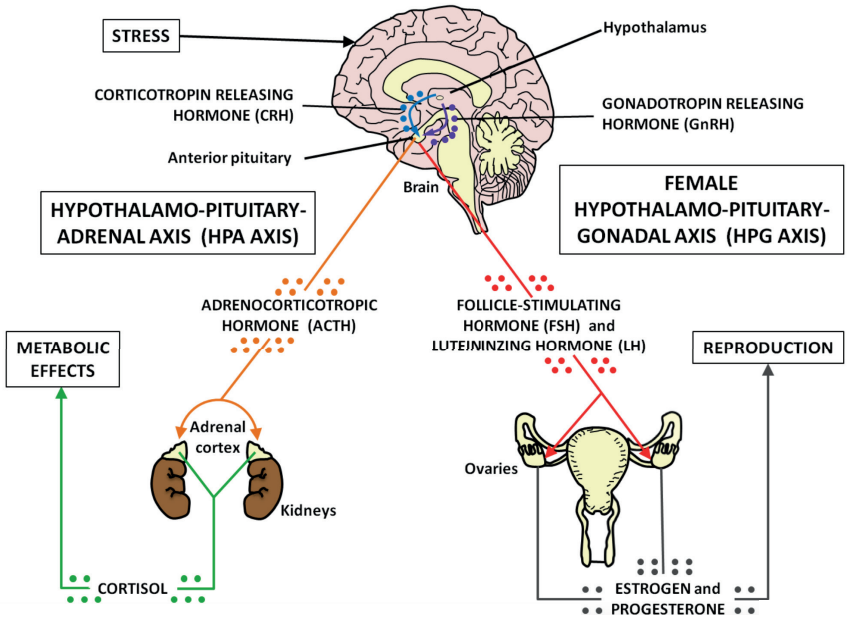

Figure 2. A schematic representation of the interaction between the HPA and the HPG axis (© 2012 Nepomnaschy & Salvante)

1.7 The corticosteroid receptors

The mineralocorticoid and glucocorticoid receptors and depression

The action of the glucocorticoids are mediated by binding to two types of corticosteroid receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) (Reul & de Kloet, 1985) (see figures 3 and 4). Depression has been attributed mostly to altered GR function (Holsboer, 2000), including evidence for GR gene polymorphisms and methylation of the GR gene promoter (Binder et al., 2004; Webster et al., 2002). Although fewer studies in major depressive disorder have focused on the MR, there is evidence of 30% decreased MR expression in depression, especially in brain regions crucially involved in cognitive and emotional function like the hippocampus and inferior frontal gyrus and cingulate gyrus (Klok et al., 2011a; Medina et al., 2012). Furthermore, an imbalance in mediated actions by the MR and the GR may result in cortisol responses of large magnitude and long duration, being indicators of an increased vulnerability to mental disorders like depression (De Kloet 2016; De Kloet et al., 2005, 2007, 2018; Holsboer, 1999, 2000).

Functioning of the GR and the MR

GR are ubiquitously expressed in neurons and glia cells with highest expression in stress-responsive brain structures (de Kloet et al., 1998). GR show a low affinity for cortisol and become only gradually occupied during the circadian peak or during higher cortisol concentrations after stress. In contrast, MR bind cortisol with a 10-fold higher affinity than the GR (see figure 3). The GR also bind potent anti-inflammatory and immunosuppressive synthetic glucocorticoids such as dexamethasone and prednisone. The synthetic glucocorticoids do not bind to the MR, but the mineralocorticoid aldosterone does. Aldosterone circulates in a 1000-fold lower concentration than cortisol. However, epithelial cells involved in Na resorbtion, express an enzyme that breaks down cortisol, rendering these cells solely responsive to aldosterone (see figure 4).

Aldosterone (Aldo) is the principal mineralocorticoid secreted in the picomolar range by the glomerulosa cells in the adrenal cortex as endproduct of the renin-angiotensin system. Aldo is engaged in the control of Na/K homeostasis and acts via the mineralocorticoid receptor (MR) in epithelial cells of kidney, intestines and sweat glands (Jaisser and Farman, 2016). The glucocorticoid cortisol (F), secreted by the adrenal cortex zona fasciculata cells in nanomolar concentrations (100 fold higher than Aldo), is the end product of the HPA axis. F is critical for stress, adaptation, immunity, inflammatory processes and energy metabolism. F can also act via MR present in non-epithelial cells of e.g. brain, heart, adipose tissue and muscle. Thus, the MR in the kidney solely responds to Aldo, while it appears that the brain MR – for instance the hippocampus – is mainly occupied by F. In vitro MR binds Aldo and F with similar affinity. Yet, the question arises how it comes that the same MR recognizes in vivo either Aldo in the kidney or F in the brain.

The solution to this question is the enzyme 11β hydroxy steroid dehydrogenase (11-HSD) which occurs in two isoforms, a reductase (11HSD-1) and an oxidase (11HSD-2) (Chapman et al. 2013). The kidney 11HSD-2 catalyzes the conversion from bioactive F to inactive cortisone (E), allowing Aldo to bind to the MR (Edwards et al. 1988; Funder et al. 1988). In the hindbrain there is a small group of a few hundred neurons in the nucleus of the solitary tract (NTS) that does express the Aldo-selective MR. These NTS neurons control salt appetite and through its innervations in the limbic-forebrain also motivational, emotional and cognitive features of salt appetite (Geerling and Loewy, 2009). In the rest of the brain and in e.g. heart and adipose tissue, 11HSD-1 does the reverse and promotes the regeneration of bioactive F, which circulates in a 100- to 1000-fold excess over aldosterone. Hence, the 'F-preferring MR' responds predominantly to bioactive F rather than Aldo. These 'F-preferring' MRs are abundantly expressed in the hippocampus, amygdala, and prefrontal cortex, limbic regions of the brain which are crucial for memory performance and emotional response (Brinks et al., 2009; de Kloet et al., 2016).

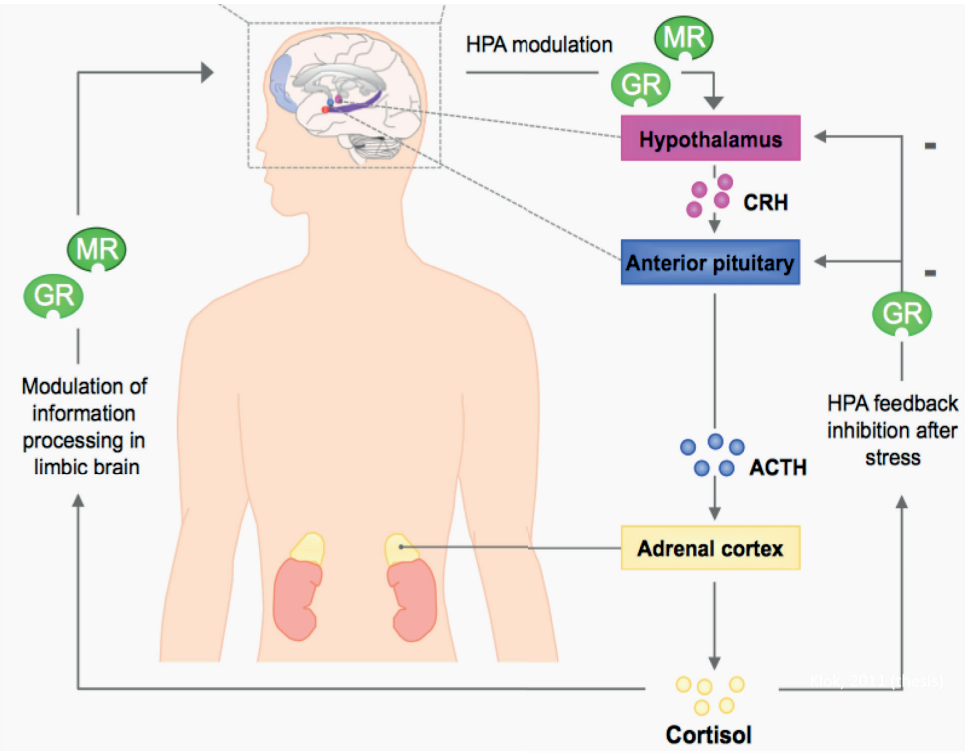

Figure 3. The MR, GR and their function in the HPA-axis (reprinted from Klok, 2011)

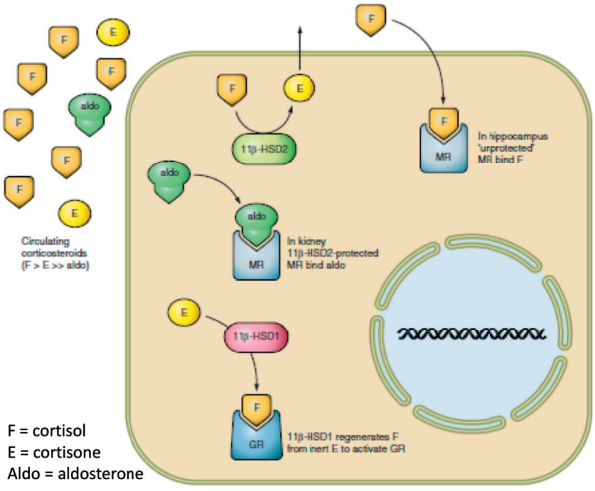

Figure 4. The role of 11β hydroxy steroid dehydrogenase in steroid specificity of the mineralocorticoid receptor (Chapman et al., 2013)

The high affinity of the MR implicates that these receptors are already sensitive to low levels of cortisol (Reul and de Kloet, 1985). Consistently, the MR is important for the initial phase of the stress response. Hence, MR activation promotes cognitive processes like selective attention, appraisal, memory retrieval, choice of coping style, encoding and formation of emotional memory (see f.i. Otte et al., 2007; Joëls et al., 2012). With rising cortisol concentrations, the GR becomes gradually activated allowing cortisol to complement the actions initiated via the MR. Hence, the GR prevents initial endocrinological defense reactions from overshooting, provides energy substrates to tissues, promotes contextualization and rationalization of the stressor and finally facilitates memory consolidation in order to be prepared for future threats (Sapolsky et al., 2004; de Kloet et al., 2005; Joëls et al., 2012; de Kloet et al. 2018) (see figure 3).

The nuclear receptor superfamily

The MR, GR, estradiol and progesterone receptors belong to the nuclear receptor superfamily and have equivalent mechanisms of action (Lu et al., 2006; Boron & Boulpaep, 2012). This also implicates that these steroids can interact on various levels: from synthesis and breakdown, to receptor binding and the transcription machinery. The steroid hormones cortisol, testosterone, estradiol (E2) and progesterone activate gene expression by binding to intracellular receptors that are themselves transcription factors. After entering the cell, these steroid hormones bind to their receptors in the cytoplasm. The steroid-receptor complex moves to the nucleus where it binds to specific response elements on the DNA leading to changes in gene transcription. For instance, endogenous estrogen and in particular progesterone, which acts as a functional MR antagonist, may affect expression and binding characteristics of the brain MR (Carey et al., 1995; Quinkler et al., 2002; Quinkler & Diederich, 2009). Furthermore, OC decrease HPA-axis activity, which results in a reduced (free) cortisol response (in saliva) to a stressor (Kirschbaum et al., 1999). Following this, OC may affect the feedback action of cortisol in the brain, which is mediated by the GR and the MR.

1.8 The human MR (NR3C2) gene and the MR haplotype

With regard to the functioning of the MR considerable interpersonal differences have been reported.

rs2070951[MR-2G/C]

The rs2070951 polymorphism is a functional SNP located 2 nucleotides before the translation site of exon 2 of the MR gene (DeRijk, 2009; van Leeuwen et al., 2011, 2010c). In vitro research revealed decreased MR protein and reduced transcriptional activation in the rs2070951 G allele compared to the C allele. Genotype GG was correlated with higher aldosterone levels in

healthy volunteers and higher systolic blood pressure in males (van Leeuwen et al., 2010a). The rs2070951 has been associated with in particular physical MR-related responses. Carriers of the G-allele of this MR polymorphism suffering from cardiac heart failure showed a better response to spironolactone – an MR-antagonist – by competitively inhibiting binding of aldosterone and cortisol (Cavallari et al., 2015). They were also more susceptible to chronic central serous chorioretinopathy, a severe eye disease associated with steroid signaling and perceived chronic stress (van Dijk et al., 2017).

rs5522 [MR-2A/G]

rs5522 (minor allele frequency 12%) is a SNP resulting in an isoleucine (A) to valine (G) amino acid change located the N-terminal domain of the protein (DeRijk et al., 2006; van Leeuwen, 2011) and has mainly been related to behavior and learning under stress. In the no stress condition reward learning was enhanced in val carriers (rs5522 G). However, after being exposed to an acute, uncontrollable stressor val carriers (rs5522 G) showed a larger learning deficit than iso homozygotes (rs5522 A) (Bogdan et al., 2010). Clinical consequences of the increased stress sensitivity in rs5522 val carriers were observed too, as they were more likely to develop substance abuse (Rovaris et al., 2015) and depression following childhood trauma (Vinkers et al., 2015).

MR-haplotype approach

Because rs5522 and rs2070951 are in linkage disequilibrium, several studies have investigated these SNPs together, following a haplotype approach (De Rijk, 2008; De Kloet at al., 2016) (see figure 5). The MR-haplotype is constructed from two single nucleotide polymorphisms (SNP) of the MR gene (NR3C2), rs2070951 (MR-2G/C]) and rs5522 (MR-2A/G), resulting in three common functional haplotypes: haplotype 1 (GA); haplotype 2 (CA) and haplotype 3 (CG) and one very rare haplotype, haplotype 4 (GG; frequency < 0.1%).

The MR-haplotypes differ in the transmission, translation and activation of the MR (Klok et al., 2011ab; van Leeuwen et al, 2011). The MR-haplotype influences the cortisol awakening response (CAR) in a gender-dependent manner, after suppression of HPA axis activity by dexamethasone and thereby depleting the brain from glucocorticoid cortisol (van Leeuwen et al., 2010b; Klok et al., 2011c). The CAR – in which the highly MR expressing hippocampus is key player – enables individuals to anticipate on daily events and possible disturbances (de Kloet et al., 2005; Fries et al., 2009). Further, MR-haplotype 2 (CA) is associated with higher dispositional optimism and fewer thoughts of hopelessness during sad mood. MR haplotype 2 carriers showed a lower risk of major depression in the Netherlands Study of Depression and Anxiety (NESDA; n = 1639), with a dose effect: the more MR-haplotype 2 alleles, the lower the risk. These effects were restricted to premenopausal women, which suggests that female sex

steroids may interact with the MR gene, thereby modulating resilience (Klok et al., 2011b). These MR-haplotype associations in adults could not be replicated, neither in a large adolescent sample, nor in a study with a case-control design with healthy subjects (n = 634) and inpatients with major depressive disorder (n = 412), however (Bouma et al., 2011; Sarubin et al., 2017).

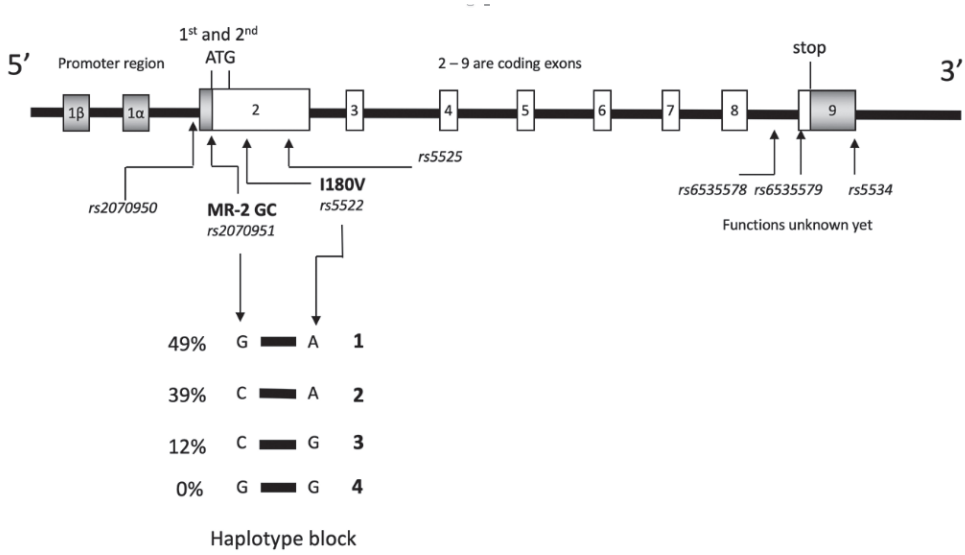

Figure 5. The human MR (NR3C2) gene with the haplotype block of rs2070951 (MR-2C/G) and rs5522 (MRI180V) in the 5' region (reprinted from van Leeuwen, 2010).

Finally, female MR haplotype 2 (CA) carriers showed fewer symptoms of depression following childhood trauma in a population-based cohort (n = 665) and the NESDA cohort (Vinkers et al., 2015). A genome wide association study (GWAS) in the same cohort revealed that the MR (NR3C2) gene was more strongly associated with amygdala volume, hippocampal volume, and MDD status in participants with a of history childhood maltreatment (Gerritsen et al., 2017). Following these previous findings, we expected to observe in our research projects that MR-haplotype 2 carriers would be less sensitive to the influence of natural and synthetic female hormones on mood (see section 1.9.4).

1.9 This PhD project

1.9.1 Rationale

During their fertile years, women are twice as likely to develop a clinical depression as men. Mood shifts towards depression are more likely to occur at female reproductive events such as menarche, menses, peri-partum and the menopausal transition. Hence, besides genetic and psychosocial factors, the female hormonal status may also play a role in the onset and course of depression. Furthermore, some women may experience mood shifts towards depression during usage of contraceptives containing synthetic female hormones, which may even lead to the diagnosis of a clinical depression. Especially young women seem to be prone to these depressogenic effects of OC (Skovlund et al., 2017). Following these reports rates of OC use are declining, putting women at risk for unintentional pregnancy. Since hormonal contraceptives are one of the safest ways for birth control, further research into determinants underlying this vulnerability to the negative mood effects of OC is needed.

In this PhD project we aimed to investigate the subtle effects of natural and induced changes in female hormonal levels on depression-related cognitive-emotional processes. Some women experience rather strong effects of taking OCs or of the various phases of their menstrual cycle, however that is a minority. Recent evidence suggests that many more women experience quite subtle effects of OCs on cognition. These biases which might not be noticed in daily life, and may only be detectable by sensitive laboratory tests. We aimed to further investigate the extent and robustness of these effects and we explored whether the effects, if any, were moderated by genotype, in particular the MR gene.

1.9.2 Factors which may affect mood

Female hormonal status

According to the 'hormonal hypothesis', fluctuation in ovarian hormones occurring around menarche and menopause, and pre- and post-partum may induce mood shifts contributing to women's increased vulnerability to mood disorders. Therefore, in research paradigms examining the processing of emotional information in mood disorders the hormonal status of female volunteers should be assessed, whenever possible.

Genetically determined differences in stress vulnerability

Many biological and genetic determinants of vulnerability to mood disorders have been reported (Kornstein & Clayton, 2002; Rachman 2004). For reasons of feasibility, we investigated in this

PhD project if one particular component of the stress-system influenced the impact of the female hormonal status on emotion processing. We decided to focus on the relatively underinvestigated MR for the following reasons. Firstly, the MR is involved in emotional information processes as vigilance, attention, appraisal and selection of coping style. Blockade of the MR induced anxiolytic responses and attenuated aggressive behavior (Korte et al., 1995; Joëls et al., 2012; Kruk et al. 2013; Schwabe et al., 2013). Secondly, in vitro and in vivo studies showed that estrogen and in particular progesterone modulate MR functioning (Carey et al., 1995; Quinkler et al., 2002, Quinkler & Diederich, 2009). Finally, 'the MR-haplotype approach' revealed among others that female carriers of MR-haplotype 2 were protected against depression, but only during their fertile years (Klok et al., 2011b), again suggestive of an interaction between the female hormonal status and the MR. Following these results, we considered the 'MR-haplotype approach' sound for an exploratory research project.

Physical and mental health

People with a temperamental vulnerability to mood disorders tend to score higher on among others neuroticism, introversion and anxiety. This affective vulnerability extends itself into a status of 'cognitive vulnerability', meaning that it also affects the perception, collection and interpretation of information (Clark et al, 1994). In order to control for differences amongst our participants, we assessed measures on personality, implicit and self-reported negative and positive affect and depression-related cognitions. In addition, involvement in a committed relationship, compared with being single, is associated with fewer depressive symptoms for college women (Whitton et al., 2013) and may therefore influence measures on depression. Finally, as physical health, use of psychopharmaca, alcohol and nicotine etc. may affect performance on emotional information processing, participants were carefully screened before inclusion and at assessment.

1.9.3 Assessment of emotional information processing by psychological tasks

The way which people interpret and predict future events may be distorted in the presence of a mood disorder. For example, people who suffer from depression, tend to overestimate the likelihood of aversive events (see f.i. Beck, 1963, 2002; Ehlers, 1993; Harvey et al., 2009). They also pay less attention to positive information and have more difficulty in avoiding negative information (Harvey et al., 2009), meaning that depression already influences their attentional focus (Ingram, 1990). The way depressed people interpret information maintains this self-reinforcing cycle: they tend to view events in a more negative way (Haaga et al., 1991), rather than applying a more flexible approach of reality (Dykman et al., 1989).[5]

[5] Normally attention will be diverted from a cue as soon as the stimulus becomes more familiar, but in depression this is not the case: depression induces a circle of repetitive negative thoughts and beliefs (Kahneman, 2011).

The information-processing approach to cognition and emotion is an empirical methodology which does not rely on self-reports. It also enables the assessment of mediating cognitive variables. Furthermore, it offers a framework for generating specific hypotheses underlying 'distorted' cognitive emotional processes involved in mood disorders (Ingram, 1978). This approach also postulates that the processing of emotional-cognitive information involves different interconnected modules, which may process information simultaneously and automatically (Teasdale, 1997). Following this approach, experimental psychological research discovered that the biased processing of emotional stimuli – facial expressions of emotions in particular – may be one of the mechanisms underlying vulnerability to mood disorders (Joormann et al., 2006). Equivalent paradigms were applied investigating the effects of a single dose of an antidepressant in healthy volunteers. For instance, a single dose of the antidepressant reboxetine facilitated within a few hours after administration the processing of positively valenced emotional information in healthy volunteers, reflecting the early effect of an antidepressant on the negative biases in perception as observed in depressed patients (Harmer et al., 2003, 2009).

In this PhD project we assessed tasks covering various stages of cognitive-emotional processing – such as the perception and the interpretation of (emotional) stimuli – and selected tasks which were applied earlier in studies investigating mechanisms underlying mood disorders. We assessed the perception of emotional cues of others (facial expression recognition paradigm, cognitive empathy) and neutral stimuli (attentional blink paradigm), the verbal memory performance following mood-inducing distracters, the recall of characteristics and risky decision making. We selected 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). In addition to these experimental paradigms, we explored MR-genotype dependent change in mood in OC- users and naturally cycling (NC) women in a masked longitudinal design with online questionnaires (see chapter 6).

1.9.4 Research projects

This PhD project involved four research projects.

Project 1

In earlier experimental research OC-users failed to report positive affect after positive mood induction (Jarva & Oinonen, 2007). Depressogenic effects remain one of the main reasons for discontinuation of OC (Oinonen & Mazmanian, 2002).

In our first project we undertook an exploratory cross-sectional parallel-group behavioral lab study in which the influence of OC on emotional information processing was investigated.

Naturally cycling (NC) women (n = 41) were tested between day 6 and 26 of their menstrual cycle and OC users (n = 44) were tested outside their pill-free week. OC brand name and chemical compound, duration of OC use and in case of NC women first day of last menses were registered. We explored additionally whether this effect was moderated by genetic variation in the MR following a haplotype approach based on rs2070951 [MR-2G/C]) and rs5522 [MR-2A/G], which were previously found to be in linkage disequilibrium. Specifically, we hypothesized that the effects of OC on emotional information processes would be more pronounced in carriers of MR haplotype 1 and 3 than in MR haplotype 2 (chapter 3).

Project 2

Previous experimental studies revealed that the menstrual cycle phase influences performance in emotional information processing. The OC-effects of OC may be due to the suppression of cyclical changes in ovarian hormones (Jones, 2012). OC decrease the HPA axis response to stress and salivary free cortisol responses to stress. The MR mediates the effect of cortisol on initial stress reactions (De Kloet et al., 2005). These rapid effects are especially mediated by the MR sited in limbic structures, regions in which early life stress, chronic stress and depression were found to downregulate MR-expression. In our second project we investigated the influence of OC and menstrual cycle phase on emotional information processing in MR-genotyped healthy volunteers in an exploratory cross-sectional parallel group lab study.

We assessed healthy NC women with a regular cycle (25 – 35 days) in the early follicular (day 2 – 5; n = 21) or mid luteal (day 18 – 25; n = 23) phase of their menstrual cycle. Only women using second generation OC (EE .03 mg; LNG .15 mg) for more than three months were included and were tested outside their pill-free week (n = 49). We assessed tasks which have shown earlier to be sensitive to the influence of OC (chapter 2 and 3) and new tasks in order to cover more stages of emotional information processing (chapter 4).

Project 3

Female hormones modulate the impact of stress on mood. The observed effects of OC and female hormones on emotional information processing may be mediated by estrogen and progesterone receptors, which are abundantly expressed in limbic brain structures (Handa and Weiser, 2014). In these limbic areas these sex steroids may modulate the function of mineralocorticoid receptors (MR). Progesterone binds to the MR with nearly the same affinity as aldosterone and cortisol, and acts as a competitive antagonist, while estrogen suppresses the synthesis and transactivation of the MR in brain and vascular endothelial cells. Consequently, the MR is of relevance in candidate gene studies investigating the influence of female hormones on emotional information processes associated with depression.

Our third project consisted of two parts in which we investigated the same sample. In the first part we studied the moderating influence of the MR-haplotype on the impact of OC-use and menstrual cycle phase in a behavioral lab study using a within subject, counterbalanced design. OC-users (EE .03 mg; LNG .15 mg; n = 57) were tested in a counterbalanced entry-order: once in the second week of active OC-use (day 8 – 14) and once during inactive OC-use (day 4 – 7 of the pill-free week). NC participants (n = 39) were tested at two counterbalanced time-points. Once in the early follicular phase (day 2 – 6), when both hormones are low, and once in the middle of the luteal phase (3–10 days before the onset of the new cycle) when the concentration of P4 is at its maximum and E2 reaches a second peak. At intake the average cycle duration of the NC participants was registered. After confirmation of the start of the new cycle, test data were scheduled and adjusted to the individual cycle-duration, reasoning that the luteal phase always lasts two weeks (Jones, 2012). Participation ended after confirmation of the start of the new cycle. This cycle onset information was used to confirm whether participants had been tested on the right moment. Participants were MR-genotyped at assessment (amount of MR-haplotype 2 alleles: 0, 1 or 2) (chapter 5). I

In the second part of this project we tested the hypothesis that NC women (n = 35) had higher scores on measures associated with reproductive depression than OC-users (n = 57). We further hypothesized that NC women with MR-haplotype 1/3 had higher scores on these measures than MR haplotype 2 carriers. This study had a prospective longitudinal design. All data were collected using an online survey tool (Qualtrics, Provo, UT). NC participants filled out questionnaires on four time-points of two consecutive menstrual cycles. Care was taken to adjust the time-points to the individual cycle-duration. OC users using OC (.03 mg EE; .15 mg LNG) for at least three months, were assessed at four equivalent time-points during two consecutive months. Their actual participation started with a new pill strip. We controlled for cycle phase and pregnancy by levels of E2, P4 and estriol in saliva at two sessions in our lab. We carefully masked our research objective to prevent artefacts caused by expectations concerning menstrual cycle symptoms (Aubuchon & Calhoun, 1985) and checked after completion of the data collection whether the masking was successful (chapter 6).

Project 4

Following our observations from the previous projects, we hypothesized that the moderating effects of the MR-haplotype on the impact of OC-use and menstrual cycle phase could be observed also on electrophysiological measures. Therefore, we undertook a within-subject, counterbalanced study in which we assessed electrophysiological processes associated with anxiety and depression, namely resting state EEG in 44 NC women and 44 OC users (chapter 7). In line with our third project, we assessed levels of estradiol and progesterone in saliva and applied a categorical approach of the MR-genotype (amount of MR-haplotype 2 alleles) in our analyses.

Chapter 2

Oral contraceptives may alter the detection of emotions in facial expressions

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Abstract

A possible effect of oral contraceptives on emotion recognition was observed in the context of a clinical trial with a corticosteroid. Users of oral contraceptives detected significantly fewer facial expressions of sadness, anger and disgust than non-users. This was true for trial participants overall as well as for those randomized to placebo. Although it is uncertain whether this is an effect of oral contraceptives or a pre-existing difference, future studies on the effect of interventions should control for the effects of oral contraceptives on emotional and cognitive outcomes.

Introduction

In many women oral contraceptives (OC) reduce the variability of affect across the menstrual cycle and reduce negative affect during menstruation. However, some women experience a worsening of mood during OC use (Oinonen & Mazmanian, 2002). It is less well-known that OC may also have more subtle but important psychological effects in women who do not experience subjective mood changes. For instance, OC use induced a preference for less masculine faces (Little et al., 2013). Furthermore, partnered women who used OC reported significantly higher levels of jealousy than naturally cycling partnered women in their non-fertile cycle phase (Cobey et al., 2012).

Here we report on a coincidental finding concerning possible effects of OC use on emotion perception. The effect was observed in the context of a clinical trial investigating the between-subject effects of the mineralocorticoid receptor (MR)-agonist fludrocortison on emotional information processing in healthy women.

Experimental procedures

Participants

Forty physically and mentally healthy, non-smoking female volunteers of north-western European origin (age: 18-35) took part in this study. Participants were tested outside their pill-free week or menstrual period (two days before until five days after the start of their period). All participants provided written informed consent before the start of the study and received financial compensation for their participation. The study was approved by the medical ethics committee (METC) of Leiden University Medical Center (LUMC).

Instruments and procedure

This single-centre study had a randomized, placebo-controlled, double-blind, between-subject design. Randomization on fludrocortisone or placebo was carried out by the pharmacy of LUMC. Use of oral contraceptives was registered, since endogenous (menstrual cycle) and exogenous (oral contraceptives) hormonal changes moderate activity of the hypothalamus-pituitary-adrenal axis (Kirschbaum et al., 1999). Forty non-smoking, mentally and physically healthy participants were recruited at various sites at Leiden University. All were physically examined at Leiden University Medical Center and screened for psychiatric past by the Mini International Neuropsychiatric Interview (Sheehan et al., 1998).

After the administration of a single dose of fludrocortisone (0.5 mg) or placebo, participants rested in a neutral research room for two hours. Subsequently the facial expression recognition task

(FERT) was assessed. The FERT displays five basic emotions (happiness, sadness, fear, anger and disgust) taken from the Pictures of Facial Affect Series (Ekman and Friesen, 1976). Male and female examples of these pictures were morphed between each prototype and neutral in 10% steps. Four trials of each emotion were presented at each intensity level. Each face was also given in a neutral expression, resulting in a total of 204 stimuli that were presented in a randomized order for 500 ms and replaced by a blank screen. Participants were asked to respond as quickly and accurately as possible by pressing the corresponding buttons of a response box (happiness, sadness, fear, anger and disgust and neutral). Personality was measured with the NEO-FFI, which assesses five dimensions: Neuroticism, Extraversion, Openness to experience, Agreeableness and Conscientiousness (Hoekstra et al, 1996). The state version of the Positive Affect Negative Affect Schedule (PANAS) was administered to assess mood at intake (Watson et al., 1988). Depression vulnerability was measured with the Leiden Index of Depression Sensitivity - Revised (LEIDS-R; Van der Does, 2002).

Statistical analyses

There were no outliers (defined as > |3 SD| from the mean), multivariate influential cases (Cooks' distances > 1) or missing data. Mean scores on personality dimensions in both conditions (FC and placebo) were compared with t-tests. To analyze overall effects on the FERT we calculated sum scores (accuracy rates) and means (reaction times) for each emotion and entered these in multivariate analyses of variance, with both condition (FC/ placebo) and OC use (yes/no) as between-subject factors. When data were not normally distributed, we followed-up the findings of parametric tests with equivalent non-parametric tests.

Results

Participant characteristics

Users and non-users of OC did not differ significantly on age, BMI and personality scores, except for Agreeableness (t = - 2.91, df = 38, p = .006; see table 1).

Facial expression recognition

Multivariate tests of the multivariate ANOVA (MANOVA) revealed a significant main effect of OC use [F (5,32) = 3.78; p = .008; η_p^2= .371; power = .89]. Tests of between-subjects effects indicated that OC influence the accuracy of emotion recognition: OC users recognized fewer expressions of anger [F(1,36) = 13.09, p = .001; η_p^2= .267; power = .94], sadness [F(1,36) = 5.09, p = .030; η_p^2 = .371 ; power = .89] and disgust [F(1,36) = 5.56, p = .024; η_p^2 = .134 ; power = .63]. OC-users also had faster RTs on correctly recognized sadness [F(1,36) = 5.78, p = .022; η_p^2 = .138; power = .65];] and disgust trials [trend; F(1,36) = 3.28; p = 0.079; η_p^2 = .083; power =.42].

Table 1. Demographic and clinical characteristics.

	Non-users (n = 14)	**OC-users (n = 26)**
Age in years	22.2 (0.7)|	20.6 (0.5)
Body mass index	21.9 (0.7)	21.8 (0.6)
Neuroticism	31.6 (2.2)	30.4 (1.4)
Extraversion	41.9 (1.3)	45.0 (1.3)
Openness to experience	33.3 (0.6)	33.7 (0.6)
Agreeableness	32.6 (0.7)*	35.2 (0.7)*
Conscientiousness	40.9 (0.7)	41.3 (0.9)
LEIDS-R Total	33.6 (4.2)	32.4 (2.7)
PANAS Positive affect	23.9 (1.23)	25.7 (1.1)
PANAS Negative affect	11.9 (.74)	10.8 (.27)

Abbreviations: OC = oral contraceptives; LEIDS-R = Leiden Index of Depression Sensitivity – Revised.
Notes: Means, standard errors in parentheses; * $p < .05$.

In order to exclude the possible influence of the administration of fludrocortisone on the outcomes of FERT, we analysed the placebo condition (n = 20; 14 OC-users) separately in a MANOVA with OC as between-subjects factor. Multivariate tests for OC were significant [$F(5,14) = 5,55$; $p = .005$; $\eta_p^2 = .665$; power = .938]. Tests of between-subjects confirmed main effects for OC-use on correct recognition of anger [$F(1,18) = 15.36$, $p = .001$; $\eta_p^2 = .460$; power = .96]; sadness [$F(1,18) = 4.53$, $p = .047$; $\eta_p^2 = .201$; power = .521] and disgust [$F(1,18) = 4.75$, $p = .043$; $\eta_p^2 = .209$; power = .541]. (see figure 1). The effect of OC-use on the speed of recognition of sadness became a trend ([$F(1,18) = 3.38$, $p = .083$; $\eta_p^2 = .158$; power = .413]. The same analyses with non-parametric Kruskal-Wallis ANOVAs largely confirmed these findings. The effects of OC on the recognition of anger ($p = .002$) remained significant, whereas the effects for disgust and sadness became trends ($p = .051$).

To control for the significant difference on Agreeableness between users and non-users of OC, we added this score as a covariate in the multivariate analysis of variance of the placebo-treated participants. The effect of OC on accuracy rates of anger remained significant [$F(1,17) = 14.19$, $p = .002$; $\eta_p^2 = .455$; power = .944], whereas the effects on disgust ($p = .096$) and sadness (accuracy, $p = .093$) became trends.

Since the FERT contains both female and male pictures as stimuli, we further explored the effect of gender of stimulus picture in a repeated measures MANOVA with gender as within-subjects factor, the five emotions as measures and OC-use as between-subjects factor. This revealed significant multivariate main effects of OC [$F(5,14) = 5.55$; $p = .005$; $\eta_p^2 = .665$; power=.938] and gender [$F(5,14) = 5.22$; $p = .007$; $\eta_p^2 = .651$; power = .922];]. In univariate tests the influence of gender on the recognition of happy was significant [$F(1,18) = 21.06$; p =

.001; η_p^2 = .539; power=.991], while the effect of disgust was a trend [F(1,18) = 3.16; p = .092; η_p^2 = .149; power = .391]. Furthermore, an interaction effect between OC-use and gender appeared for the recognition of disgust [F(1,18) = 4.85; p = .041; η_p^2 = .212; power = .550]. In non-parametric tests, happiness was recognised better on a male face than on a female face by both OC-users (trend; p = .058) and non-users (p = .002). A disgusted expression, however, was recognized better in female faces by participants who were not using OC (p = .039).

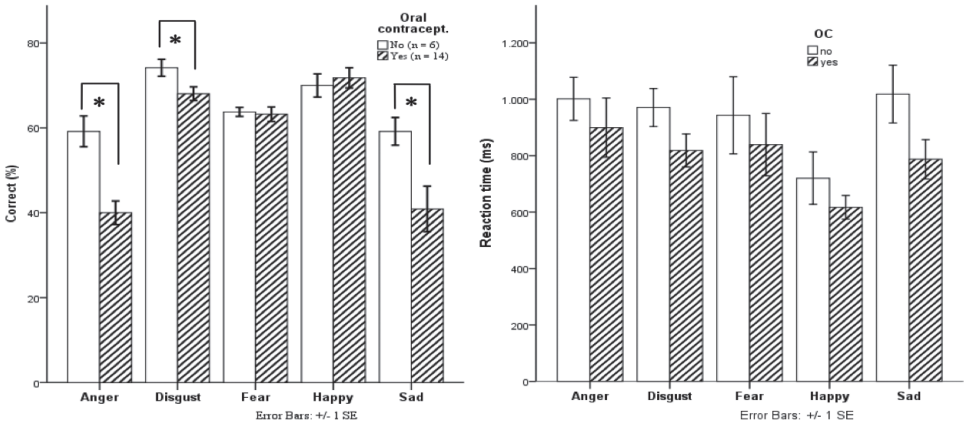

Figure 1a and 1b. Emotion recognition accuracy (left panel) and reaction time (right panel) scores of OC-users and non-users randomized to placebo. *Notes:* OC = oral contraceptives; * p < 0.05.

Discussion

We observed a possible effect of oral contraceptives use on emotion recognition: OC-users detected significantly fewer facial expressions of sadness, anger and disgust, but no effects were seen on the detection of happy or fearful facial expressions. An explorative analysis of the placebo condition confirmed these findings. Furthermore, the influence of OC use on the recognition of disgust was moderated by the gender of the poser of the facial expression.

It is uncertain whether these observed differences can be ascribed to OC use or were pre-existing. For instance, differences in personality traits may influence performance on facial expression recognition. In our study (non-)users of OC did not differ significantly from each other in terms of cognitive vulnerability to depression, positive and negative affect and personality traits, except for Agreeableness. High scorers on Agreeableness in NEO-FFI tend to be compassionate and cooperative rather than suspicious and hostile (Hoekstra et al, 1996). This positive attitude towards our fellow man may result that one is also less likely to recognize antagonistic facial expressions. However, after controlling for Agreeableness (which may actually

be overcorrection, if OC have an effect on both Agreeableness and emotion recognition), OC-users still recognized significantly fewer expressions of anger.

Hormonal contraceptive pills are used by approximately 40% of the Dutch women between 18 and 45 years. In the present sample of university students, the rate was 66%. Given the fact that students frequently serve as participants in psychological research, these subtle psychological consequences may act as confounders in many studies on social behavior and cognition. Although oral contraceptives have been studied quite extensively, little of this research has been directed towards the potential psychological consequences resulting from use (Cobey & Buunk, 2012). In view of the extensive research on the influence of fluctuations of ovarian hormones across the menstrual cycle on social behavior and cognition, the paucity of research on similar effects of OC is surprising. For instance, young naturally cycling women recognize facial expressions of emotion better during their follicular than the luteal phase (Derntl et al., 2008). Recently it was found that the partners of women who started using OC at relationship formation have less masculine faces than the partners of women who did not start using OC (Little et al., 2013).

The most frequently used OC contain a synthetic progestin and an estrogen. These synthetic hormones inhibit the rise of estrogen and maturation of the ovarian follicle that occurs during the follicular phase of the menstrual cycle by altering the hypothalamic-pituary-ovarian feedback loop (Rivera et al., 1999). Furthermore, the rise of the progesterone level after ovulation is mainly caused by the empty follicle. Hence, in pill-taking women the endogenous levels on both estrogen and progesterone are lower compared with naturally cycling women (Fleischmann et al, 2010). Possibly, the suppressing effect of OC on endogenous female hormones mimics a condition in which the higher social sensitivity that females experience during their follicular phase is not needed, since users of OC recognized fewer facial expressions of disgust, fear and anger. In the follicular phase, social interaction needs to be facilitated which may favorably influence mating behavior as well (Derntl et al., 2008). Since we neither registered menstrual cycle phase nor collected hormonal measures, we were not able to explore the association between endogenous female hormones on our findings.

We also observed a possible effect of OC on the speed of recognition of sadness. Although the effect was non-significant in non-parametric testing, similar effects have been found in naturally cycling women: women in their luteal phase recognized sadness faster in a working memory task for emotional facial expressions (Gasbarri et al, 2008). The luteal phase is characterized by a lower level of estrogen. Estrogens mediate signaling cascades in individual hippocampal neurons (Wu et al., 2011), which suggests functional consequences of estrogen levels for these central limbic structures. A multimodal, translational study in female humans and rodents revealed that both endogenous and exogenous estrogens improve the ability to retain extinguished fear memories by modulating neuronal activity and synaptic plasticity in the ventromedial prefrontal

cortex and amygdala - regions involved in fear expression and extinction in both species (Zeidan et al., 2011). These effects were not found in a study on the effects of progesterone on fear extinction or its recall (Milad et al, 2010). Furthermore, a placebo-controlled fMRI study in women with previous OC-induced mood deterioration who reinitiated OC-use revealed that OC affected amygdala habituation in an emotion processing task (Gingnell, 2013). Hormonal contraceptives may also affect brain structure, including areas involved in face processing and emotional memory (Pletzer et al., 2010; De Bondt et al., 2013).

As our study was not aimed primarily at assessing the influence of OC on emotional information processing, we ask for replication of our findings. Future studies should not only investigate bigger sample sizes, but should also register menstrual cycle phase, OC-type and duration of use. Last but not least, interpersonal differences in sensitivity to side-effects of OC need closer investigation.

Chapter 3

Mineralocorticoid receptor haplotype, oral contraceptives and emotional information processing

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Abstract

Background

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

Aim

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

Methods

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

Results

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

Limitations

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

Conclusions

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

Introduction

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

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

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

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

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

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

More specifically, the MR seems to be involved in the regulation of initial psychological stress reactions like vigilance, selective attention, emotional expressions and formation of emotional memory (Otte et al., 2007). This is consistent with the fact that the MR is located especially in limbic structures such as the subgenual anterior cingulate cortex, amygdala and hippocampus, which are crucial for processing of stressful information (De Kloet, 2005). In depressed individuals, MR expression is approximately 30% lower in the hippocampus, inferior frontal gyrus and cingulate gyrus than in non-depressed controls (Klok et al., 2011b). Recent studies have revealed that MR haplotype 2 is associated with higher dispositional optimism, fewer thoughts of hopelessness during sad moods and lower risk of depression. These effects are restricted to premenopausal women suggesting a moderating effect of female gonadal steroids on the function of the MR (Klok et al., 2011b). The observed differences in sensitivity between different MR-haplotypes may also explain why some women experience depression-like side-effects of OC whereas others do not (Boron & Boulpaep, 2012; Oinonen & Mazmanian, 2002).

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

Experimental procedures

Participants

Eligible participants were women of North-Western European origin with a regular menstrual cycle (between 25 and 35 days). Age limits were between 18 and 35 years. Users of hormonal contraceptives other than hormonal pills were excluded. Further exclusion criteria were pregnancy or lactation, dyslexia, alcoholism, habitual smoking, a history of regular use of (hard) drugs including XTC and cannabis and use of medication likely to interfere with the study (e.g. antidepressants, St John's Wort, benzodiazepines, ADHD medication). Participants were recruited at various sites at Leiden University. All participants provided written informed consent before the start of the study and received course credit or 15 euro. The study was approved by the Ethics Committee Psychology of Leiden University (CEP 7099926055).

Design and procedure

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

Instruments

Clinical characteristics

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

Information processing tests

Facial expression recognition task (FERT)

The FERT displays five basic emotions taken from the Pictures of Facial Affect Series (Ekman and Friesen, 1976; see figure 1). Male and female examples of these pictures expressing happiness, sadness, fear, anger and disgust were morphed at ten intensity levels (10–100%). This morphing procedure involved taking a percentage of the shape and texture differences between the two standard images 0% (neutral) and 100% (full emotion) in 10% steps (Young et al., 2007). Each face was also given in a neutral expression, resulting in a total of 204 stimuli that were presented in a randomized order for 500 ms and replaced by a blank screen. Participants were asked to respond as quickly and accurately as possible by pressing one of the buttons of a response box corresponding with the perceived facial expression: anger, sad, fear, happy, disgust or neutral.

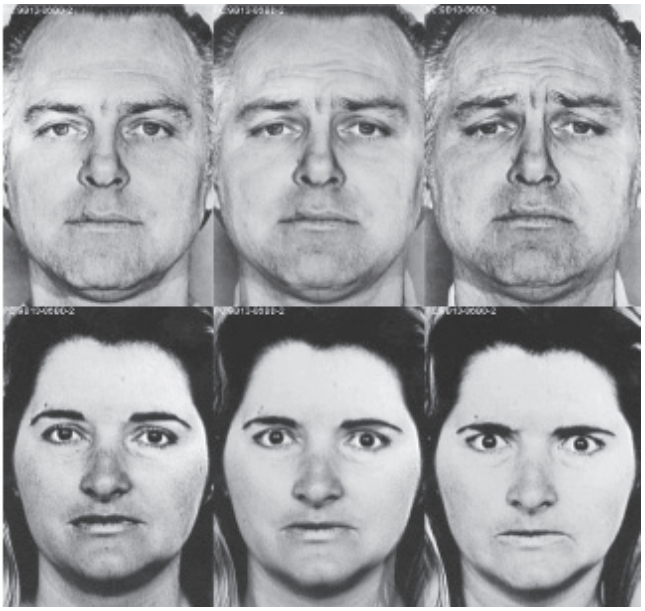

Figure 1. Sample stimuli form Ekman's facial expression dataset

Word categorization and memory task (WCMT)

The WCMT holds sixty personality characteristics, that are generally rated as either disagreeable (e.g. domineering, untidy, hostile) or agreeable (e.g. cheerful, honest, optimistic) (Anderson, 1968). All characteristics were presented on a computer screen for 500 ms. Disagreeable and agreeable words were matched in terms of word length, ratings of frequency used and familiarity ('meaningfulness'). Participants were asked to classify as quickly as possible whether they would

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

Decision-making task (DMT)

The DMT (Rogers et al., 2003) is an adaptation of the Iowa Gambling Test and consists of 80 trials. Each participant started with 100 points (eurocents) and in each trial she was asked to choose between two simultaneously presented options: a control and an experimental gamble. In the control gamble, participants had a 0.5 probability of winning or losing ten points (the 'safe' option). Experimental gambles came in eight types, that vary in the probability of winning (.4 vs .6), the magnitude of the expected gains (30 vs 70 points) and the magnitude of the expected losses (30 vs 70 points). Additionally, eight 'wins or losses only' trials were presented: in these conditions the participant chose between a certain loss (or win) of 30 points and a gamble with a .5 probability of losing (or winning) nothing and .5 probability of losing (or winning) 60 points. The order of trials was randomized within four blocks of 20 trials, yielding each trial type twice per block. The aim of the test was to gain as many points as possible and participants were to keep the amount gained at the end of the test (on average between €1 and €3).

Genotyping

DNA isolation

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

PCR amplification

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

were used: a forward primer (5'-GGAGGSCTGGAAATTGAGGA–3') and a reverse primer (5'- CGACAAGCTGTAGTCAATACTC-3'). The PCR reactions contained 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 2.5U of Paq5000 DNA polymerase (Agilent Technologies) in a total volume of 30 μl using the following cycling conditions: initial denaturation step of 4 min at 95°C, followed by 40 cycles of 30 sec 94°C, 30 sec 50°C, 120 sec 72°C and a final extension step of 10 min 72°C.

Analysis of the rs2070951 and rs5522 polymorphisms

To determine the rs2070951 and rs5522 polymorphisms, PCR fragments were sequenced using the forward primers (5'-GTTCCYTAGATTCCAGCTCAG-3') respectively (5'-AGAGGAGTTCCCTGGGTGAT-3') and dye terminator chemistry (BigDye v3.1, Applied Biosystems). Sequence reactions were run on an ABI-3730 automated sequencer and sequence data was analysed using SeqScape software (Applied Biosystems). According to the observed frequency in the population (DeRijk et al., 2008), MR-haplotype 1 (GA) was constituted by MR-2 G and MRI180V A, MR-haplotype 2 (CA) by MR-2 C and MRI180V A, MR-haplotype 3 (CG) by MR-2 C and MRI180V G and the in vivo seldom observed MR-haplotype 4 (GG) by MR-2 G and MRI180V G.

Statistical analyses

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

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

Outcomes on the EMT were analysed with valence (positive vs. negative characteristics) as within- subject factors and correct and intrusive recall as measures. The dependent variables of the DMT were the number of trials in which participants chose the experimental (risky) gamble. We applied analyses of variance to control for the effects of OC use and (interaction with) MR haplotype. Since the DMT scores remained significantly different from normal distribution after log-, arcsine- and Poisson-transformation, we applied non-parametric tests (Mann-Whitney U tests) subsequently.

Results

Participant characteristics

Hundred and thirty-three volunteers signed up, 26 of whom withdrew before inclusion and 19 of whom did not meet inclusion criteria. Eighty-five women took part, 44 of whom were using OC. Forty-one NC volunteers were included, of whom eleven were tested in the follicular phase (day 6 – 14) and 29 in the luteal phase (day 15 - 26) of the menstrual cycle. Since there were few homozygous MR-haplotype 2 carriers in both groups (see table 1), we compared all homo- and heterozygous MR-haplotype 2 carriers with carriers of MR-haplotypes 1 or 3. MR-haplotypes (2 vs. 1/3) were equally distributed across users and non-users of OC (χ^2 = 1.872; df = 1; p =.171).

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

Table 1. Participants' characteristics

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

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

Table 2. Type of OC used

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

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

Facial expression recognition task

Accuracy

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

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

Reaction times

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

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

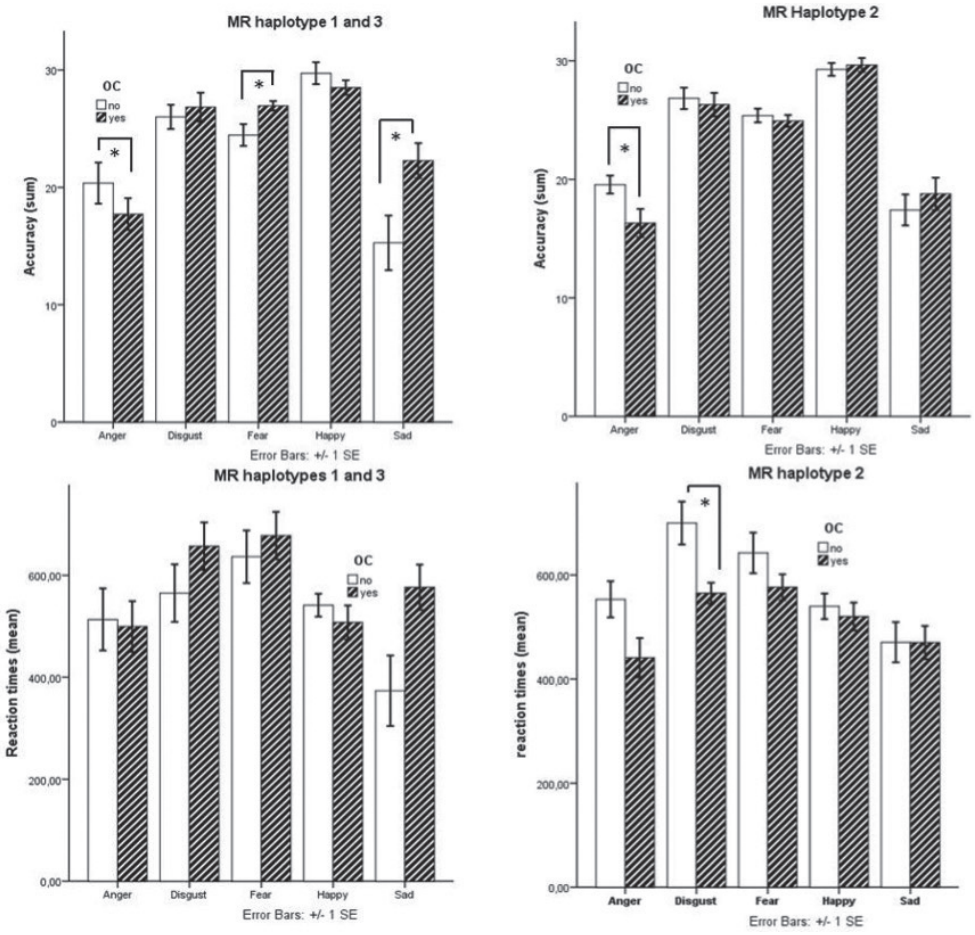

Figure 2. The impact of OC use on emotion recognition (top panels) and reaction times (bottom panels) in MR-haplotypes 1 and 3 (left panels) and haplotype 2 (right panels); * $p < .05$.

Word categorization and memory task

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

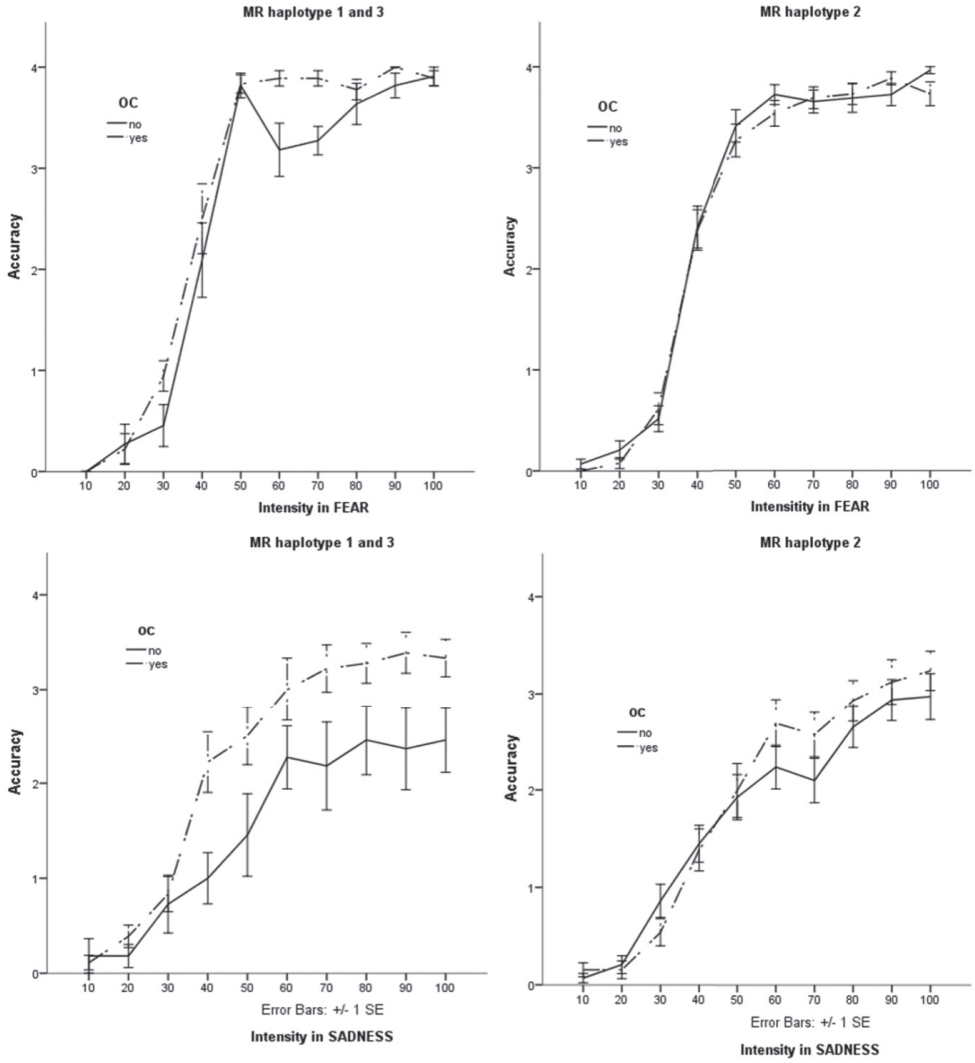

Figure 3. Recognition of fear and sadness by carriers of haplotype 1/3 (left panel) and haplotype 2 (right panel); * $p < .05$.

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

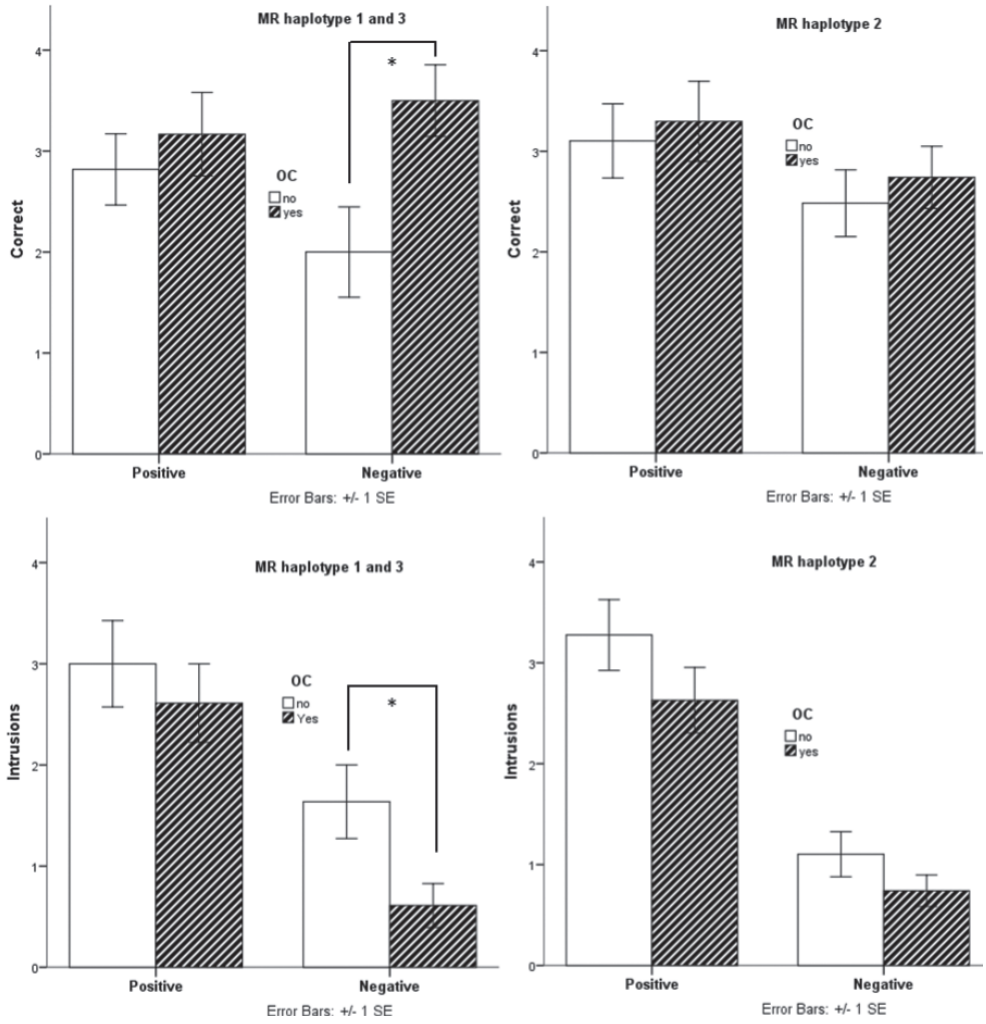

Figure 4. Influence of OC use on correctly recalled (top panels) and intrusive characteristics (bottom panels) in carriers of MR haplotype 1 or 3 (left panel) and haplotype 2 (right panel); * $p < .05$.

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

Decision-making task (DMT)

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

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

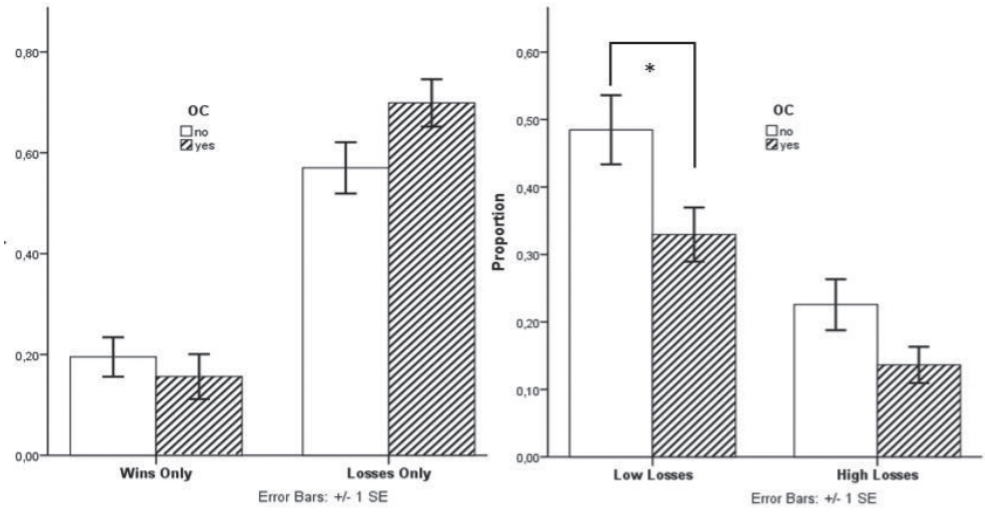

Figure 5. Proportion of choice for the experimental gamble in the wins- and losses-only conditions (left panel) and in conditions with a small chance to gain a large amount in the context of low or high potential losses (right panel); * $p < .05$.

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

Chapter 4

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

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Abstract

Rationale

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

Objectives

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

Methods

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

Results

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

Conclusion

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

Introduction

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

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

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

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

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

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

Experimental procedures

Study population

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

Design and procedure

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

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

Instruments

Clinical characteristics

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

Mineralocorticoid haplotype (MR-haplotype)

Analysis of the rs2070951 and rs5522 polymorphisms.

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

DNA isolation

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

PCR amplification.

The rs2070951 and rs5522 regions were amplified by PCR using the following primers: a forward primer (5'- GCTGGAAACAGAGCACCTTG -3') and a reverse primer (5'-GCAAGCCACCCACTTCACTA-3'). Typical PCR reactions contained between 10 to 100 ng genomic DNA template, 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 2.5U of Paq5000 DNA polymerase (Agilent Technologies)

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

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

Emotional cognition

Implicit affect

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

Facial expression recognition task (FERT)

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

Emotional working memory

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

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

Decision-making task (DMT)

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

Reading the mind in the eye task (RMET)

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

Statistical analyses

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

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

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

Results

Participants

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

Emotional cognition

Implicit positive and negative affect

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

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

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

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

Facial expression recognition

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

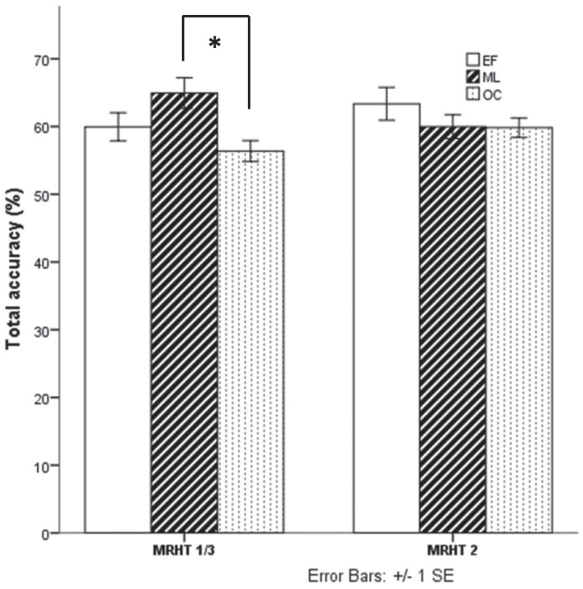

Figure 1. MR-haplotype, hormonal status and facial expression recognition scores.
Error bars represent ± 1 SE; MRHT = Mineralocorticoid receptor haplotype; * = $p < .05$

Emotional working memory

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

Decision-making task

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

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

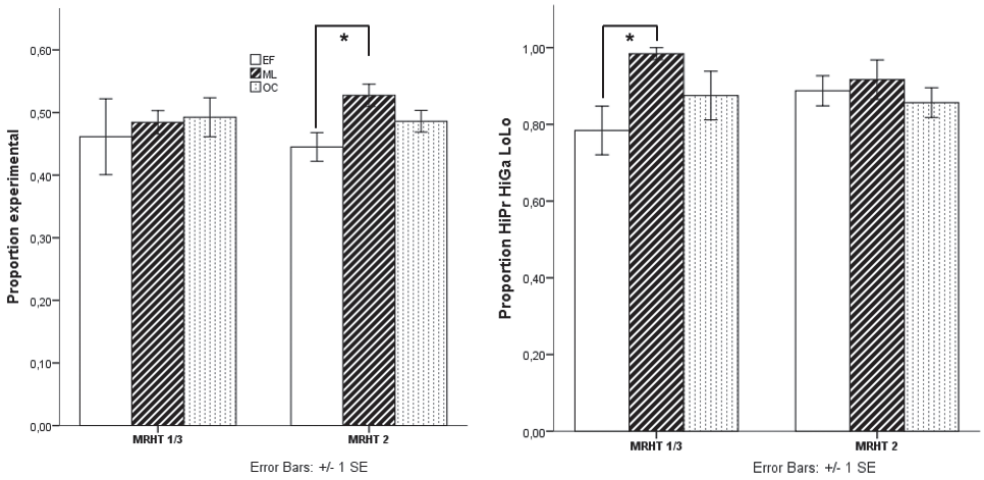

Figure 2. MR-haplotype, hormonal status and gambling behavior in all trials (left panel) and in the most favorable gambling conditions (high probability to win high gains risking low losses; right panel). *Notes*: Error bars represent ± 1 SE. *Abbreviations*: MRHT = Mineralocorticoid receptor haplotype;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

Reading the mind in the eye task

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

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

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

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

Discussion

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

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

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

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

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

Chapter 5

Mineralocorticoid receptor haplotype, estradiol, progesterone and emotional information processing

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Abstract

Background

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

Method

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

Results

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

Conclusion

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

Introduction

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

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

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

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

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

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

Methods

Design

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

Participants

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

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

Clinical characteristics

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

Procedure

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

Biological measures

Hormonal assessment

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

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

Mineralocorticoid haplotype (MR-haplotype)

Analysis of the rs2070951 and rs5522 polymorphisms.

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

DNA isolation.

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

PCR amplification.

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

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

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

Test battery

Implicit affect

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

Word categorization and memory task (WCMT)

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

Attentional blink

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

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

Facial expression recognition task (FERT)

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

Reading the mind in the eye task (RMET)

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

Risky decision-making task (DMT)

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

Statistical analyses

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

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

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

Results

Participant characteristics

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

Table 1. Sample characteristics

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

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

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

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

Abbreviations: E2=estradiol; P4=progesterone.

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

Hormonal measures

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

Test battery

Implicit positive and negative affect

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

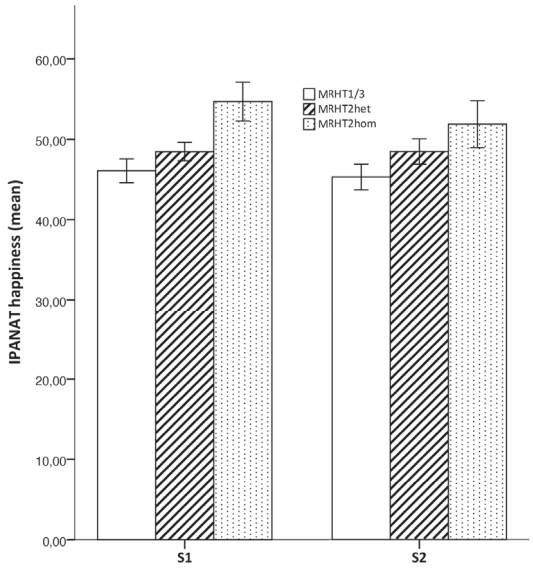

Figure 1. Implicit happiness scores by MR-haplotype.
Notes: Error bars: +/- 1 SE. *Abbreviations:* MRHT = mineralocorticoid receptor haplotype; S1 = session 1; S2 = session 2; IPANAT = implicit positive and negative affect.

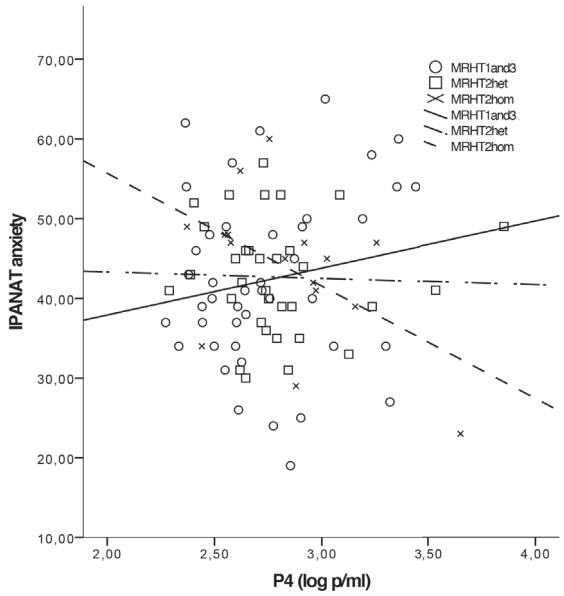

Figure 2. Progesterone (P4) correlations with implicit anxiety scores (session 1).
Abbreviations: MRHT = mineralocorticoid receptor haplotype; IPANAT = implicit positive and negative affect.

Word categorization and memory task

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

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

Attentional blink

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

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

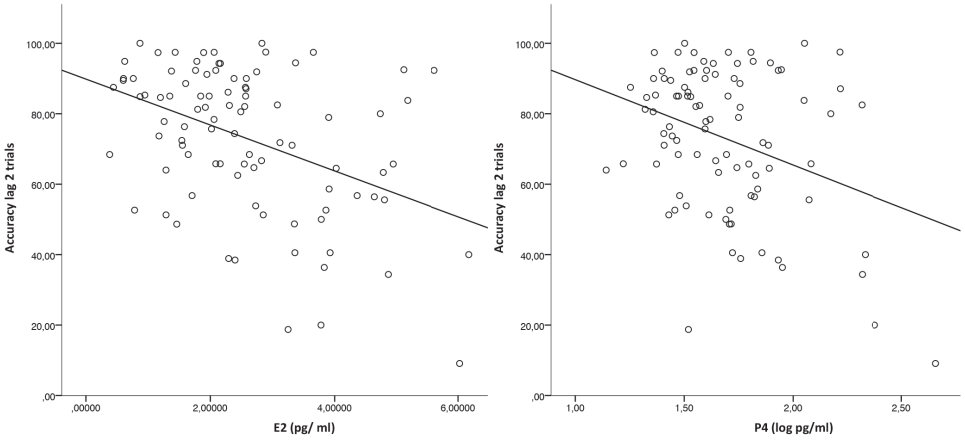

Figure 3. Estradiol (E2) and progesterone (P4) correlations with performance in lag 2 trials (session 2)

Facial expression recognition test

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

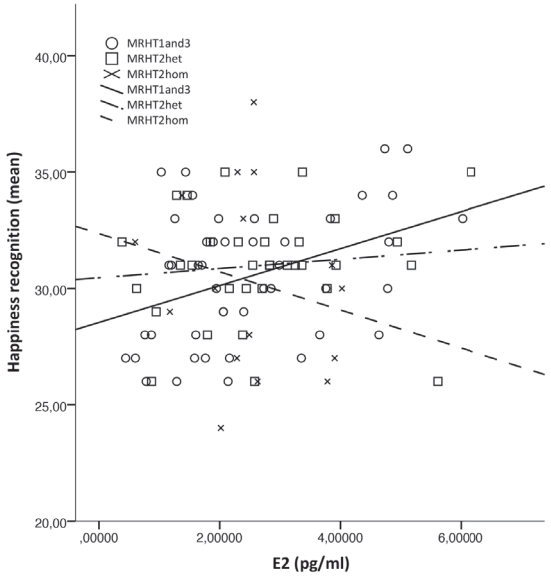

Figure 4. Estradiol (E2) correlates with happiness recognition accuracy (session 2)
Abbreviations: MRHT = mineralocorticoid receptor haplotype

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

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

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

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

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

Reading the mind in the eye test

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

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

Risky decision-making task

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

Table 3. Mean outcomes (SE)

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

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

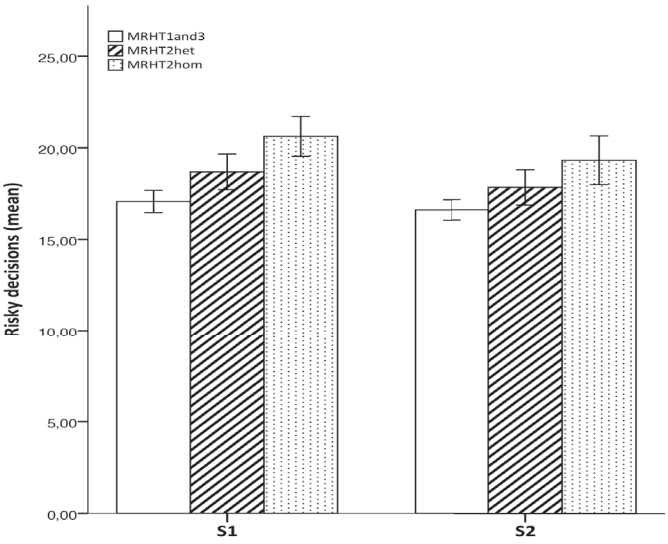

Figure 5. Risky decision-making in trials with low losses by MR-haplotype.
Notes: Error bars: +/- 1 SE. *Abbreviations:* MRHT = mineralocorticoid receptor haplotype; S1=session 1; S2 =session 2;

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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.

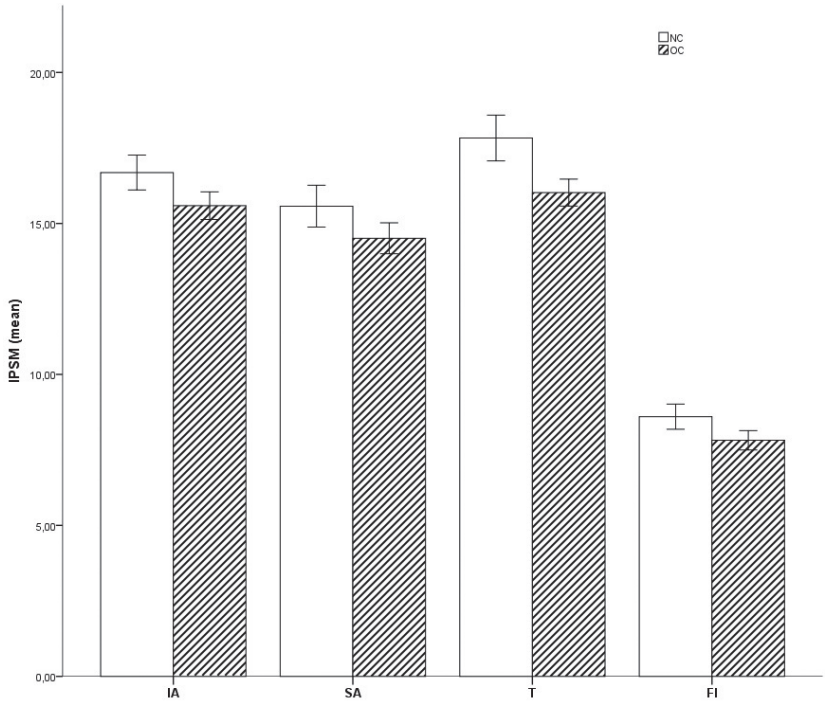

Figure 1. Overall mean scores on interpersonal sensitivity per subscale of NC women compared with OC-users. *Notes.* Error bars represent ± 1 SE. *Abbreviations.* NC = naturally cycling women; OC = oral contraceptives users; IPSM = interpersonal sensitivity measure; IA = interpersonal awareness; SA =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

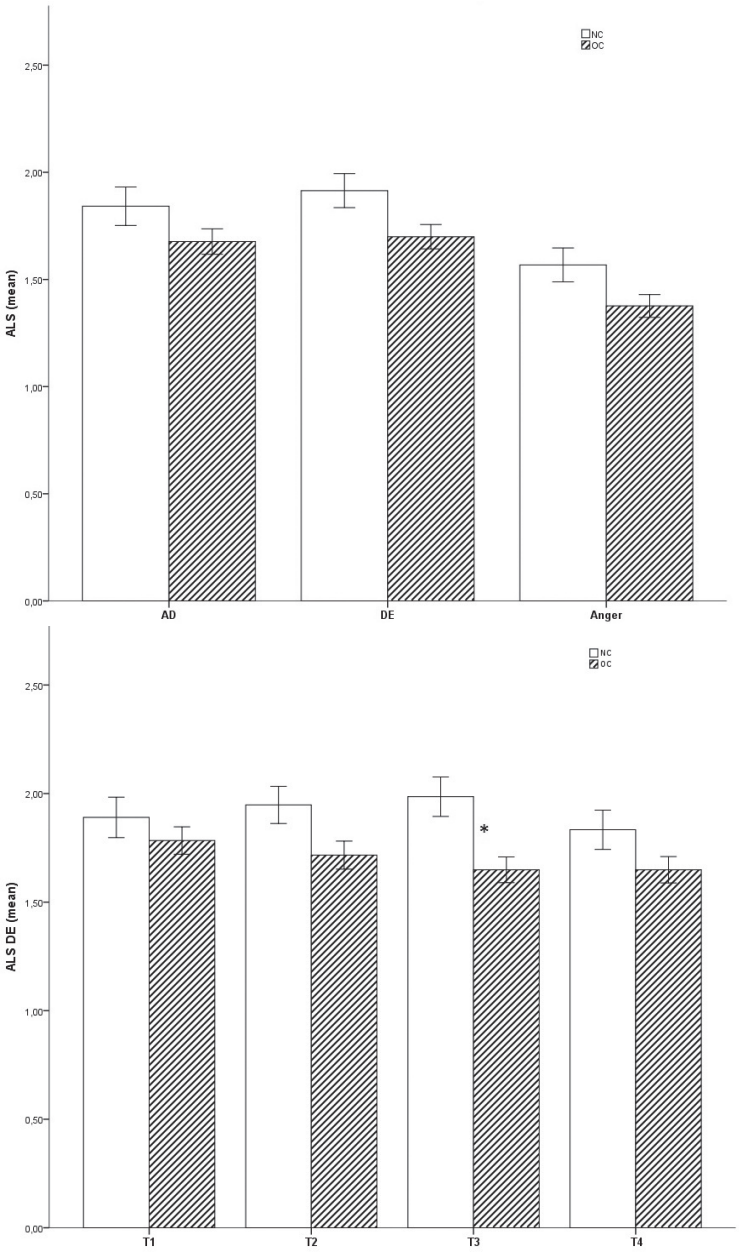

Figure 2. Affect lability scores. *First panel*: overall mean affect lability scores of NC women compared to OC-users. *Second panel:* Scores on mood shifts between depression and elation per time point of NC women compared to OC-users. *Notes.* * p < .05; Error bars represent ± 1 SE. *Abbreviations.*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

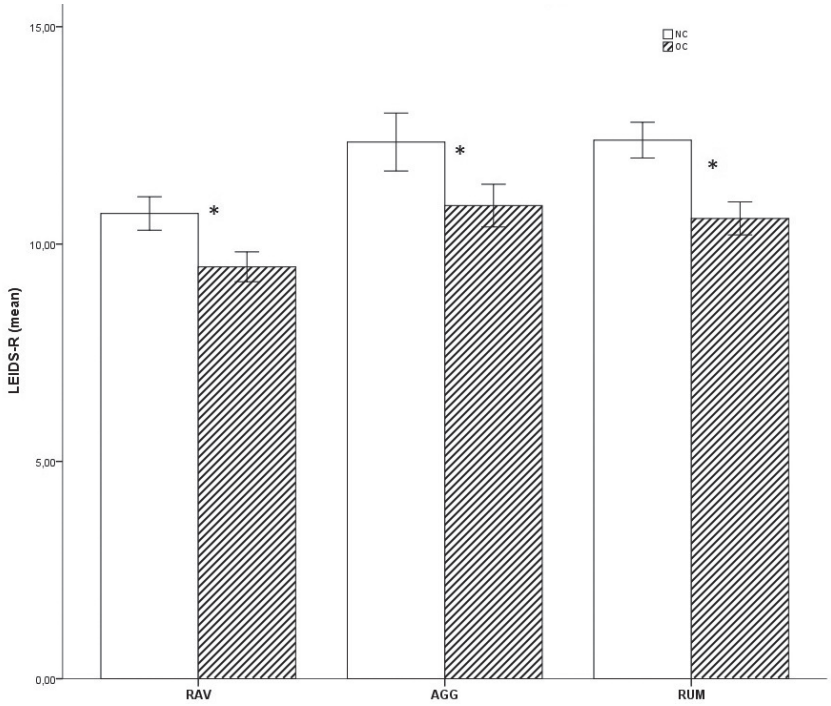

Figure 3. Overall mean scores on depression-congruent cognitions.

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).

Chapter 7

The role of the mineralocorticoid receptor haplotype and oral contraceptives use in resting state EEG theta/beta ratio

Under review

Abstract

Objective

Resting-state electroencephalography (rs EEG) theta/beta ratio (TBR) is a biomarker of cognitive control over the affective system. Theta power may be associated with prefrontal cortex (PFC)-mediated goal-directed behavior and beta power with arousal. Female hormones modulate cortical-subcortical neural circuits. We examined whether users of oral contraceptives (OC) and naturally cycling (NC) women differ in TBR. Since the mineralocorticoid receptor (MR) haplotype moderates the influence of the female hormonal status on emotional information processing, we also investigated whether any effect of OC-use on rs EEG TBR was moderated by MR-haplotype.

Method

Frontal and parietal rs EEG recordings were acquired in 44 OC-users and 44 NC women in a counterbalanced mixed within-subject design. At both sessions estradiol and progesterone concentrations were assessed.

Results

OC-users and NC women did not differ in rs EEG TBR ($p > .58$). TBR was different between MR-haplotypes ($p = .022$). MR-haplotype 2 homozygotes had lower TBR scores than MR-haplotype 2 heterozygotes ($p = .006$).

Conclusion

Genetic variation associated with enhanced MR expression may modulate regulation of arousal in females irrespective of oral contraceptive use or menstrual cycle phase. Lower parietal TBR in MR-haplotype 2 homozygotes may reflect improved PFC-mediated effortful control and resilience to stressful events.

Introduction

Emotion regulation involves the control of emotional responses to external stimuli or internal mental representations by regulating their occurrence, intensity, and expression (Ochnsner & Gross, 2005). Neural systems involved in emotion regulation comprise cortical and subcortical regions. A cortical regulatory network involving the prefrontal cortex (PFC) and the anterior cingulate cortex (ACC), exerts a top-down influence on an emotional appraisal system residing in subcortical structures, including the amygdala (Ochsner et al., 2012).

The connectivity and plasticity of the limbic-cortical circuits is regulated by sex- and stress hormones (Arnsten & Rubia, 2012; Sacher et al., 2013). Estrogen and progesterone receptors, which mediate these central actions of the sex steroids, are expressed amongst others in the PFC, the amygdala, and the hippocampus (McEwen et al, 2012). Accordingly, estrogen and progesterone enhance cortical-subcortical communication, which may contribute to improved emotion regulation [for a review, see Peper et al., 2011; van Wingen et al., 2011). For instance, estrogen contributed to higher resting-state connectivity between the amygdala and PFC regions (Ottowitz et al., 2008; Engman et al., 2016) and progesterone increased functional connectivity between the amygdala and dorsal ACC in response to emotional faces (van Wingen et al., 2008). OC, however, contain synthetic versions of estrogen and progesterone that suppress endogenous estrogen and progesterone levels (Fleischman et al., 2010). So, OC-use may influence neural circuits underlying emotion regulation and has also been associated with modified emotion recognition (Hamstra et al., 2014, 2015, 2016, 2017a, 2017b, Osorio et al., 2018). A prospective resting-state fMRI study showed that OC-use contributed to a negative connectivity between the amygdala and the dorsolateral PFC (Lisoksky et al., 2016). In addition, both menstrual cycle phase and OC-use have been related to variation in the default mode and executive control networks (Petersen et al., 2014). Literature on how resting-state networks are affected by the female hormonal status is still limited, however (Engman et al., 2018).

Resting-state (rs) theta/beta ratio (TBR) is an electroencephalographic (EEG) marker suggested to represent cortical regulation over subcortical affective systems (Schutter & van Honk, 2005; Knyazev, 2007) and has repeatedly been found to be associated with executive control over threat-related responses in healthy individuals, cross-sectionally and with a one-week predictive interval (Putman et al., 2010; Angelidis et al., 2016). TBR predicted interpersonal differences in (mal)adaptive emotional information processing after exposure to stimuli with different threat levels (Putman et al., 2014; Angelidis et al., 2018; van Son et al., 2018ab) and results from dividing the slow-wave theta power (4-7 Hz) by the fast-wave beta power (13-30 Hz). Theta may stem from emotion-oriented and evolutionary old subcortical structures such as the amygdala and has been associated with goal directed behavior and motivational sensitivity (Knyazev, 2007;

Siegel & Sapru, 201; Massar et al., 2014; Cavanagh & Shackman, 2015). Beta may originate from more complex cognitively-oriented cortical structures (Knyazev & Slobodskaya, 2003) and is associated with higher-order cognitive processes (Singer, 1993; Hofman et al., 2013). Hence, cortical control over subcortical systems may be reduced if theta dominates over beta, resulting in an increased TBR (Schutter & van Honk, 2005; Putman et al., 2014), which in turn may impair down-regulation of negative emotions like anxiety (Tortella-Feliu et al., 2014) and relates to inhibited attentional control (Angelidis et al., 2016). Theta and beta power correlated negatively with progesterone levels (Becker et al, 1982) and differed between menstrual cycle phases (Creutzfeld et al., 1976; Solis-Ortiz et al., 1994). Accordingly, OC-use may influence TBR: an effect which, as far as we know, has not been investigated yet.

Female hormones also act upon the mineralocorticoid receptor (MR) (Handa & Weiser, 2014). MRs and glucocorticoid receptors (GRs) are crucial for the regulation of the hypothalamic-pituitary-adrenal (HPA) axis by mediating the actions of cortisol (Joels et al., 2008). MR synthesis in the brain is suppressed by estrogen (Carey et al., 1995). Progesterone is a competitive antagonist of the MR, because it binds to the MR with almost the same affinity as cortisol and aldosterone (Carey et al., 1995; Quinkler et al, 2002; Handa & Weiser, 2014). The MR is abundantly expressed in the hippocampus and amygdala and also in the PFC (Joels et al., 2008; De Kloet et al, 2005). Pharmacological and genetic studies have shown that 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 (De Loet et al, 2005; Cornelisse et al., 2011; Henckens et al., 2012).

Genetic variation in the MR was found to be associated with behavior, autonomic function and neuroendocrine responses to stress (DeRijk et al., 2006). The most widely studied MR haplotype is based on two common functional single nucleotide polymorphisms (SNP's): the rs5522 (A/G) is located in codon 180 of exon 2 and causes an amino acid change from isoleucine (ATT) to valine (GTT), and rs2070951 (G/C) located in the MR promoter region two nucleotides before the translation start site (DeRijk et al., 2006; van Leeuwen et al, 2010, 2011). Three common MR-haplotypes were identified: haplotype 1 (GA) frequency ~ 49 %, haplotype 2 (CA) frequency ~ 42%, and haplotype 3 (CG) frequency ~ 9% and a very rare haplotype 4 (GG) (DeRijk et al., 2006). *In vitro* in a cell line MR-haplotype 2 showed the highest transcriptional, translational and transactivational activity of the MR-gene variants (DeRijk et al., 2006, 2007; van Leeuwen et al., 2010, 2011; Kumsta et al., 2018). Female carriers of MR-haplotype 2 reported higher dispositional optimism, less rumination and fewer thoughts of hopelessness, and were also less vulnerable to depression than MR-haplotype 1/3 carriers and following childhood maltreatment (Klok et al., 2011; Vinkers et al., 2015; Hamstra

et al., 2017a). MR promotes the stress-induced shift from cognitive towards 'habitual' learning (Schwabe et al., 2010, 2013), particularly in MR-haplotype 2 carriers (Wirz et al., 2017).

We have previously reported that these MR-haplotypes moderate the influence of the menstrual cycle phase, OC- use, estrogen and progesterone on emotional information processing, as assessed with both behavioral measures and self-reports (Hamstra et al., 2015, 2016, 2017a, 2017b). Psychophysiological studies, however, show effects related to cortical arousal and may reveal subtle changes in information processing that may remain unnoticed in self-reported arousal or behavioral studies. In sum, the aim of the present study was to examine rs EEG TBR in healthy OC-users and naturally cycling women. We also investigated whether any effect of OC-use and levels on estrogen of progesterone was moderated by MR-haplotype. The same counterbalanced within-subject design was applied as in our earlier behavioral study (see Hamstra et al., 2017a).

Materials and methods

Participants

Data was collected from May 2016 till April 2017 at Leiden University. Participants were healthy, Dutch-speaking, right-handed female students of Northwestern European origin, aged between 18 and 35 years. Participants were recruited at the local university campus and through social media. All participants provided written informed consent and received €50 or course credits for participation. The study was approved by the Psychology Research Ethics Committee at Leiden University (approval number: CEP16-0318/139).

Inclusion and exclusion criteria

In this study we recruited a new sample of 106 women, of whom 60 were using oral contraceptives (OC) and 46 had a natural menstrual cycle (NC). OC-users used second generation monophasic OC containing Ethinylestradiol (EE; 0.03 mg)/ Levonorgestrel (LNG; 0.15 mg) and applied a pill-free week. Only NC women with a regular menstrual cycle between 25 and 35 days were included. All participants were in their current hormonal status for three months or longer. Participants were excluded in case of pregnancy, lactation, and use of abortifacients or morning-after pill, current physical or psychological illness. Additionally, participants were excluded when screened positive for psychiatric present and past by the MINI International Neuropsychiatric Interview (van Vliet & de Beurs, 2007) or premenstrual syndrome [as determined by the Menstrual Distress Questionnaire (MDQ; Moos, 1968). Lastly, participants were excluded in case of excessive alcohol use (> 14 units per week), smoking, regular soft- or hard-drug use, and use of prescribed medication.

Procedure

Design

This study had a within-subject counterbalanced design. OC-users and NC women were tested in a counterbalanced entry-order. OC-users were tested during active OC-use (day 8–14), and during the pill-free week (day 4–7). NC women were tested during the early follicular phase (day 2–6), when both estrogen and progesterone levels are low, and in the mid-luteal phase (3–10 days prior to onset of the new cycle), when progesterone levels are at its maximum and E2 reaches a second peak (Jones et al., 2012). Information on the onset of the next cycle – day 1 of the new pill-strip for OC-users, start of menses for NC participants – was used to confirm whether participants had been tested at the right moment.

Clinical characteristics

Vulnerability for depression was assessed with the revised version of the Leiden Index of Depression Sensitivity (LEIDS-R; (van der Does, 2002). Explicit affect was assessed with the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988). Implicit affect was assessed with the Implicit Positive and Negative Affect Test (IPANAT; Quirin et al., 2009). Personality traits were assessed with the NEO-Five Factor Inventory (NEO-FFI; McCrae & Costa, 1987).

Biological measures

Hormonal assessment

Estradiol (E2) and progesterone (P4) were assessed in saliva, collected at three time points during the experiment with at least a 30 min interval (see 2.5). In order to control for pregnancy, the estriol level in saliva was assessed as well. Participants were not allowed to eat or chew gum 30 minutes prior to participation nor to drink coffee before participation. After rinsing their mouth with water, participants directly expectorated 1ml of saliva into a sterile tube (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. Reference values of free estradiol (E2) in saliva were: follicular phase 0.2–10.4 pg/ml; ovulation 5.8–21.2 pg/ml; luteal phase 0.8–10.8 pg/ml. For free progesterone (P4) in saliva: follicular phase 28–82 pg/ml; luteal phase 127–445 pg/ml; post-menopause and OC: 18–51 pg/ml.

Mineralocorticoid (MR)-haplotype

Analysis of the rs2070951 and rs5522 polymorphisms.

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

DNA isolation.

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

PCR amplification.

The rs2070951 and rs5522 regions were amplified by PCR using the following primers: a forward primer (5′-GCTGGAAACAGAGCACCTTG-3′) and a reverse primer (5′-GCAAGCCACCCACTTCACTA-3′). Typical PCR reactions contained between 10–100 ng genomic DNA template, 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 2.5U of Paq5000 DNA polymerase (Agilent Technologies) in a total volume of 30µl using the following cycling conditions: initial denaturation step of 4 min at 95C, followed by 40 cycles of 30 s 94°C, 30 s 50°C, 120 s 72°C and a final extension step of 10 min 72°C. After the first PCR 1ul of the amplification product was used directly in a second PCR amplification with nested primers. The following primers were used: a forward primer (5′ GGAGGSCTGGAAATTGAGGA-3′) and a reverse primer (5′-CGACAAGCTGTAGTCAATACTC-3′). The PCR reactions contained 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 2.5U of Paq5000 DNA polymerase (Agilent Technologies) in a total volume of 30µl using the following cycling conditions: initial denaturation step of 4 min at 95°C, followed by 40 cycles of 30 sec 94°C, 30 sec 50°C, 120 sec 72°C and a final extension step of 10 min 72°C. According to the observed frequency in the population (DeRijk et al., 2006, 2008), MR-haplotype 1 (GA) is composed by MR-2 (G) and MR-I180 V (A), MR-haplotype 2 (CA) by MR-2 (C) and MR-I180 V (A), MR-haplotype 3 (CG) by MR-2 (C) and MR-I180 V (G) and the in vivo seldom observed MR-haplotype 4 (GG) by MR-2 (G) and MR-I180 V (G).

EEG measures

Recording and software

EEG was recorded from 15 Ag/AgCl scalp electrodes (F_3, F_Z, F_4, C_Z, CP_1, CP_Z, CP_2, P_3, P_1, P_Z, P_2, P_4, PO_3, PO_4, O_Z) placed in accordance with the International 10/20 System. Scalp electrodes were referenced online to CMS/DRL positions and mastoid electrodes were used as offline reference channels. Vertical electro-oculogram (EOG) was recorded from electrodes placed at the supraorbital and the infraorbital ridge of the right eye. Horizontal EOG was recorded from the outer canthi of the left and the right eye. EEG signal was pre-amplified at the electrode to improve the signal-to-noise ratio and amplified with a gain of 16x by a BioSemi ActiveTwo system (BioSemi B.V., Amsterdam).

Resting-state paradigm

RsEEG was recorded for eight minutes, in alternating one-minute trials of eyes open/eyes closed. During eyes open trials, participants were instructed to look at a fixation cross on the computer screen (see also Angelidis et al., 2016).

Procedure

Screening procedure

Interested participants completed an online questionnaire screening for in- and exclusion criteria. Subsequently participants were screened with the MINI by phone interview. Lastly, the average cycle duration of the NC participants was registered. This cycle onset information was used to confirm whether participants had been tested at the right moment.

First session

Participants were seated in a room adjacent to the experimenters' room. All participants read the study's information letter and provided written informed consent and were interviewed for inclusion and exclusion criteria. Consecutively mucus and saliva were collected, and the PANAS, LEIDS-R, NEO-FFI, and IPANAT were administered. After the self-report measures, EEG electrodes were applied by two experimenters. Following another saliva sample, participants underwent an eight-minute resting-state EEG recording. The first session ended after the collection of a third saliva sample.

Second session

Procedures of the second session were identical, except for the absence of mucus collection and assessment of the NEO-FFI and LEIDS-R. Furthermore, at the end of the session, the MDQ was administered and participants were debriefed on the study objectives. Participation ended after confirmation of the start of the new menstrual cycle or pill-strip.

EEG processing

Data processing was performed with Brain Vision Analyzer V2.0.4 (Brain Products GmbH, Germany). Data were sampled at 500 Hz. The data were high-pass filtered at 0.1-Hz, low-pass filtered at 100-Hz low-pass filter, and a 50-Hz notch filter was applied (as in Putman et al., 2014). Ocular correction was done automatically (Gratton & Coles, 1983). Segments containing remaining muscle movements, amplitudes above 200 μV [as in Putman et al., 2014 (25)], or other artifacts were rejected automatically. A fast Fourier transformation with a resolution of 0.25 Hz and a 10% Hamming window length was used to estimate the spectral power density (μV2/Hz) for the frontal (F3, Fz, F4) and parietal (P3, Pz, P4) electrodes in the theta (4-7 Hz) and beta (13-30 Hz) bands.

Following Putman et al. (2010, 2016), all eyes-open/eyes-closed segments were collapsed for the analysis. Frontal power density averages were obtained from frontal electrodes at F3, Fz, and F4 positions. Parietal power density averages were obtained from parietal electrodes at P3, Pz, and P4 positions. Frontal and parietal averages were calculated over both the theta and the beta power density. Non-normally distributed theta and beta power densities from frontal and parietal positions were log-normalized. Frontal and parietal TBR were calculated by dividing the log-normalized theta power density by the log-normalized beta power density (Angelidis et al., 2016).

Statistical analyses

Background variables

OC and NC groups, and MR-haplotype subgroups within the OC and NC groups, were characterized in terms of their average age, cognitive vulnerability to depression (LEIDS-R), personality traits (NEO-FFI), and explicit and implicit affect (PANAS; IPANAT). The distribution of MR-haplotypes over naturally cycling and OC groups was analyzed by chi-square tests.

Analyses of variance (ANOVA)

Outcomes on frontal and parietal TBR, theta, and beta power were analyzed with repeated measures (rm) ANOVAS, with session (1, 2) as a within-subject factor. Between-subject factors were hormonal status (OC, NC) and MR-haplotype (amount MR-haplotype 2 alleles: 0, 1, 2). Counterbalancing-order (AB, BA) was added as a covariate to all (rm) ANOVAS. Significant session effects were reported but not further interpreted (Hamstra et al., 2017a). Univariate outliers on EEG outcomes ($Z \leq -3$ or ≥ 3), influential cases (Cook's distance ≥ 1) and bivariate outliers (Mahalanobis distance significant at $p > .001$) were excluded from analyses (Tabachnik & Fidell, 2013). Effects on MR-haplotype ($p < .05$) were investigated with contrasts. Partial eta squared (η_p^2) and power are reported as estimates of effect size. Effects were tested on a two-tailed alpha of .05.

Moderated regression analyses

We applied the same approach as in our previous study (Hamstra et al., 2017a). We performed moderated regression analyses to explore the correlations between sex steroids (estradiol, progesterone), MR-haplotype on the data from the first and second sessions separately. Dependent variables were frontal and parietal EEG outcomes. Main predictor variables in the regression models were mean-centered sex steroid values (estrogen/ progesterone), MR-haplotype, and corresponding interaction-terms. For reasons of multicollinearity, estrogen (E2) and progesterone (P4) were investigated in separate analyses. Progesterone outcomes were log-transformed. Assumptions of multicollinearity were satisfied when Tolerance was >.01 and VIF < 10. Cases were identified as multivariate outliers and excluded from analyses if Mahalanobis distance was significant at $p > .001$, and if Cook's distance ≥ 1. Regression estimates were deemed statistically different at $p < .05$. Semi-partial correlations (*sr*) were reported as an indication of the unique contribution of the variable to the model (Aiken & West, 1991).

Results

Hormonal measures

Hormonal analyses from the first session failed of one NC MR-haplotype 2 heterozygous participant. Six participants exceeded laboratory reference values and sample mean by 3 *SD*s on progesterone (P4) or estrogen (E2). These participants were included in the remaining analyses, since their estriol levels did not indicate pregnancy. The outcomes were within the expected laboratory pattern (see table 1).

Table 1. Means (SE) hormonal levels per phase.

	E2 pg/ml	P4 pg/ml
Early follicular phase (EF)	3.0 (.3)	80.4 (11.2)
Inactive OC-use (IU)	2.1 (.2)	61.3 (7.2)
Mid-luteal phase (ML)	3.8 (.2)	201.8 (19.9)
Active OC-use (AU)	2.0 (.3)	56.6 (10.0)

Participant characteristics

A total of 455 women showed interest in the study and were screened for eligibility, of whom 107 signed informed consent. After inclusion, 12 participants were excluded due use of medication on prescription (n = 3), valerian (n = 1), soft drugs (n = 1), the abortion pill (n = 1), change of hormonal status after inclusion (n = 2), irregular menstrual cycle > 35 days (n = 1) and scheduling difficulties (n = 3). After participation, seven participants had to be excluded because they had not been tested in the intended cycle phase. Two participants (one OC and one NC participant) were excluded because genotyping failed. No participant was screened positive for premenstrual dysphoric disorder (Moos, 1968). The final sample consisted of 86 participants.

Table 2 displays general characteristics of the sample. Background information (NEO-FFI; PANAS; IPANAT; LEIDS-R) of the first session was lost from one participant due to technical problems. Participant's ages ranged from 18 to 26. Scores on cognitive vulnerability to depression (LEIDS-R) were within a non-depressed range (Solis et al., 2017). There were no statistically significant differences between groups in age, personality traits (NEO-FFI), explicit affect (PANAS), LEIDS-R scores and implicit affect (IPANAT).

Table 2. Participant general background information (n = 85)

	Hormonal status		**NC**			**OC**		
	NC	**OC**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**
N (% total sample)	43 (50)	43 (50)	16 (18.6)	20 (22.3)	7 (8.1)	17 (19.8)	19 (22.1)	7 (8.1)
Age	21.79 (0.29)	22.05 (0.28)	22.06 (0.54)	21.60 (0.44)	21.71 (0.52)	22.00 (0.50)	22.05 (0.40)	22.14(0.59)
NEO-FFI								
Agreeableness	33.95 (0.60)	34.51 (0.61)	34.69 (0.90)	33.95 (0.94)	32.29 (1.55)	33.65 (0.83)	34.89 (0.91)	35.57 (2.00)
Conscientiousness	40.40 (0.48)	40.77 (0.48)	41.19 (0.87)	40.05 (0.60)	39.57 (1.27)	40.18 (0.71)	40.74 (0.79)	42.29 (0.87)
Extraversion	43.31 (0.95)	44.33 (0.69)	44.19 (1.25)	42.68 (1.50)	43.00 (2.99)	44.59 (0.84)	44.00 (1.11)	44.57 (2.34)
Neuroticism	32.74 (1.03)	30.47(1.03)	32.69 (1.57)	33.47 (1.68)	30.86 (2.44)	30.47 (1.24)	30.68 (1.46)	29.86 (4.27)
Openness	33.55 (0.51)	34.09 (0.56)	34.31 (0.85)	32.58 (0.77)	34.43 (0.95)	33.88 (0.90)	34.58 (0.68)	33.29 (2.07)
LEIDS-R								
Total	39.4 (2.34)	38.09 (1.82)	39.69 (3.05)	38.00 (3.72)	42.57 (7.50)	37.29 (1.85)	38.37 (2.92)	39.29 (7.05)
PANAS								
PA S1	24.68 (0.80)	26.60 (.78)	25.06 (1.04)	24.22(1.17)	25.00 (2.81)	26.35 (1.16)	27.32(1.22)	25.29 (2.24)
PA S2	24.29 (0.95)	25.58 (1.04)	24.44 (1.29)	24.28 (1.70)	24.00 (2.07)	25.24 (1.83)	25.32 (1.39)	27.14 (3.01)
NA S1	12.93 (0.52)	12.30 (0.39)	14.31 (0.99)*	11.94 (0.53)	12.29 (1.34)	11.76 (0.63)*	12.89 (0.58)	12.00 (1.00)
NA S2	12.49 (0.34)	12.37(0.35)	13.31 (0.69)	12.11 (1.11)	11.57 (0.53)	11.88 (0.52)	12.74 (0.52)	12.57 (1.13)
IPANAT								
PA S1	47.33 (1.59)	46.35 (1.45)	46.25 (2.52)	45.95 (2.43)	53.57 (3.33)	48.41 (1.67)	45.68 (2.63)	43.14 (3.48)
PA S2	47.21 (1.56)	45.56 (1.62)	47.13 (2.30)	45.21 (2.45)	52.86 (4.00)	46.41 (2.44)	44.53 (2.81)	46.29 (3.06)
NA S1	133.29 (3.39)	134.33 (3.54)	129.50 (4.82)	132.84 (5.81)	143.14 (6.43)	137.12 (5.20)	131.79 (5.95)	134.43 (8.28)
NA S2	132.86 (4.10)	134.23 (3.43)	127.81 (6.82)	135.21 (6.26)	138.00 (9.52)	132.18 (4.75)	132.05 (5.32)	145.14 (10.21)

Notes: N (%) or means (SE). *p <0.05. Abbreviations: OC = oral contraceptive users; NC = naturally cycling women; MRHT 1/3 = mineralocorticoid receptor haplotype 1/3; MRHT 2 het = mineralocorticoid receptor haplotype 2 heterozygotes; MRHT 2 hom = mineralocorticoid receptor haplotype 2 homozygotes; NEO-FFI = NEO Five-Factor Inventory; LEIDS-R = Leiden Index of Depression Sensitivity; S1 = session 1; S2 = session 2; PANAS = Positive Affect Negative Affect Scale; IPANAT = Implicit Positive Affect Negative Affect Scale; PA = Positive Affect; NA = Negative Affect.

Theta beta ratio

Two NC participants had aberrant EEG signals (Z > 3) and were excluded from analyses on TBR scores. Eighty-four participants remained for frontal and parietal analyses. See for mean outcomes on TBR per session Table 3.

Repeated measures ANOVA

Repeated measures ANOVAs with log-normalized TBR as outcome, revealed no significant between-subject effects for hormonal status (OC, NC) on frontal [F (1, 77) = .016; p = .901; η_p^2 <.001; power = .05] or parietal sites [F (1, 77) = .304; p = .583; η_p^2 = .004; power = .09]. A main-effect of MR-haplotype on parietal TBR was observed, however [F (2, 77) = 4.03; p = .022; η_p^2 = .095; power = .70]. Contrast analyses confirmed lower parietal TBR scores in MR-haplotype 2 homozygotes than in MR-haplotype 2 heterozygotes (p = .006) and MR-haplotype 1/3 carriers (p = .052). Although not significant, the same pattern of results was observed on frontal sites [F (2, 77) = .829; p = .441; η_p^2 =.021; power = .19], see figure 1. There were no significant interaction effects between hormonal status and MR-haplotypes on frontal [F (2, 77) = .065; p = .937; η_p^2 =.002; power = .06] or parietal sites [F (2, 77) = .244; p = .784; η_p^2 =.006; power = .09].

Moderated regression analyses on estrogen and progesterone

Regression analyses on TBR scores revealed no significant main or interaction effects between estrogen, progesterone, and the MR-haplotypes (*srs* ranged between -.17 and .10; p > .122).

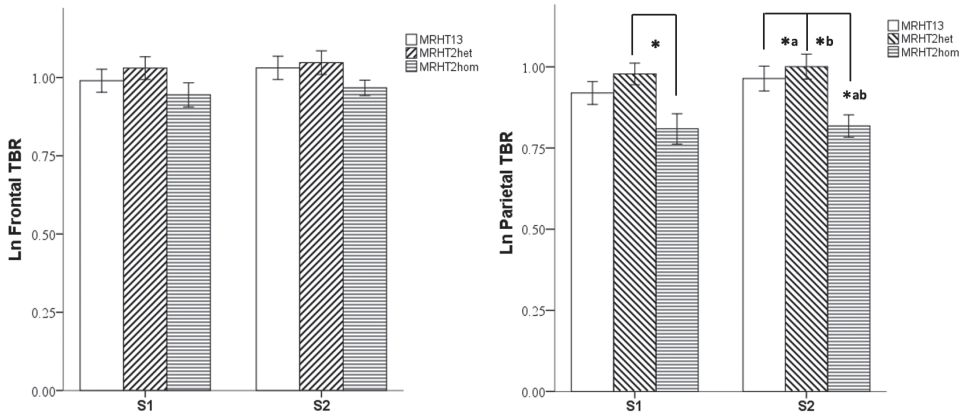

Figure 1. Log-normalized frontal (left graph) and parietal (right graph) TBR scores by MR-haplotype.
Abbreviations. Ln = log-normalized; MRHT = mineralocorticoid receptor haplotype; het = heterozygotes; hom = homozygotes; Error bars: +/- 1 SE.* p < .05.

Table 3. Means (SE) for frontal and parietal EEG data per session

	Hormonal status		**NC**			**OC**			**MRHT**		
	NC	**OC**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**	**MRHT 1/3**	**MRHT 2 het**	**MRHT 2 hom**
Frontal											
TBR S1	1.13 (0.09)	1.06 (0.09)	1.16 (0.16)	1.19 (015)	0.91 (0.13)	1.02 (0.13)	1.14 (0.17)	0.91 (0.13)	1.08 (0.10)	1.17 (0.11)	0.91 (0.09)
TBR S2	1.17 (0.10)	1.14 (0.10)	1.26 (0.17)	1.21 (0.16)	0.87 (0.07)	1.12 (0.14)	1.22 (0.17)	0.99 (0.09)	1.19 (0.11)	1.21 (0.11)	0.93 (0.06)
Parietal											
TBR S1	1.03 (0.08)	0.90 (0.06)	1.05 (0.16)	1.14 (0.12)	0.73 (0.13)	0.95 (0.11)	0.92 (0.08)	0.72 (0.10)	1.00 (0.09)	1.03 (0.07)*	0.73 (0.08)*
TBR S2	1.11 (0.10)	0.97 (0.07)	1.21 (0.20)	1.17 (0.14)	0.73 (0.09)	1.04 (0.10)	1.01 (0.11)	0.70 (.07)	1.12 (0.11)*[a]	1.09 (0.09)*[b]	0.71 (0.06)*[ab]

Notes: Means without univariate outliers (Z > 3). Abbreviations: OC = oral contraceptive users; NC = naturally cycling women; MRHT 1/3 = mineralocorticoid receptor haplotype 1/3; MRHT 2 het = mineralocorticoid receptor haplotype 2 heterozygotes; MRHT 2 hom = mineralocorticoid receptor haplotype 2 homozygotes ; TBR = theta/beta ratio. * $p < .05$.

Discussion

We investigated the impact of OC and menstrual cycle phase and the moderating effect of the MR-haplotypes on rsEEG theta/ beta ratio (TBR) in a counterbalanced within-subject design.

Contrary to our expectations, neither OC-use nor menstrual cycle phase were associated with TBR. Comparable resting state functional connectivity patterns in both OC-users and NC women were not only observed in earlier studies (DeBondt et al., 2013), but also in a recently published study (20) using a double-blind randomized placebo-controlled design, investigating the influence of OC (.03 EE; .15 LNG) on amygdala and salience network resting-state functional connectivity. In this study menstrual cycle phase (in the placebo condition) and (in)active OC-use influenced amygdala and salience resting-state networks comparably. Higher endogenous and exogenous hormone levels resulted in higher rs functional connectivity, although the effect of endogenous hormones was slightly more pronounced (Engman et al., 2018).

MR-haplotype 2 homozygotes had the lowest parietal TBR compared with MR-haplotype 2 heterozygotes and MR-haplotype 1/3 carriers. Low TBR is associated with better attentional control, resilience to stressful events (Putman et al., 2010; Tortella-Feliu, 2014; Angelidis et al., 2016) and a reduced tendency to worry (van Son et al., 2018c). Worry is sometimes referred to as a 'negative form' of mind wandering and can be seen as self-generated off-task thought (Ottaviani et al., 2015). Improved cognitive control over the affective system results in a more adaptive appraisal of stressors, decreasing the odds on developing a major depressive disorder and other stress-related psychopathology (Joormann & Stanton, 2016; Mogg & Bradley, 2016). This is in line with reports of lower vulnerability to major depressive disorder, less ruminative cognitions and higher explicit optimism scores in MR-haplotype 2 carriers in a cohort study (Klok et al., 2011) and higher implicit happiness scores in MR-haplotype 2 homozygotes in an experimental study (Hamstra et al., 2017a). The finding of lower self-reported chronic stress in MR-haplotype 2 homozygotes (van Leeuwen et al., 2011) and resilience to childhood trauma (Vinkers et al., 2015) supports the hypothesis of an improved coping ability with stressors in this group as well.

Interpretations on the clinical and cognitive implications of differences in TBR may diverge. Lower TBR in MR-haplotype 2 homozygotes might represent a lower baseline reactivity to affective stimuli (theta) and an improved ability to down-regulate amygdala reactivity (beta), thus a more 'cognitive' way of coping with potential stressors. Consistently, in a study of Wirz et al. (Wirz et al., 2017) MR-haplotype 2 carriers showed in two experiments less hippocampal activity and reduced amygdala connectivity with the parahippocampal cortex after stress. Only in MR-haplotype 2 carriers stress led to a more pronounced shift from hippocampal to dorsal striatal learning, and was followed by distinctive alterations in memory networks, reflecting a stress-induced shift toward habit memory.

Additionally, MR-haplotype 2 carriers had reduced amplitudes on ERP components associated with early attentional processing, irrespective of stress. Wirz et al (2017) treated homo- and heterozygous carriers of MR-haplotype 2 as one group, however. Our study revealed that all MR-haplotypes constitute distinct phenotypes on TBR, with significant lower base-line TBR in MR-haplotype 2 homozygotes. Earlier studies, which found lower self-reported chronic stress and higher implicit happiness only in MR-haplotype 2 homozygotes, but not in MR-haplotype 2 heterozygotes (van Leeuwen et al., 2011; Hamstra et al., 2017a), agree on this idea. Future studies may benefit from treating MR-haplotype 1/3, MR-haplotype 2 heterozygotes and homozygotes as separate groups or adding the amount of MR-haplotype 2 alleles (0, 1, 2) as a covariate to the model.

Our research has a number of potential limitations. We did not exclude NC participants for prior OC-use. Although TBR was previously related to performance on an emotion regulation task (e.g., Tortella-Feliu et al., 2014) we did not assess self-reported or behavioral emotion regulation abilities in our sample. Furthermore, we have no direct neuroimaging evidence of the assumption that TBR reflects prefrontal control over subcortical areas. This should be investigated with methods with a higher spatial resolution than EEG, such as magnetoencephalography, or supplement rs EEG with rs fMRI. Lastly, our sample was small for a genetic association study. Our sample size was too small to detect if differences on TBR, theta and beta power between MR-haplotype 2 hetero- and homozygotes were driven by the G-allele. This would have been of scientific interest, because MR-haplotype 2 homozygotes lack the G-allele from the rs5522 (A/G) and rs2070951 (C/G) SNPs. Carriers of G-allele from the rs5522 SNP displayed higher stress-reactivity (Kuningas et al., 2007; Van Leeuwen et al., 2011), and increased threat-related amygdala reactivity (Joels et al., 2007; Bogdan et al., 2010).

The strengths of our study include the fact that we assessed TBR in a counterbalanced, mixed between-subject (OC/NC) design. We observed subtle physiological effects in emotional processing which remained unnoticed in self-reports on affective state and personality, as the MR-haplotypes did not differ in scores on neuroticism, depressive cognitions, implicit anxiety or negative affect. This is the first report on TBR in a homogenous sample of OC-users during (in) active use and NC women on personalized time-points in their menstrual cycle. Additionally, we verified hormonal status with the saliva concentration of estrogen and progesterone. To conclude, our work suggests that MR-haplotype 2 homozygotes had lower TBR than the other haplotype groups, possibly reflecting an improved regulatory cortical activity over the affective system. MR-haplotype 2 homozygotes may therefore be better in regulating their emotions, allowing them to cope better with stress and negative events.

Conclusion

Neither OC-use nor menstrual cycle phase were associated with rsEEG theta/ beta ratio (TBR) in healthy premenopausal women. Lower parietal TBR in MR-haplotype 2 homozygotes may reflect improved PFC-mediated effortful control and resilience to stressful events. Genetic variation associated with enhanced MR expression may modulate regulation of arousal in females.

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 4th 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.

Chapter 9

References
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References

A

Aalto, A. M., Elovainio, M., Kivimäki, M., Uutela, A., & Pirkola, S. (2012). The Beck Depression Inventory and General Health Questionnaire as measures of depression in the general population: a validation study using the Composite International Diagnostic Interview as the gold standard. *Psychiatry research*, 197(1-2), 163-171.

Aas, M., Pedersen, G., Henry, C., Bjella, T., Bellivier, F., Leboyer, M., ... & Lagerberg, T. V. (2015). Psychometric properties of the Affective Lability Scale (54 and 18-item version) in patients with bipolar disorder, first-degree relatives, and healthy controls. *Journal of affective disorders*, 172, 375-380.

Adair, J. G. (1984). The Hawthorne effect: a reconsideration of the methodological artifact. *Journal of applied psychology*, 69(2), 334.

Adams, T., Pounder, Z., Preston, S., Hanson, A., Gallagher, P., Harmer, C. J., & McAllister-Williams, R. H. (2016). Test–retest reliability and task order effects of emotional cognitive tests in healthy subjects. *Cognition and emotion*, 30(7), 1247-1259.

Aiken, L & West, S. (1991). *Multiple regression: testing and interpreting interactions*. Sage Publications.

Akyürek, E. G., Eshuis, S. A., Nieuwenstein, M. R., Saija, J. D., Başkent, D., & Hommel, B. (2012). Temporal target integration underlies performance at lag 1 in the attentional blink. *Journal of Experimental psychology: human perception and performance*, 38(6), 1448.

Albert, K., Pruessner, J., & Newhouse, P. (2015). Estradiol levels modulate brain activity and negative responses to psychosocial stress across the menstrual cycle. *Psychoneuroendocrinology*, 59, 14-24.

Aleknaviciute, J., Tulen, J. H., De Rijke, Y. B., Bouwkamp, C. G., Van der Kroeg, M., Timmermans, M., ... & van Rossum, E. F. (2017). The levonorgestrel-releasing intrauterine device potentiates stress reactivity. *Psychoneuroendocrinology,* 80, 39-45.

Alexander, G. M., Sherwin, B. B., Bancroft, J., & Davidson, D. W. (1990). Testosterone and sexual behavior in oral contraceptive users and nonusers: A prospective study. *Hormones and behavior*, 24(3), 388-402.

American Psychiatric Association, & Nederlandse Vereniging voor Psychiatrie. (2014). Hengeveld, M.W. (ed.). *Handboek voor de classificatie van psychische stoornissen: DSM-5™*. Vijfde oplage. Amsterdam: Uitgeverij Boom.

Anderson, N. H. (1968). Likableness ratings of 555 personality-trait words. *Journal of personality and social psychology*, 9(3), 272.

Angelidis, A., Hagenaars, M., van Son, D., van der Does, W., & Putman, P. (2018). Do not look away! Spontaneous frontal EEG theta/beta ratio as a marker for cognitive control over attention to mild and high threat. *Biological psychology*, 135, 8-17.

Angelidis, A., van der Does, W., Schakel, L., & Putman, P. (2016). Frontal EEG theta/beta ratio as an electrophysiological marker for attentional control and its test-retest reliability. *Biological psychology*, 121, 49-52.

Antypa, N. (2011). *Cognitive vulnerability to depression: genetic and environmental influences. (*Doctoral dissertation, Faculty of Social and Behavioural Sciences, Leiden University, The Netherlands).

Arnsten, A. F., & Rubia, K. (2012). Neurobiological circuits regulating attention, cognitive control, motivation, and emotion: disruptions in neurodevelopmental psychiatric disorders. *Journal of the american academy of child & adolescent psychiatry*, 51(4), 356-367.

Arp, J. M., ter Horst, J. P., Kanatsou, S., Fernández, G., Joëls, M., Krugers, H. J., & Oitzl, M. S. (2014). Mineralocorticoid receptors guide spatial and stimulus-response learning in mice. *PLoS one*, 9(1), e86236.

Aubuchon, P. G., & Calhoun, K. S. (1985). Menstrual cycle symptomatology: the role of social expectancy and experimental demand characteristics. *Psychosomatic medicine*, 47, 35-46.

B

Bancroft, J., & Rennie, D. (1993). The impact of oral contraceptives on the experience of peri-menstrual mood, clumsiness, food craving and other symptoms. *Journal of psychosomatic research*, 37(2), 195–202.

Barrett Mueller, K., Lu, Q., Mohammad, N. N., Luu, V., McCurley, A., Williams, G. H., ... & Jaffe, I. Z. (2014). Estrogen receptor inhibits mineralocorticoid receptor transcriptional regulatory function. *Endocrinology*, 155(11), 4461-4472.

Bayer, J., Bandurski, P., & Sommer, T. (2013). Differential modulation of activity related to the anticipation of monetary gains and losses across the menstrual cycle. *European journal of neuroscience*, 38(10), 3519-3526.

Bayer, J., Schultz, H., Gamer, M., & Sommer, T. (2014). Menstrual-cycle dependent fluctuations in ovarian hormones affect emotional memory. *Neurobiology of learning and memory*, 110, 55-63.

Beaton, D. (2017). *Implementing appropriate multivariate methods for higher quality results from genetic association studies in substance abuse populations.* Doctoral dissertation. University of Texas at Dallas.

Beck, A. T. (1963). Thinking and depression: I. Idiosyncratic content and cognitive distortions. *Archives of general psychiatry,* 9(4), 324-333.

Beck, A. T. (2002). Cognitive models of depression. *Clinical advances in cognitive psychotherapy: Theory and application*, 14(1), 29-61.

Beck, A. T., & Clark, D. A. (1997). An information processing model of anxiety: Automatic and strategic processes. *Behaviour research and therapy*, 35(1), 49-58.

Becker, D., Creutzfeldt, O. D., Schwibbe, M., & Wuttke, W. (1982). Changes in physiological, EEG and psychological parameters in women during the spontaneous menstrual cycle and following oral contraceptives. *Psychoneuroendocrinology*, 7(1), 75-90.

Binder, E.B., Salyakina, D., Lichtner, P., Wochnik, G. M., Ising, M., Pütz, B. & Muller-Myhsok, B. (2004). Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nature genetics*, 36(12), 1319-1325.

Bitran, D., Shiekh, M., & McLeod, M. (1995). Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABAA receptors. *Journal of neuroendocrinology*, 7(3), 171-177.

Bobst, C., Sauter, S., Foppa, A., & Lobmaier, J. S. (2014). Early follicular testosterone level predicts preference for masculinity in male faces–but not for women taking hormonal contraception. *Psychoneuroendocrinology*, 41, 142-150.

Bogdan, R., Perlis, R. H., Fagerness, J., & Pizzagalli, D. A. (2010). The impact of mineralocorticoid receptor ISO/VAL genotype (rs5522) and stress on reward learning. *Genes, brain and behavior*, 9(6), 658-667.

Bogdan, R., Perlis, R.H., Fagerness, J. & Pizzagalli, D.A (2010). The impact of mineralocorticoid receptor ISO/VAL genotype (rs5522) and stress on reward learning. *Genes brain behavior*, 9, 658-667.

Bogdan, R., Williamson, D. E., & Hariri, A. R. (2012). Mineralocorticoid receptor Iso/Val (rs5522) genotype moderates the association between previous childhood emotional neglect and amygdala reactivity. *American journal of psychiatry*, 169(5), 515-522.

Bone, J. K., Lewis, G., Button, K. S., Duffy, L., Harmer, C. J., Munafò, M. R., ... & Lewis, G. (2019). Variation in recognition of happy and sad facial expressions and self-reported depressive symptom severity: A prospective cohort study. *Journal of affective disorders*, 257, 461-469.

Boron, E.L., Boulpaep, W.F. (Eds.) (2012). *Medical physiology*, Second Ed., Saunders Ed, Philadelphia, USA.

Bosman, R. C., Jung, S. E., Miloserdov, K., Schoevers, R. A., & aan het Rot, M. (2016). Daily symptom ratings for studying premenstrual dysphoric disorder: A review. *Journal of affective disorders*, 189, 43-53.

Bouma, E., Riese, M., Nolte, C., Oosterom, H., Verhulst, I., Ormel, E., & Oldehinkel, F. (2011). No associations between single nucleotide polymorphisms in corticoid receptor genes and heart rate and cortisol responses to a standardized social stress test in adolescents: the TRAILS study. *Behavior genetics*, 41(2), 253-261.

Boyce, P. & Parker, G. (1989). Development of a scale to measure interpersonal sensitivity. *Australian new zealand journal of psychiatry* 23, 341-51.

Boyce, P., Parker, G., Barnett, B., Cooney, M., & Smith, F. (1991). Personality as a vulnerability factor to depression. *The british journal of psychiatry*, 159(1), 106-114.

Brinks, V., Berger, S., Gass, P., De Kloet, E. R., & Oitzl, M. S. (2009). Mineralocorticoid receptors in control of emotional arousal and fear memory. *Hormones and behavior*, 56(2), 232-238.

Brody, L.R., Hall, J. & Stokes, L.R. (2016). Gender and emotion: Theory, findings and context, in: Feldman Barrett, L., Lewis, M., Haviland-Jones, J.M. (Eds.), *Handbook of emotions*. The Guilford Press, New York, pp. 369-392.

Broome, M. R., He, Z., Iftikhar, M., Eyden, J., & Marwaha, S. (2015). Neurobiological and behavioural studies of affective instability in clinical populations: a systematic review. *Neuroscience & biobehavioral reviews*, 51, 243-254.

Brosschot, J. F., Geurts, S. A. E., Kruizinga, I., Radstaak, M., Verkuil, B., Quirin, M., & Kompier, M. A. J. (2014). Does unconscious stress play a role in prolonged cardiovascular stress recovery? *Stress and health*, 30(3), 179-187.

C

Carey, M. P., Deterd, C. H., De Koning, J., Helmerhorst, F., & De Kloet, E. R. (1995). The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. *Journal of endocrinology*, 144(2), 311-321.

Carr, B.R., Parker, C.R., Madden, J.D., MacDonald, P.C. & Porter JC (1979). Plasma levels of adrenocorticotropin and cortisol in women receiving oral contraceptive steroid treatment. *The journal of clinical endocrinology and metabolism,* 49, 346-349.

Carver, C.S. & Scheier, M.S. (2014). Dispositional optimism. *Trends in cognitive sciences*, 6, 293-299.

Cavallari, L. H., Groo, V. L., Viana, M. A., Dai, Y., Patel, S. R., & Stamos, T. D. (2010). Association of aldosterone concentration and mineralocorticoid receptor genotype with potassium response to spironolactone in patients with heart failure. *Pharmacotherapy: the journal of human pharmacology and drug therapy,* 30(1), 1-9.

Cavanagh, J. F., & Shackman, A. J. (2015). Frontal midline theta reflects anxiety and cognitive control: meta-analytic evidence. *Journal of physiology-Paris*, 109(1-3), 3-15.

Champagne, D. L., Bagot, R. C., van Hasselt, F., Ramakers, G., Meaney, M. J., De Kloet, E. R., ... & Krugers, H. (2008). Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. *Journal of neuroscience*, 28(23), 6037-6045.

Chapman, K., Holmes, M., & Seckl, J. (2013). 11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiological reviews*, 93(3), 1139-1206.

Charney, D. S., & Manji, H. K. (2004). Life stress, genes, and depression: multiple pathways lead to increased risk and new opportunities for intervention. *Science's STKE*, 2004 (225), re5-re5.

Cheslack-Postava, K., Keyes, K. M., Lowe, S. R., & Koenen, K. C. (2015). Oral contraceptive use and psychiatric disorders in a nationally representative sample of women. *Archives of women's mental health*, 18, 103-111.

Clance, P., Imes, S., & Kovacs, Arthur L. (1978). The imposter phenomenon in high achieving women: Dynamics and therapeutic intervention. *Psychotherapy: theory, research & practice*, 15(3), 241-247.

Clark, L. A., Watson, D., & Mineka, S. (1994). Temperament, personality, and the mood and anxiety disorders. *Journal of abnormal psychology*, 103(1), 103.

Cobey, K., Buunk, A. (2012). Conducting high-quality research on the psychological impact of oral contraceptive use. *Contraception, 86*, 330-331.

Cobey, K. D., Buunk, A. P., Roberts, S. C., Klipping, C., Appels, N., Zimmerman, Y., et al. (2012). Reported jealousy differs as a function of menstrual cycle stage and contraceptive pill use: A within-subjects investigation. *Evolution and human Behavior, 33,* 395-401.

Cokley, K., Awad, G., Smith, L., Jackson, S., Awosogba, O., Hurst, A., … & Roberts, D. (2015). The roles of gender stigma consciousness, impostor phenomenon and academic self-concept in the academic outcomes of women and men. *Sex roles*, 73(9), 414-426.

Colzato, L. S., & Hommel, B. (2014). Effects of estrogen on higher-order cognitive functions in unstressed human females may depend on individual variation in dopamine baseline levels. *Frontiers in neuroscience*, 8, 65.

Cools, R. (2006). Dopaminergic modulation of cognitive function-implications for L-DOPA treatment in Parkinson's disease. *Neuroscience & biobehavioral reviews*, 30(1), 1-23.

Cooper, S. E., Goings, S. P., Kim, J. Y., & Wood, R. I. (2014). Testosterone enhances risk tolerance without altering motor impulsivity in male rats. *Psychoneuroendocrinology*, 40, 201-212.

Cornelisse, S., Joëls, M. & Smeets T (2011). A randomized trial on mineralocorticoid receptor blockade in men: effects on stress responses, selective attention, and memory. *Neuropsychopharmacology*, 36, 2720-2728.

Creutzfeldt, O. D., Arnold, P. M., Becker, D., Langenstein, S., Tirsch, W., Wilhelm, H., & Wuttke, W. (1976). EEG changes during spontaneous and controlled menstrual cycles and their correlation with psychological performance. *Electroencephalography and clinical neurophysiology*, 40(2), 113-131.

Cullberg, J. (1972). Mood changes and menstrual symptoms with different gestagen/estrogen combinations. A double-blind comparison with a placebo. *Acta psychiatrica sScandinavica. Supplementum*, 236, 1.

D

De Bondt, T., Jacquemyn, Y., Van Hecke, W., Sijbers, J., Sunaert, S., & Parizel, P. M. (2013). Regional gray matter volume differences and sex-hormone correlations as a function of menstrual cycle phase and hormonal contraceptives use. *Brain research*, 1530, 22-31.

De Kloet, E. R. (2016). Corticosteroid receptor balance hypothesis: implications for stress-adaptation. In *Stress: concepts, cognition, emotion, and behavior* (pp. 21-31). Academic Press.

De Kloet, E. R., DeRijk, R. H., & Meijer, O. C. (2007). Therapy Insight: is there an imbalanced response of mineralocorticoid and glucocorticoid receptors in depression? *Nature clinical practice endocrinology & metabolism*, 3, 168-179.

De Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature reviews neuroscience*, 6(6), 463-475.

De Kloet, E. R., Meijer, O. C., de Nicola, A. F., de Rijk, R. H., & Joels, M. (2018). Importance of the brain corticosteroid receptor balance in metaplasticity, cognitive performance and neuro-inflammation. *Frontiers in neuroendocrinology*, 49, 124-145.

De Kloet, E. R., Otte, C., Kumsta, R., Kok, L., Hillegers, M. H. J., Hasselmann, H., ... & Joels, M. (2016). Stress and depression: a crucial role of the mineralocorticoid receptor. *Journal of neuroendocrinology*, 28(8).

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocrine reviews*, 19(3), 269-301.

De Kloet, E., Otte, C., Kumsta, R., Kok, L., Hillegers, M., Hasselmann, H, Kliegel., D., Joëls, M. (2016). Stress and Depression: A crucial role of the mineralocorticoid receptor. *Journal of neuroendocrinology*, 28(8).

De Kloet, E., Vreugdenhil, E., Oitzl, M., & Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocrine reviews*, 19(3), 269-301.

De Kloet, E.R., Joëls, M. & Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nature reviews neuroscience*, 6: 463-475.

De Kloet, E.R., Otte, C., Kumsta, R., Kok, L., Hillegers, M.H., Hasselmann, H., Kliegel, D. & Joëls, M. (2016). Stress and depression: a crucial role of the mineralocorticoid receptor. *Journal of neuroendocrinology*, 28(8).

DeBondt, T. Jacquemyn, Y., VanHecke, W., Sijbers, J., Sunaert, S., Parizel, P. (2013). Regional gray matter volume differences and sex-hormone correlations as a function of menstrual cycle phase and hormonal contraceptives use. *Brain research, 1530,* 22-31.

Deecher, D., Andree, T. H., Sloan, D., & Schechter, L. E. (2008). From menarche to menopause: exploring the underlying biology of depression in women experiencing hormonal changes. *Psychoneuroendocrinology*, 33(1), 3-17.

Deijen, J. B., Duyn, K. J., Jansen, W. A., & Klitsie, J. W. (1992). Use of a monophasic, low-dose oral contraceptive in relation to mental functioning. *Contraception*, 46(4), 359-367.

DeRijk, R. H. (2009). Single nucleotide polymorphisms related to HPA axis reactivity. *Neuroimmunomodulation*, 16(5), 340-352.

DeRijk, R. H., & de Kloet, E. R. (2008). Corticosteroid receptor polymorphisms: determinants of vulnerability and resilience. *European journal of pharmacology,* 583(2-3), 303-311.

DeRijk, R. H., van Leeuwen, N., Klok, M. D., & Zitman, F. G. (2008). Corticosteroid receptor-gene variants: modulators of the stress-response and implications for mental health. *European journal of pharmacology*, 585(2-3), 492-501.

DeRijk, R. H., Wüst, S., Meijer, O. C., Zennaro, M.C., Federenko, I. S., Hellhammer, D. H., Giacchetti, G., Vreugdenhil, E., Zitman, F.G., & de Kloet, E. R. (2006). A common polymorphism in the mineralocorticoid receptor modulates stress responsiveness. *Journal of clinical endocrinology and metaboli*sm, 91(12), 5083-5089.

Derntl, B., Kryspin-Exner, I., Fernbach, E., Moser, E., & Habel, U. (2008). Emotion recognition accuracy in healthy young females is associated with cycle phase. *Hormones and Behavior*, 53(1), 90-95.

DeSoto, M. C., Geary, D. C., Hoard, M. K., Sheldon, M. S., & Cooper, L. (2003). Estrogen fluctuations, oral contraceptives and borderline personality. *Psychoneuroendocrinology*, 28(6), 751-766.

Deuter, C. E., Wingenfeld, K., Schultebraucks, K., Hellmann-Regen, J., Piber, D., & Otte, C. (2017). Effects of mineralocorticoid-receptor stimulation on risk taking behavior in young healthy men and women. *Psychoneuroendocrinology*, 75, 132-140.

Dougherty, D.M., Bjork, J.M., Huang, D. & Moeller, F.G. (1997). The relationship between self-reported menstrual symptomatology and aggression measured in the laboratory, *Personal and individual differences*, 3, 381-391.

Dreher, J. C., Schmidt, P. J., Kohn, P., Furman, D., Rubinow, D., & Berman, K. F. (2007). Menstrual cycle phase modulates reward-related neural function in women. *Proceedings of the national academy of sciences*, 104(7), 2465-2470.

Durand, G. (2018a). Influence of allelic variations in relation to norepinephrine and mineralocorticoid receptors on psychopathic traits: A pilot study. *PeerJ,* 6(3), E4528.

Durand, G. (2018b). Demystification of the Relationship Between Psychopathy and Happiness. *Journal of happiness Studies*, 19(2), 381-395.

Dykman, B., Abramson, L., Alloy, L., Hartlage, S., & Sarason, Irwin G. (1989). Processing of ambiguous and unambiguous feedback by depressed and non-depressed college students: schematic biases and their Implications for depressive realism. *Journal of personality and social psychology*, 56(3), 431-445.

E

Edwards, C. R. W., Burt, D., McIntyre, M. A., De Kloet, E. R., Stewart, P. M., Brett, L., ... & Monder, C. (1988). Localisation of 11β-hydroxysteroid dehydrogenase-tissue specific protector of the mineralocorticoid receptor. *The lancet*, 332(8618), 986-989.

Ehlers, A. (1993). Interoception and panic disorder. *Advances in behaviour research and therapy*, 15(1), 3-21.

Eig, J. (2014). *The birth of the pill: How four crusaders reinvented sex and launched a revolution*. New York: W.W. Norton & Company.

Ekman, P., & Friesen, W. V. (1976). Measuring facial movement. *Environmental psychology and nonverbal behavior*, 1(1), 56-75.

Ellis, A. J., Wells, T. T., Vanderlind, W. M., & Beevers, C. G. (2014). The role of controlled attention on recall in major depression. *Cognition & emotion*, 28(3), 520-529.

Endedijk, H., Nelemans, S., Schür, R., Boks, M., Lier, P., Meeus, W., ... & Vinkers, C. (2020). The role of stress and mineralocorticoid receptor haplotypes in the development of symptoms of depression and anxiety during adolescence. *Frontiers in ps*ychiatry, 11, 367.

Endedijk, H., Nelemans, Schür, R., Boks, M., Meeus, W., Vinkers, C… & Branje. (2019). The role of stressful parenting and mineralocorticoid receptor haplotypes on social development during adolescence and young adulthood. *Journal of youth and adolescence*, 1-18.

Engman, J., Linnman, C., Van Dijk, K. R., & Milad, M. R. (2016). Amygdala subnuclei resting-state functional connectivity sex and estrogen differences. *Psychoneuroendocrinology*, 63, 34-42.

Engman, J., Poromaa, I. S., Moby, L., Wikström, J., Fredrikson, M., & Gingnell, M. (2018). Hormonal cycle and contraceptive effects on amygdala and salience resting-state networks in women with previous affective side effects on the pill. *Neuropsychopharmacology*, 43(3), 555-563.

Eysenck, M. (1992). Anxiety: The cognitive perspective. *Essays in cognitive psychology*, 47332328). Hove [etc.]: Lawrence Erlbaum Associates.

F

Fauser, B.C., Lagro-Janssen, A.L., Bos, A.M. (2013). *Handboek vrouwspecifieke geneeskunde*. Houten: Prelum Uitgevers.

Fava, M., Hwang, I., Rush, A. J., Sampson, N., Walters, E. E., & Kessler, R. C. (2010). The importance of irritability as a symptom of major depressive disorder: results from the National Comorbidity Survey Replication. *Molecular psychiatry*, 15(8), 856-867.

Felitti, V. J., Anda, R. F., Nordenberg, D., Williamson, D. F., Spitz, A. M., Edwards, V., . . . Marks, J. S. (1998). Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The Adverse Childhood Experiences (ACE) Study. *American journal of preventive medicine*, 14, 245-258.

Felten, D. L., O'Banion, M. K., & Maida, M. E. (2015). *Netter's atlas of neuroscience*. Elsevier Health Sciences.

Figueroa, C. A., Ruhé, H. G., Koeter, M. W., Spinhoven, P., Van der Does, W., Bockting, C. L., & Schene, A. H. (2015). Cognitive reactivity versus dysfunctional cognitions and the prediction of relapse in recurrent major depressive disorder. *The Journal of clinical Psychiatry*, 10E1306.

Fink, G. (Ed.) (2016). *Stress: concepts, cognition, emotion, and behavior: Handbook of stress series (Vol.1)*. Academic Press.

Fleischman, D. S., Navarrete, C. D., & Fessler, D. M. (2010). Oral contraceptives suppress ovarian hormone production. *Psychological science,* 21(5), 750-752.

Franke, L.K. (2017). *Mineralocorticoid receptor genotype influences resting state EEG theta/beta ratio in women*. (Research master thesis, Leiden University, The Netherlands).

Fries, E., Dettenborn, L., Kirschbaum, C. (2009). The cortisol awakening response (CAR): facts and future directions. *International journal of psychophysiology*, 72, 67-73.

Fritzsche, A., Dahme, B., Gotlib, I. H., Joormann, J., Magnussen, H., Watz, H., ... & von Leupoldt, A. (2010). Specificity of cognitive biases in patients with current depression and remitted depression and in patients with asthma. *Psychological medicine*, 40(5), 815.

Frokjaer, V.G., Pinborg, A., Holst, K.K., Overgaard, A., Henningsson, S., Heede, M., Larsen, E.C., … & Knudsen, G.M. (2015). Role of serotonin transporter changes in depressive responses to sex-steroid hormone manipulation: a positron emission tomography study. *Biological psychiatry*, 78, 534-543.

FSRH *Clinical Guidance: Combined Hormonal Contraception – March 2021*. Retrieved on the Internet on https://www.fsrh.org/standards-and-guidance/documents/combined-hormonal-contraception/.

Funder, J. W., Pearce, P. T., Smith, R., & Smith, A. I. (1988). Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science*, 242(4878), 583-585.

G

Gasbarri, A., Pompili, A., d'Onofrio, A., Cifariello, A., Tavares, M., Tomaz, C. (2008). Working memory for emotional facial expressions: role of the estrogen in young women. *Psychoneuroendocrinology*, 33, 964-972.

Geerling, J. C., & Loewy, A. D. (2009). Aldosterone in the brain. *American journal of physiology-renal physiology*, 297(3), F559-F576.

Gerritsen, L., Milaneschi, Y., Vinkers, C. H., Van Hemert, A.M., Van Velzen, L., Schmaal, L., & Penninx, B. W. (2017). HPA axis genes, and their interaction with childhood maltreatment, are related to cortisol levels and stress-related phenotypes. *Neuropsychopharmacology*, 42(12), 2446.

Gillies, G. E., Virdee, K., McArthur, S., & Dalley, J. W. (2014). Sex-dependent diversity in ventral tegmental dopaminergic neurons and developmental programing: a molecular, cellular and behavioral analysis. *Neuroscience*, 282, 69-85.

Gingnell, M., Engman, J., Frick, A., Moby, L., Wikström, J., Fredrikson, M., & Sundström-Poromaa, I. (2013). Oral contraceptive use changes brain activity and mood in women with previous negative affect on the pill—a double-blinded, placebo-controlled randomized trial of a levonorgestrel-containing combined oral contraceptive. *Psychoneuroendocrinology*, 38(7), 1133-1144.

Gogos, A. (2013). Natural and synthetic sex hormones: Effects on higher-order cognitive function and prepulse inhibition. *Biological psychology*, 93(1), 17-23.

Gogos, A., Wu, Y. C., Williams, A. S., & Byrne, L. K. (2014). The effects of ethinylestradiol and progestins ("the pill") on cognitive function in pre-menopausal women. *Neurochemical research*, 39(12), 2288-2300.

Goldberg, D. P., Gater, R., Sartorius, N., Ustun, T. B., Piccinelli, M., Gureje, O., & Rutter, C. (1997). The validity of two versions of the GHQ in the WHO study of mental illness in general health care. *Psychological medicine*, 27(1), 191-197.

Gonda, X., Telek, T., Juhasz, G., Lazary, J., Vargha, A., & Bagdy, G. (2008). Patterns of mood changes throughout the reproductive cycle in healthy women without premenstrual dysphoric disorders. *Progress in neuro-psychopharmacology and biological psychiatry*, 32(8), 1782-1788.

Goodstein, D. (2010). *On fact and fraud: Cautionary tales from the front lines of science*. Princeton University Press.

Gratton, G., Coles, M. G., & Donchin, E. (1983). A new method for off-line removal of ocular artifact. *Electroencephalography and clinical neurophysiology*, 55(4), 468-484.

Groeneweg, F. L., Karst, H., de Kloet, E. R., & Joëls, M. (2012). Mineralocorticoid and glucocorticoid receptors at the neuronal membrane, regulators of nongenomic corticosteroid signalling. *Molecular and cellular endocrinology*, 350(2), 299-309.

Guapo, V. G., Graeff, F. G., Zani, A. C. T., Labate, C. M., dos Reis, R. M., & Del-Ben, C. M. (2009). Effects of sex hormonal levels and phases of the menstrual cycle in the processing of emotional faces. *Psychoneuroendocrinology*, 34(7), 1087-1094.

H

Haaga, D., Dyck, M., Ernst, D., & Steinberg, Robert J. (1991). Empirical Status of Cognitive Theory of Depression. *Psychological bulletin*, 110(2), 215-236.

Haig, M. (2015). *Reasons to stay alive*. Edinburgh: Canongate Books.

Hall, J. R., & Benning, S. D. (2006). The "successful" psychopath. *Handbook of pPsychopathy*, 459-478.

Haller, J., Mikics, É., & Makara, G. B. (2008). The effects of non-genomic glucocorticoid mechanisms on bodily functions and the central neural system. A critical evaluation of findings. *Frontiers in neuroendocrinology*, 29(2), 273-291.

Hampson, E., van Anders, S. M., & Mullin, L. I. (2006). A female advantage in the recognition of emotional facial expressions: Test of an evolutionary hypothesis. *Evolution and human behavior*, 27(6), 401-416.

Hamstra, D. (2013). *The effect of a single dose fludrocortisone, a MR-agonist, on emotional information processing in healthy female volunteers*. (Research master thesis, Leiden University, The Netherlands).

Hamstra, D. A., de Kloet, E. R., de Rover, M., & Van der Does, W. (2017b). Oral contraceptives positively affect mood in healthy PMS-free women: A longitudinal study. *Journal of Psychosomatic Research*, 103, 119-126.

Hamstra, D. A., De Kloet, E. R., Van Hemert, A. M., De Rijk, R. H., & Van der Does, A. J. W. (2015). Mineralocorticoid receptor haplotype, oral contraceptives and emotional information processing. *Neuroscience*, 286, 412-422.

Hamstra, D. A., De Rover, M., De Rijk, R. H., & Van der Does, W. (2014). Oral contraceptives may alter the detection of emotions in facial expressions. *European neuropsychopharmacology*, 24(11), 1855-1859.

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

Hamstra, D.A., de Kloet, E.R., Quataert, I. Jansen, M. & Van der Does, W. (2017). Mineralocorticoid receptor haplotype, estradiol, progesterone and emotional information processing. *Psychoneuroendocrinology*, 76, 162-173.

Handa, R. J., & Weiser, M. J. (2014). Gonadal steroid hormones and the hypothalamo–pituitary–adrenal axis. *Frontiers in neuroendocrinology*, 35(2), 197-220.

Handa, Robert J., Burgess, Loyd H., Kerr, J.E., & Keefe, J. A. (1994). Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. *Hormones and behavior*, 28(4), 464-476.

Harald, B., & Gordon, P. (2012). Meta-review of depressive subtyping models. *Journal of affective disorders*, 139(2), 126–140.

Harkness, K., Sabbagh, M., Jacobson, J., Chowdrey, N., & Chen, T. (2005). Enhanced accuracy of mental state decoding in dysphoric college students. *Cognition & emotion*, 19(7), 999-1025.

Harmer, C. J., Bhagwagar, Z., Perrett, D. I., Völlm, B. A., Cowen, P. J., & Goodwin, G. M. (2003). Acute SSRI administration affects the processing of social cues in healthy volunteers. *Neuropsychopharmacology*, 28(1), 148-152.

Harmer, C. J., Goodwin, G. M., & Cowen, P. J. (2009). Why do antidepressants take so long to work? A cognitive neuropsychological model of antidepressant drug action. *The british journal of psychiatry*, 195(2), 102-108.

Harmer, C. J., Hill, S. A., Taylor, M. J., Cowen, P. J., & Goodwin, G. M. (2003). Toward a neuropsychological theory of antidepressant drug action: increase in positive emotional bias after potentiation of norepinephrine activity. *American journal of psychiatry*, 160(5), 990-992.

Harmer, C. J., Shelley, N. C., Cowen, P. J., & Goodwin, G. M. (2004). Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *American journal of psychiatry*, 161(7), 1256-1263.

Harmer, C., Cowen, P., Goodwin, G. (2011). Efficacy markers in depression. *Journal of psychopharmacology*, 25, 1148.

Harmer, C., Goodwin, G. & Cowen, P. (2009). Why do antidepressants take so long to work? A cognitive neuropsychological model of antidepressant action. *British journal of psychiatry*, 195, 102-108.

Harris, R. (2017). *Rigor Mortis: How sloppy science creates worthless cures, crushes hope, and wastes billion*s. Hachette Book Group, New York.

Harvey, A. (2004). *Cognitive behavioral processes across psychological disorders: A transdiagnostic approach to research and treatment*. Chapter 2 and 3. Oxford [etc.]: Oxford University Press.

Hather, R., Trussel, J., Cates, W., Stewart, F., Kowal, D. (2007). *Contraceptive technology*. 19th revised ed. New York: Ardent Media.

Heck, A. L., & Handa, R. J. (2019). Sex differences in the hypothalamic–pituitary–adrenal axis' response to stress: an important role for gonadal hormones. *Neuropsychopharmacology*, 44(1), 45-58.

Henckens, M. J., van Wingen, G. A., Joëls, M., & Fernández, G. (2012). Time-dependent effects of cortisol on selective attention and emotional interference: a functional MRI study. *Frontiers in integrative neuroscience*, 6, 66.

Hermans, E. J., Henckens, M. J., Joëls, M., & Fernández, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in neurosciences*, 37(6), 304-314.

Herzberg, B. N., Johnson, A. L., & Brown, S. (1970). Depressive symptoms and oral contraceptives. *British medical journal*, 4(5728), 142-145.

Hjelmervik, H., Hausmann, M., Osnes, B., Westerhausen, R., & Specht, K. (2014). Resting states are resting traits–an FMRI study of sex differences and menstrual cycle effects in resting state cognitive control networks. *PloS one*, 9(7), e103492.

Hoekstra, H.A., Ormel, J. & de Fruyt, F. (1996). *Handleiding NEO persoonlijkheids-vragenlijsten NEO-PI-R en NEO-FFI.* Swets Test Services, Lisse.

Hofman, D., Terburg, D., van Wielink, L., & Schutter, D. J. (2013). Coalescence of dominance motivation and responses to facial anger in resting-state and event-related electrophysiology. *NeuroImage*, 79, 138-144.

Holländer, A., Hausmann, M., Hamm, J.P., Corballis, M.C. (2005). Sex hormonal modulation of hemispheric asymmetries in the attentional blink. *Journal of the international neuropsychological society*, 11, 263-272

Holsboer, F. (1999). The rationale for corticotropin-releasing hormone receptor (CRH-R) antagonists to treat depression and anxiety. *Journal of psychiatric research*, 33(3), 181-214.

Holsboer, F. (2000). The corticosteroid receptor hypothesis of depression. *Neuropsychopharmacology*, 23(5), 477-501.

Homberg, J. (2012). Genetic sensitivity to the environment, across lifetime. *Behavioral and brain sciences*, 35(5), 368.

Homberg, J. R., & Lesch, K. P. (2011). Looking on the bright side of variation. *Biological psychiatry*, 69(6), 513-519.

Hoogendijk, W. & de Rek, W. (2017). *Van big bang tot burnout*. First ed. Amsterdam: Balans.

I

Ingram, R. (1986). *Information processing approaches to clinical psychology* (Personality, psychopathology and psychotherapy. 041574028). Orlando [etc.]: Academic Press.

Ingram, R. (1990). Self-focused attention in clinical disorders: Review and a conceptual model. *Psychological bulletin*, 107(Mar 90), 156-176.

Ioannidis, J.P.A. (2005). Why most published research findings are false'. *PLoS Medicine*, 2.8, e124.

J

Jack, D. (2019). *Silencing the self: Women and depression*. Cambridge, Mass. [etc.]: Harvard University Press.

Jaisser, F., & Farman, N. (2016). Emerging roles of the mineralocorticoid receptor in pathology: toward new paradigms in clinical pharmacology. *Pharmacological reviews*, 68(1), 49-75.

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., ... & Abdollahpour, I. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The lancet,* 392(10159), 1789-1858.

Jarva, J. A., & Oinonen, K. A. (2007). Do oral contraceptives act as mood stabilizers? Evidence of positive affect stabilization. *Archives of women's mental health*, 10(5), 225-234.

Joëls, M., Karst, H., DeRijk, R., & de Kloet, E. R. (2008). The coming out of the brain mineralocorticoid receptor. *Trends in neurosciences*, 31(1), 1-7.

Joëls, M., Sarabdjitsingh, R. A., & Karst, H. (2012). Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. *Pharmacological reviews*, 64(4), 901-938.

Jones E, Boron E, Walter F, Boulpaep (Eds.), *Medical Physiology*, Second Ed., Saunders Ed, Phil, USA; 2012: 1150-1160.

Joormann J, Talbot L, Gotlib I (2007). Biased processing of emotional information in girls at risk for depression. *Journal of abnormal psychology,* 116(1) 135-143.

Joormann, & Stanton. (2016). Examining emotion regulation in depression: A review and future directions. *Behaviour research and therapy*, 86, 35-49.

Joormann, J., & Gotlib, I. H. (2007). Selective attention to emotional faces following recovery from depression. *Journal of abnormal psychology*, 116(1), 80.

Joormann, J., & Stanton, C. H. (2016). Examining emotion regulation in depression: A review and future directions. *Behaviour research and therapy*, 86, 35-49.

Joormann, J., Gotlib, I., & Watson, David. (2006). Is this happiness I see? Biases in the identification of emotional facial expressions in depression and social phobia. *Journal of abnormal psychology*, 115(4), 705-714.

Junod, S., & Marks, L. (2002). Women's trials: The approval of the first oral contraceptive pill in the United States and Great Britain. *Journal of the history of medicine and allied sciences*, 57(2), 117-160.

K

Kahneman, D. (2011). *Thinking, fast and slow* (1st ed.). Chapter 38. New York: Farra, Straus and Giroux.

Kamboj, S. K., Krol, K. M., & Curran, H. V. (2015). A specific association between facial disgust recognition and estradiol levels in naturally cycling women. *PloS One*, 10(4), e0122311.

Karst, H., Berger, S., Erdmann, G., Schütz, G., & Joëls, M. (2010). Metaplasticity of amygdalar responses to the stress hormone corticosterone. *Proceedings of the national academy of sciences*, 107(32), 14449-14454.

Karst, H., Berger, S., Turiault, M., Tronche, F., Schütz, G., & Joëls, M. (2005). Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *Proceedings of the national academy of sciences*, 102(52), 19204-19207.

Kay, C. R. (1984). The royal college of general practitioners' oral contraception study: some recent observations. *Clinics in obstetrics and gynaecology*, 11(3), 759-786.

Keller, J., Gomez, R., Williams, G., Lembke, A., Lazzeroni, L., Murphy, G., & Schatzberg, A. (2016). HPA Axis in Major Depression: Cortisol, Clinical Symptomatology, and Genetic Variation Predict Cognition. *Molecular psychiatry*, 22(4), 527-536.

Kendler, K.S., Gatz, M., Gardner, C.O. et al. (2006). A Swedish national twin study of lifetime major depression. *American journal of psychiatry,* 163(1); 109-114.

Kennis, M., Gerritsen, L., van Dalen, M., Williams, A., Cuijpers, P., & Bockting, C. (2019). Prospective biomarkers of major depressive disorder: a systematic review and meta-analysis. *Molecular psychiatry,* 1-18.

Keyes, K. M., Cheslack-Postava, K., Westhoff, C., Heim, C. M., Haloossim, M., Walsh, K., & Koenen, K. (2013). Keyes et al. Respond to "hormonal contraception and mood". *American journal of epidemiology*, 178(9), 1392-1393.

Kirmayer, L. J. (2001). Cultural variations in the clinical presentation of depression and anxiety: implications for diagnosis and treatment. *Journal of clinical psychiatry*, 62, 22-3.

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic medicine*, 61(2), 154-162.

Kirschbaum, C., Pirke, K.M., & Hellhammer, D.H. (1993). The 'Trier Social Stress Test'– a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology*, 28, 76-81.

Klok, M. D. (2011). *Mineralocorticoid receptor in human brain: a key player in resilience*. Doctoral thesis. Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, Faculty of Science, Leiden University.

Klok, M. D., Alt, S. R., Lafitte, A. J. I., Turner, J. D., Lakke, E. A., Huitinga, I., ... & DeRijk, R. H. (2011). Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *Journal of psychiatric research*, 45(7), 871-878.

Klok, M. D., Giltay, E. J., van der Does, A. J. W., Geleijnse, J. M., Antypa, N., Penninx, B. W. J. H., de Geus, E.J., Willemse, G., Boomsma, D.I., van Leeuwen, N., Zitman, F.G., deKloet, E.R., & DeRijk, R. H. (2011b). A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. *Translational psychiatry*, 1, e62.

Klok, M. D., Vreeburg, S. A., Penninx, B. W. J. H., Zitman, F. G., de Kloet, E. R., & DeRijk, R. H. (2011c). Common functional mineralocorticoid receptor polymorphisms modulate the cortisol awakening response: Interaction with SSRIs. *Psychoneuroendocrinology*, 36(4), 484-494.

Klok, M., Giltay, E., van der Does, A., Geleijnse, J., Antypa, N., Penninx, B., de Geus, E., Willemsen, G., Boomsma, D., van Leeuwen, N., Zitman, F., de Kloet, R., DeRijk, R. (2011a). A common and functional mineralocorticoid receptor haplotype enhances optimism and protects against depression in females. *Translatational psychiatry*, 1, 1-8.

Klok, M.D., Alt, S.R., Irurzun Lafitte, A.J., Turner, J.D., Lakke, E.A., Huiting,a I., Muller, C.P., Zitman, F.G., de Kloet, E.R. & Derijk, R.H. (2011a). Decreased expression of mineralocorticoid receptor mRNA and its splice variants in postmortem brain regions of patients with major depressive disorder. *Journal of psychiatric research*, 45, 871-878.

Knyazev, G. G. (2007). Motivation, emotion, and their inhibitory control mirrored in brain oscillations. *Neuroscience & biobehavioral reviews*, 31(3), 377-395.

Knyazev, G. G., & Slobodskaya, H. R. (2003). Personality trait of behavioral inhibition is associated with oscillatory systems reciprocal relationships. *International journal of psychophysiology*, 48(3), 247-261.

Kornstein, S.G. & Clayton, A.H. (eds) (2002). *Women's health*. First Ed. Guilford Press, New York, USA.

Korte, S. M., De Boer, S. F., De Kloet, E. R., & Bohus, B. (1995). Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus-maze. *Psychoneuroendocrinology*, 20(4), 385-39

Krattenmacher, R. (2000). Drospirenone: pharmacology and pharmacokinetics of a unique progestogen. *Contraception*, 62(1), 29-38.

Krause-Utz, A., Oei, N. Y., Niedtfeld, I., Bohus, M., Spinhoven, P., Schmahl, C., & Elzinga, B. M. (2012). Influence of emotional distraction on working memory performance in borderline personality disorder. *Psychological medicine*, 42(10), 2181.

Kruijt, A. W., Antypa, N., Booij, L., de Jong, P. J., Glashouwer, K., Penninx, B. W., & Van der Does, W. (2013). Cognitive reactivity, implicit associations, and the incidence of depression: a two-year prospective study. *Plos one*, 8(7), e70245.

Kruk, M. R., Haller, J., Meelis, W., & de Kloet, E. R. (2013). Mineralocorticoid receptor blockade during a rat's first violent encounter inhibits its subsequent propensity for violence. *Behavioral neuroscience*, 127(4), 505.

Kudielka, B.M. & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: a review. *Biological psychology*, 69, 113-132.

Kuehner, C., & Weber, I. (1999). Responses to depression in unipolar depressed patients: An investigation of Nolen-Hoeksema's response styles theory. *Psychological medicine*, 29(6), 1323-1333.

Kulkarni, J. (2007). Depression as a side effect of the contraceptive pill. *Expert opinion on drug safety*, 6(4), 371-374.

Kumsta, R., Kliegel, D., Linden, M., DeRijk, R., & de Kloet, E. R. (2019). Genetic variation of the mineralocorticoid receptor gene (MR, NR3C2) is associated with a conceptual endophenotype of "CRF-hypoactivity". *Psychoneuroendocrinology*, 105, 79-85.

Kuningas, M., De Rijk, R. H., Westendorp, R. G., Jolles, J., Slagboom, P. E., & Van Heemst, D. (2007). Mental performance in old age dependent on cortisol and genetic variance in the mineralocorticoid and glucocorticoid receptors. *Neuropsychopharmacology*, 32(6), 1295-1301.

Kurver, M., Van Der Wijden, C., & Burgers, J. (2012). Summary of the Dutch College of General Practitioners' practice guideline 'Contraception'. *Nederlands tijdschrift voor geneeskunde*, 156(41), A5083.

Kutner, S. & Brown, W. (1972). Types of oral contraceptives, depression and premenstrual symptoms. *Journal of nervous and mental disease,* 155, 153-162.

L

Lang, P.J., Bradley, M.M. & Cuthbert, B.N. (2005). International Affective Picture System (IPS): digitized photographs, instruction manual and affective ratings. *Technical report A-6*, Gainesville: University of Florida.

Lawrie, T., Helmerhorst, F., Maitra, N., Kulier, R., Bloemenkamp, K., & Glmezoglu, A. (2004). Types of progestogens in combined oral contraception: Effectiveness and side-effects. *Cochrane fertility regulation group*, (3), CD004861.

Lebron-Milad, K., & Milad, M. R. (2012). Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders. *Biology of mood & anxiety disorders*, 2(1), 3.

Lethaby, A., Hogervorst, E., Richards, M., Yesufu, A., & Yaffe, K. (2008). Hormone replacement therapy for cognitive function in postmenopausal women. *Cochrane database of systematic reviews*, (1).

Liao, P. V., & Dollin, J. (2012). Half a century of the oral contraceptive pill: historical review and view to the future. *Canadian family physician*, 58(12), e757–e760.

Lisofsky, N., Riediger, M., Gallinat, J., Lindenberger, U. & Kuhn, S., (2016). Hormonal contraceptive use is associated with neural and affective changes in healthy young women. *Neuroimage*, 134, 597-606.

Little, A. C., Burriss, R. P., Petrie, M., Jones, B. C., & Roberts, S. C. (2013). Oral contraceptive use in women changes preferences for male facial masculinity and is associated with partner facial masculinity. *Psychoneuroendocrinology*, 38(9), 1777-1785.

Lösel, R. M., & Wehling, M. (2008). Classic versus non-classic receptors for nongenomic mineralocorticoid responses: emerging evidence. *Frontiers in neuroendocrinology*, 29(2), 258-267.

Lu, N. Z., Wardell, S. E., Burnstein, K. L., Defranco, D., Fuller, P. J., Giguere, V., ... & Wilson, E. M. (2006). International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. *Pharmacological reviews*, 58(4), 782-797.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature reviews neuroscience*, 10(6), 434-445.

M

Macoveanu, J., Henningsson, S., Pinborg, A., Jensen, P., Knudsen, G., Frokjaer, V., & Siebner, H. (2016). Sex-steroid hormone manipulation reduces brain response to reward. *Neuropsychopharmacology*, 41(4), 1057-1065.

Mallareddy, M., Hanes, V., & White, W. B. (2007). Drospirenone, a new progestogen, for postmenopausal women with hypertension. *Drugs & aging*, 24(6), 453-466.

Maner, J. K., & Miller, S. L. (2014). Hormones and social monitoring: Menstrual cycle shifts in progesterone underlie women's sensitivity to social information. *Evolution and human behavior*, 35(1), 9-16.

Maner, J. K., Miller, S. L., Schmidt, N. B., & Eckel, L. A. (2010). The endocrinology of exclusion: Rejection elicits motivationally tuned changes in progesterone. *Psychological science*, 21(4), 581-588.

Marcus, S.M., Kerber, K.B., Rush, A.J., … Trivedi (2008). Sex differences in depression symptoms in treatment-seeking adults: confirmatory analyses from the sequenced treatment alternatives to relieve depression study. *Comprehensive psychiatry*, 49(3), 238-246.

Marjoribanks, J., Brown, J., O'Brien, P., & Wyatt, K. (2013). Selective serotonin reuptake inhibitors for premenstrual syndrome. *Cochrane database of systematic reviews*, 2013(6), CD001396.

Massar, S. A., Kenemans, J. L., & Schutter, D. J. (2014). Resting-state EEG theta activity and risk learning: sensitivity to reward or punishment? *International journal of psychophysiology*, 91(3), 172-177.

Matthews, G. (2016). Distress. In *Stress: Concepts, cognition, emotion, and behavior* (pp. 219-226). Academic Press.

Matthews, G., Deary, I. J., & Whiteman, M. C. (2003). *Personality traits*. Cambridge University Press.

McCambridge, J., Witton, J., & Elbourne, D. R. (2014). Systematic review of the Hawthorne effect: new concepts are needed to study research participation effects. *Journal of clinical epidemiology*, 67(3), 267-277.

McCarney, R., Warner, J., Iliffe, S., Van Haselen, R., Griffin, M., & Fisher, P. (2007). The Hawthorne Effect: a randomised, controlled trial. *BMC medical research methodology*, 7(1), 30.

Mccarthy, G., Puce, A., Gore, J., Truett, T. (1997). Face-specific processing in the human fusiform gyrus. *Journal of cognitive neuroscience,* 9(5), 605-610.

McCrae, R. R., & Costa, P. T. (1987). Validation of the five-factor model of personality across instruments and observers. *Journal of personality and social psychology*, 52(1), 81.

McEwen, B. (2002). Estrogen actions throughout the brain. *Recent progress in hormone research*, 57, 357-384.

McEwen, B. S. (2014). Sex, stress and the brain: interactive actions of hormones on the developing and adult brain. *Climacteric*, 17(sup2), 18-25.

McEwen, B. S. (2016). Central role of the brain in stress and adaptation: Allostasis, biological embedding, and cumulative change. In *Stress: concepts, cognition, emotion, and behavior* (pp. 39-55). Academic Press.

McEwen, B. S., & Alves, S. E. (1999). Estrogen actions in the central nervous system. *Endocrine reviews*, 20(3), 279-307.

McEwen, B. S., Akama, K. T., Spencer-Segal, J. L., Milner, T. A., & Waters, E. M. (2012). Estrogen effects on the brain: actions beyond the hypothalamus via novel mechanisms. *Behavioral neuroscience,* 126(1), 4.

McEwen, B., Gray, J., & Nasca, C. (2015). 60 Years of neuroendocrinology: Redefining neuroendocrinology: stress, sex and cognitive and emotional regulation. *Journal of endocrinology,* 226(2), T67-T83.

McVay, M. A., Copeland, A. L., & Geiselman, P. J. (2011). Eating disorder pathology and menstrual cycle fluctuations in eating variables in oral contraceptive users and non-users. *Eating behaviors*, 12(1), 49-55.

Medina, A., Seasholtz, A. F., Sharma, V., Burke, S., Bunney, W., Myers, R.M ... Watson, S. J. (2012). Glucocorticoid and mineralocorticoid receptor expression in the human hippocampus in major depressive disorder. Journal of psychiatric research, 47(3), 307-314.

Mehta, D., Newport, D.J., Frishman, G., Kraus, L., Rex- Haffner, M., Ritchie, J.C., Lori, A., Knight, B.T., Stagnaro, E., Ruepp, A., Stowe, Z.N. & Binder, E.B. (2014). Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. *Psychological medicine*, 44, 2309-2322.

Mehta, D., Rex-Haffner, M., Søndergaard, H. B., Pinborg, A., Binder, E., & Frokjaer, V. G. (2019). Biological changes in a pharmacologically–induced depression model confirm the role of estrogen sensitivity in perinatal depression. *European neuropsychopharmacology*, 29, S45-S46.

Milad, M., Zeidan, M., Contero, A., Pitman, R., Klibanski, A., Rauch, S., Goldstein, J. (2010). The influence of gonadal hormones on conditioned fear extinction in healthy humans. *Neuroscience,* 168, 652- 658.

Milak, M. S., Parsey, R. V., Keilp, J., Oquendo, M. A., Malone, K. M., & Mann, J. J. (2005). Neuroanatomic correlates of psychopathologic components of major depressive disorder. *Archives of general psychiatry*, 62(4), 397-408.

Milders, M., Bell, S., Platt, J., Serrano, R., & Runcie, O. (2010). Stable expression recognition abnormalities in unipolar depression. *Psychiatry research*, 179(1), 38-42.

Miller, E. K. (2000). The prefontral cortex and cognitive control. *Nature reviews neuroscience*, 1(1), 59.

Mitchell, V. E., & Welling, L. L. (2020). Not all progestins are created equally: considering unique progestins individually in psychobehavioral research. *Adaptive human behavior and physiology*, 6(1), 1-32.

Mogg, K. & Bradley, B.P. (2016). Anxiety and attention to threat: Cognitive mechanisms and treatment with attention bias modification. *Behaviour research and therapy*, 87, 76-108.

Montoya, E. R., & Bos, P. A. (2017). How oral contraceptives impact social-emotional behavior and brain function. *Trends in cognitive sciences*, 21(2), 125-136.

Moos, R. H. (1968). The development of a menstrual distress questionnaire. *Psychosomatic medicine*, 30(6), 853-867.

Mordecai, Rubin, & Maki. (2008). Effects of menstrual cycle phase and oral contraceptive use on verbal memory. *Hormones and behavior*, 54(2), 286-293.

Morrison, A. S., Brozovich, F. A., Lakhan-Pal, S., Jazaieri, H., Goldin, P. R., Heimberg, R. G., & Gross, J. J. (2016). Attentional blink impairment in social anxiety disorder: Depression comorbidity matters. *Journal of behavior therapy and experimental psychiatry*, 50, 209-214.

Mukherjee, S. (2016). *The gene, an intimate history*. Scribner, New York.

Murphy, F. C., Rubinsztein, J. S., Michael, A., Rogers, R. D., Robbins, T. W., Paykel, E. S., & Sahakian, B. J. (2001). Decision-making cognition in mania and depression. *Psychological medicine*, 31(4), 679.

N

Nielsen, S. E., Segal, S. K., Worden, I. V., Yim, I. S., & Cahill, L. (2013). Hormonal contraception use alters stress responses and emotional memory. *Biological psychology*, 92(2), 257-266.

Nolen-Hoeksema S. (1995). Epidemiology and theories of gender differences in unipolar depression. In: Seeman, M.V., ed. *Gender and psychopathology*. Washington: American Psychiatric Press.

Nolen-Hoeksema, S. (1991). Responses to depression and their effects on the duration of depressive episodes. *Journal of abnormal psychology*, 100(4), 569.

Nolen-Hoeksema, S. (2012). Emotion regulation and psychopathology: The role of gender. *Annual review of clinical psychology*, 8, 161-187.

O

Ochsner, K. N. & Gross, J. J. (2014). Chapter 2: The neural bases of emotion and emotion regulation: a valuation perspective. In: *Handbook of emotion regulation*. Second edition. Guilford publications, New York.

Ochsner, K. N., & Gross, J. J. (2005). The cognitive control of emotion. *Trends in cognitive sciences*, 9(5), 242-249.

Ochsner, K. N., Silvers, J. A., & Buhle, J. T. (2012). Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. *Annals of the new york academy of sciences*, 1251, E1.

Oelkers, W. (2002). Antimineralocorticoid activity of a novel oral contraceptive containing drospirenone, a unique progestogen resembling natural progesterone. *The european journal of contraception & reproductive health care*, 7, 19-26.

Oinonen, K. & Mazmanian, D. (2002) To what extent do oral contraceptives influence mood and affect? *Journal of affective disorders*, 70, 229-240.

Oitzl, M. S., & De Kloet, E. R. (1992). Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. *Behavioral neuroscience*, 106(1), 62.

Oliver, M. N., & Simons, J. S. (2004). The affective lability scales: Development of a short-form measure. *Personality and individual differences*, 37(6), 1279-1288.

Opmeer, E. M. (2013). *Linking depression: longitudinal and neuroimaging genetic studies in major depressive disorder*. (Doctoral dissertation, Groningen University, The Netherlands).

Osório, F.L., de Paula Cassis, J.M., Machado de Sousa, J.P., Poli-Neto, O. & Martín-Santos, R. (2018) Sex hormones and processing of facial expressions of emotion: a systematic literature review. *Frontiers in psychology*, (9)529, 1-14.

Ossewaarde, L., Hermans, E. J., van Wingen, G. A., Kooijman, S. C., Johansson, I. M., Bäckström, T., & Fernández, G. (2010). Neural mechanisms underlying changes in stress-sensitivity across the menstrual cycle. *Psychoneuroendocrinology*, 35(1), 47-55.

Ossewaarde, L., Van Wingen, G. A., Kooijman, S. C., Bäckström, T., Fernández, G., & Hermans, E. J. (2011). Changes in functioning of mesolimbic incentive processing circuits during the premenstrual phase. *Social cognitive and affective neuroscience*, 6(5), 612-620.

Ott, M. A., Shew, M. L., Ofner, S., Tu, W., & Fortenberry, J. D. (2008). The influence of hormonal contraception on mood and sexual interest among adolescents. *Archives of sexual behavior*, 37, 605-613.

Ottaviani, C., Shahabi, L., Tarvainen, M., Cook, I., Abrams, M., & Shapiro, D. (2015). Cognitive, behavioral, and autonomic correlates of mind wandering and perseverative cognition in major depression. *Frontiers in neuroscience*, 8, 433.

Otte, C., Hinkelmann, K., Moritz, S., Yassouridis, A., Jahn, H., Wiedemann, K., & Kellner, M. (2010). Modulation of the mineralocorticoid receptor as add-on treatment in depression: a randomized, double-blind, placebo-controlled proof-of-concept study. *Journal of psychiatric res*earch, 44(6), 339-346.

Otte, C., Moritz, S., Yassouridis, A., Koop, M., Madrischewski, A. M., Wiedemann, K., & Kellner, M. (2007). Blockade of the mineralocorticoid receptor in healthy men: effects on experimentally induced panic symptoms, stress hormones, and cognition. *Neuropsychopharmacology*, 32(1), 232-238.

Otte, C., Wingenfeld, K., Kuehl, L. K., Kaczmarczyk, M., Richter, S., Quante, A., ... & Hinkelmann, K. (2015). Mineralocorticoid receptor stimulation improves cognitive function and decreases cortisol secretion in depressed patients and healthy individuals. *Neuropsychopharmacology*, 40(2), 386-393.

Ottowitz, W. E., Derro, D., Dougherty, D. D., Lindquist, M. A., Fischman, A. J., & Hall, J. E. (2008). Analysis of amygdalar-cortical network covariance during pre-versus post-menopausal estrogen levels: potential relevance to resting state networks, mood, and cognition. *Neuro endocrinology letters*, 29(4), 467.

P

Pahnke, R., Mau-Moeller, A., Junge, M., Wendt, J., Weymar, M., Hamm, A. & Lischke, A. (2019) Oral contraceptives impair complex emotion recognition in healthy women. *Frontiers in neuroscience*, 12, 1041.

Patchev, V.K., Shoaib, M., Holsboer, F., Almeida, O. (1994). The neurosteroid tetra-hydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. *Neuroscience*, 62, 265 -271.

Payne, J. L., Palmer, J. T. & Joffe, H. (2009). A reproductive subtype of depression: conceptualizing models and moving toward etiology. *Harvard review of psychiatry*, 17(2), 72-86.

Pearlstein, T., Yonkers, K.A., Fayyad, R., & Gillespie, J.A. (2005). Pretreatment pattern of symptom expression in premenstrual dysphoric disorder. *Journal of affective disorders*, 3, 272-282.

Pearson, R. M., Lightman, S. L., & Evans, J. (2009). Emotional sensitivity for motherhood: late pregnancy is associated with enhanced accuracy to encode emotional faces. *Hormones and behavior*, 56(5), 557-563.

Peper, J. S., van den Heuvel, M. P., Mandl, R. C., Pol, H. E. H., & van Honk, J. (2011). Sex steroids and connectivity in the human brain: a review of neuroimaging studies. *Psychoneuroendocrinology*, 36(8), 1101-1113.

Petersen, N., Kilpatrick, L. A., Goharzad, A., & Cahill, L. (2014). Oral contraceptive pill use and menstrual cycle phase are associated with altered resting state functional connectivity. *Neuroimage*, 90, 24-32.

Plath, S. (1972). *The bell jar* (1963). London: Faber & Faber.

Pletzer, B. A., & Kerschbaum, H. H. (2014). 50 years of hormonal contraception – time to find out, what it does to our brain. *Frontiers in neuroscience*, 8, 256.

Pletzer, B., Kronbichler, M., & Kerschbaum, H. (2015). Differential effects of androgenic and anti-androgenic progestins on fusiform and frontal gray matter volume and face recognition performance. *Brain research*, 1596, 108-115.

Pletzer, B., Kronbichler, M., Aichhorn, M., Bergmann, J., Ladurner, G., Kerschbaum, H. (2010). Menstrual cycle and hormonal contraceptive use modulate human brain structure. *Brain research,* 1348, 55-62.

Pletzer, B., Kronbichler, M., Nuerk, H. C., & Kerschbaum, H. (2014). Hormonal contraceptives masculinize brain activation patterns in the absence of behavioral changes in two numerical tasks. *Brain research*, 1543, 128-142.

Plotsky, P. M., Owens, M. J., & Nemeroff, C. B. (1998). Psychoneuroendocrinology of depression: hypothalamic-pituitary-adrenal axis. *Psychiatric clinics of north america*, 21, 293-307.

Poromaa, I. S., & Segebladh, B. (2012). Adverse mood symptoms with oral contraceptives. *Acta obstetricia et gynecologica scandinavica*, 91(4), 420-427.

Putman, P., van Peer, J., Maimari, I. & van der Werff, S. (2010). EEG theta/beta ratio in relation to fear-modulated response-inhibition, attentional control, and affective traits. *Biological Psychology,* 83, 73-78.

Putman, P., Verkuil, B., Arias-Garcia, E., Pantazi, I., & van Schie, C. (2014). EEG theta/beta ratio as a potential biomarker for attentional control and resilience against deleterious effects of stress on attention. *Cognitive, affective, & behavioral neuroscience*, 14(2), 782-791.

Putnam, K. T., Harris, W. W., & Putnam, F. W. (2013). Synergistic childhood adversities and complex adult psychopathology. *Journal of traumatic stress*, 26(4), 435-442.

Q

Quinkler, M., & Diederich, S. (2002). Difference of in vivo and in vitro antimineralocorticoid potency of progesterone. *Endocrine research*, 28(4), 465-470.

Quinkler, M., Meyer, B., Bumke-Vogt, C., Grossmann, C., Gruber, U., Oelkers, W., & Bähr, V. (2002). Agonistic and antagonistic properties of progesterone metabolites at the human mineralocorticoid receptor. *European journal of endocrinology*, 146(6), 789-800.

Quinkler, M., Meyer, B., Bumke-Vogt, C., Grossmann, C., Gruber, U., Oelkers, W., ... & Bahr, V. (2002). Agonistic and antagonistic properties of progesterone metabolites at the human mineralocorticoid receptor. *European journal of endocrinology*, 146(6), 789-799.

Quirin, M., Kazén, M., & Kuhl, J. (2009). When nonsense sounds happy or helpless: the implicit positive and negative affect test (IPANAT). *Journal of personality and social psychology*, 97(3), 500.

R

Raadsheer, F. C., Hoogendijk, W. J., Stam, F. C., Tilders, F. J., & Swaab, D. F. (1994). Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology*, 60(4), 436-444.

Raap, D., Doncarlos, L., Garcia, F., Muma, N., Wolf, W., … & Battaglia, G. (2000). Estrogen desensitizes 5-HT(1A) receptors and reduces levels of G(z), G(i1) and G(i3) proteins in the hypothalamus. *Neuropharmacology*, 39, 1823-1832.

Rachman, S. (2004). *Anxiety* (2nd ed., Clinical Psychology. 175563357). Hove [etc.]: Psychology Press.

Radke, & Derntl. (2016). Affective responsiveness is influenced by intake of oral contraceptives. *European neuropsychopharmacology*, 26(6), 1014-1019.

Raymer, K. A., Waters, R. F., & Price, C. R. (2005). Proposed multigenic composite inheritance in major depression. *Medical hypotheses*, 65(1), 158-172.

Reul, J. M & De Kloet, E.R. (1985). Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology*, 117(6), 2505-2511.

Ritter, D. (2003). Effects of menstrual cycle phase on reporting levels of aggression using the Buss and Perry Aggression Questionnaire. *Aggression and behavior*, 29, 531-538.

Rivera, R., Yacobson, I., & Grimes, D. (1999). The mechanism of action of hormonal contraceptives and intrauterine contraceptive devices. American *journal of obstetrics and gynecology*, 181(5), 1263-1269.

Robakis, T. K., Holtzman, J., Stemmle, P. G., Reynolds-May, M. F., Kenna, H. A., & Rasgon, N. L. (2015). Lamotrigine and GABAA receptor modulators interact with menstrual cycle phase and oral contraceptives to regulate mood in women with bipolar disorder. *Journal of affective disorders*, 175, 108-115.

Rogers, R. D., Tunbridge, E. M., Bhagwagar, Z., Drevets, W. C., Sahakian, B. J., & Carter, C. S. (2003). Tryptophan depletion alters the decision-making of healthy volunteers through altered processing of reward cues. *Neuropsychopharmacology*, 28(1), 153-162.

Rohleder N, Schommer NC, Hellhammer DH, Engel R, & Kirschbaum C. (2001). Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosomatic medicine*, 63, 966-972.

Roos, A., Lochner, C., Kidd, M., van Honk, J., Vythilingum, B., & Stein, D. J. (2012). Selective attention to fearful faces during pregnancy. *Progress in neuro-psychopharmacology and biological psychiatry*, 37(1), 76-80.

Rovaris, D. L., Mota, N. R., Bertuzzi, G. P., Aroche, A. P., Callegari-Jacques, S. M., Guimarães, L. S., ... & Grassi-Oliveira, R. (2015). Corticosteroid receptor genes and childhood neglect influence susceptibility to crack/cocaine addiction and response to detoxification treatment. *Journal of psychiatric research*, 68, 83-90.

Rozeboom, A. M., Akil, H., & Seasholtz, A. F. (2007). Mineralocorticoid receptor overexpression in forebrain decreases anxiety-like behavior and alters the stress response in mice. *Proceedings of the national academy of sciences*, 104(11), 4688-4693.

Rudolph, K.D., Hammen, C., Burge, D. (1994). Interpersonal functioning and depressive symptoms in childhood: addressing the issues of specificity and comorbidity. *Journal of abnormal child psychology*, 22, 355-371.

S

Sacher, J., Okon-Singer, H., & Villringer, A. (2013). Evidence from neuroimaging for the role of the menstrual cycle in the interplay of emotion and cognition. *Frontiers in human neuroscience*, 7, 374.

Sapolsky, R. M. (2004). *Why zebras don't get ulcers: The acclaimed guide to stress, stress-related diseases, and coping-now revised and updated*. Holt paperbacks.

Sarubin, N., Hilbert, S., Naumann, F., Zill, P., Wimmer, A., Nothdurfter, C.& Schüle, M. (2017). The sex-dependent role of the glucocorticoid receptor in depression: Variations in the NR3C1 gene are associated with major depressive disorder in women but not in men. *European archives of psychiatry and clinical neuroscience*, 267(2), 123-133.

Schaffir, J., Worly, B. L., & Gur, T. L. (2016). Combined hormonal contraception and its effects on mood: a critical review. *The european journal of contraception & reproductive health care*, 21(5), 347-355.

Schmidt, P., Nieman, L., Danaceau, M., Adams, L., & Rubinow, D. (1998). Differential behavioral effects of gonadal steroids in women with and in those without premenstrual syndrome. *The new England journal of medicine*, 338(4), 209-216.

Schultheiss, O. C., Dargel, A., & Rohde, W. (2003). Implicit motives and gonadal steroid hormones: Effects of menstrual cycle phase, oral contraceptive use, and relationship status. *Hormones and behavior*, 43(2), 293-301.

Schutter, D. J., & Van Honk, J. (2005). Electrophysiological ratio markers for the balance between reward and punishment. *Cognitive brain research*, 24(3), 685-690.

Schwabe, L., Schächinger, H., de Kloet, E. R., & Oitzl, M. S. (2010). Corticosteroids operate as a switch between memory systems. *Journal of cognitive neuroscience*, 22(7), 1362-1372.

Schwabe, L., Tegenthoff, M., Höffken, O., & Wolf, O. T. (2013). Mineralocorticoid receptor blockade prevents stress-induced modulation of multiple memory systems in the human brain. *Biological psychiatry*, 74(11), 801-808.

Segerstrom, S. C. (2001). Optimism and attentional bias for negative and positive stimuli. *Personality and social psychology bulletin*, 27(10), 1334-1343.

Sheehan, D., Lecrubier, Y., Sheehan, K., Aamarim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G. (1998). The MINI-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatruic interview for DSM-IV and ICD-10. *Journal of clinical pychiatry,* 59, Suppl 20, 22-33.

Shiffman, S., Stone, A. A. & Hufford, M. R. (2008). Ecological momentary assessment. *Annual Reviews on Clinical Psychology*, 4, 1-32.

Shirazi, T. N., Rosenfield, K. A., Cárdenas, R. A., Breedlove, S. M., & Puts, D. A. (2020). No evidence that hormonal contraceptive use or circulating sex steroids predict complex emotion recognition. *Hormones and behavior*, 119, 104647.

Shull, A., Mayer, S. E., McGinnis, E., Geiss, E., Vargas, I., & Lopez-Duran, N. L. (2016). Trait and state rumination interact to prolong cortisol activation to psychosocial stress in females. *Psychoneuroendocrinology*, 74, 324-332

Siegel A, Sapru H. (2011). *Essential Neuroscience*, Second Edition, Lippincott Williams & Wilkins, Baltimore: USA.

Singer, W. (1993). Synchronization of cortical activity and its putative role in information processing and learning. *Annual review of physiology*, 55(1), 349-374.

Sitruk-Ware, R. (2005). New progestagens for contraceptive use. *Human reproduction update*, 12(2), 169-178.

Sitruk-Ware, R. (2005). Pharmacology of different progestogens: the special case of drospirenone. *Climacteric*, 8(sup3), 4-12.

Skovlund, C. W., Mørch, L. S., Kessing, L. V., & Lidegaard, Ø. (2016). Association of hormonal contraception with depression. *JAMA psychiatry*, 73(11), 1154-1162.

Slagter, H.A., Tomer, R., Christian, B.T., Fox, A.S., Colzato, L.S., King, C.R., ... & Davidson, R.J. (2012). PET evidence for a role for striatal dopamine in the attentional blink: Functional implications. *Journal of cognitive neuroscience*, 24(9), 1932-1940.

Smith, R., & Lane, R. D. (2016). Unconscious emotion: A cognitive neuroscientific perspective. *Neuroscience & biobehavioral reviews*, 69, 216-238.

Soares, C. N., & Zitek, B. (2008). Reproductive hormone sensitivity and risk for depression across the female life cycle: a continuum of vulnerability? *Journal of psychiatry & neuroscience,* 4, 331-343.

Solberg Nes, L. S. (2016). Optimism, pessimism, and stress. In *Stress: concepts, cognition, emotion, and behavior* (pp. 405-411). Academic Press.

Solis, E, Antypa, N., Conijn, J., Kelderman H., Van der Does, W. (2017). Psychometric properties of the Leiden Index of Depression Sensitivity (LEIDS). *Psychological Assessment*, 29(2), 158.

Solis-Ortiz, S., Ramos, J., Arce, C., Guevara, M. A., & Corsi-Cabrera, M. (1994). EEG oscillations during menstrual cycle. *International journal of neuroscience*, 76(3-4), 279-292.

Solis, E., Antypa, N., Conijn, J. M., Kelderman, H., & Van Der Does, W. (2016). Psychometric properties of the Leiden index of depression. *Psychological assessment. Advance online publication*.

Solomon, A. (2001). *The Noonday Demon*. An atlas of depression. Scribner, New York, NY.

Sommer, T., Richter, K., Singer, F., Derntl, B., Rune, G., Diekhof, E., & Bayer, J. (2018). Effects of the experimental administration of oral estrogen on prefrontal functions in healthy young women. *Psychopharmacology*, 235(12), 3465-3477.

Stahl, M. (2013). Antidepressants. In *Stahl's essential psychopharmacology*. Neuroscientific basis and practical application. Cambridge University Press. New York.

Stanczyk, F. Z. (2003). All progestins are not created equal. *Steroids*, 68(10–13), 879-890.

Stanczyk, F. Z., Hapgood, J. P., Winer, S., & Mishell Jr, D. R. (2013). Progestogens used in postmenopausal hormone therapy: differences in their pharmacological properties, intracellular actions, and clinical effects. *Endocrine reviews*, 34(2), 171-208.

Stanton, S. J., Liening, S. H., & Schultheiss, O. C. (2011). Testosterone is positively associated with risk taking in the Iowa Gambling Task. *Hormones and behavior*, 59(2), 252-256.

Sundström Poromaa, I., & Gingnell, M. (2014). Menstrual cycle influence on cognitive function and emotion processing–from a reproductive perspective. *Frontiers in neuroscience*, 8, 380, 1-16.

Sundström-Poromaa, I & Segebladh, B. (2012). Adverse mood effects with oral contraceptives. *Acta obstetricia et gynecologica Scandinavica*, 91, 420-427.

Sundström-Poromaa, I. (2018). The menstrual cycle influences emotion but has limited effect on cognitive function. *Vitamins and hormones*, 107, 349-376.

Suslow, T., Ihme, K., Quirin, M., Lichev, V., Rosenberg, N., Bauer, J., ... & Lobsien, D. (2015). Implicit affectivity and rapid processing of affective body language: an fMRI study. *Scandinavian journal of psychology*, 56(5), 545-552.

Svendal, G., Berk, M., Pasco, J. A., Jacka, F. N., Lund, A., Williams, L. J. (2012). The use of hormonal contraceptive agents and mood disorders in women. *Journal of affective disorders*, 140(1), 92-96.

T

Tabachnick, B. G., Fidell, L. S., & Ullman, J. B. (2007). *Using multivariate statistics*. Boston, MA: Pearson.

Teasdale, J. D. (1997). The relationship between cognition and emotion: The mind-in-place in mood disorders. In D. M. Clark & C. G. Fairburn (Eds.), *Science and practice of cognitive behaviour therapy* (p. 67-93). Oxford University Press.

Teatero, M. L., Mazmanian, D., & Sharma, V. (2014). Effects of the menstrual cycle on bipolar disorder. *Bipolar disorders*, 16(1), 22-36.

Ter Heegde, F., De Rijk, R. H., & Vinkers, C. H. (2015). The brain mineralocorticoid receptor and stress resilience. *Psychoneuroendocrinology*, 52, 92-110.

Ter Horst, J. P., Carobrez, A. P., Van Der Mark, M. H., De Kloet, E. R., & Oitzl, M. S. (2012). Sex differences in fear memory and extinction of mice with forebrain-specific disruption of the mineralocorticoid receptor. *European journal of neuroscience*, 36(8), 3096-3102.

Ter Horst, J. P., Kentrop, J., Arp, M., Hubens, C. J., de Kloet, E. R., & Oitzl, M. S. (2013). Spatial learning of female mice: a role of the mineralocorticoid receptor during stress and the estrous cycle. *Frontiers in behavioral neuroscience*, 7, 56.

Terburg, D., Aarts, H., & van Honk, J. (2012). Testosterone affects gaze aversion from angry faces outside of conscious awareness. *Psychological science*, 23(5), 459-463.

Thayer, R. E., Newman, J. R., & McClain, T. M. (1994). Self-regulation of mood: Strategies for changing a bad mood, raising energy, and reducing tension. *Journal of personality and social psychology*, 67(5), 910.

Thomas, J. M., Higgs, S., & Dourish, C. T. (2016). Test–retest reliability and effects of repeated testing and satiety on performance of an Emotional Test Battery. *Journal of clinical and experimental neuropsychology*, 38(4), 416-433.

Thomas, L. A., De Bellis, M. D., Graham, R., & LaBar, K. S. (2007). Development of emotional facial recognition in late childhood and adolescence. *Developmental science*, 10(5), 547-558.

Toffol, E., Heikinheimo, O., Koponen, P., Luoto, R., & Partonen, T. (2012). Further evidence for lack of negative associations between hormonal contraception and mental health. *Contraception*, 86(5), 470-480.

Tops, M. & Wijers, A.A. (2011). Letter to the editor Re: "The effect of cortisol on emotional responses depends on order of cortisol and placebo administration in a within-subject design" by Wirth et al. *Psychoneuroendocrinology,* 36, 1097-1099.

Tops, M., Huffmeijer, R., Linting, M., Grewen, K.M., Light, K.C., Koole, S.L., Bakermans-Kranenburg, M.J. & van IJzendoorn, M.H. (2013). The role of oxytocin in familiarization-habituation responses to social novelty. *Frontiers in Psychology*, 4, 761.

Tortella-Feliu, M., Morillas-Romero, A., Balle, M., Llabrés, J., Bornas, X., & Putman, P. (2014). Spontaneous EEG activity and spontaneous emotion regulation. *International journal of psychophysiology*, 94(3), 365-372.

Trussell, J., & Kost, K. (1987). Contraceptive failure in the United States: a critical review of the literature. S*tudies in family planning*, 18(5), 237-283.

U

Upton, D. J., Bishara, A. J., Ahn, W. Y., & Stout, J. C. (2011). Propensity for risk taking and trait impulsivity in the Iowa Gambling Task. *Personality and individual differences*, 50(4), 492-495.

V

Van de Kar, L. & Blair M. (1999). Forebrain pathways mediating stress-induced hormone secretion. *Frontiers in neuroendocrinology*, 20, 1-48.

Van den Broeck (2014). *De echte vader van de pil. Het verhaal van de man die de vrouw bevrijdde*. De Bezige Bij, Antwerpen.

Van der Does, W. (2002). Cognitive reactivity to sad mood: structure and validity of a new measure. *Behaviour research and therapy*, 40(1), 105-119.

Van der Kolk, B. A. (2015). *The body keeps the score: Brain, mind, and body in the healing of trauma.* Penguin Books, USA.

Van Dijk, E. H., Schellevis, R. L., van Bergen, M. G., Breukink, M. B., Altay, L., Scholz, P., ... & Boon, C. J. (2017). Association of a haplotype in the NR3C2 gene, encoding the mineralocorticoid receptor, with chronic central serous chorioretinopathy. *JAMA ophthalmology*, 135(5), 446-451

Van Heusden, A. M., & Fauser, B. C. J. M. (1999). Activity of the pituitary-ovarian axis in the pill-free interval during use of low-dose combined oral contraceptives. *Contraception*, 59(4), 237-243.

Van Heusden, A. M., & Fauser, B. C. J. M. (2002). Residual ovarian activity during oral steroid contraception. *Human reproduction update*, 8(4), 345-358.

Van Leeuwen, N. (2010c). *Mineralocorticoid receptor variants. Implications for stress, blood pressure and personality*. Doctoral thesis. Division of Medical Pharmacology, Leiden/Amsterdam Center for Drug Research, Faculty of Science, Leiden University.

Van Leeuwen, N., Bellingrath, S., de Kloet, E. R., Zitman, F. G., DeRijk, R. H., Kudielka, B. M. & Wüst, S. (2011). Human mineralocorticoid receptor (MR) gene haplotypes modulate MR expression and transactivation: implication for the stress response. *Psychoneuroendocrinology*, 36(5), 699-709.

Van Leeuwen, N., Caprio, M., Blaya, C., Fumeron, F., Sartorato, P., Ronconi, V., G, …& Zennaro, M. (2010a). The functional c.-2G > G variant of the mineralocorticoid receptor modulates blood pressure, renin, and aldosterone levels. *Hypertension*, 56(5), 995-1002.

Van Leeuwen, N., Kumsta, R., Entringer, S., de Kloet, E. R., Zitman, F. G., DeRijk, R. H., & Wust, S. (2010b). Functional mineralocorticoid receptor (MR) gene variation influences the cortisol awakening response after dexamethasone. *Psychoneuroendocrinology*, 35(3), 339-349.

Van Praag, H. M. (1996). Serotonin-related, anxiety/aggression-driven, stressor-precipitated depression. A psycho-biological hypothesis. *European psychiatry*, 11(2), 57-67.

Van Son, D. (2019). *EEG theta/beta ratio: a marker of executive control and its relation with anxiety-linked attentional bias for threat*. (Doctoral dissertation, Faculty of Social and Behavioural Sciences, Leiden University).

Van Son, D., Angelidis, A., Hagenaars, M. A., van der Does, W., & Putman, P. (2018). Early and late dot-probe attentional bias to mild and high threat pictures: Relations with EEG theta/beta ratio, self-reported trait attentional control, and trait anxiety. *Psychophysiology*, 55(12), e13274.

Van Son, D., De Blasio, F. M., Fogarty, J. S., Angelidis, A., Barry, R. J., & Putman, P. (2019). Frontal EEG theta/beta ratio during mind wandering episodes. *Biological Psychology*, 140, 19-27.

Van Son, D., Schalbroeck, R., Angelidis, A., van der Wee, N. J., van der Does, W., & Putman, P. (2018). Acute effects of caffeine on threat-selective attention: moderation by anxiety and EEG theta/beta ratio. *Biological psychology*, 136, 100-110.

Van Vliet, I. M., & De Beurs, E. (2007). The MINI-International Neuropsychiatric Interview. A brief structured diagnostic psychiatric interview for DSM-IV en ICD-10 psychiatric disorders. *Tijdschrift voor psychiatrie*, 49(6), 393-397.

Van Wingen, G. A., Ossewaarde, L., Bäckström, T., Hermans, E. J., & Fernández, G. (2011). Gonadal hormone regulation of the emotion circuitry in humans. *Neuroscience*, 191, 38-45.

Van Wingen, G. A., Van Broekhoven, F., Verkes, R. J., Petersson, K. M., Bäckström, T., Buitelaar, J., & Fernandez, G. (2008). Progesterone selectively increases amygdala reactivity in women. *Molecular psychiatry*, 13(3), 325-333.

Verhoeven, F. E. (2014). *Rain with chances of a thunderstorm: on the role of anger in depression* (Doctoral dissertation, Faculty of Social and Behavioural Sciences, Leiden University).

Vessey, M. P., McPherson, K., Lawless, M., & Yeates, D. (1985). Oral contraception and serious psychiatric illness: absence of an association. *The British journal of psychiatry*, 146(1), 45-49.

Viau, V., & Meaney, M.J. (1991). Variations in the hypothalamic-pituitary-adrenal response to stress during the estrous cycle in the rat. *Endocrinology*, 129, 2503-2511.

Vinkers, C. H., Joëls, M., Milaneschi, Y., Gerritsen, L., Kahn, R. S., Penninx, B. W., & Boks, M. P. (2015). Mineralocorticoid receptor haplotypes sex-dependently moderate depression susceptibility following childhood maltreatment. *Psychoneuroendocrinology*, 54, 90-102.

Vogel, S., Fernández, G., Joëls, M., & Schwabe, L. (2016). Cognitive adaptation under stress: a case for the mineralocorticoid receptor. *Trends in cognitive sciences*, 20(3), 192-203.

Vogel, S., Gerritsen, L., van Oostrom, I., Arias-Vásquez, A., Rijpkema, M., Joëls, M., ... & Fernández, G. (2014). Linking genetic variants of the mineralocorticoid receptor and negative memory bias: interaction with prior life adversity. *Psychoneuroendocrinology*, 40, 181-190.

Vogel, S., Klumpers, F., Krugers, H. J., Fang, Z., Oplaat, K. T., Oitzl, M. S., ... & Fernández, G. (2015). Blocking the mineralocorticoid receptor in humans prevents the stress-induced enhancement of centromedial amygdala connectivity with the dorsal striatum. *Neuropsychopharmacology*, 40(4), 947-956.

W

Wambach, G., & Higgins, J. R. (1978). Antimineralocorticoid action of progesterone in the rat: correlation of the effect on electrolyte excretion and interaction with renal mineralocorticoid receptors. *Endocrinology*, 102(6), 1686-1693.

Warren, A. M., Gurvich, C., Worsley, R., & Kulkarni, J. (2014). A systematic review of the impact of oral contraceptives on cognition. *Contraception*, 90(2), 111-116.

Warren, M. B., Cowen, P. J., & Harmer, C. J. (2019). Subchronic treatment with St John's wort produces a positive shift in emotional processing in healthy volunteers. *Journal of psychopharmacology*, 33(2), 194-201.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of personality and social psychology*, 54(6), 1063.

Wax, R. (2013). *Sane new world: taming the mind*. London: Hodder & Stoughton.

Webster, M.J., Knable, M.B., O'Grady, J., Orthmann, J. & Weickert, C.S. (2002). Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. *Molecular psychiatry*, 7(9), 985-994.

Weissman, M. M.; Klerman, G.L. (1977). Sex differences and the epidemiology of depression. *Archives of general psychiatry*, 34, 98-111.

Welling, L. L. M. (2013). Psychobehavioral effects of hormonal contraceptive use. *Evolutionary psychology*, 11(3), 718–742.

Whitton, S. W., Weitbrecht, E. M., Kuryluk, A. D., & Bruner, M. R. (2013). Committed dating relationships and mental health among college students. *Journal of american college healt*h, 61(3), 176-183.

WHO. (n.d.). *Depression.* Fact sheet. Updated February 2017. Retrieved March 2021, from http://www.who.int/mediacentre/factsheets/fs369/en/

Wickstrom, G. & Bendix, T. (2000): The "Hawthorne effect": what did the original Hawthorne studies actually show? *Scandinavian journal of work, environment & health*, 26, 363-367.

Wiebe, E. R. (2013). Invited commentary: how can we reconcile the findings of Keyes et al.'s study with the experience of our patients in clinical practice? *American journal of epidemiology*, 178(9), 1389-1391.

Wilhelm, K., Boyce, P., & Brownhill, S. (2004). The relationship between interpersonal sensitivity, anxiety disorders and major depression. *Journal of affective disorders*, 79(1-3), 33-41.

Wilhelm, O., Hildebrandt, A., Manske, K., Schacht, A., & Sommer, W. (2014). Test battery for measuring the perception and recognition of facial expressions of emotion. *Frontiers in psychology*, 5, 404.

Wingenfeld, K., & Otte, C. (2019). Mineralocorticoid receptor function and cognition in health and disease. *Psychoneuroendocrinology*, 105, 25-35.

Wirth, M. (2011). Beyond the HPA axis: Progesterone-derived neuroactive steroids in human stress and emotion. *Frontiers in endocrinology*, 2, 19.

Wirth, M. M., & Schultheiss, O. C. (2006). Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol. *Hormones and behavior*, 50(5), 786-795.

Wirth, M. M., & Schultheiss, O. C. (2007). Basal testosterone moderates responses to anger faces in humans. *Physiology & behavior*, 90(2-3), 496-505.

Wirz, L., Reuter, M., Wacker, J., Felten, A., & Schwabe, L. (2017). A haplotype associated with enhanced mineralocorticoid receptor expression facilitates the stress-induced shift from "cognitive" to "habit" learning. *eNeuro*, 4(6).

Wood, R. I., Armstrong, A., Fridkin, V., Shah, V., Najafi, A., & Jakowec, M. (2013). 'Roid rage in rats? Testosterone effects on aggressive motivation, impulsivity and tyrosine hydroxylase. *Physiology & behavior*, 110, 6-12.

Wray, N. R., Ripke, S., Mattheisen, M., Trzaskowski, M., Byrne, E. M., Abdellaoui, A., ... & Bacanu, S. A. (2018). Genome-wide association analyses identify 44 risk variants and refine the genetic architecture of major depression. *Nature genetics*, 50(5), 668.

Wu, T., Chen, S., Brinton, R. (2011). Membrane estrogen receptors mediate calcium signaling and MAP kinase activation in individual hippocampal neurons. *Brain research,* 1379, 34-43.

Y

Yonkers, K. A., Cameron, B., Gueorguieva, R., Altemus, M., & Kornstein, S. G. (2017). The influence of cyclic hormonal contraception on expression of premenstrual syndrome. *Journal of women's health*, 26(4), 321-328.

Young, A. W., Rowland, D., Calder, A. J., Etcoff, N. L., Seth, A., & Perrett, D. I. (1997). Facial expression megamix: Tests of dimensional and category accounts of emotion recognition. *Cognition*, 63(3), 271-313.

Young, E. A., Kornstein, S. G., Harvey, A. T., Wisniewski, S. R., Barkin, J., Fava, M., ... & Rush, A. J. (2007). Influences of hormone-based contraception on depressive symptoms in premenopausal women with major depression. *Psychoneuroendocrinology*, 32, 843-853.

Z

Zeidan, M., Igoe, S., Linnman, C., Vitalo, A., Levine, J., Klibanski, A., Goldstein, J., Milad, M. (2011). Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. *Biological psychiatry,* 70, 920–927.

Zethraeus, N., Dreber, A., Ranehill, E., Blomberg, L., Labrie, F., von Schoultz, B., ... & Hirschberg, A. L. (2017). A first-choice combined oral contraceptive influences general well-being in healthy women: a double-blind, randomized, placebo-controlled trial. *Fertility and sterility*, 107(5), 1238-1245.

Zhou, M., Bakker, E. H., Velzing, E. H., Berger, S., Oitzl, M., Joëls, M., & Krugers, H. J. (2010). Both mineralocorticoid and glucocorticoid receptors regulate emotional memory in mice. *Neurobiology of learning and memory*, 94(4), 530-537.

Zimmerman, Y., Eijkemans, M., Coelingh Bennink, H., Blankenstein, M., & Fauser, B. (2014). The effect of combined oral contraception on testosterone levels in healthy women: a systematic review and meta-analysis. *Human reproduction update*, 20(1), 76-105.

Zoccola, P. M., Quas, J. A., & Yim, I. (2010). Salivary cortisol responses to a psychosocial laboratory stressor and later verbal recall of the stressor: The role of trait and state rumination. *Stress*, 13(5), 435-443.

Summary

Chapter 1 Introduction

Background

During their fertile years, women are twice as likely to develop a clinical depression as men. Some women may experience mood shifts towards depression during usage of contraceptives containing synthetic female hormones, which may even lead to the diagnosis of a clinical depression. Especially young women seem to be prone to these depressogenic effects of OC (Skovlund et al., 2017). Since hormonal contraceptives still are one of the safest ways for birth control, further research after specific determinants underlying this vulnerability to the negative mood effects of OC is needed.

Rationale

In this PhD project we investigated whether OC use and menstrual cycle phase influence emotional information processes associated with depression in healthy pre-menopausal women. We aimed at detecting subtle biases which might not be noticed in daily life. In addition, we explored whether any effects of the female hormonal status would be influenced by genetic variation in one particular component of cortisol feedback regulation in the stress response system – the mineralocorticoid receptor (MR) (De Kloet et al., 2005; Boron & Boulpaep, 2012).[32] The focus is on the MR because previous research showed that genetic variation in this receptor predicted stress-coping and resilience (Klok et al. 2011; Vinkers et al 2015). In pre-menopausal women carriers of MR haplotype 2 were less vulnerable to mood disorders following of childhood trauma than carriers MR haplotype 1/3, suggesting an interaction between the female hormonal status and genetic variation in the MR (Klok et al., 2011; Vinkers et al., 2015). In the next sections the content of Chapters 2 – 7 is summarized and followed by the general conclusion.

Chapter 2 Oral contraceptives may alter the detection of emotions in facial expressions

Background

This PhD project started with a first-of-its-kind study in which we investigated the potential antidepressant effects of the potent synthetic mineralocorticoid fludrocortisone (FC) in a neuropsychological model of drug action. In this model (emotional) information processing indices serve as a proxy for a potential therapeutic response. The model has been validated using a number of registered antidepressants in various designs, ranging from the effect of a single dose

[32] See for specificity of the MR Chapter 1, figure 4.

in healthy volunteers to repeated administration in depressed or remitted depressed patients. The findings depend on antidepressant drug, dosage and target population, but have in common that subtle antidepressant effects have subtle effects on emotional information processing are noted which cannot easily be detected by self-report. It remains to be demonstrated that the (size of the) effects are related to subsequent clinical response to antidepressants, but the immediate effects of single doses in healthy volunteers have been repeatedly demonstrated and may be a fast way to assess the antidepressant potential of new substances (see a.o. Harmer et al., 2009; Warren et al., 2019). 11

We used FC in this model because it is a potent mineralocorticoid agonist with some glucocorticoid activity. Hence, FC can also suppress HPA axis activity by synergy of its central MR- and pituitary GR-mediated actions, which may be of pharmacological interest in the treatment of disorders like depression (Lembke et al., 2013; Buckley et al., 2007). For instance, FC accelerated the antidepressant effects of the SSRI escitalopram (Otte et al., 2010), while spironolactone, a MR antagonist, decreased the efficacy of the antidepressant amitriptyline in depressed patients (Holsboer et al., 1999). We considered but decided not to exclude participants who used OC and registered OC use instead. The reason is that we expected a relatively high dose of FC to override possible confounding effects of oral contraceptives.

Procedure

We studied 40 healthy female volunteers in a randomized, double-blind, parallel-group, placebo-controlled design. Healthy pre-menopausal volunteers were included after physical examination and being screened for psychiatric present and past. In order to ensure relatively stable levels on cortisol, two hours after a standardized lunch 0,5 mg FC or placebo was administered. Subsequently participants spent two hours in sedentary activities between the intervention and assessment of the facial expression recognition test (FERT) and the emotional categorization and memory task. Participants and researchers were blind for their condition and MR-haplotypes.

Results

Contrary to expectations, we found no effect of FC administration on the accuracy in the FERT and the emotional categorization and memory task. Except for happiness trials, FC administration was associated with decreased reaction times in the FERT. Unexpectedly, users of OC detected significantly fewer facial expressions of sadness, anger and disgust than naturally cycling (NC) women in the FERT. This was true for all trial participants as well as those individuals who were randomized to the placebo condition.

Exploratory analyses in the NC group (n = 14) revealed that the administration of one dose of FC (0.5 mg) was associated with better and quicker recognition of facial expressions

of fear and happiness in carriers of MR-haplotype 1/3. They also remembered more positive characteristics after FC administration. This effect was not observed in MR-haplotype 2 carriers (homo- and heterozygotes).

Conclusion

Clearly, FC did not override the OC effects, thus confounding effects of OC may underlie the lack of effect of FC administration in general. Exploratory analyses in the NC group revealed that only in MR-haplotype 1/3 carriers a single dose of FC was associated with improved emotional information processing. The effect of OC on emotion recognition is a chance finding and if replicated, could either be an effect of OC use or a pre-existing difference between OC-users and NC women. Future studies on the effect of neuropsychiatric interventions should control for the effects of oral contraceptives on emotional and cognitive outcomes.

Chapter 3 Mineralocorticoid receptor haplotype, oral contraceptives and emotional information processing

Background

In experimental research OC-users failed to report positive affect after positive mood induction (Jarva & Oinonen, 2007). Depressogenic effects remain one of the main reasons for discontinuation of OC (Oinonen & Mazmanian, 2002). The goal of the current study was to further investigate the effects of OC use on information processing (Hamstra et al., 2014), considering potential moderating effects of MR- haplotypes. Firstly, OC-users were compared with NC women on indices of emotional information processing. Secondly, we hypothesized that performance on these tasks would be moderated by MR-haplotype. Specifically, we hypothesized that the effects of OC on emotional information processes would be more pronounced in carriers of MR haplotype 1/3 than in MR haplotype 2.

Procedure

This exploratory study had a cross-sectional, parallel-group design. Healthy pre-menopausal volunteers completed a test battery in a psychology laboratory. NC women (n = 41; mean age = 20.2) were tested between day 6 and 26 of their menstrual cycle. OC users (n = 44; mean age = 20.4) were tested outside their pill-free week. OC brand name and chemical compound, duration of OC use and in case of NC women first day of last menses were registered. Participants were genotyped at assessment.

Results

In line with the previous study, OC-users recognized fewer facial expressions of disgust, happiness, sadness and anger (only anger became significant). Further, OC-users made fewer

risky decisions in a gambling paradigm. A significant interaction effect between OC (yes/no) and MR-haplotype revealed that OC-users carrying MR-haplotype 1/3 recognized more sad and fearful faces and remembered more negative characteristics. OC-use did not affect MR-haplotype 2 carriers (homo- and heterozygotes).

Conclusion

Although the effects became less pronounced, we replicated that OC-users recognize fewer expressions of anger, disgust and sadness. Decreased sensitivity for facial expressions together with risk-avoidant behavior may reflect a blunting effect of OC on affect. Furthermore, only OC-users carrying MR-haplotype 1/3 displayed a depressogenic bias on emotional information processing. This study may shed light on why some, but not all women experience negative effects of OC on mood.

Chapter 4 Mineralocorticoid receptor haplotype moderates the effects of oral contraceptives and menstrual cycle on emotional information processing

Background

Emotional information processing is influenced during the menstrual cycle phase. The effects of OC may be due to the suppression of cyclical changes in ovarian hormones (Jones, 2012). OC decrease the HPA axis response to stress and salivary free cortisol responses to stress. The MR mediates the effect of cortisol on initial stress reactions (De Kloet et al., 2005). These rapid effects are especially mediated by the MR sited in limbic structures, regions in which early life stress, chronic stress and depression were found to downregulate MR-expression. In this exploratory study we investigated the influence of OC and menstrual cycle phase on emotional information processing in MR-genotyped healthy volunteers. We assessed tasks which have shown earlier to be sensitive to the influence of OC (chapter 2 and 3) and new tasks in order to cover more stages of emotional information processing.

Procedure

This study had a cross-sectional, parallel-group design. Tasks were assessed in a fixed order and the duration per task was 5 – 15 minutes. NC women with a regular cycle (25 – 35 days) were tested in the early follicular (day 2 – 5; n = 21) or mid luteal (day 18 – 25; n = 23) phase of their menstrual cycle. Only women using second generation OC (Ethinyl estradiol .03 mg; Levonorgestrel .15 mg) for more than three months were included and were tested outside their pill-free week (n = 49). Participants were genotyped at assessment.

Results

The female hormonal status affected the recognition of facial expressions of emotion in general, an effect that was driven by differences in the MR-haplotype 1/3 group. Only in MR-haplotype 1/3 carriers OC-users recognized significantly fewer facial expressions than mid luteal women. No effects on specific emotions were observed, however.

OC-users carrying MR-haplotype 1/3 reported more depressive cognitions following sad mood, than other groups. Regardless their hormonal status, MR-haplotype 1/3 carriers had higher scores on implicit anger and sadness than MR-haplotype 2 carriers (homo- and heterozygotes).

In general, women in the ML phase gambled more than women in the early follicular phase. Mid luteal women carrying MR-haplotype 2 took more risky-decisions in general, while mid luteal women carrying MR-haplotype 1/3 gambled more only in conditions with a low risk to lose. Performance on the reading the mind in the eye task was affected neither by hormonal status nor by MR-haplotype.

Conclusion

We observed higher negative implicit affect and more depressive cognitions following sad mood in OC-users carrying MR-haplotype 1/3, which may reflect subliminal depressive affect and is in line with our previous study (chapter 2). Furthermore, we also observed a possibly increased sensitivity to the female hormonal status in MR-haplotype 1/3 carriers in the emotion recognition paradigm. In line with earlier studies we observed that women in the mid luteal phase gambled more than in the early follicular phase (Bayer et al., 2013). This effect became pronounced in mid luteal MR-haplotype 2 carriers in all trials, while mid luteal MR 1/3 carriers only gambled more in low-risk conditions. This risky-decision making behavior of MR-haplotype 2 carriers may reflect an optimistic expectation about the outcomes of one's decisions. Signs of dispositional optimism in MR-haplotype 2-carriers have been observed earlier in a naturalistic cohort study (Klok et al., 2011).

Chapter 5 Mineralocorticoid receptor haplotype, estradiol, progesterone and emotional information processing

Background

Female hormones modulate the impact of stress on mood. The observed effects of OC and female hormones on emotional information processing may be mediated by estrogen and progesterone receptors, which are abundantly expressed in limbic brain structures (Handa and Weiser, 2014). In these limbic areas the sex steroids may modulate the function of the MR. Progesterone (P4) binds to the MR with nearly the same affinity as aldosterone and cortisol, and acts as a competitive antagonist (Quinkler et al., 2002; Carey et al., 1995). Estradiol (E2) suppresses the

synthesis and transactivation of the MR in brain and vascular endothelial cells (Barrett Mueller et al., 2014; Carey et al., 1995). Consequently, the MR is of relevance in candidate gene studies investigating the influence of female hormones on emotional information processes associated with depression (De Kloet et al., 2008).

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

Procedure

This study had a counterbalanced within-subject design. OC-users (Ethinylestradiol .03 mg; Levonorgestrel .15 mg; n = 57) were tested in a counterbalanced entry-order: once in the second week of active OC-use (day 8 – 14) and once during inactive OC-use (day 4 – 7 of the pill-free week). Naturally cycling (NC) participants (n = 39) were tested at two counterbalanced time-points that are characterized by relatively stable hormone levels of E2 and P4. Once in the early follicular phase (day 2–6), when both hormones are low, and once in the middle of the luteal phase (3–10 days before the onset of the new cycle) when the concentration of P4 is at its maximum and E2 reaches a second peak (Bayer et al., 2014; Jones, 2012). At intake the average cycle duration of the NC participants was registered. After confirmation of the start of the new cycle, test data were scheduled and adjusted to the individual cycle-duration, reasoning that the luteal phase always lasts two weeks (Jones, 2012). Participation ended after confirmation of the start of the new cycle. This cycle onset information was used to confirm whether participants had been tested on the right moment. Participants were MR-genotyped at assessment (amount of MR-haplotype 2 alleles: 0, 1 or 2).

Results

We replicated partially a blunting effect of OC-use on the recognition of facial expressions of emotions, as OC-users recognized fewer but quicker expressions of happiness and sadness. OC-users had also fewer attentional blinks, thus performed better at the attentional blink task (all $p < .05$). These effects were no longer significant after correction for multiple comparisons ($p < .005$), however. In the second session – thus after habituation – lower levels on E2 and P4 were significantly ($p < .001$) associated with fewer attentional blinks. Finally, in contrast with our previous study (chapter 4), OC-users recognized significantly more positive characteristics in the reading the mind in the eye task ($p = .001$). Furthermore, in the first session of our study P4 was associated with an increase in implicit anxiety ($sr = 0.25$; $p = 0.015$).

We observed the following main effects on the MR-genotype, regardless their hormonal status. MR-haplotype 2 homozygotes had significantly higher scores on implicit happiness than MR-haplotype 1/3 carriers ($p < .001$). We also observed a linear relationship: the more MR-haplotype 2 alleles, the higher the scores on implicit happiness. They also reacted slower to correctly recognized happy faces ($p = .001$). Furthermore, MR-haplotype 2 homozygotes gambled more than MR-haplotype 1/3 carriers in trials with low expected losses ($p < .01$). Although this effect did not remain after corrections for multiple comparisons, the pattern was in line with our previous study (chapter 4). We also observed a linear relationship: the more MR-haplotype 2 alleles, the more risky decisions.

Finally, exploratory analyses revealed that the MR-haplotype may moderate the impact of E2 and P4 on emotional information processing. In the first session – thus in a situation of novelty – P4 was associated with an increase in implicit anxiety ($sr = 0.25$; $p = 0.015$). A significant interaction effect revealed that only in MR-haplotype 2 homozygotes higher levels of P4 were associated with reduced implicit anxiety ($sr = -.61$; $p = .012$). In the second session – thus after habituation – higher levels on E2 were associated with better happiness recognition, but only in MR-haplotype 1/3 carriers ($sr = .39$; $p = .008$).

Conclusion

We replicated that OC-users recognize fewer facial expressions of happiness and sadness. In addition, performance on the attentional blink task may be influenced by OC-use, an effect which may be explained by the suppressive effects of OC on natural estrogen and progesterone levels and has been reported earlier (Hollander et al., 2005).

Higher implicit happiness scores, slower reaction times in happiness trials and increased risky-decision making in MR-haplotype 2 homozygotes are in line with previous reports (Bogdan et al., 2010; Klok et al. 2011; chapter 3 and 4) and may be summated by the term 'dispositional optimism'. Interestingly, this study revealed a linear relationship: the more MR-haplotype 2 alleles, the more dispositional optimism was displayed. The fact that MR-haplotype 2 carriers gambled more, but only in low-risk trials may reflect a more rational attitude, too.

In this study we found indications that the MR-haplotype may moderate the influence of E2 and P4 on emotional information processing. Regardless the MR-haplotype P4 was weakly associated with an increase in implicit anxiety, but in MR-haplotype 2 homozygotes P4 was strongly associated with reduced implicit anxiety in a novel situation. These observations may reflect the controversial role of P4 in anxiety.

After habituation to the research paradigm, only in MR-haplotype 1/3 carriers E2 was positively correlated with the recognition of happy expressions. Better happiness recognition may reflect a subtle shift towards a more positive information processing bias, which has also been

observed in healthy volunteers after single doses of an antidepressant (Harmer et al., 2003, 2009). We previously found effects of OC-use on information processing in the opposite direction in MR- haplotype 1/3 carriers (chapter 3 and 4). Although we did not measure hormone levels in these studies, the observed effects may have been caused by OC's suppressing E2 or by an intrinsic effect of the synthetic estrogens.

Against expectation, important practice effects were observed and the pattern of correlations differed between the sessions. Future studies in NC women should take care to verify cycle-phase by confirmation of the next cycle onset. We had to exclude 15 cases because their cur- rent cycle was shorter or longer than expected (mean age = 20.7) and menstrual irregularities are more frequently observed in young women (Boron and Boulpaep, 2012).

Chapter 6 Oral contraceptives positively affect mood in healthy PMS-free women: a longitudinal study

Background

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).

The mood effects of OC and the menstrual cycle phase may be induced by an interaction of (synthetic) hormones on the hypothalamic-pituitary-adrenal axis (Jones, 2012; Handa and Weiser, 2014). Endogenous estrogen may suppress the expression of the MR in the limbic brain (Carey et al., 1995). Progesterone binds to the MR with nearly the same affinity as cortisol and aldosterone, but acts as an antagonist (Carey et al., 1995). The MR-genotype moderates the effect of the female hormonal status on the appraisal and processing of emotionally relevant information (chapter 3, 4 and 5). Following this we hypothesized that hormonal changes during the cycle may trigger mood changes in a MR-genotype dependent manner. In this study we tested the hypothesis that NC women had higher scores on measures associated with reproductive depression than OC-users. We further hypothesized that NC 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 (Aubuchon & Calhoun, 1985).

Procedure

This study had a prospective longitudinal design. All data were collected using an online survey tool (Qualtrics, Provo, UT) in which we assessed in consecutive order chronotype and quality of sleep (outcomes not reported), positive and negative affect (Watson et al., 1998), interpersonal

sensitivity (Boyce & Parker, 1989), affect lability (Oliver & Simons, 2004) and cognitive reactivity following sad mood (Van der Does et al, 2002). Naturally cycling (NC) participants (n = 35) filled out questionnaires on four time-points of two consecutive menstrual cycles. Care was taken to adjust the time-points to the individual cycle-duration. OC users using OC (.03 mg Ethinylestradiol; .15 mg Levonorgestrel; N = 57) for at least three months, were assessed at four equivalent time-points during two consecutive months. Their actual participation started with a new pill strip. We controlled for cycle phase and pregnancy by levels of E2, P4 and estriol in saliva at two sessions in our lab.

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

Results

Masking of the research question was successful. Both OC-users and NC women reported shifts in anger in the first cycle week ($p < .001$), thus at menses or in the pill-free week. OC-users reported fewer mood-shifts between depression and elation than NC women in the ML phase ($p = .002$), when natural hormones (E2 and P4) peak. Compared to NC women, OC-users also reported also fewer ruminating thoughts in all phases ($p = .001$). These effects remained after we controlled for committed relationship status.

Conclusion

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. Not only rumination, but also interpersonal sensitivity, affect lability and depressive cognitions associated with anger and risk avoidance tended to be reduced in OC-users. Hence, OC-users scored overall more favorably on measures associated with reproductive depression. Importantly, OC-users did not differ significantly from NC women in personal characteristics (e.g. neuroticism), mental and physical health and positive and negative affect at the time of assessment. Hence, OC-users showed decreased affect variability which is in line with previous reports on affect-stabilizing effects of OC.

Chapter 7 Mineralocorticoid receptor haplotype, not oral contraceptives use, influences resting state EEG theta/ beta ratio in healthy women

Background

The MR-haplotype may moderate the influence of the menstrual cycle phase, OC-use, estrogen and progesterone on emotional information processing associated with depression as assessed with both behavioral and self-reports (chapter, 3, 4, 5 and 6). Psychophysical studies however, show effects related to cortical arousal and may reveal subtle changes in information processing that remain unnoticed in behavioral studies.

Estrogen and progesterone enhance cortical-subcortical communication, which may contribute to improved emotion regulation (Peper et al., 2011). Resting-state electro-encephalography (rsEEG) theta/beta ratio (TBR) is a biomarker of cognitive control over the affective system. Theta power may be associated with prefrontal cortex (PFC) mediated goal-directed behavior and beta power with arousal. The aim of this study was to examine rsEEG TBR in healthy OC-users and NC women. We also investigated whether any effect of OC-use and levels on E2 and P4 was moderated by MR-haplotype.

Procedure

This study had the same counter-balanced within subject design as applied as in chapter 5.

Results

OC-users (N = 44) and NC women (N = 44) did not differ in rsEEG TBR. TBR was different between MR-haplotypes: MR-haplotype 2 homozygotes had lower TBR scores than MR-haplotype 2 heterozygotes, indicating more cognitive control.

Conclusion

Variation in the MR gene modulates cognitive control of arousal in healthy females, irrespective of OC-use. In particular, MR-haplotype 2 homozygotes have improved cognitive control which decreases the odds on developing mood-disorders.

Chapter 8 General Discussion

This PhD project revealed that the female hormonal status – including OC use – and stress vulnerability – as defined by the MR-haplotype – have practical implications in experimental psychological research. Furthermore, incorporation of these variables in models of emotional information processing may be of help in understanding and treating mood disorders in women. Namely, even small biases may affect information processing and may contribute to the resilience or proneness to mood-disorders.

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. Healthy female MR-haplotype 1/3 carriers may be more prone to distress, and may also be more sensitive to (pharmacological) changes which may counteract or sustain their vulnerability. Consistently, we observed subtle markers of depressogenic side-effects of OC only in MR-haplotype 1/3 carriers. Our findings regarding the MR-haplotypes 2 carriers are generally in line with earlier observations. We observed that MR-haplotype 2 carriers – especially homozygotes – are the less susceptible, more optimistic and more rational individuals, also in 'unstressed' conditions. 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.

OC-users had lower affect variability and reduced sensitivity to interpersonal emotional cues. This 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 our 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.

Samenvatting

Hoofdstuk 1 Introductie

Tijdens hun vruchtbare jaren hebben vrouwen twee keer zoveel kans om een klinische depressie te ontwikkelen als mannen. Sommige vrouwen ervaren dusdanige stemmingswisselingen tijdens het gebruik van de anticonceptiepil, dat dit zelfs een klinische depressie kan worden vastgesteld. Vooral jonge vrouwen lijken vatbaar te zijn voor deze depressogene effecten van de pil (Skovlund et al., 2017). Aangezien de pil nog steeds één van de meest gebruikte anticonceptiemethoden is, is verder onderzoek nodig naar specifieke determinanten die hieraan ten grondslag kunnen liggen.

Achtergrond

In dit promotieonderzoek onderzochten we of pilgebruik en de menstruele cyclusfase emotionele informatieprocessen geassocieerd met depressie bij gezonde premenopauzale vrouwen beïnvloedden. Wij richtten ons daarbij op de detectie van subtiele invloeden die in het dagelijks leven misschien niet opgemerkt worden. Daarnaast hebben we onderzocht of effecten van de vrouwelijke hormonale status zouden worden beïnvloed door genetische variatie in een bepaald onderdeel van de cortisol-feedbackregulatie in het stressresponssysteem, namelijk de mineralocorticoïd receptor (MR) (De Kloet et al., 2005; Boron & Boulpaep, 2012).

De focus ligt op de MR omdat eerder onderzoek heeft aangetoond dat genetische variatie in deze receptor stressbestendigheid en veerkracht na psychotrauma in de kinderjaren voorspelde. Zo bleken vrouwen met MR-haplotype 2 minder vaak stemmingsproblematiek als gevolg van jeugdtrauma te ontwikkelen dan vrouwen met MR-haplotype 1/3. Dit verschil tussen de MR-haplotypes is vooral zichtbaar in pre-menopausale vrouwen, hetgeen een interactie tussen de vrouwelijke hormonale status en genetische variatie in de MR doet vermoeden (Klok et al., 2011; Vinkers et al., 2015). In de volgende paragrafen wordt de inhoud van de hoofdstukken 2 tot en met 7 samengevat en gevolgd door een algemene conclusie.

Hoofdstuk 2 De pil kan de detectie van emoties in gezichtsuitdrukkingen beïnvloeden

Achtergrond

Dit PhD project begon met een gerandomiseerd placebo-gecontroleerd onderzoek waarin we de mogelijke antidepressieve effecten van de krachtige synthetische mineralocorticoïd fludrocortison (FC) onderzochten. In dit onderzoek werden verschillende computertaken afgenomen die geassocieerd worden met verschillende aspecten van de (emotionele) informatieverwerking. De gebruikte testbatterij dient als onderzoeksmodel voor een mogelijke therapeutische respons op

antidepressiva en is gevalideerd in meerdere onderzoeken waarbij het effect van verschillende geregistreerde antidepressiva is onderzocht. Ook al liepen de uitkomsten van eerdere studies uiteen – ze verschilden afhankelijk van het design, het onderzochte antidepressivum, de dosering en de doelgroep – toch is in deze studies aannemelijk gemaakt dat antidepressiva subtiele effecten hebben op aspecten van de emotionele informatieverwerking die niet met behulp van vragenlijsten kunnen worden onderzocht. Het moet nog worden aangetoond dat de (omvang van de) effecten op de testbatterij verband houden met de daaropvolgende klinische respons op antidepressiva, maar de onmiddellijke effecten van enkelvoudige doses bij gezonde vrijwilligers zijn herhaaldelijk aangetoond en kunnen een efficiënte manier zijn om het antidepressieve potentieel van nieuwe middelen te beoordelen (zie onder andere Harmer et al., 2009; Warren et al., 2019).

We hebben FC in dit model gebruikt omdat het een krachtige mineralocorticoïde agonist is met ook glucocorticoïde activiteit. Daardoor kan FC de activiteit van de hypothalamus-hypofyse-bijnier (HPA) as onderdrukken, wat van farmacologisch belang kan zijn bij de behandeling van aandoeningen zoals depressie (Lembke et al., 2013; Buckley et al., 2007). FC versnelde bijvoorbeeld de antidepressieve effecten van het SSRI-escitalopram (Otte et al., 2010), terwijl spironolacton, een mineralocorticoid receptor (MR) antagonist, de werkzaamheid van het antidepressivum amitriptyline bij depressieve patiënten verminderde (Holsboer et al., 1999). We hebben besloten om deelnemers die de anticonceptiepil gebruikten niet uit te sluiten. We verwachtten namelijk dat een relatief hoge dosis FC de mogelijke verstorende effecten van de pil zou opheffen.

Procedure

We onderzochten 40 gezonde vrouwelijke vrijwilligers in een gerandomiseerde, dubbelblinde, placebo-gecontroleerde onderzoeksopzet met parallelle groepen. Gezonde premenopauzale vrijwilligers werden geïncludeerd na lichamelijk onderzoek en gescreend op huidige en eerdere psychische klachten. Na inclusie werd wat bloed afgenomen ter bepaling van het MR-haplotype, zodat MR-haplotype 2 draagsters konden worden vergeleken met MR-haplotype 1/3 draagsters. Om een relatief stabiele cortisolspiegel te garanderen, werd twee uur na een gestandaardiseerde lunch 0,5 mg FC of placebo ingenomen. Vervolgens rustten de deelnemers twee uur, waarna de gezichtsuitdrukkings-herkenningstaak (FERT) en de karaktereigenschappen taak (ECAT) werden afgenomen.

Resultaten

In tegenstelling tot de verwachtingen vonden we geen effect van FC op de accuratesse in de FERT en de ECAT. Wel was er sprake van een interactie-effect tussen pilgebruik (ja vs. nee) en conditie (FC vs. placebo). Bij follow-up analyses bleek dat in pilgebruiksters geen effect van FC

werd gevonden, maar in vrouwen met een natuurlijke cyclus (NC-vrouwen) wel. Ook bleek FC geassocieerd met kortere reactietijden in de FERT. Onverwacht vonden we dat de pilgebruiksters significant minder gezichtsuitdrukkingen van verdriet, woede en walging herkenden dan NC-vrouwen.

Verkennende analyses in de groep NC-vrouwen (n = 14) lieten zien dat de toediening van één dosis FC alleen in draagsters van MR-haplotype 1/3 geassocieerd was met een betere en snellere herkenning van gezichtsuitdrukkingen van angst en blijdschap. Ook herinnerden ze zich meer positieve karaktereigenschappen. Deze effecten werden niet waargenomen bij MR-haplotype 2 draagsters (n = 26; homo- en heterozygoten samen).

Conclusie

In ons onderzoek werden de effecten van een enkele dosis FC verstoord door het gebruik van de anticonceptiepil. Verkennende analyses in de NC-groep lieten zien dat alleen bij MR-haplotype 1/3 draagsters een enkele dosis FC geassocieerd was met een positieve bias in de emotionele informatieverwerking, zoals deze ook wordt gezien na de inname van een enkele dosis antidepressivum in gezonde vrijwilligers. Het effect van de pil op emotieherkenning is een toevalsbevinding en kan, indien gerepliceerd, ofwel een effect zijn van pilgebruik of een reeds bestaand verschil betreffen tussen pilgebruiksters en NC-vrouwen. Toekomstige studies die gebruik maken van neuropsychologische testbatterijen zouden moeten controleren voor de invloed van de pil op de emotionele informatieverwerking (Hamstra, 2013; Hamstra et al., 2014).

Hoofdstuk 3 Mineralocorticoïd receptor haplotype, de pil en de emotionele informatieverwerking

Achtergrond

In eerder experimenteel psychologisch onderzoek konden pilgebruiksters geen toename in positief affect rapporteren na positieve stemmingsinductie (Jarva & Oinonen, 2007). Dit effect op de affectmodulatie is één van de belangrijkste redenen om te stoppen met de pil (Oinonen & Mazmanian, 2002). Het doel van de huidige studie was om de effecten van de pil op de emotionele informatieverwerking verder te onderzoeken (hoofdstuk 2), rekening houdend met een mogelijke modererende invloed van de genetisch bepaalde gevoeligheid voor stress (MR-haplotypes). Prestaties van pilgebruiksters op verschillende aspecten van de emotionele informatieverwerking werden vergeleken met die van NC-vrouwen. Ook onderzochten we of deze resultaten zouden worden beïnvloed door het MR-haplotype. We veronderstelden te vinden dat de effecten van de pil meer uitgesproken zouden zijn bij draagsters van MR-haplotype 1/3 dan bij draagsters van MR-haplotype 2.

Procedure

In deze verkennende studie met een cross-sectioneel design werden de verschillende groepen gelijktijdig onderzocht. Gezonde premenopauzale vrijwilligers voltooiden een testbatterij in het psychologisch laboratorium van de Universiteit Leiden. NC-vrouwen (n = 41; gemiddelde leeftijd 20,2 jaar) werden getest tussen dag 6 en 26 van hun menstruatiecyclus. Pilgebruiksters (n = 44; gemiddelde leeftijd 20,4 jaar) werden getest buiten hun pilvrije week. Merknaam en de samenstelling van de gebruikte anticonceptiepil, de duur van het pilgebruik en – in het geval van de NC-vrouwen – de eerste dag van de laatste menstruatie werden geregistreerd. Wangslijm ten behoeve van MR-genotypering werd bij de deelneemsters afgenomen op de dag van het onderzoek.

Resultaten

Overeenkomstig de resultaten van het vorige onderzoek herkenden pilgebruiksters minder gezichtsuitdrukkingen van walging, geluk, verdriet en woede. Echter, dit verschil was alleen voor woede significant. Verder namen pilgebruiksters minder risicovolle beslissingen in een gokparadigma. Een significant interactie-effect tussen hormonale status (pil vs. NC) en MR-haplotype onthulde dat pilgebruiksters met MR-haplotype 1/3 meer verdrietige en angstige gezichten herkenden en zich meer negatieve karaktereigenschappen herinnerden dan NC-vrouwen met MR-haplotype 1/3. Dit effect werd niet gevonden in vrouwen met MR-haplotype 2 (homo- en heterozygoten samen).

Conclusie

Hoewel de effecten minder uitgesproken waren, repliceerden we dat pilgebruiksters minder uitingen van woede, walging en verdriet herkennen. Een verminderde accuratesse in de herkenning van emoties in combinatie met risicomijdend gedrag kan een afstompend effect van de pil op emotionele informatieverwerking weerspiegelen. Bovendien vertoonden alleen pilgebruiksters met MR-haplotype 1/3 een depressogene bias op emotionele informatie. De uitkomsten van deze studie kunnen mogelijkerwijs in een gedeeltelijke verklaring voorzien hoe het komt dat sommige, maar niet alle vrouwen negatieve effecten van de anticonceptiepil op hun stemming ervaren.

Hoofdstuk 4 Mineralocorticoïd receptor haplotype modereert de invloed van de pil en de menstruatiecyclus op de emotionele informatieverwerking

Achtergrond

De menstruele cyclusfase beïnvloedt de emotionele informatieverwerking. De pil onderdrukt niet alleen de maandelijkse variatie in lichaamseigen geslachtshormonen, maar beïnvloedt ook de respons van de HPA-as op stress en dan in het bijzonder de ongebonden cortisolspiegel in speeksel (Jones, 2012; Kirschbaum et al., 1999). De MR medieert het effect van cortisol op initiële stressreacties. Deze snelle effecten worden vooral gemedieerd door de MR die zich in de limbische structuren van het brein bevindt. Dit zijn regio's waarvan gevonden is dat zij na vroege levensstress, chronische stress en depressie de MR-expressie verminderen. In deze verkennende studie onderzochten we de invloed van de pil en de menstruele cyclusfase op de emotionele informatieverwerking bij MR-gegenotypeerde gezonde vrijwilligers. Onze testbatterij bevatte taken waarvan eerder is aangetoond dat ze gevoelig zijn voor de invloed van de pil (zie hoofdstukken 2 en 3) en nieuwe taken om meer aspecten van de emotionele informatieverwerking te onderzoeken.

Procedure

Deze studie had een cross-sectioneel design waarbij de verschillende groepen gelijktijdig werden onderzocht. Taken werden gemaakt in een vaste volgorde en de duur per taak was 5 tot 15 minuten. NC-vrouwen met een regelmatige cyclus (25 – 35 dagen) werden getest in de vroege folliculaire (EF; dag 2 – 5; n = 21) of midluteale (ML; dag 18 – 25; n = 23) fase van hun menstruatiecyclus. Alleen vrouwen die de tweede generatie anticonceptiepil (Ethinylestradiol 0,03 mg; Levonorgestrel 0,15 mg) langer dan drie maanden gebruikten, werden geïncludeerd en werden getest buiten hun pilvrije week (n = 49). Wangslijm ten behoeve van MR-genotypering werd bij de deelnemers afgenomen op de dag van het onderzoek.

Resultaten

De vrouwelijke hormonale status beïnvloedde de accuratesse in de gezichtsuitdrukkings-herkenningstaak, dit effect werd gedragen door verschillen in de MR-haplotype 1/3 groep. Alleen bij MR-haplotype 1/3 draagsters herkenden pilgebruiksters significant minder gezichtsuitdrukkingen dan midluteale vrouwen. Er werden echter geen emotie-specifieke effecten waargenomen. Pilgebruiksters met MR-haplotype 1/3 rapporteerden meer depressieve cognities na een verdrietige ervaring dan draagsters van MR-haplotype 2. MR-haplotype 1/3 draagsters (pilgebruiksters en NC- vrouwen) scoorden hoger op impliciete woede en verdriet dan MR-haplotype 2 draagsters (homo- en heterozygoten).

Over het algemeen gokten vrouwen in de ML-fase meer dan vrouwen in de EF-fase. Midluteale vrouwen met MR-haplotype 2 namen in het algemeen meer risicovolle beslissingen, terwijl ML-vrouwen met MR-haplotype 1/3 alleen meer gokten in omstandigheden met een klein risico om te verliezen. De prestatie op de 'reading the mind in the eye' taak werd noch door de hormonale status, noch door het MR-haplotype beïnvloed.

Conclusie

Pilgebruiksters met MR-haplotype 1/3 rapporteerden een hoger negatief impliciet affect en meer depressieve cognities na een verdrietige ervaring, hetgeen overeenkomt met de bevindingen in onze voorgaande studie (hoofdstuk 3). Bovendien bleek alleen in MR-haplotype 1/3 draagsters de hormonale status van invloed te zijn op hun prestaties in de gezichtsuitdrukkings-herkenningstaak. In lijn met eerdere onderzoeken zagen we dat vrouwen in de midluteale fase meer gokten dan vrouwen in de vroege folliculaire fase (Bayer et al., 2013). Midluteale MR 1/3-draagsters gokten alleen meer in trials met een laag risico, midluteale MR-haplotype 2 draagsters gokten meer in algemene zin, dus onafhankelijk van het risico dat ze daarbij liepen om te verliezen. Dit gedrag van de MR-haplotype 2-draagsters weerspiegelt mogelijk een optimistische verwachting over de uitkomsten van hun beslissingen. Tekenen van dispositioneel optimisme bij MR-haplotype 2-draagsters zijn eerder waargenomen in een naturalistische cohortstudie (Klok et al., 2011).

Hoofdstuk 5 Mineralocorticoïd receptor haplotype, estradiol, progesteron en emotionele informatieverwerking

Achtergrond

Vrouwelijke hormonen moduleren de impact van stress op stemming. Mogelijk kunnen de waargenomen effecten van de pil en menstruele cyclus op de emotionele informatieverwerking (zie hoofdstukken 2, 3 en 4) gedeeltelijk worden verklaard door variërende oestrogeen en progesteron spiegels. Bovendien komen oestrogeen- en progesteronreceptoren rijkelijk tot expressie in limbische hersenstructuren, gebieden waarin de geslachtshormonen ook de functie van de MR kunnen moduleren (Handa en Weiser, 2014). Zo bindt progesteron (P4) aan de MR met bijna dezelfde affiniteit als aldosteron en cortisol, en werkt P4 als een competitieve antagonist op de MR (Quinkler et al., 2002; Carey et al., 1995). Estradiol (E2) onderdrukt de synthese en transactivatie van de MR in hersen- en vasculaire endotheelcellen (Barrett Mueller et al., 2014; Carey et al., 1995). Dit maakt de MR een relevante factor in genetische studies die de invloed van vrouwelijke hormonen op emotionele informatieprocessen gerelateerd aan depressie onderzoeken (De Kloet et al., 2008).

Het doel van deze studie was om het effect van menstruele cyclus en pilgebruik op de emotionele informatieverwerking en de mogelijke beïnvloeding van dit effect door het MR-

haplotype te onderzoeken. In tegenstelling tot de meeste eerdere onderzoeken, gebruikten we een longitudinaal, 'within-participant' design, waarbij ook E2- en P4-spiegels in speeksel werden gemeten. Onze hypothese was dat variaties in E2 en P4 een grotere invloed hebben op emotionele informatieprocessen gerelateerd aan depressie in vrouwen met MR-haplotype 1/3 dan met MR-haplotype 2.

Procedure

Deze studie had een 'counterbalanced within-partipant' design: alle deelnemers moesten dus twee keer dezelfde testbatterij voltooien. Pilgebruiksters (Ethinylestradiol 0,03 mg; Levonorgestrel 0,15 mg; n = 57) werden eenmaal in de tweede week van actief pilgebruik (dag 8 – 14) en eenmaal tijdens inactief pilgebruik (dag 4 - 7 van de pilvrije week) getest. NC-deelnemers (n = 39) werden getest op twee momenten in de menstruele cyclus die worden gekenmerkt door relatief stabiele hormoonspiegels van E2 en P4. Eenmaal vroeg in de folliculaire fase (dag 2 – 6), wanneer de E2 en P4 spiegels laag zijn, en eenmaal in het midden van de luteale fase (3 – 10 dagen voor het begin van de nieuwe cyclus), wanneer de concentratie van P4 maximaal is en E2 een tweede piek bereikt (Bayer et al., 2014; Jones, 2012). Bij de intake werd de gemiddelde cyclusduur van de NC-deelnemers geregistreerd. Na bevestiging van de start van de nieuwe cyclus, werden testgegevens gepland en aangepast aan de individuele cyclusduur, redenerend dat de luteale fase altijd twee weken duurt (Jones, 2012). Deelname eindigde na bevestiging van de start van de nieuwe cyclus. Deze informatie over het begin van de cyclus werd gebruikt om te bevestigen of deelnemers op het juiste moment waren getest. Wangslijm ten behoeve van MR-genotypering (aantal MR-haplotype 2 allelen: 0, 1 of 2) werd bij de deelnemers afgenomen op de dag van het onderzoek.

Resultaten

We repliceerden gedeeltelijk onze eerdere bevinding dat pilgebruiksters minder goed presteren op de gezichtsuitdrukkingsherkenningstaak: ze herkenden minder uitdrukkingen van geluk en verdriet. Verder hadden ze significant kortere reactietijden op de correct herkende trials. Pilgebruiksters presteerden significant beter op de aandachtstaak. Deze effecten waren echter niet langer significant na correctie voor herhaalde vergelijkingen. In tegenstelling tot onze vorige studie (zie hoofdstuk 4), herkenden pilgebruiksters significant meer positieve karaktereigenschappen in de 'reading the mind in the eye' taak ($p = .001$).

Vrouwen met lagere E2 en P4 spiegels presteerden significant ($p < .001$) beter op de aandachtstaak in de tweede sessie, dus nadat men gewend was aan de testsituatie. Ook vonden we indicaties dat het MR-haplotype de invloed van E2 en P4 op de emotionele informatieverwerking modereert. Zo scoorden vrouwen met hogere P4 spiegels hoger op impliciete angst in de eerste

sessie (*sr* = 0.25; p = 0.015). Echter, na opvolging van een significant interactie-effect vonden we dat alleen MR-haplotype 2 homozygoten lager scoorden op impliciete angst als ze hogere P4 spiegels hadden (*sr* = -.61; p = .012). Uitkomsten van de tweede sessie lieten zien dat MR-haplotype 1/3 draagsters meer gezichtsuitdrukkingen van blijdschap herkenden als ze hogere E2 spiegels hadden (*sr* = .39; p = .008).

Conclusie

We repliceerden de bevinding dat pilgebruiksters minder gezichtsuitdrukkingen van geluk en verdriet herkennen. Ook presteerden zij beter op de aandachtstaak. Dit laatste is in overeenstemming met eerdere bevindingen (Hollander et al., 2005). Homozygoten met MR-haplotype 2 hadden hogere scores op impliciete blijdschap en langzamere reactietijden op gezichtsuitdrukkingen van blijdschap, ook bleken zij significant meer risico-preferent, hetgeen aansluit op andere onderzoeksbevindingen (Bogdan et al., 2010; Klok et al. 2011; hoofdstukken 3 en 4). Het feit dat MR-haplotype 2 draagsters meer gokten, maar alleen in trials waarin men weinig risico liep te verliezen, weerspiegelt mogelijk ook een meer rationele houding. Bovendien zagen wij een lineair verband: hoe meer MR-haplotype 2-allelen de deelnemer had, hoe hoger men scoorde op impliciete blijdschap en hoe meer risicovolle beslissingen men nam. Ook vonden we aanwijzingen dat het MR-haplotype de invloed van E2 en P4 op de emotionele informatieverwerking kan beïnvloeden. Bijvoorbeeld, hogere P4 spiegels bleken positief gecorreleerd met een toename in impliciete angst, maar in MR-haplotype 2 homozygoten bleek P4 sterk gecorreleerd met verminderde impliciete angst in de eerste sessie, dus als men nog moet wennen aan de testsituatie. Deze ogenschijnlijk tegenstrijdige bevindingen sluiten aan op andere onderzoeken waarin een controversiële rol van P4 bij angst is geobserveerd.

Alleen in vrouwen met MR-haplotype 1/3 bleek een hogere E2 spiegel geassocieerd met een betere herkenning van gezichtsuitdrukkingen van blijdschap. Gezonde vrijwilligers herkenden ook meer blijdschap op dezelfde taak na de inname van een enkele dosis van een antidepressivum (Harmer et al., 2003, 2009). Een beter herkenning van blijdschap weerspiegelt mogelijkerwijs een positieve bias in de emotionele informatieverwerking. Eerder vonden we een 'depressogene' bias op dezelfde taak bij pilgebruiksters met MR-haplotype 1/3 (hoofdstukken 3 en 4). Mogelijk kan deze negatieve bias worden verklaard doordat de pil de afgifte van E2 onderdrukt of door een intrinsiek effect van het synthetisch oestrogeen dat één van de bestanddelen is van de anticonceptiepil.

Tegen onze verwachting in vonden we belangrijke leereffecten. Ook bleek het patroon van de correlaties te verschillen per sessie. Voor toekomstig experimenteel psychologisch onderzoek naar de invloed van de menstruele fase verdient het aanbeveling dat de cyclusfase wordt geverifieerd met behulp van een bevestiging van het begin van de volgende cyclus. We moesten

namelijk 15 deelnemers (gemiddelde leeftijd = 20.7 jaar) excluderen omdat hun huidige cyclus korter of langer was dan verwacht. Menstruele onregelmatigheden worden vaker waargenomen bij jonge vrouwen (Jones, 2012).

Hoofdstuk 6 De pil heeft een positieve invloed op de stemming bij gezonde PMS-vrije vrouwen: een longitudinale studie

Achtergrond

De term reproductieve depressie verwijst naar een verhoogde affectieve gevoeligheid als gevolg van veranderingen in de vrouwelijke hormonale status. Deze kan worden gekenmerkt door verhoogde prikkelbaarheid en interpersoonlijke gevoeligheid, depressieve stemming en cognitieve disfunctie (Deecher et al., 2008; Harald & Gordon, 2012). Eerder vonden wij dat de anticonceptiepil en de menstruele cyclus invloed hebben op sommige aspecten van de emotionele informatieverwerking geassocieerd met depressie. Ook zagen wij dat het MR-haplotype een modererende invloed hierop heeft (hoofdstukken 2 – 5). Hierop aansluitend wilden we onderzoeken of hormonale schommelingen tijdens de cyclus stemmingsveranderingen kunnen veroorzaken op een MR-haplotype-afhankelijke wijze. In deze studie hebben we de hypothese getest dat NC-vrouwen hogere scores hadden op metingen die verband houden met reproductieve depressie dan pilgebruiksters. Ook veronderstelden we te vinden dat NC-vrouwen met MR-haplotype 1/3 hogere scores hadden op deze metingen dan draagsters van MR-haplotype 2. We hebben ons onderzoeksdoel zorgvuldig gemaskeerd om te voorkomen dat de waarneming van de deelnemers zou worden beïnvloed door – al dan niet veronderstelde –menstruatiecyclus gerelateerde symptomen (Aubuchon & Calhoun, 1985).

Procedure

Deze studie had een prospectieve longitudinale opzet. Alle gegevens werden verzameld met behulp van een online enquêtetool (Qualtrics, Provo, UT). In chronologische volgorde werden vragenlijsten afgenomen over het chronotype en de slaapkwaliteit (uitkomsten niet gerapporteerd), positief en negatief affect (Watson et al., 1998), interpersoonlijke gevoeligheid (Boyce & Parker, 1989), affect labiliteit (Oliver & Simons, 2004) en depressieve cognities na een verdrietige gebeurtenis (Van der Does et al, 2002).

NC-vrouwen (n = 35) vulden vragenlijsten in op vier tijdstippen van twee opeenvolgende menstruatiecycli. Er werd voor gezorgd dat de tijdstippen werden aangepast aan de individuele cyclusduur. Pilgebruiksters die de pil (0,03 mg Ethinylestradiol; 0,15 mg Levonorgestrel; N = 57) gedurende ten minste drie maanden gebruikten, beantwoordden gedurende twee opeenvolgende maanden op vier vergelijkbare tijdstippen dezelfde vragenlijsten. We controleerden cyclusfase en zwangerschap aan de hand van E2, P4 en oestriol spiegels in speeksel afgenomen tijdens twee sessies in ons laboratorium.

We maskeerden ons onderzoeksdoel door de deelnemers te informeren dat we de impact van chronotype en slaap op de stemming onderzochten bij gezonde MR-gegenotypeerde vrouwen. Na afronding van de achtste sessie vroegen we de deelnemers om de onderliggende onderzoeksvraag (open vraag) te raden, waarmee we konden controleren of de maskering succesvol was. Vervolgens vulden de deelnemers een vragenlijst in gericht op de screening van het premenstrueel syndroom, waarna ze werden geïnformeerd over de daadwerkelijke onderzoeksvraag.

Resultaten

Het maskeren van de onderzoeksvraag was succesvol. Zowel pilgebruiksters als NC-vrouwen rapporteerden last te hebben van driftbuien in de eerste cyclusweek ($p < .001$), dus tijdens de menstruatie of in de pilvrije week. Pilgebruiksters rapporteerden minder stemmingswisselingen tussen somberheid en opgetogenheid dan NC-vrouwen in de midluteale fase, dus als de E2 en P4 spiegels maximaal zijn ($p = .002$). In vergelijking met NC-vrouwen rapporteerden pilgebruiksters ook minder te piekeren ($p = .001$). Deze effecten bleven bestaan nadat we hadden gecontroleerd voor het feit of men een vaste relatie had of niet.

Conclusie

De uitkomsten suggereerden wederom dat het MR-haplotype een modererende invloed heeft op de impact van de hormonale status op de stemming, maar deze effecten waren na correctie voor herhaalde vergelijkingen niet meer significant. Pilgebruiksters rapporteerden minder stemmingsklachten geassocieerd met reproductieve depressie dan NC-vrouwen. Ook leek er bij pilgebruiksters minder sprake van stemmingswisselingen, hetgeen in lijn is met eerder onderzoek. Daarbij is het goed om op te merken dat beide groepen niet significant van elkaar verschilden in persoonlijkheidskenmerken zoals neuroticisme, psychische en fysieke gezondheid en positieve en negatieve stemming op het moment dat zij de vragenlijsten invulden.

Hoofdstuk 7 Mineralocorticoïd receptor haplotype, maar niet de pil, beïnvloedt de EEG theta/ bèta ratio in rust bij gezonde vrouwen

Achtergrond

Het MR-haplotype kan de invloed van de menstruele cyclusfase, pilgebruik, oestrogeen en progesteron op emotionele informatieverwerking geassocieerd met depressie modereren, zoals gezien in onderzoeken waarbij gebruik is gemaakt van zowel computertaken als zelfrapportages (hoofdstuk 3, 4, 5 en 6). Psychofysiologische studies kunnen subtiele veranderingen in de informatieverwerking signaleren die onopgemerkt blijven in gedragsstudies. Oestrogeen en progesteron versterken de corticale-subcorticale communicatie, wat kan bijdragen aan verbeterde

emotieregulatie (Peper et al., 2011). Elektro-encefalografie in rusttoestand (rsEEG) theta / beta-ratio (TBR) is een biomarker van cognitieve controle over het affectieve systeem. Thetagolven worden geassocieerd met door de prefrontale cortex (PFC) gemedieerd doelgericht gedrag en bètagolven met agitatie. Het doel van deze studie was om rsEEG TBR te onderzoeken in gezonde pilgebruiksters en NC-vrouwen. We onderzochten daarbij ook of de mogelijke invloed van de hormonale status werd gemodereerd door het MR-haplotype.

Procedure

Deze studie had hetzelfde onderzoeksdesign als de studie in *hoofdstuk 5*.

Resultaten

Pilgebruiksters (N = 44) en NC-vrouwen (N = 44) verschilden niet in rsEEG TBR. TBR was verschillend tussen MR-haplotypes: MR-haplotype 2 homozygoten hadden een kleinere TBR-ratio dan MR-haplotype 2 heterozygoten, wat duidt op meer cognitieve controle.

Conclusie

Variatie in het MR-haplotype moduleert de cognitieve controle over agitatie in gezonde vrouwen, ongeacht hun hormonale status. Met name MR-haplotype 2 homozygoten hebben een verbeterde cognitieve controle. Mensen met een betere cognitieve controle lijken minder kwetsbaar te zijn voor (de ontwikkeling van) stemmingsstoornissen.

Hoofdstuk 8 Algemene discussie

In dit PHD-project is gebleken dat de vrouwelijke hormonale status en de genetisch bepaalde stressgevoeligheid - zoals in dit project gedefinieerd door het MR-haplotype - de uitkomsten van experimenteel psychologisch onderzoek kunnen beïnvloeden. Bovendien kan de inclusie van deze variabelen in modellen van emotionele informatieverwerking helpen bij het begrijpen en behandelen van stemmingsstoornissen bij vrouwen. Zelfs subtiele veranderingen in de verwerking van emotionele informatie kunnen al van invloed zijn op de perceptie van onze sociale omgeving en de gevoeligheid voor stemmingsstoornissen.

Ons onderzoek heeft laten zien dat de genetisch bepaalde gevoeligheid voor stress een modererende invloed kan hebben op de impact van de vrouwelijke hormonale status op emotionele informatieprocessen geassocieerd met depressie in gezonde premenopauzale vrouwen. Gezonde vrouwelijke draagsters van MR-haplotype 1/3 zijn mogelijk gevoeliger. Zo zijn zij gevoeliger voor stress en de gevolgen daarvan zoals stemmingsstoornissen. Mogelijkerwijs zijn zij ook gevoeliger voor (farmacologische) interventies die hun kwetsbaarheid kunnen tegengaan of in stand houden. In lijn hiermee hebben we subtiele markers van depressogene bijwerkingen van

de pil alleen waargenomen bij MR-haplotype 1/3 draagsters. Onze bevindingen met betrekking tot vrouwen met MR-haplotype 2 zijn over het algemeen in overeenstemming met eerder onderzoek. Vooral de MR-haplotype 2 homozygoten zijn minder stressgevoelig en hebben een optimistischer denkpatroon. Daarnaast blijkt bij hen ook sprake te zijn van een wat rationeler, minder empathische mindset. Deze associaties moeten echter met de nodige voorzichtigheid worden geïnterpreteerd. Stress-gerelateerde psychopathologie is zeer heterogeen van aard en eiwitten van meerdere genen kunnen een interactie aangaan in het stressgevoelige fenotype. Tevens is de verhoogde kwetsbaarheid van vrouwen voor stemmingsstoornissen het gevolg van een geheel aan biologische, psychologische en vooral ook sociologische factoren.

Pilgebruiksters lieten een lagere affectvariabiliteit zien en een verminderde gevoeligheid voor interpersoonlijke emotionele signalen. Dit kan door de ene pilgebruikster als een prettig stemming stabiliserend effect worden ervaren, en door de andere als een afstompend effect op de affectmodulatie. De lagere scores van pilgebruiksters op symptomen van reproductieve depressie in onze longitudinale studie suggereren een beschermend effect van de monofasische anticonceptiepil. Toekomstige studies zouden deze effecten in grotere cohorten van pilgebruiksters nader moeten onderzoeken, waarbij onderscheid moet worden gemaakt tussen nieuwe gebruiksters, tevreden gebruiksters en pilwisselaars.

Over de auteur

Danielle Hamstra werd geboren op 28 december in 1965 in Utrecht. Nadat zij in 1984 haar diploma had behaald aan het Stedelijk Gymnasium te Utrecht, ging zij in dezelfde stad psychologie studeren. Hierbij werd zij geïnspireerd door de invloed van reclame op menselijk gedrag. Om financiële redenen is zij in 1985 overgestapt naar de avond- HEAO richting marketing en communicatie, die zij in 1989 afrondde. Na een loopbaan in de marketing (communicatie) is zij in 2006 gestart als zij-instromer in het basisonderwijs en behaalde zij in 2008 haar PABO-diploma.

In 2009 is zij begonnen met de premaster Klinische Psychologie aan de Universiteit Leiden met de wens om meer te leren over (differentiaal) diagnostiek en de behandeling van psychopathologie. Tijdens deze premaster besloot ze zich te ontwikkelen tot scientist practitioner. Tijdens haar afstudeeronderzoek ontdekte zij bij toeval dat de pilgebruiksters minder emoties herkenden in een gezichtsherkenningstaak (hoofdstuk 2 van dit proefschrift). Zij rondde haar (Research) Master Clinical & Health Psychology aan de Universiteit Leiden af in 2013. Studie en later promotieonderzoek combineerde zij van 2010 tot 2019 met docentschap bij de Universiteit Leiden en later ook werk in de hulpverlening. Sinds 2019 is zij werkzaam als psycholoog met als aandachtsgebied persoonlijkheidsproblematiek en psychotrauma.

Publicaties

Hamstra, D. A., De Rover, M., De Rijk, R. H., & Van der Does, W. (2014). Oral contraceptives may alter the detection of emotions in facial expressions. *European neuropsychopharmacology,* 24(11), 1855-1859.

Hamstra, D. A., De Kloet, E. R., Van Hemert, A. M., De Rijk, R. H., & Van der Does, A. J. W. (2015). Mineralocorticoid receptor haplotype, oral contraceptives and emotional information processing. *Neuroscience,* 286, 412-422.

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

Hamstra, D. A., de Kloet, E. R., Quataert, I., Jansen, M., & Van der Does, W. (2017). Mineralocorticoid receptor haplotype, estradiol, progesterone and emotional information processing. *Psychoneuroendocrinology*, 76, 162-173.

Hamstra, D. A., de Kloet, E. R., de Rover, M., & Van der Does, W. (2017). Oral contraceptives positively affect mood in healthy PMS-free women: A longitudinal study. *Journal of psychosomatic research*, 103, 119-126.

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