Mood and the pill

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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).

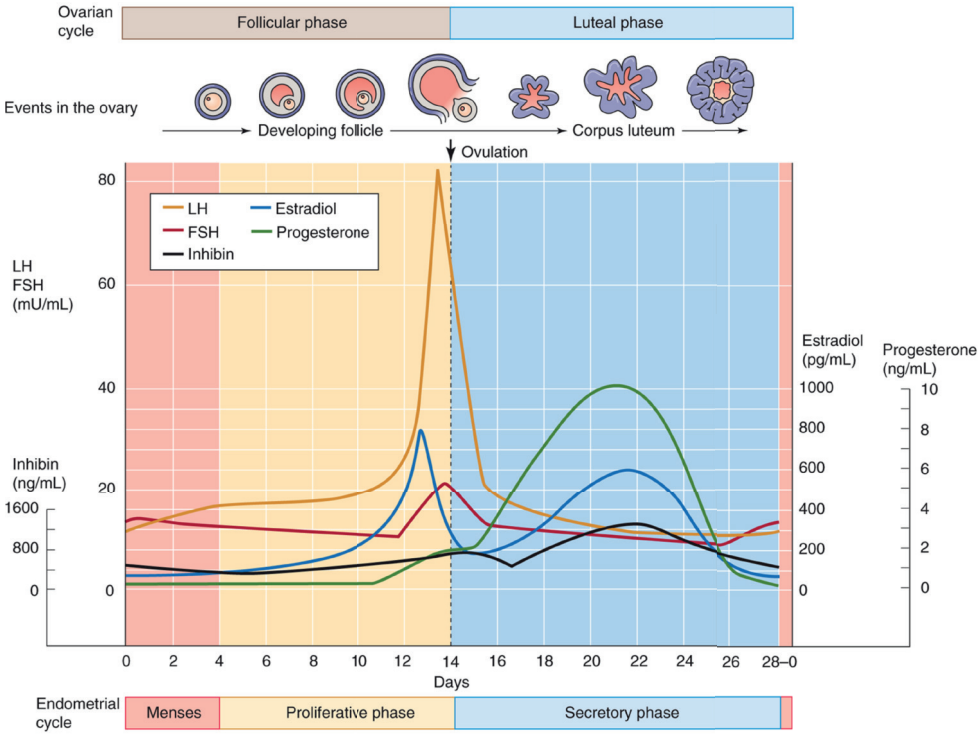

Figure 1. Hormonal changes during the menstrual cycle (Boron & Boulpaep, 2012).
FSH = follicle-stimulating hormone; LH = luteinizing hormone

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).

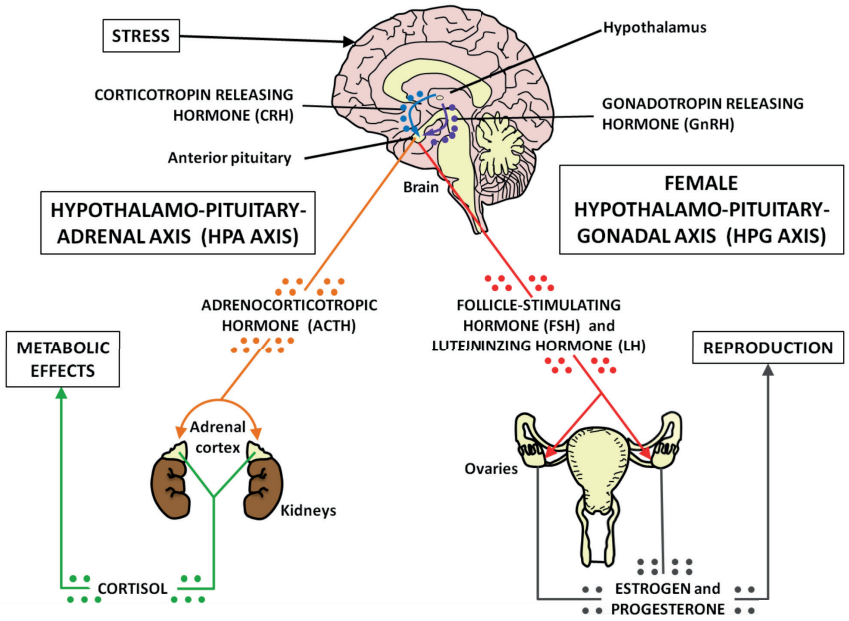

Figure 2. A schematic representation of the interaction between the HPA and the HPG axis (© 2012 Nepomnaschy & Salvante)

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).

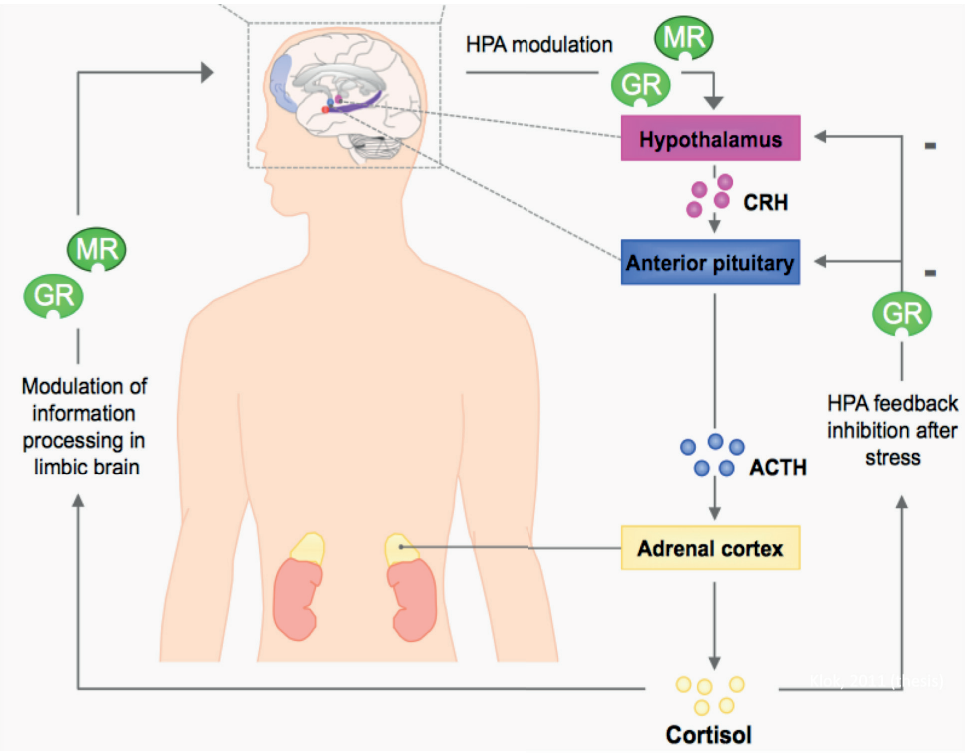

Figure 3. The MR, GR and their function in the HPA-axis (reprinted from Klok, 2011)

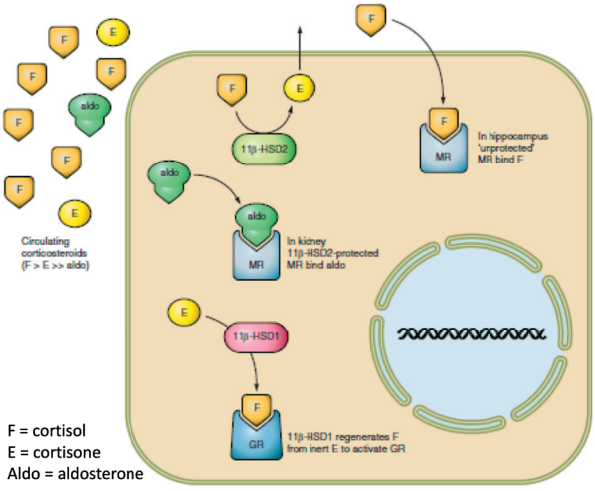

Figure 4. The role of 11β hydroxy steroid dehydrogenase in steroid specificity of the mineralocorticoid receptor (Chapman et al., 2013)

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).

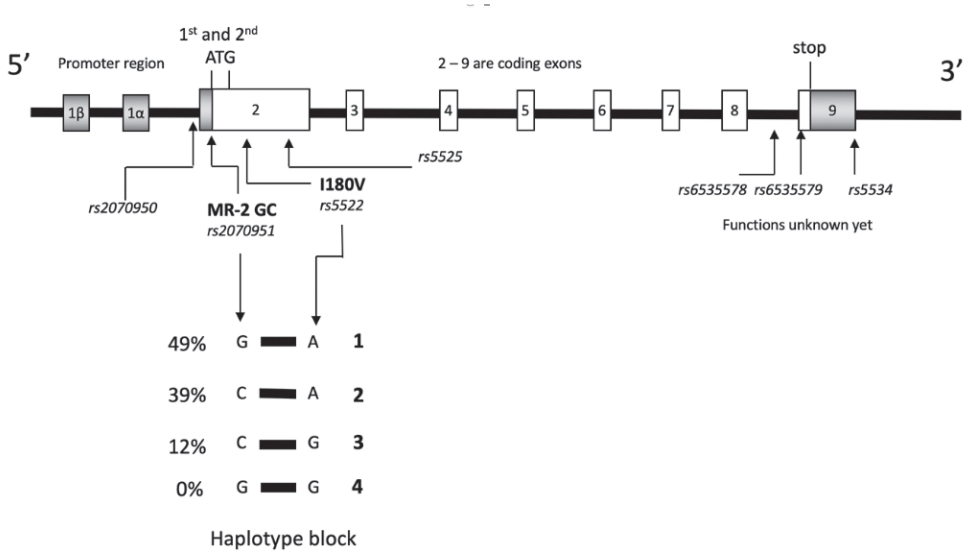

Figure 5. The human MR (NR3C2) gene with the haplotype block of rs2070951 (MR-2C/G) and rs5522 (MRI180V) in the 5' region (reprinted from van Leeuwen, 2010).

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.