Ovarian Steroid Hormones, Emotion Processing and Mood

MALIN GINGNELL
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Abstract

It is known that some psychiatric disorders may deteriorate in relation to the menstrual cycle. However, in some conditions, such as premenstrual dysphoric disorder (PMDD), symptomatology is triggered mainly by the variations in ovarian steroid hormones. Although symptoms induced by fluctuations in ovarian steroids often are affective, little is known about how emotion processing in women is influenced by variations, or actual levels, of ovarian steroid hormones.

The general aim of this thesis was to evaluate menstrual cycle effects on reactivity in emotion generating and controlling areas in the corticolimbic system to emotional stimulation and anticipation, in healthy controls and women with PMDD. A second aim was to evaluate corticolimbic reactivity during long-term administration of exogenous ovarian steroids.

In study I, III and IV effects of the menstrual cycle on emotional reactivity in women with PMDD was studied. In study I, women with PMDD in displayed higher amygdala reactivity than healthy controls to emotional faces, not in the luteal phase as was hypothesised, but in the follicular phase. No difference between menstrual cycle phases was obtained in women with PMDD, while healthy controls had an increased reactivity in the luteal phase. The results of study I was further elaborated in study III, where women with PMDD were observed to have an increased anticipatory reactivity to negative emotional stimuli. However, no differences in amygdala reactivity to emotional stimuli were obtained across the menstrual cycle. Finally, in study IV the hypothesis that amygdala reactivity increase in the luteal phase in women with PMDD is linked to social stimuli rather than generally arousing stimuli was suggested, tested and supported.

In study II, re-exposure to COC induced mood symptoms de novo in women with a previous history of COC-induced adverse mood. Women treated with COC reported increased levels of mood symptoms both as compared to before treatment, and as compared to the placebo group. There was a relatively strong correlation between depressive scores before and during treatment. The effects of repeated COC administration on subjective measures and brain function were however dissociated with increased aversive experiences accompanied by reduced reactivity in the insular cortex.

Keywords: premenstrual dysphoric disorder, menstrual cycle, combined oral contraceptives, estrogen, estradiol, progesterone, ethinyl-estradiol, levonorgestrel, randomized clinical trial, placebo, fMRI, amygdala, ACC, insula, dLPFC, mPFC, IFG, MFG

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Knowledge is power – share it.
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV Gingnell M, Bannbers E, Ahlstedt V, Wikström J, Sundström-Poromaa I, Fredrikson M (in manuscript): Social stimulation, amygdala reactivity and connectivity in premenstrual dysphoric disorder.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>BA</td>
<td>Brodmann Area</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood Oxygenation Level Dependent</td>
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<tr>
<td>CD scale</td>
<td>Cyclicity Diagnoser Scale</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>COC</td>
<td>Combined Oral Contraceptives</td>
</tr>
<tr>
<td>CSF</td>
<td>Corticospinal Fluid</td>
</tr>
<tr>
<td>dmPFC</td>
<td>Dorsomedial Prefrontal Cortex</td>
</tr>
<tr>
<td>dPFC</td>
<td>Dorsolateral Prefrontal cortex</td>
</tr>
<tr>
<td>DICOM</td>
<td>Digital Imaging and Communications in Medicine</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Module of Mental Disorders</td>
</tr>
<tr>
<td>EE</td>
<td>Ethinyl Estradiol</td>
</tr>
<tr>
<td>EPI</td>
<td>Echo Planar Imaging</td>
</tr>
<tr>
<td>EPT</td>
<td>Estradiol and Progestagen Therapy</td>
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<tr>
<td>FFA</td>
<td>Fusiform Face Area</td>
</tr>
<tr>
<td>FWE</td>
<td>Family Wise Error</td>
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<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
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<tr>
<td>FP</td>
<td>Frontopolar cortex</td>
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<tr>
<td>FSH</td>
<td>Follicle Stimulating Hormone</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma Aminobutyric Acid</td>
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<tr>
<td>GnRH</td>
<td>Gonadotropin Releasing Hormone</td>
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<tr>
<td>GLM</td>
<td>General Linear Model</td>
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<tr>
<td>HAMD</td>
<td>Hamilton rating scale for Depression</td>
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<tr>
<td>HC</td>
<td>Healthy Control</td>
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<td>HRF</td>
<td>Haemodynamic Response Function</td>
</tr>
<tr>
<td>IAPS</td>
<td>International Affective Pictures System</td>
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<tr>
<td>IFG</td>
<td>Inferior Frontal Gyrus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>ITI</td>
<td>Inter Trial Interval</td>
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<td>k</td>
<td>Cluster size</td>
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<tr>
<td>LH</td>
<td>Luteinizing Hormone</td>
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<tr>
<td>MADRS</td>
<td>Montgomery-Åsberg Depression Rating Scale</td>
</tr>
<tr>
<td>M.I.N.I.</td>
<td>Mini International Neuropsychiatric Interview</td>
</tr>
<tr>
<td>MFG</td>
<td>Middle Frontal Gyrus</td>
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<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
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<tr>
<td>MR</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NIIITI</td>
<td>Neuroimaging Informatics Technology Initiative</td>
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<tr>
<td>OFC</td>
<td>Orbitofrontal Cortex</td>
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<tr>
<td>PCC</td>
<td>Posterior Cingulate Cortex</td>
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<td>PET</td>
<td>Positron Emitting Tomography</td>
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<tr>
<td>PFC</td>
<td>Prefrontal Cortex</td>
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<tr>
<td>PMDD</td>
<td>Premenstrual Dysphoric Disorder</td>
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<td>PMS</td>
<td>Premenstrual Syndrome</td>
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<td>POP</td>
<td>Progesterone Only Pill</td>
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<tr>
<td>PTSD</td>
<td>Post Traumatic Stress Disorder</td>
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<td>ROI</td>
<td>Regions of Interest</td>
</tr>
<tr>
<td>SAD</td>
<td>Seasonal Depressive Disorder</td>
</tr>
<tr>
<td>SCID</td>
<td>Structured Clinical Interview for DSM III-R</td>
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<tr>
<td>SEM</td>
<td>Standard Error of Mean</td>
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<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
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<td>STAI</td>
<td>State-Trait Anxiety Inventory</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
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<tr>
<td>T</td>
<td>Tesla</td>
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<tr>
<td>TA</td>
<td>Acquisition Time</td>
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<td>TE</td>
<td>Echo Time</td>
</tr>
<tr>
<td>TR</td>
<td>Time of Repetition</td>
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<tr>
<td>vPFC</td>
<td>Ventrolateral Prefrontal Cortex</td>
</tr>
<tr>
<td>vmPFC</td>
<td>Ventromedial Prefrontal Cortex</td>
</tr>
</tbody>
</table>
Introduction

Ovarian steroid hormones and mood disorders

Some psychiatric disorders such as depression, anxiety, bipolar disorders or eating disorders may deteriorate in relation to the menstrual cycle (Pinkerton et al., 2010; Bäckström et al., 2006; Kornstein et al., 2005). However, there are also conditions where symptomatology is triggered merely by the variations in ovarian steroid hormones across the menstrual cycle such as premenstrual dysphoric disorder (PMDD) (American Psychiatric Association, 2000). Although the symptoms induced by fluctuations in ovarian steroid related symptoms often are affective (Sundström et al., 1999a; Segebladh et al., 2009), relatively little is known about how emotion processing in women is influenced by variations, or actual levels, of ovarian steroid hormones (van Wingen et al., 2011). In this thesis, functional magnetic resonance imaging (fMRI) is used to study brain reactivity during different hormonal and emotional states in women with PMDD (American Psychiatric Association, 2000) and in a sub-group of women who had experience of emotional side effects on combined oral contraceptives (COC) (Poromaa and Segebladh, 2012; Böttcher et al., 2012; Ernst et al., 2002; Kelly et al., 2010; Oionen and Mazmanian, 2002). In the backgrounds section, PMDD and mood-related side effects of COC are first briefly introduced; a short background is thereafter given on ovarian steroid hormones, emotion processing and fMRI. Finally, an overview of previous studies of neuroimaging in PMDD and during COC use is given.

Premenstrual dysphoric disorder

Premenstrual dysphoric disorder affects 3-5% of women in childbearing ages (Sveindóttir and Bäckström, 2000) and diagnostic criteria for the disorder are described in Appendix B of the DSM-IV (American Psychiatric Association, 2000). PMDD is characterized by a cluster of distressing symptoms that regularly appear during the luteal phase of the menstrual cycle (Figure 1, Table 1). Symptom onset is usually in the early or mid-luteal phase with a gradual worsening in the late luteal phase. In the majority of cases symptoms remain during the first 2-3 days of menses (Hartlage et al., 2012), after which complete remission is experienced. Affected individuals report a sig-
significant impact on daily functioning, most often expressed as impaired family, social and occupational relationships (Halbreich et al., 2003).

Figure 1. Variation (mean and standard error of mean (SEM)) in symptomatology over the menstrual cycle in two groups of women with PMDD, one with relatively low severity and one group with high severity of PMDD (Gingnell et al., 2010). Daily symptom severity in each group is measured as summed scores on the CD scale for the four core PMDD symptoms (depressed mood, anxiety, affective lability and irritability) during each day.

Symptoms (Table 1) must be at least five and include at least one of the following: depression, anxiety, irritability or affective lability. Other symptoms are decreased interest or pleasure in usual activities, difficulty in concentrating, lack of energy, change in appetite, changed sleeping patterns and feeling of being overwhelmed or out of control. Somatic symptoms such as breast tenderness, headache or bloating are also commonly reported. Furthermore symptoms must not be due to premenstrual exacerbation of concurrent depressive, anxiety or personality disorder and the diagnosis must be confirmed by prospective daily ratings during at least two consecutive menstrual cycles.

Table 1. Summary of symptoms included in PMDD.

<table>
<thead>
<tr>
<th>Core symptoms</th>
<th>Additional symptoms</th>
<th>Somatic symptoms</th>
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<tbody>
<tr>
<td>Depression or feeling of hopelessness</td>
<td>Decreased interest in usual activities</td>
<td>Breast tenderness</td>
</tr>
<tr>
<td>Anxiety or tension</td>
<td>Difficulty concentrating</td>
<td>Headaches</td>
</tr>
<tr>
<td>Irritability</td>
<td>Lack of energy</td>
<td>Bloating</td>
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<tr>
<td>Affective lability</td>
<td>Changed sleep pattern</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Changed appetite</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feeling of being overwhelmed or out of control</td>
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</table>

PMDD symptoms are defined by their relation to the luteal phase of the menstrual cycle. As progesterone is only present in the luteal phase, PMDD
is commonly regarded as a disorder caused by the variation in (or mere presence) of progesterone levels. Research in support of this include findings of symptom relief during anovulatory cycles (Sundström et al., 1999a; Wyatt et al., 2004), the reinstatement of symptoms when add-back hormone therapy is administered together with gonadotropin releasing hormone (GnRH) agonists (Segebladh et al., 2009), and findings of progestagen-induced mood symptoms in postmenopausal women (Andréen et al., 2005; Andréen et al., 2006; Björn et al., 2000; Björn et al., 2002). However, previous findings also suggest that it may in fact be the combined effect of high estradiol and progesterone levels during the luteal phase that contribute to the symptoms (Segebladh et al., 2009). Notably, no consistent hormonal differences between women with PMDD and healthy controls have been reported (Bäckström et al., 1983; Sundström et al., 1999b). Thus, it is hypothesized that the recurring mood symptoms are due to increased sensitivity to hormonal exposure rather than disturbances of the hormonal synthesis or secretion (Bäckström, 2003). In line with this, women with PMDD have been described to have an altered sensitivity to progesterone metabolites in the brain’s major inhibitory system, the gamma aminobutyric acid system (GABA) which might be affected through binding of progesterone metabolites to the GABA_A receptor (Sundström et al., 1998).

As the core symptoms of PMDD, depression, anxiety, irritability and affective lability, all are affective it is likely that PMDD patients, exhibit differential brain reactivity during emotion processing. Prior studies on corticolimbic reactivity to emotional stimuli in PMDD patients are thus far limited to one report of increased amygdala reactivity in the luteal phase of women with PMDD (Protopopesceu et al., 2008).

**Combined oral contraceptives and mood**

Combined oral contraceptives containing both synthetic estrogen and progesterone are used by 22-50% of Swedish women between age of 19 and 29 (Lindh et al., 2010). Because of high contraceptive efficacy, COCs have generally been considered first line choice in adolescents and young women. While the majority of COC users report high levels of satisfaction with treatment (Skouby, 2010), some users report emotional side effects. In clinical trials 4-10% of women on COC report mood-related side effects (Ernst et al., 2002; Kelly et al., 2010), whereas the retrospective of previous mood-related side effects among previous users may be as high as 15% (Oddens, 1999). Emotional side effects are also among the most common reasons for discontinuation of combined oral contraceptives. Approximately 15-30% of women who discontinue oral contraceptives report emotional side effects as the reason for discontinuation (Lindh et al., 2009; Sanders et al., 2001), and mood-related discontinuations appear to have increased over the last 30 years (Lindh et al., 2009). Although the mood symptoms most commonly
reported by COC users include depressive symptoms, affective lability and irritability, clinical depression and suicidal attempts have also been reported (Ramcharan, 1981; Wagner 1996). COC side effects in general also include bleeding irregularities, nausea and bloating. Side effects are usually most intense in the first treatment cycles (Graham et al., 1995) and during the pill-free intervals (Coffee et al., 2007; Sulak et al., 2000; Kelly et al., 2010). The emotional side effects differ between contraceptive brands and has been attributed to the progestagen component. For instance, COCs with anti-androgenic progestagens such as drospirenone and desogestrel appear more favourable in terms of mood symptoms than progestagens with a more androgenic profile such as levonorgestrel (Poromaa and Segebladh, 2012). However, the concentrations of estrogen also influence the risk for mood-related side effects. COCs with lower levels of estrogen associated with a more favourable mood profile than preparations with higher estrogen doses (Greco et al., 2007). While the symptom profile and the hormonal exposure of COC-induced mood-related side effects appear similar to PMDD, the true drug-related causality is far from established. Thus far, three placebo-controlled COC trials with mood symptoms as primary outcome have been performed in healthy women (Leeton et al., 1978; Graham et al., 1995; O’Connell et al., 2007). However, as these studies only included sterilized women (Leeton et al., 1978; Graham et al., 1995) or dysmenorrhea patients (O’Connell et al., 2007), the results may not be valid for typical users. Furthermore, as the majority of COC users report unchanged or improved mood (Ernst et al., 2002), the mechanisms for how mood is affected by COC may only be present in particularly vulnerable individuals. To the best of my knowledge, no placebo-controlled studies of COC-induced mood effects in individuals with prior mood-related side effects have been published and no prior study has followed the effect on corticolimbic reactivity to emotional stimuli during combined oral contraceptive use in younger women.

Risk factors for ovarian steroid associated mood symptoms
Risk factors for both PMDD and mood deterioration during COC use include previous history of mood disorders and anxiety related personality traits (Cohen et al., 2002; Segebladh et al., 2009; Gingnell et al., 2010; Borgström et al., 2008).

Previous mood disorders
Previous history of depressive episodes, including postpartum depression, is common in women with PMDD (Pearlstein et al., 1990; Cohen et al., 2002), and individuals with previous major depression have a three-fold increased risk of developing PMDD (Cohen et al., 2002). Vice versa, PMDD has also been suggested to be a risk factor for major depression and postpartum depression, although conclusions from available prospective studies are limited.
by relatively small sample sizes or by the use of retrospective PMDD reports (Hartlage et al., 2001; De Ronchi et al., 2000; Sylven et al., 2013). Co-morbidity with depressive and anxiety disorders is common in women with PMDD. In a longitudinal community survey, co-morbidity rates for anxiety disorders were 47.7% and for mood disorders 22.9%. Only 26.5% of the investigated cases had only PMDD (Wittchen et al., 2002). Finally, as women with PMDD are more prone than healthy controls to react with a panic attack to the anxiety provoking substances lactate, cholecystokinin and CO$_2$, an association between PMDD and panic disorder has been suggested (Gorman et al., 2001; Harrison et al., 1989; Sandberg et al., 1993; Landen and Eriksson, 2003). Previous depressive episodes are also more common in COC users who develop COC-induced mood worsening than in women who report unchanged or improved mood on COC (Segebladh et al., 2009; Joffe et al., 2003).

**Personality traits**

Personality traits are characteristic ways of thinking, feeling and behaving. Once adulthood is reached, personality traits are considered to be fairly stable throughout life, although some studies indicate that the level of stability may differ between traits (Billstedt et al., 2013; Hampson and Goldberg, 2006). Certain personality traits may be associated with the development of psychiatric disorders, and one of the most established associations is between high scores of neuroticism and the risk of developing depressive or anxiety disorders (Kotov et al., 2010; Bagby et al., 2008; Foster and Mac-Queen, 2008). Neuroticism can be described as a tendency of experiencing negative emotions such as stress, anxiety and anger, and is a stable, heritable personality trait with approximately 50% of the variance accounted for by genetic factors (Lesch et al., 1996; Kendler et al., 2006; Canli, 2008). Women with PMDD are overrepresented among individuals with high scores on neuroticism-related traits (Critchlow et al., 2001; Freeman et al., 1995; Gingnell et al., 2010) and women who report mood effects of COC use have increased scores on somatic trait anxiety and stress susceptibility (Borgström et al., 2008). High levels of neuroticism or trait anxiety have been associated with increased reactivity in the corticolimbic system (Chan et al., 2008; Chan et al., 2009; Haas et al., 2007; Hooker et al., 2008; Feinstein et al., 2006), but to the best of my knowledge, no previous study have reported the influence of trait anxiety on corticolimbic reactivity across the menstrual cycle.
Ovarian steroid hormones
Steroid hormones such as cortisol, aldosterone, progesterone, testosterone and estrogen are synthesized from cholesterol in the adrenal glands, adipose tissue and gonads. The major female sex steroid hormones are estradiol and progesterone (Speroff et al., 2004).

Ovarian steroid hormones and reproduction
During the menstrual cycle the hormones estradiol and progesterone are synthesised in the ovaries. The main source for both estradiol and progesterone in the luteal phase is the corpus luteum but estradiol is also synthesised during the follicular phase in the growing follicle. Levels of ovarian steroids vary over the menstrual cycle, with estradiol being high in the late follicular and luteal phase and progesterone reaching its peak during the mid-luteal phase. Both hormones are low during the early follicular phase (Figure 2).

Figure 2. Schematic figure of variations in estradiol and progesterone during the menstrual cycle. Both hormones are low in the early follicular phase. Estradiol is increased in the late follicular phase and in the luteal phase, while progesterone has its peak in the luteal phase.

The main effects of ovarian steroid hormones are related to reproduction with changes affecting development and release of oocytes from the ovaries and the endometrial preparation for implantation. Ovarian steroids are also important during pregnancy. The growth of a follicle as well as the cyclic variations in ovarian steroid hormones are regulated by release in the hypothalamus by a pulsatile activity of GnRH that affects levels of follicle stimulation hormone (FSH) and luteinizing hormone (LH). The release of GnRH is regulated by a feedback mechanism, where circulating levels of estradiol and progesterone inhibits or triggers GnRH release over the menstrual cycle. The fact that high levels of circulating progesterone inhibits GnRH release
(and thereby the growth of new follicles) is used during treatment with oral contraceptives (Speroff et al., 2004).

Ovarian steroid hormones and the brain

Sex steroid hormones play fundamental roles in the development and function of the central nervous system (CNS). In women, ovarian hormones govern the fertility process by influencing the hypothalamic-pituitary-gonadal axis, but ovarian steroids also modulate a number of CNS functions such as cognition (Maki, 2012), pain perception (Vincent and Tracey, 2010), appetite (Asarian and Geary, 2006), sexual function (at least in animals) (Dixson, 2001) and mood (Poromaa and Segebladh, 2012; Bäckström et al., 2006).

The CNS acts both as a source and a target of sex steroids. The ovarian steroids, estradiol, progesterone and the progesterone metabolites (3α-hydroxy-5β-pregnan-20-on allopregnanolone and 3α-hydroxy-5α-pregnan-20-on pregnanolone) all pass through the blood brain barrier and may also be synthesised within the CNS (Joëls, 1997; McEwen et al., 2012; Melcangi et al., 2011). Animal studies and post-mortem studies in reproductive and postmenopausal women indicate that estradiol, progesterone and allopregnanolone are accumulated in the brain (Bixo et al., 1986; Bixo et al., 1995; Bixo et al., 1997). For instance, the highest levels of allopregnanolone are found in the substantia nigra and hypothalamus, whereas the highest concentration of progesterone is found in the amygdala (Bixo et al., 1997).

Receptors for estradiol (α and β) and progesterone are present throughout the brain, including cortical areas, although the highest receptor densities are found in the hypothalamus and other parts of the limbic system (Österlund et al., 2000a; Österlund et al., 2000b). Estradiol and progesterone act through genomic regulation via intracellular or membrane-bound receptors (McEwen et al., 2012), and the progesterone metabolites also interact with the GABA-system (Majewska et al., 1986; Lambert et al., 2005). Other major neurotransmitter systems are also modulated by ovarian steroids; serotonin neurons of the dorsal raphe contain β-receptors for estrogen and progestin receptors whereby ovarian steroids have the potential to increase the cellular resilience of serotonin neurons (Bethea et al., 2009) and estradiol and progesterone may influence serotonergic neurotransmission at several levels including the synthesis and degradation of serotonin, as well as gene expression of pre- and postsynaptic serotonin receptors (Bethea et al., 2002; Kugaya et al., 2003). Estradiol also modulates dopaminergic neurotransmission (Becker and Hu, 2008) and noradrenergic neurotransmission (Kasturi et al., 2013). Finally, estradiol may also exert some of its actions through binding to ligand-gated ion channels, like the N-methyl d-aspartate (NMDA) receptors in the hippocampus (Smith et al., 2009).
Ovarian steroid hormones and mood

Even if the majority of women remain unaffected by the hormonal changes of the menstrual cycle (Sveindóttir and Bäckström, 2000), approximately 20% of women experience some sort of sub-threshold PMDD (Wittchen et al., 2002). Mood effects of ovarian steroids may be both dose-dependent and influenced by how the individual hormones are combined.

Mood enhancing effects of estrogen in postmenopausal women have been suggested to be dose-dependent, with higher estrogen doses associated with improved well-being (Sherwin and Gelfand, 1989). Spurred by this finding, estrogen treatment (with or without progestagen addition) has been evaluated for use in women with clinical depression, although with mixed results (Soares and Frey, 2010). However, when estrogen is combined with progestagens, lower estrogen doses generally associate with better mood outcomes both in postmenopausal women (Björn et al., 2003), healthy fertile women (Poromaa and Segebladh, 2012), and women with PMDD (Segebladh et al., 2009). The effect of progesterone-only is rarely ever studied; it is either given together with estrogen or administered in the presence of endogenous estradiol levels. Synthetic progestagens have different side effect profiles depending on their specificity and affinity to the progesterone, androgen and glucocorticoid receptors (Burkman et al., 2010). In addition, dose and especially duration, of progesterone treatment matter for the effects on mood. While single administration of progesterone in animals is reliably anxiolytic (an effect attributed to the GABAergic progesterone metabolites), long-term treatment with progesterone may instead precipitate anxiety-like symptoms (Sundström et al., 2003), and discontinuation of progesterone treatment consistently results in withdrawal symptoms including both anxiety- and depression-like behaviors (Li et al., 2012; Smith et al., 1998). Corresponding human anxiolytic effects of progesterone or allopregnanolone have not been possible to establish, in most cases probably because baseline anxiety levels have been low (Freeman et al., 1993), but the postpartum blues which occurs three days after delivery and affects up to 70% of all women is the most typical manifestation of progesterone withdrawal (Nappi et al., 2001). Finally, in a series of clinical trials using vaginal as well as oral progesterone treatment, Andréen and colleagues were able to show that intermediate, as opposed to low or high (and sedative) allopregnanolone concentrations were associated with the most intense mood symptoms in postmenopausal women (Andréen et al., 2005; Andréen et al., 2006).

Emotions and mood

An emotion is a positive or negative feeling or state that includes cognitive, physiological and behavioural reactions. Emotions are usually transient (sec-
onds to minutes) and usually triggered by external stimuli (such as viewing a threatening situation), cognitive mechanisms (such as recalling a memory) or physical events (like pain or induction of panic attacks by inhalation of carbon dioxide) (Esquivel et al., 2010). The concept of emotions is found throughout the world and the description of emotions remains fairly stable, although cultural display rules might differ (Scherer and Wallbott, 1994; Matsumoto et al., 2007). The physiological emotional response, or arousal, is relatively similar for positive and negative emotions and includes activation of the sympathetic nervous system with palpitations, vasoconstriction and increased sweating, while the subjective experience varies greatly between positive and negative emotions. In this thesis a dimensional approach in which emotions are classified according to continuous scales of valence (negative – positive) and arousal (low – high) is used (Figure 3).

![Figure 3](image-url)

*Figure 3.* A schematic representation of a dimensional approach to classification of emotions (Russell, 1980). The different emotions are rated according to degree of negative or positive valence and level of experienced arousal.

Mood, as compared to transient emotions, is generally less intense and more prolonged; the experience is present over hours or days. In mood disorders both the experience and expression of emotions are affected (American Psychiatric Association, 2000). Depressed subjects reports prolonged feelings of hopelessness, sadness and inability to experience normally positive emotions such as happiness (American Psychiatric Association, 2000) or at least an inability to retain positive emotions for longer periods of time (Heller et al., 2009). In this thesis external stimuli (images) are used to elicit emotions in individuals with mood alterations associated with variation in ovarian steroid levels. As PMDD and mood-related side effects of COC most often include negative mood and negative emotional symptoms, the main focus of this thesis is on negative emotional stimuli. Stimuli with a more positive valence are mainly included to maximize the difference in valence between trials while keeping arousal levels relatively constant.
Emotions and the brain

From an anatomical view, emotions are often attributed to activity in the brain's so-called limbic system (McLean, 1949, 1952 and 1970). The limbic system is an evolutionary old part of the brain and includes the hypothalamus, amygdala, insula, fornix, septum and anterior cingulate cortex (ACC) (Shin and Liberzon, 2010; Davidson et al., 2000). Parts of the limbic system form a hypothesized corticolimbic emotional network where the amygdala and insula is activated by stimuli-induced bottom-up emotional processes, whereas the ACC is involved in top-down automatic and cognitive regulation (Shin and Liberzon, 2010; Bush et al., 2000; Ray and Zald, 2012). There is a close connection between the limbic and the autonomic nerve system (Fischer et al., 2004; Critchley, 2009). A situation or stimuli that induce fear will also be associated with an increased activity in the sympathetic nervous system resulting in palpitations, sweating and dilated eye pupils (Lang et al., 2000). In the present thesis, the concept corticolimbic system is used to refer to the parts of the limbic system included in the fear circuit (Shin and Liberzon, 2010).

Reactivity to emotional stimuli has also been associated with altered brain reactivity in the prefrontal cortex (PFC) (Fusar-poli et al., 2009; Pessoa and Adolphs, 2010, Etkin et al., 2011). The PFC is often subdivided into dorsolateral (dlPFC), ventrolateral (vlPFC), frontopolar (FP), ventromedial (vmPFC), dorsomedial (dmPFC) and orbitofrontal cortex (OFC) (Ray et al., 2012) (Figure 4). In general, dorsomedial areas are associated with experience of emotions (Etkin et al., 2011) while ventromedial areas are involved in more automatic regulation of emotional reactivity (Phillips et al., 2008). DlPFC and vlPFC, have been suggested to mediate cognitive regulation of emotions, perhaps through connection with the ACC (Ray and Zald, 2012). The PFC is also involved in the anticipation of emotions. Anticipation tunes the experience of the upcoming emotional event and may thus affect emotional processing and experiences (Onoda et al., 2008; Sarinopoulus et al., 2010; Denny et al., 2013). Anticipation of emotional events is generally associated with activation of the vmPFC, dmPFC, and the ACC (Bermpohl et al., 2006; Herwig et al., 2007; Ueda et al., 2003; Nitschke et al., 2006) but a role for the insula in emotional anticipation has also been suggested, at least in individuals with a predisposition for anxiety (Simmons et al., 2006; Simmons et al., 2011) and in women with post traumatic stress disorder (PTSD) (Simmons et al., 2008). Furthermore, increased amygdala reactivity during exposure to anticipated negative emotional stimuli (Ueda et al., 2003) or anticipation of phobic stimuli (Lorberbaum et al., 2004; Tillfors et al., 2002) has also been observed.
While increased reactivity in emotion generating areas such as the amygdala and insula almost invariably are associated with anxiety disorders (Etkin and Wager, 2007; Ressler and Mayberg 2007; Freitas-Ferrari et al., 2010; Del Casale et al., 2012; Hayes et al., 2012; Linares et al., 2012; Patel et al., 2012; Fredrikson and Faria, in press; Klump et al., 2013), reduced activity in emotion controlling prefrontal areas are sometimes but not always present (Fredrikson and Faria, in press; Klump et al., 2013). Some disorders, such as PTSD, are characterized by increased reactivity to several types of emotional stimuli (Rauch et al., 2000; Rauch et al., 2006; Shin et al., 2001; Shin et al., 2005), while emotion-induced reactivity in other disorders are restricted to disorder-specific stimuli (Wright et al., 2003). When mood and emotional symptoms are triggered in PMDD and by COC, areas in the brain related to emotional processing are hypothetically involved, but prior neuroimaging reports on PMDD are limited to one report using a combined cognitive and emotional task (Protopopesceu et al., 2008). One question of the present thesis is if reactivity to emotional stimuli is altered during periods of mood symptoms in emotion generating or controlling areas, or both. If so, an attempt is made to disentangle whether alternated reactivity is general or restricted to certain types of stimuli.

Mood, ovarian steroid hormones and neuroimaging

In a recent review it was suggested that ovarian steroid hormones have opposing effects on reactivity in areas generating and controlling emotions, exemplified by progesterone-induced reactivity increases in emotion generating areas (i.e. the amygdala) and reactivity decreases in emotion control-
ling areas (van Wingen et al., 2011). However, given the variable mood effects associated with ovarian steroids and the relative scarcity of publications in the field, it appears premature at this stage to generalize about hormonal effects on emotion-induced brain reactivity. In addition, the types of paradigms that have been used, targeting cognitive as well as emotional processes vary between studies. A description of menstrual cycle studies (Amin et al., 2006; Protopopescu et al., 2005; Rupp et al., 2009; Derntl et al., 2008; Derntl et al., 2010; Goldstein et al., 2005; Andreano and Cahill, 2010) and hormonal administration studies (van Wingen et al., 2008; Love et al., 2010) that use emotional tasks is given below. Studies across the menstrual cycle are also presented in table 2.

Neuroimaging across the menstrual cycle in healthy women

Besides the emotional tasks, menstrual cycle variations in brain reactivity have been assessed in response to food stimuli (Frank et al., 2010; Alonso-Alonso et al., 2011), reward delivery (Dreher et al., 2007) and working memory tasks (Jacobs and D’Esposito, 2011; Baller et al., 2013). For anticipation of emotional stimuli, no previous study has been performed, but a study of pain anticipation by Choi et al. (2006) reported increased anticipatory reactivity in the inferior, medial and middle frontal gyri during the follicular phase and increased luteal phase reactivity in the cerebellum, precentral gyrus, uncus, superior temporal gyrus, middle temporal gyrus, parahippocampal gyrus and amygdala.

Response inhibition

As one symptom of PMDD is compromised inhibitory control, it is interesting that two studies have used a response inhibition task (Go/NoGo) with emotional words of positive, negative and neutral valences to study inhibitory control in healthy women across the menstrual cycle (Amin et al., 2006; Protopopescu et al., 2005). In the Go/NoGo task, subjects are requested to give a response for every Go-target but to withhold a response to the NoGo-targets.

Amin et al., (2006) studied the emotional component of attention by comparing reactivity to emotional words that were NoGo-targets (as contrasted to non-targets) in the early follicular and mid-luteal phase. Increased reactivity in the dIPFC and ACC was observed when positive words were used as NoGo-targets in the luteal phase. Reactivity correlated positively with estradiol levels in the luteal phase. No differences across the menstrual cycle were reported for negative emotional words.

Protopopescu et al. (2005) studied a mixture of emotion and cognition using an emotional Go/NoGo-task in healthy women. By comparing NoGo-trials OFC reactivity to negative (as compared to neutral) words was reported to be lower in the luteal than in the follicular phase.
Facial perception

Three studies have reported menstrual cycle brain reactivity changes in response to facial perception (Rupp et al., 2009; Derntl et al., 2008; Derntl et al., 2010).

Rupp et al. (2009) reported higher OFC reactivity in the follicular than in the luteal phase, and a correlation between reactivity and the estradiol/progesterone ratio regardless of phase. The paradigm consisted of evaluation of potential partners (male faces) as contrasted against potential homes (pictures of buildings). However, eight out of ten participants performed their first scan in the luteal phase, indicating that order effects might have affected the results.

Derntl et al. (2010) used several tasks for identifying and naming facial emotions as contrasted against rest and reported increased amygdala reactivity to emotional stimuli in women scanned in the follicular as compared to the luteal phase. However, the study used a cross-sectional, instead of the preferred longitudinal, design for menstrual cycle studies and the sample sizes were small (n=6 in each group).

The other study by Derntl et al. (2008) is slightly larger (n=11 in each group), but still cross-sectional. In this study only facial emotion recognition was studied and a higher reactivity in the amygdala was reported in the follicular phase group when naming emotions in faces expressing disgust and sadness.

In conclusion, studies of facial perception indicate that brain reactivity in healthy controls to emotional faces is increased in the follicular phase, but order effects and cross-sectional designs may be affecting the results.

Emotionally valenced images

Emotional images from the International Affective Pictures System (IAPS) (Lang et al., 2005) have been used by two studies (Goldstein et al., 2005; Andreano and Cahill, 2010). In both studies, negatively valenced highly arousing images were contrasted against low arousing neutral images.

Goldstein et al. (2005) studied changes across the follicular phase. They reported amygdala, hypothalamus, hippocampus, brainstem and mPFC reactivity to be lower in the late follicular than in the early follicular phase.

Andreano and Cahill (2010) studied the effect of active perception of IAPS-images, on amygdala, hippocampus and neighboring areas. Increased reactivity was observed for negative (as contrasted against neutral) images in the luteal phase for amygdala, fusiform face area (FFA), hippocampus, cerebellum, caudate nucleus and the inferior frontal gyrus (IFG). Hippocampal activity during both negative and neutral stimuli as contrasted against passive viewing of hair crosses correlated negatively with estradiol and there was also a trend for a negative correlation between estradiol and change in amygdala reactivity. As progesterone levels differed between sessions, while
estradiol levels were stable, it was proposed that progesterone might affect amygdala reactivity more than estradiol.

**Table 2.** A summary of fMRI studies of emotional processing over the menstrual cycle.

<table>
<thead>
<tr>
<th>Study</th>
<th>Task</th>
<th>Menstrual cycle phase</th>
<th>Number of participants</th>
<th>Main finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amin et al., 2006</td>
<td>Attention to emotional words</td>
<td>Follicular vs. luteal</td>
<td>20</td>
<td>Luteal phase, positive inhibition &gt; neutral: ↑ dlPFC, ACC</td>
</tr>
<tr>
<td>Protopopesceu et al., 2005</td>
<td>Inhibition to emotional words</td>
<td>Follicular vs. luteal</td>
<td>12</td>
<td>Luteal phase, negative &gt; neutral: ↓ lateral OFC, ACC and insula, ↑ medial OFC</td>
</tr>
<tr>
<td>Rupp et al., 2009</td>
<td>Evaluation of attractiveness</td>
<td>Follicular vs. luteal</td>
<td>10</td>
<td>Follicular phase, male faces &gt; buildings: ↑ OFC</td>
</tr>
<tr>
<td>Derntl et al., 2008</td>
<td>Emotional recognition of facial expressions</td>
<td>Follicular and luteal</td>
<td>11/11</td>
<td>Follicular phase, recognition &gt; rest: ↑ amygdala</td>
</tr>
<tr>
<td>Derntl et al., 2010</td>
<td>Emotional recognition of facial expressions</td>
<td>Follicular and luteal</td>
<td>6/6</td>
<td>Follicular phase, identification &gt; rest: ↑ amygdala</td>
</tr>
<tr>
<td>Goldstein et al., 2005</td>
<td>Emotional images</td>
<td>Early vs. late follicular</td>
<td>12</td>
<td>Late follicular, negative &gt; neutral: ↓ amygdala, hypothalamus, hippocampus, brain stem, mPFC</td>
</tr>
<tr>
<td>Andreano and Cahill, 2010</td>
<td>Emotional images</td>
<td>Follicular vs. luteal</td>
<td>17</td>
<td>Luteal, negative &gt; neutral: ↑ amygdala, FFA, hippocampus, cerebellum, caudate nucleus, IFG.</td>
</tr>
</tbody>
</table>
Two studies have examined change in corticolimbic reactivity during emotional processing after short-term (van Wingen et al., 2008) and long-term (Love et al., 2010) administration of ovarian steroids.

The study by van Wingen et al. (2008) used an emotion task with emotional faces and geometrical shapes (similar to the task used in study I and II in this thesis) to compare amygdala and FFA reactivity and connectivity during the follicular phase before and after a single progesterone injection. Increased amygdala reactivity to emotional faces was observed after progesterone administration. A whole brain search for connectivity to the amygdala during facial (as opposed to geometrical) perception also indicated that progesterone decreased functional connectivity between amygdala and FFA and, with a more lenient statistical threshold, increased connectivity between amygdala and the dorsal ACC.

Finally, Love et al. (2010) performed a placebo-controlled, cross-over study of continuous combined estrogen and progestagen treatment (EPT) in ten postmenopausal women. FMRI was registered as participants rated the valence for negative, neutral and positive IAPS-images. The reactivity to negative (as contrasted against neutral) images was higher during EPT in the OFC, precentral gyrus, posterior cingulate cortex (PCC) and occipital cortex, but lower in the dIPFC, postcentral gyrus and dorsal ACC. EPT also lowered reactivity to positive (as contrasted against neutral) images in the mPFC. As this study included both estrogen and progesterone it is not possible to disentangle the contribution of each hormone.

Direct comparison of these two studies is not possible, because age (and menopausal status) differed among participants. In addition, van Wingen et al. (2008) evaluated acute administration while the study by Love et al. (2010) studied long-term effects.

Neuroimaging and premenstrual dysphoric disorder

The core symptoms of PMDD are affective in nature and corticolimbic brain areas are thus likely to be involved in the disorder. Increased amygdala reactivity characterize negative affective states like anxiety and depression (Etkin and Wager, 2007; Ressler and Mayberg, 2007; Freitas-Ferrari et al., 2010; Del Casale et al., 2012; Hayes et al., 2012; Linares et al., 2012; Patel et al., 2012; Fredrikson and Faria, in press), and amygdala reactivity has been suggested to mediate negative affect also in PMDD patients (van Wingen et al., 2008).

Prior studies on amygdala reactivity across the menstrual cycle in PMDD patients are limited to one report of increased amygdala reactivity in the luteal phase (Protopopesceu et al., 2008). However, the results of the Proto-
popescu study are difficult to interpret, as the major changes across the menstrual cycle were found in the healthy controls and not among the PMDD women.

Two structural studies using fMRI and positron emitting tomography (PET) have suggested that cerebellar volume and glucose consumption might be affected in women with PMDD (Rapkin et al. 2011, Berman et al., 2012). In addition, lower menstrual cycle variability in serotonin 5-HT_{1A} binding (Jovanovic et al., 2006) and altered cortical GABA levels have been reported in women with PMDD (Epperson et al., 2002). Women with PMDD have also been reported to have an inverse correlation between mood symptoms and 5-hydroxy-l-tryptophan-trapping (Eriksson et al., 2006).

With the use of a cognitive paradigm, Baller et al., (2013) reported increased activity in the dlPFC of women with PMDD as compared to healthy controls during an n-back task. This effect was unrelated to hormonal levels. Using a non-emotional Go/NoGo-task, women with PMDD have also been reported to have lower parietal reactivity than healthy controls regardless of menstrual cycle phase (Bannbers et al., 2012).

In conclusion, no prior studies of facial perception, emotionally valenced pictorial stimuli or emotional anticipation have been performed in women with PMDD. Prior studies in mood disorders (Etkin and Wager, 2007; Ressler and Mayberg, 2007; Freitas-Ferrari et al., 2010; Del Casale et al., 2012; Hayes et al., 2012; Linares et al., 2012; Patel et al., 2012; Fredrikson and Faria, in press) and healthy controls (Andreano and Cahill, 2010; van Wingen et al., 2008) suggest that an increased reactivity in emotion processing areas, such as the amygdala and insular cortex, may be present in women with PMDD during the luteal phase. Some support is also given that emotion controlling areas might be involved (Protoposesceu et al., 2005 and 2008). However, it has not been reported whether an increased activity in emotion generating areas are paralleled by a decrease in emotion regulation areas or if these presumed changes are general to several types of emotional stimuli (such as in PTSD (Rauch et al., 2000; Rauch et al., 2006; Shin et al., 2001; Shin et al., 2005)) or more restricted to symptom provoking stimuli (such as in specific phobia (Wright et al., 2003)).

Neuroimaging and combined oral contraceptives

Neuroimaging studies of COC-induced brain activity changes are even scarcer. In general, previous studies have focused on structural changes and compared individuals already on COC to non-users. In a structural MR study, COC use was associated with increased gray matter volumes in the prefrontal and temporal cortices as well as pre- and postcentral gyri, parahippocampal and fusiform gyri (Pletzer et al., 2010). COC use has also been associated with structural changes in the fornix, an important white matter tract in the corticolimbic circuit (de Bondt et al., 2013). By the use of a cog-
nitive paradigm, one study reports increased reactivity in the IFG and tem-
poral cortex in COC users (Rumberg et al., 2010).

Taken together, studies of hormonal administration as well as the menstrual
cycle studies indicate that amygdala reactivity to emotional (and especially
negatively valenced) stimuli is increased during progesterone exposure, such
as the luteal phase in healthy controls (Andreano and Cahill, 2010) or in
response to single-dose administration (van Wingen et al., 2008). Mixed
results have been reported for other emotion processing and regulatory areas
such as OFC, ACC, mPFC and dIPFC.

The general aim of this thesis was thus to evaluate menstrual cycle effects on
reactivity in emotion generating and controlling areas in the corticolimbic
system to emotional stimulation and anticipation in healthy controls and
women with PMDD. A second aim was to evaluate corticolimbic reactivity
during combined oral contraceptive use.
Aims

Study I
To test if women with PMDD display increased amygdala reactivity in the luteal as compared to their follicular phase, and if the reactivity is higher in women with PMDD than in controls during the luteal phase. A secondary aim was to evaluate the influence of trait anxiety levels and ovarian steroid serum concentrations on amygdala reactivity.

Study II
To investigate if COC use would induce more pronounced mood symptoms than placebo in women with a previous history of COC-induced adverse mood. Secondly, to determine if COC use is associated with altered brain reactivity in regions previously associated with emotion processing and responsiveness to ovarian steroid hormones.

Study III
To test if women with PMDD display increased PFC and corticolimbic reactivity during anticipation of negative emotional stimuli in the luteal as compared to the follicular phase, and if reactivity is higher in women with PMDD than in healthy controls during the luteal phase. To further evaluate if women with PMDD have altered reactivity during exposure to emotional stimuli across the menstrual cycle.

Study IV
To test the hypothesis that altered corticolimbic reactivity across the menstrual cycle in PMDD is not generalized, but symptom-specific and linked to socially relevant stimulation.
Materials and methods

Participants and study protocols

In **study I, III** and **IV**, women with PMDD were compared to healthy controls across the menstrual cycle. Participants were scanned twice, once in the follicular phase (day 6-12 after the onset of menstrual bleeding) and once in the late luteal phase (postovulatory day 8-13). To protect against order effects, half of the participant started the study in the follicular phase while the other half started in the luteal phase. Luteal phase scanning was scheduled according to self-administered home-tests of LH-peaks and was confirmed by progesterone serum concentrations and records of the next menstrual bleeding on the CD scale.

Thirty-seven women with self-experienced PMDD were recruited through newspaper advertisement and among women seeking help for premenstrual symptoms at the out-patient ward of the Department of Obstetrics and Gynecology, Uppsala University Hospital. Upon screening, seventeen women were excluded (no informed consent (n = 12), ongoing treatment for PMDD or immediate request for treatment (n = 3), or MR-incompatible implants (n = 2)). Of the remaining twenty subjects, 18 women met the criteria for PMDD diagnosis, defined in the DSM-IV (American Psychiatric Association, 2000). In addition 16 asymptomatic controls were recruited through newspaper advertisement. During the studies, one woman with PMDD and one healthy control dropped out after the first scanning session due to personal reasons. Seventeen women with PMDD and 15 healthy controls thus completed the studies.

**Study II** was a clinical trial in which women who previously had discontinued oral contraceptives due to emotional side effects were re-exposed to treatment with a COC. The study was an investigator-initiated, double-blinded, randomized, parallel-group clinical trial during which the participants were treated with either an oral COC containing ethinyl estradiol (EE) 0.03 mg and 0.15 mg levonorgestrel (Bayer Pharma AB) or placebo (Bayer Pharma AB) during one treatment cycle. Following a pretreatment cycle (allowing for baseline assessments), women started taking the COC or placebo tablets once daily on the first day of menses and continued treatment for 21 days. Compliance was assessed by counting the remaining capsules at the final visit.
Participants were recruited through newspaper advertisement. Forty women were screened for the study. Of these, four did not fulfill inclusion and exclusion criteria and one subject dropped out prior to randomization. Hence 35 women were randomized to COC or placebo. One woman dropped out of the study immediately after randomization, leaving 34 participants in the study.

**Exclusion criteria** in all studies were ongoing pregnancy or breastfeeding, treatment with hormonal contraceptives, benzodiazepines or other psychotropic drugs within 3 months prior to study start, ongoing depression, anxiety and other psychiatric illness as evaluated with M.I.N.I.. Due to the use of MRI, individuals with pacemakers, internal defibrillators, aneurysm clips, metal implants, visual impairment >5 degrees or profound astigmatism or weight over 150 kg were also excluded. All participants had negative pregnancy tests. During analyses, exclusion from the fMRI analyses was also done of individuals with large movement artifacts (peaks of movement in the x/y/z-axis of more than 3 mm or more than 2 degrees of rotation) or incomplete scanning sessions due to hardware problems. An overview of the inclusion of participants can be seen in table 3.

**Table 3.** Overview of recruitment to studies.

<table>
<thead>
<tr>
<th>Study I, III and IV</th>
<th>Study II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruited</td>
<td>53</td>
</tr>
<tr>
<td>Screening</td>
<td>M.I.N.I.</td>
</tr>
<tr>
<td>CD scale for 1-2 months</td>
<td></td>
</tr>
<tr>
<td>Included</td>
<td>PMDD (n=17); HC (n=15)</td>
</tr>
</tbody>
</table>

M.I.N.I.: Mini International Neuropsychiatric Interview; CD scale: Cyclicity Diagnoser Scale; PMDD: premenstrual dysphoric disorder; HC: Healthy controls; COC: Combined Oral Contraceptives.
Ovarian steroid level assessments

Progesterone and estradiol serum concentrations were analyzed by competitive immunometry electrochemistry luminescence detection at the Department of Clinical Chemistry, Uppsala University hospital. The samples were run on a Roche Cobas e601 with Cobas Elecsys estradiol and progesterone reagent kits respectively (Roche Diagnostics, Bromma, Sweden). For progesterone the measurement interval was 0.1 – 191 nmol/l and for estradiol 18.4 – 15781 pmol/l. Progesterone intra-assay coefficient of variation was 2.21 % at 2.39 nmol/l and 2.82 % at 31.56 nmol/l. Estradiol intra-assay coefficient of variation was 6.8 % at 85.5 pmol/l and 2.8 % at 1640 pmol/l.

Mini International Neuropsychiatric Interview

The Swedish version of the Mini International Neuropsychiatric Interview (M.I.N.I.) was used to exclude individuals with ongoing psychiatric illness. The M.I.N.I. is short (approximately 15 min) but validated against the Structured Clinical Interview for DSM III-R (SCID). Unlike SCID, the M.I.N.I. is designed in order to be possible to perform with good diagnostic value even for an experiment leader with only limited training. M.I.N.I. includes the possibility to assess presence of mental disorders such as depression, phobia, or alcohol or drug dependence. History of past illness is however only assessed for mania, psychosis, antisocial personality disorder and panic disorder, (Sheehan et al., 1998). In study I, III and IV, the interviews were done by one of the co-authors (E.B). Likewise, one of the co-authors (I.S.) performed the interviews in study II.

Mood rating scales

Three mood rating scales were used, the Cyclicity Diagnoser Scale (Sundström et al., 1999a), the Montgomery-Åsberg Depression Rating Scale (Montgomery and Åsberg, 1979) and the Stait-Trait Anxiety Inventory (Spielberger et al., 1983).

Cyclicity diagnoser scale

The Cyclicity Diagnoser scale (CD scale) is a self-administered scale for prospective ratings of daily symptoms that participants complete at home (Figure 5). Sixteen parameters are rated each day: nine negative mood pa-
rameters (depression, decreased interest in usual activities, fatigue, irritability, tension, mood swings, lability, difficulties in concentrating, and sleeping disturbances), two positive mood parameters (cheerfulness, energy), and four somatic symptoms (food cravings, swelling, breast tenderness and menstrual bleeding). The sixteenth parameter is a score for measuring the every-day social functioning and work performance. The CD scale is a Likert scale ranging from 0-8, with 0 as complete absence of a particular parameter or symptom, and 8 as the maximal severity (Sundström et al., 1999a).

![Figure 5. An example of the Cyclicity Diagnoser scale. The participants use one column for each day and rate the perceived intensity of each symptom from 0 to 8.](image-url)

**CD scale in study I, III and IV**

Patients were considered to have PMDD if they had a 100 % increase in at least five negative mood parameters during seven premenstrual days as compared to seven mid-follicular days. The increase in scores also had to be associated with a clinically significant social and occupational impairment. The threshold score for impact on daily life was thus set to a score of four or more for more than two days during the luteal phase, indicating that subjects avoided social interaction during these days. All PMDD patients displayed at least one week of sparse symptoms (scores less than two) in the mid-follicular phase. Number of days during the 10 days before menses when subjects reported a score of two or more on the four core symptoms of PMDD (irritability, depression, anxiety and mood swings) (i.e. a scale 0-40) (Wang et al., 1996), and number of days when social interaction was avoided (0-10) were used as measures of severity of PMDD. The asymptomatic controls were physically healthy women with regular menstrual cycles and no significant premenstrual dysphoric symptoms in daily prospective ratings on the CD scale.

**CD scale in study II**

In the clinical trial of COC, the CD scale was used as an outcome measure for the changes in mood by treatment.
Montgomery-Åsberg Depression Rating Scale

All studies included the self-administered version of the Montgomery-Åsberg Depression Rating Scale (MADRS-S). The MADRS scale is a 9 item Likert scale that reflects depressive symptoms during the past three days on a scale ranging from 0 to 54. Originally, MADRS was designed as a development of the 17-item Hamilton Rating Scale for Depression (HAMD), with better sensitivity to change in symptomatology during treatment (Montgomery and Åsberg, 1979).

MADRS-S was administered at each scanning session in both the PMDD and COC studies.

State-Trait Anxiety Inventory

The State-Trait Anxiety Inventory (STAI) was used to assess anxiety. STAI exists in two versions, one for assessment of state anxiety in specific situations and one for trait anxiety. The scale is a 4-point scale with 20 items ranging from 20-80 (Spielberger et al., 1983). Higher scores correspond to higher anxiety.

In both the PMDD and COC studies STAI-S was administered during the two lab visits and STAI-T once for each participant.

FMRI-paradigms

Facial emotion task

In study I and II an emotion processing task based on Hariri and co-workers was used (Hariri et al., 2002). This paradigm involved a contrast between a task that required matching of emotional facial expressions and a simple sensory-motor control task. The emotion task consisted of three images of faces (angry and afraid Ekman-faces (Ekman and Frisen, 1976)) and the sensory-motor control task of three geometrical shapes (vertical or horizontal ellipses) (Figure 6). The target face or shape was displayed at the top and the participants were instructed to compare the target with the two images below and decide which one displayed the same emotion or orientation as the target image. The participants responded by pressing a button with the left or right index finger. Emotion and sensory-motor control task trials were presented in blocks of 6, in which images were presented for 4 seconds, interspaced with a fixation cross (for 2 seconds for the sensory-motor control task and a randomly selected duration of 2, 4 or 6 seconds for the emotion task). The expressed emotion or spatial orientation of the target, varied from trial to trial and each emotion block had an equal mix of emotions as well as sex of the actors. Accuracy and reaction times were registered for each trial.
Figure 6. Schematic representation of the emotional task based on Hariri et al. (2002). Participants were instructed to compare the top image of emotional faces (A) or geometrical shapes (B), with the two images below and decide if the left or right image displayed a similar emotional expression or geometrical orientation/shape.

Anticipatory task

In study III an emotional anticipation task was used (Figure 7). Images of positive or negative valence were presented on a screen. Each picture was preceded by a color cue indicating the valence of the subsequent picture. The color cues were red slides for negatively valenced pictures and green slides for pictures of positive valence. The red or green slide was presented for 5 seconds, immediately followed by a black screen with a duration of 2.5 - 3.5 seconds, after which the picture was presented for 2 seconds. After each color cue-picture combination, a black screen was displayed for 9-11 seconds before the next color cue-picture pair appeared. The presentation order sequence was pseudo-randomised. As emotional stimuli, 15 negative and 15 positive pictures were selected from the IAPS (Lang et al., 2005). The pictures were matched for valence and arousal according to the normative ratings in the IAPS-material. According to the normative ratings, mean arousal was approximately the same for both categories. The mean valence ratings of positive and negative pictures were clearly separated and at an approximately equal distance from the center of the rating scale. Following the fMRI session the participants reviewed and rated the valence and arousal of each picture on the Self-Assessment Manikin used in the IAPS-material. Valence was thereby rated on a scale from 1 to 9 where low scores indicate unpleasant and high scores pleasant experiences. Arousal was likewise rated on a scale from 1 to 9 where higher numbers indicated higher experienced arousal.
Figure 7. Schematic illustration of the anticipatory paradigm. A color cue was displayed for 5 seconds, and after a brief period 2.5-3.5 seconds an emotional image was displayed for 2 seconds. Images could be either positive (preceded by green color cues) or negative (preceded by red color cues) in valence.

Social task

In study IV, BOLD-data from the paradigm in study III was analysed with focus on only the social and non-social content of the negative images (Figure 8). The paradigm analysed thus included 15 negative IAPS-images (Lang et al., 2005). Brain reactivity to the eight images displaying negative social situations (IAPS: 3320, 2710, 3051, 3160, 6312, 6570, 8230, 9042) was compared with the seven images containing negative but non-social stimuli (IAPS: 1050, 1201, 1111, 1525, 1274, 1052, 9620). Images were matched for arousal (6.3 for social images and 6.4 for non-social stimuli) and valence (2.2 for the social images and 2.8 for the non-social images) according to the normative ratings in the IAPS-material (Lang et al., 2005).

Figure 8. Example of images with social (A) and non-social (B) negatively valenced stimuli.
MR imaging

MR imaging was performed using a 3T whole body scanner (Achieva 3T X Philips scanner Philips Medical Systems, Best, The Netherlands) equipped with an 8-channel head coil. An anatomical T1-weighted reference data set to a voxel size of 0.8×1.0×2.0 mm³ and 60 slices was acquired at the beginning of each scanning session. During stimulus presentation BOLD imaging was performed using a single shot EPI sequence with parameters TE/TR 35/3000 ms, flip angle 90°, acquisition matrix 76×77, acquired voxel size 3.0×3.0×3.0 mm³ and 30 slices.

In all studies, subjects were lying on their back in the scanner with the head lightly fixated with Velcro strips. Visual stimuli were presented through goggles mounted on the head coil (VisualSystem, NordicNeuroLab, Bergen, Norway). The stimulus paradigm was displayed using the commercial software package E-prime (Psychology Software Tools, Sharpsburg, PA, USA). In order to synchronize the paradigm and the MR-scanner, trigger pulses from the scanner were fed to the paradigm controlling PC through SyncBox (NordicNeuroLab, Bergen, Norway).

Preprocessing of fMRI data

Except for the conversion of DICOM files, and MNI-coordinates, all pre-processing were performed in MatLab (MathWorks, Natick, Massachusetts, United States of America) with the statistical parametric mapping software package SPM (SPM, Wellcome Department of Cognitive Neurology, London, UK). In study I, III and IV, SPM 5 was used, while SPM 8 was used in study II.

The MR-scanner used in the following studies stores raw data in the DICOM-format consisting of consecutive slices of the brain ranging from bottom to top. To allow analysis in SPM, data have been converted into the NIfTI-format which stores the data as consecutive volumes of the whole brain. For study I, III and IV, this was done in MRicron (available at http://www.cabiatl.com/mricro/mricron/install.html), while for study II, the conversion software provided in the scanner (Achieva 3T X Philips scanner Philips Medical Systems, Best, The Netherlands) was used.

The following steps were included in preprocessing:

1. Realignment of each brain volume relative to the mean of all scans during each session using an affine rigid-body transformation to the mean of all scans.
2. Within each brain volume, a correction for differences in acquisition time between the consecutive slices.
3. Adjustment of the individual brain into standard space was done using a three-steps procedure:
   a. First the anatomical scan of the brain for each individual was co-registered to the BOLD scans.
   b. The anatomical scan was then passed through a unified segmentation into gray matter, white matter and corticospinal fluid (CSF), during which parameters for how the individual brain was related to standardized space was obtained.
   c. The parameters from the segmentation step were then used to normalize the BOLD scans into standard space.
4. Finally, a Gaussian smoothing with a kernel of 8 mm and 18 edges was applied to data. It should also be acknowledged that some of the above steps (such as realignment) also include small smoothing steps.

Analyses of fMRI data

First level analysis
In first level analysis, data sets from each individual was analysed separately. The onsets and durations of experimental events (or stimuli presentations) were used as box-car regressors in a general linear model (GLM) to explain changes in the BOLD signal within each voxel. This step also included convolving the BOLD signal with a Haemodynamic Response Function (HRF). In the following studies, the canonical HRF provided by SPM (Figure 9) was used for this purpose. The result of this procedure was statistical maps of how well the variability in each individual voxel of the brain was explained by the experimental paradigm.

Second level analysis
The statistical maps obtained during first level analyses were used for within- and between-group comparisons producing coordinates and statistical magnitudes for voxels, or clusters of voxels, where differences in BOLD-reactivity between groups (or sessions) were obtained. In SPM, the standard space is defined in MNI-coordinates and the reported locations for results are transformed to Talairach-coordinates using a non-linear transformation.
Statistical considerations in fMRI analyses

Univariate fMRI analyses require a statistical test for every voxel. If used at a whole brain level, the risk for discoveries of false positives will be high. FMRI-analyses are therefore often performed in restricted areas of the brain; regions of interests (ROI). ROIs included in this thesis are the bilateral amygdala (study I, II, III and IV), insula (study II, III and IV), ACC (study II, III and IV), IFG (study II), MFG (study II) and Brodmann areas 6, 8, 9 and 10 (study III). Another protection against false positive findings is use of the co-variation between voxels. Activity changes in response to a certain stimuli are most likely often present in anatomical regions extending into several voxels. If the voxel is part of a cluster the likelihood for a real activation increases (Forman et al., 1995).

In this thesis, a combined method of restricting the comparisons to a priori hypothesized ROIs and the use of p-values corrected for volume of interest (i.e. the size of the ROI) as well as a cluster threshold (i.e. a number of voxels that need to be co-activated) is used. The choice of p-values and cluster thresholds varies based on how well established the studied phenomenon is on a behavioural and brain processing level.

Figure 9. The canonical HRF used by SPM to estimate the BOLD signal change induced by a compensatory increase in blood flow after stimulus-induced neuronal activity. Plotted in MatLab from SPM with the commando: RT = 1; hrf = spm_hrf(RT); plot(0:RT:32, hrf).
Summary of results

Study I

Among the 32 included women, three were excluded because of excessive movement during fMRI-acquisition. The final sample thus consisted of 15 healthy controls and 14 women with PMDD.

Women with PMDD had significantly higher STAI-T scores than healthy controls and increased scores on the MADRS-S in the luteal phase. Otherwise, no significant differences between the groups regarding age, hormonal levels, day of the menstrual cycle during testing, number of incorrect responses or reaction times were found. Both groups displayed a significant increase in progesterone serum concentrations in the luteal phase, while estradiol serum concentrations remained similar between cycle phases.

![Increased amygdala reactivity to facial stimuli in the follicular phase, in women with PMDD as compared to healthy controls. Brighter colors indicate higher T-scores.](image)

The main difference between women with PMDD and healthy controls was observed in the follicular phase, where women with PMDD had a higher bilateral amygdala reactivity than healthy controls (Figure 10). Follicular phase amygdala reactivity in PMDD women was also positively correlated to progesterone levels (Figure 11). An increase in amygdala reactivity between the follicular and luteal phase was observed among healthy controls and in women with PMDD who had high levels of trait anxiety (Table 4). In women with PMDD, reactivity in the amygdala during the luteal phase was positively correlated with MADRS-S scores. Significant group by phase
interactions were also found for habituation of bilateral amygdala reactivity. Women with PMDD had increased bilateral amygdala reactivity at the first session in comparison with healthy controls, if they entered the study in the follicular phase (but not if they entered the study in the luteal phase). Similarly, women with PMDD had increased bilateral amygdala reactivity during their first as compared to their second session (especially if they entered the study in the follicular phase). In the within-group comparison, controls also had higher right amygdala reactivity at their first as compared to their second session, but higher left amygdala reactivity at their second as compared to their first session. Also for healthy controls, differences were mainly observed for individuals who entered the study in the follicular phase.

![Figure 11](image-url) Positive correlations between amygdala reactivity in the follicular phase and progesterone levels in women with PMDD.

Table 4. Amygdala reactivity in PMDD patients reporting high (n = 7) as compared to low trait anxiety (n = 6) across the follicular and luteal phases of the menstrual cycle.

<table>
<thead>
<tr>
<th>Anxiety group x phase interaction</th>
<th>Tailarach coordinates</th>
<th>Voxels</th>
<th>Z-score</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right amygdala</td>
<td>X Y Z</td>
<td>27 2 -18 69</td>
<td>3.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Main effects

| Luteal phase High > Low anxiety | X Y Z | 30 2 -15 37 | 3.0 | 0.001 |
| Follicular phase Low > High anxiety | X Y Z | 30 2 -20 24 | 2.7 | 0.004 |

Change over phases

| High anxiety Luteal > Follicular | X Y Z | 21 2 -15 42 | 3.9 | <0.0001 |

No change over phase was present in the low anxiety group.
Study II

The scans of two women in the placebo group (pre-treatment) and two women in the COC group (one at both sessions and one at pre-treatment) were excluded from the fMRI analyses due to excessive movement during the fMRI acquisition. The final pre-treatment fMRI sample thus consisted of 15 women randomized to placebo and 15 women randomized to the COC. During treatment 17 women randomized to placebo and 16 women randomized to the COC were available for analyses. Data from both scanning sessions were thus available for 15 placebo and 15 COC users.

Women randomized to placebo or COC did not differ in terms of age, parity, educational level or previous history of COC use or previous experience of premenstrual syndrome. With the exception of higher scores of disordered sleep in women randomized to COC, no differences in daily mood symptom scores, self-rated anxiety or self-rated depression were evident between the COC and placebo group during the pre-treatment cycle.

Re-exposure to COC increased reports of mood-related side effects. During the last week of the treatment cycle COC users had higher scores of depressed mood, mood swings, and fatigue, in comparison with placebo users (Table 5). Compared to the pretreatment cycle, COC users also increased their scores of depressed mood, mood swings and fatigue, whereas placebo users virtually had unaltered mood (Table 5). As predicted, COC users also had higher scores of physical symptoms such as breast tenderness and bloating in comparison with the pretreatment assessment. Women randomized to COC also had significantly higher MADRS-S scores during the last week of treatment while there was no difference in STAI-S scores (Table 5). There was a positive correlation between pre-treatment scores on MADRS-S and scores after three weeks of treatment $R = .54 \ p < 0.05$ (Figure 12).

![Figure 12](image)

*Figure 12.* The correlation between MADRS-S scores before and during treatment in the two groups in study II.

The change in mood was accompanied by changes in brain reactivity to the emotional task. During pre-treatment no differences were detected between
women subsequently randomized to placebo or COC. During the last week of treatment COC users had lower reactivity in the left insula (Figure 13), left MFG, and bilateral IFG than placebo users. Compared to the pre-treatment cycle, the COC users had decreased reactivity bilaterally in the IFG. Placebo users, on the other hand, had decreased reactivity in the right amygdala in comparison with pre-treatment reactivity.

*Figure 13.* Lower reactivity in the insula in women re-exposed to COC as compared to placebo after three weeks of treatment. Brighter colors indicate higher T-scores.
Table 5. Mean daily ratings on the CD scale during the last week of the pre-treatment cycle, during the last week of the treatment cycle and change from pre-treatment cycle in women randomized to a levonorgestrel-containing COC (n = 17) or placebo (n = 17). Data are reported as mean ± SD.

<table>
<thead>
<tr>
<th>Mood symptoms</th>
<th>Pretreatment cycle</th>
<th>Treatment cycle</th>
<th>Change from corresponding pre-treatment week&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week four</td>
<td>Week three</td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 17)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressed mood</td>
<td>1.0 ± 0.7</td>
<td>1.4 ± 0.8</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td>Mood swings</td>
<td>1.7 ± 1.5</td>
<td>2.1 ± 1.6</td>
<td>1.1 ± 1.0</td>
</tr>
<tr>
<td>Irritability</td>
<td>1.4 ± 1.4</td>
<td>2.0 ± 1.4</td>
<td>1.2 ± 0.9</td>
</tr>
<tr>
<td>Anxious, worried</td>
<td>1.4 ± 1.3</td>
<td>1.6 ± 1.0</td>
<td>1.0 ± 1.1</td>
</tr>
<tr>
<td>Difficulties concentrating</td>
<td>1.5 ± 1.0</td>
<td>1.8 ± 1.2</td>
<td>1.2 ± 1.0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1.6 ± 0.9</td>
<td>1.7 ± 1.2</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td>Disordered sleep</td>
<td>0.8 ± 0.7</td>
<td>1.9 ± 1.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.7 ± 0.5</td>
</tr>
<tr>
<td>Out of control</td>
<td>0.6 ± 1.0</td>
<td>1.2 ± 1.3</td>
<td>0.5 ± 0.6</td>
</tr>
<tr>
<td>STAI-S&lt;sup&gt;e&lt;/sup&gt;</td>
<td>29.4 ± 5.4</td>
<td>28.9 ± 4.9</td>
<td>28.4 ± 5.2</td>
</tr>
<tr>
<td>MADRS-S&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.3 ± 1.3</td>
<td>5.2 ± 4.8</td>
<td>5.4 ± 3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8.9 ± 6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.1 ± 3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.7 ± 6.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> significantly greater than the placebo group, p < 0.05 – 0.01

<sup>b</sup> significantly greater than corresponding week during the pretreatment cycle, p < 0.05 – 0.01

<sup>c</sup> two women in the placebo group never returned their CD ratings, but STAI-S and MADRS-S scores are available for all participants

<sup>d</sup> mean difference in mood scores between the third week of treatment and the third week of the pre-treatment cycle.

<sup>e</sup> STAI-S and MADRS-S were obtained at the time of the fMRI scannings, in the follicular phase of the pre-treatment cycle and in the third week of the treatment cycle.
Study III

One healthy control and three women with PMDD were excluded due to movement artifacts or incomplete scanning sessions due to hardware problems. The final study sample consisted of 14 women with PMDD and 14 healthy controls.

Negative pictures induced greater feelings of unpleasantness than positive pictures but no differences between groups or cycle phases were evident (Table 6). Both women with PMDD and healthy controls rated the negative images as more arousing than the positive images in the follicular phase. No significant difference in arousal between negative and positive stimuli was obtained in the luteal phase.

Table 6. Valence and arousal ratings across the menstrual cycle. Valence and arousal ratings were scored on a scale ranging from 1-9. For valence, low scores indicate negative valence and high scores indicate positive valence. For arousal 1 indicates low and 9 high arousal. Mean normative ratings for the IAPS pictures used are given in brackets ().

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 14)</th>
<th>Women with PMDD (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Follicular phase</td>
<td>Luteal phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Follicular phase</td>
</tr>
<tr>
<td>Negative valence (2.5)</td>
<td>2.8 ± 0.9</td>
<td>3.0 ± 1.3</td>
</tr>
<tr>
<td>Positive valence (7.6)</td>
<td>6.6 ± 1.0</td>
<td>6.3 ± 1.0</td>
</tr>
<tr>
<td>Negative arousal (6.3)</td>
<td>5.2 ± 1.5</td>
<td>5.3 ± 1.6</td>
</tr>
<tr>
<td>Positive arousal (6.3)</td>
<td>4.3 ± 1.8</td>
<td>4.8 ± 2.3</td>
</tr>
</tbody>
</table>

Anticipatory reactivity in the anterior mPFC was increased in women with PMDD during the luteal phase (Figure 14, Table 7). No differences between groups or phases were observed for emotional stimuli. Among women with PMDD emotional reactivity in the dIPFC was positively correlated to progesterone in the luteal phase.
Figure 14. Increased luteal phase mPFC reactivity during anticipation of negative stimulation in women with PMDD as compared to healthy controls. Brighter colors indicate higher $T$-scores.

Table 7. BOLD signal changes during anticipation of emotional stimuli in women with PMDD ($n=14$) and healthy controls ($n=14$), examined in the mid-follicular and late luteal phase of the menstrual cycle.

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Brodmann area</th>
<th>Tailarach coordinates</th>
<th>Voxels</th>
<th>Z</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
<td></td>
</tr>
<tr>
<td><strong>Main effect of contrast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red color &gt; green color</td>
<td>Right ACC</td>
<td>BA 32</td>
<td>6</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>Green color &gt; red color</td>
<td>No significant clusters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group x phase interactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red color &gt; green color</td>
<td>Left mPFC</td>
<td>BA 6</td>
<td>-3</td>
<td>42</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Right dLPFC</td>
<td>BA 9</td>
<td>39</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Right mPFC</td>
<td>BA 8</td>
<td>3</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Right mPFC</td>
<td>BA 9</td>
<td>9</td>
<td>54</td>
<td>30</td>
</tr>
<tr>
<td>Green color &gt; red color</td>
<td>No significant clusters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Study IV

Two healthy controls and three women with PMDD were excluded due to movement artifacts, or incomplete scanning sessions due to hardware problems. The final sample consisted of 14 women with PMDD and 13 healthy controls.

When compared to healthy controls women with PMDD scored higher on MADRS-S and STAI-S in the luteal but not in the follicular phase (Figure 15).

![Figure 15](image)

*Figure 15.* Women with had PMDD significantly increased ratings on both A) MADRS-S and B) STAI-S during the luteal as compared to the follicular phase. The luteal phase ratings were significantly higher than in healthy controls.

Women with PMDD rated the social images significantly more negative than healthy controls during the luteal phase (Figure 16), but with no significant difference in the follicular phase. Also, PMDD women rated the social stimuli as more negative than the non-social stimuli both in the follicular and the luteal phase, while healthy controls gave similar ratings for social and non-social stimuli during both phases.

![Figure 16](image)

*Figure 16.* Women with PMDD, in the luteal phase, rated images with social content as more negative than images without any social content and more negative than did healthy controls.
Women with PMDD had increased reactivity in the amygdala and insular cortex, paralleled by decreased reactivity in the ACC when compared to healthy controls in the luteal phase (Figure 17). Among women with PMDD reactivity in the amygdala increased between the follicular and luteal phase and was positively correlated to the progesterone increase across cycle phases (Figure 18). Connectivity between the ACC and the insula or amygdala in the luteal phase was positive in PMDD but trended towards negative in healthy controls.

*Figure 17.* Women with PMDD had higher reactivity than healthy controls to social as compared to non-social stimuli negative stimuli in A) the amygdala and B) the insular cortex in the luteal phase, but lower reactivity in C) the ACC.

*Figure 18.* In women with PMDD, the increase in amygdala reactivity from the follicular to the luteal phase correlated positively with the corresponding change in progesterone.
Table 8. An overview of amygdala reactivity in study I, III and IV

<table>
<thead>
<tr>
<th>Study</th>
<th>Task</th>
<th>Amygdala reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Emotional faces</td>
<td>Increased in the luteal phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased in the luteal phase in women with high anxiety; higher than HC in the follicular phase</td>
</tr>
<tr>
<td>III</td>
<td>IAPS-images and anticipation</td>
<td>no difference</td>
</tr>
<tr>
<td>IV</td>
<td>Social stimuli</td>
<td>no difference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased in the luteal phase; higher than HC in the luteal phase</td>
</tr>
</tbody>
</table>

Table 9. Summary of correlations between brain reactivity and progesterone in study I, III and IV.

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>Contrast</th>
<th>Region</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>fol.</td>
<td>emotional faces &gt; shapes</td>
<td>Amygdala</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>lut.</td>
<td>positiv &gt; negative images</td>
<td>dlPFC</td>
<td>+</td>
</tr>
<tr>
<td>IV</td>
<td>lut. &gt; fol.</td>
<td>social &gt; non-social images</td>
<td>Amygdala</td>
<td>+</td>
</tr>
</tbody>
</table>

fol: Follicular phase; lut: Luteal phase
Discussion

Main findings

In study I, women with PMDD displayed higher amygdala reactivity than healthy controls, not in the luteal phase as was hypothesised, but in the follicular phase. No change between phases was observed among women with PMDD, but amygdala reactivity in the luteal phase was positively correlated with depressive scores and trait anxiety. As predicted, healthy controls had an increased reactivity in the luteal as compared to the follicular phase. Surprisingly, the relatively low levels of progesterone in the follicular phase were positively correlated with amygdala reactivity in the follicular phase.

In study II, re-exposure to COC induced mood symptoms *de novo* in women with a previous history of COC-induced adverse mood. Women treated with COC reported increased levels of mood symptoms both as compared to before treatment, and as compared to the placebo group. There was a relatively strong correlation between depressive scores before and during treatment. The effects of repeated COC administration on subjective measures and brain function were dissociated with increased aversive experiences and accompanied by reduced reactivity in the insular cortex.

In study III, the hypothesis that women with PMDD would display increased luteal phase reactivity in the mPFC during anticipation of negative emotional stimuli was supported, but no differences between groups or phases was observed during exposure to emotional stimuli. Progesterone levels in the luteal phase were positively correlated with dlPFC reactivity to positive stimuli in the PMDD group.

Study IV demonstrated that amygdala hyper-reactivity in PMDD is symptom-specific and linked to socially relevant stimulation, which affects reactivity and connectivity in the fear circuit.

An overview of the aims, hypotheses and main findings of the included studies can also be seen in table 10.

The studies included in this thesis contribute with several important findings regarding influence of ovarian steroid hormones on emotional brain reactivity.

- Supporting earlier reports (Andreano and Cahill, 2010; van Wingen et al., 2008) study I demonstrated increased amygdala reactivity in healthy controls during periods of increased ovarian steroid levels.
• Follicular phase progesterone correlated positively with amygdala reactivity in study I, consistent with previous reports of increased sensitivity to progesterone in PMDD (Segebladh et al., 2009).
• A clinical trial was performed suggesting that re-insertment of COC to women with previous reports of mood-related side effects induces symptoms de novo and that insular reactivity to emotional stimuli decreased during treatment (study II).
• A neurobiological model for PMDD, in which symptomatology is held to be triggered mainly by social stimuli was suggested, tested and supported by reactivity and connectivity results (study IV).

Amygdala reactivity in PMDD

One of the main hypotheses prior to the studies included in this thesis was that women with PMDD would display increased amygdala reactivity in the luteal as compared to the follicular phase and that luteal phase amygdala reactivity would be more pronounced in PMDD women than in healthy controls.

Study I focused on amygdala reactivity using a well-established paradigm with emotional facial displays (Fusar-Poli et al., 2009; Sabatellini et al., 2011). There was a positive correlation between amygdala reactivity and depressive scores in women with PMDD during the luteal phase that might indicate a coupling between depressed mood and amygdala reactivity in the luteal phase. Trait anxiety also correlated positively with amygdala reactivity in PMDD patients during the luteal phase. This finding is in line with numerous reports that suggest anxiety to boost amygdala reactivity, especially in response to negative events (Ball et al., 2012; Dilger et al., 2003; Etkin and Wager, 2007; Klump et al., 2010; Shin et al., 2004; Shin et al., 2005; Tillfors et al., 2001). However, women with PMDD did not display any change between cycle phases and no difference in comparison with controls in the luteal phase. Instead women with PMDD had higher amygdala reactivity in the follicular phase than healthy controls. In line with previous reports (van Wingen et al., 2008; Cahill and Andreano, 2010) healthy controls had increased amygdala reactivity in the luteal phase, when progesterone levels were high.

In study III neither healthy controls, nor women with PMDD displayed any changes in amygdala reactivity across the menstrual cycle phase. This is contrary to the study by Andreano and Cahill (2010), which also used emotional scenes from the IAPS. While the differences in controls may be explained by the chosen time points for scannings, a possible explanation for the results in women with PMDD is given in study IV which aimed at elucidating whether enhanced emotionally determined reactivity is elicited by social rather than non-social stimulation. If so, the non-observed luteal phase
reactivity in the amygdala of PMDD women in study I and III could be due to the chosen stimuli. Using a paradigm that contrasted brain reactivity in response to social and non-social stimuli, women with PMDD during the luteal phase displayed increased amygdala reactivity which was paralleled with a reduced reactivity in areas previously reported to be involved in emotion regulation (Roy et al., 2008; Milad et al., 2007; Ochsner et al., 2012). Reactivity alterations in PMDD to social stimuli might thus be similar to anxiety disorders with increased reactivity in the amygdala and insula but decreased reactivity in the regulatory parts of ACC which is restricted to symptom provoking situations (Shin and Liberzon, 2010). This might indicate that PMDD is an affective sensitivity disorder triggered by symptom-related emotions in the luteal phase. Further studies using paradigms comparing emotional threats involving social, other persons, or non-social, dangerous animals, might substantiate this hypothesis.

**Amygdala reactivity and progesterone**

In study I, the follicular phase amygdala reactivity in women with PMDD correlated positively with progesterone, while no such correlation was observed in healthy controls. Progesterone levels are low during the follicular phase, and from a reproductive perspective negligible. The source for progesterone during the follicular phase is most likely not the ovaries but rather the adrenals, possibly in response to stress. Progesterone produced by the adrenals is in fact considered a stress hormone in many species including rats and cats (Axner, 2008; Fajer et al., 1971; Frye, 2007). It is possible that the progesterone levels in study I reflects distress in response to the experimental procedure. Nevertheless, women with PMDD are in general more sensitive to progesterone than controls (Schmidt et al., 1998), and the findings of study I suggest that this may occur already at very low levels. Indeed, in comparison with the healthy controls in the study by van Wingen et al. (2008), the progesterone levels that induced amygdala reactivity in healthy women were approximately ten-fold higher than the progesterone levels of the PMDD women in this thesis. As progesterone receptors are present throughout the limbic system (Österlund et al., 2000a; Österlund et al., 2000b) and progesterone concentrations are high in the amygdala (Bixo et al., 1997) the follicular phase increase in amygdala reactivity might reflect a trait-like sensitivity in the amygdala to low levels of progesterone, which is abolished in the luteal phase when ovarian steroid levels are increased. Bäckström et al., (2006) has suggested that CNS may develop a tolerance to progesterone during the luteal phase and that women with PMDD have an aberrant tolerance development. Although this hypothesis mainly relies on findings of GABAergic progesterone metabolites (Martijena et al., 2002), it is equally possible that progesterone receptors in the CNS may be saturated.
during the luteal phase and direct associative couplings between amygdala reactivity and small stress-induced changes in progesterone may not be detectable. Instead results from study IV indicates that the prolonged progesterone exposure of the luteal phase mainly trigger amygdala reactivity to social stimuli. However, it should also be acknowledged that two parallel processes may be captured by study I and IV and that women with PMDD have an increased sensitivity to low levels of progesterone, but that this sensitivity not is related to the amygdala reactivity in the luteal phase.

Taken together, data suggest that women with PMDD are characterized by altered emotional brain reactivity, particularly in the amygdala and in response to social emotions, which is variable over the menstrual cycle.

PMDD and anticipation

Based on previous studies of affect modulated startle (Bannbers et al., 2011) where increased startle modulation in women with PMDD was present during emotional anticipation in the luteal phase, we hypothesized that anticipatory reactivity might be altered in PMDD. In study III, women with PMDD had indeed increased anticipatory reactivity of the anterior mPFC and dlPFC in the luteal phase. The mPFC is activated in paradigms of instructed or conditioned fear (Etkin et al., 2011) and is associated with sympathetic nervous system regulation (Critchley et al., 2001). Reactivity in the dorsal mPFC also increases in response to stress in the presence of progesterone (Ossewaarde et al., 2010). In addition, these areas have been implicated in conscious experience and expression of emotion (Etkin et al., 2011). In study III, the increased reactivity in the anterior mPFC in women with PMDD during negative anticipation most likely reflects the experience of emotions rather than any regulatory function. This assumption is based first on the finding that almost all significant clusters in the anterior mPFC during negative anticipation also were activated to a larger extent by viewing negative than positive emotional stimuli, and secondly, that regulatory prefrontal areas are in general located more ventrally than was observed here (Etkin et al., 2011). Anticipation of negative events may be adaptive and promote behavior that increases the possibility for survival in response to threat. However, increased anticipation and worry could also be dysfunctional and initiate anxious responses also to non-threatening stimuli. The observed luteal phase increase in anticipatory reactivity in women with PMDD may be associated with the possibility to treat PMDD with selective serotonin reuptake inhibitors (SSRI) (Brown et al., 2009) as SSRI has been reported to decrease anticipatory brain reactivity, at least in healthy controls (Simmons et al., 2009).

The estradiol and progesterone correlations in study III were present in areas where positive emotional stimuli induce larger reactivity than negative emotional stimuli. The mPFC correlation with estradiol was located in areas
that usually associate with the experience of emotion (Etkin et al., 2011) while the dIPFC reactivity that correlated with progesterone was located in areas which have been proposed to have a more regulatory role, perhaps through connectivity with the ACC (Ochsner et al., 2012; Ray et al., 2012). As there were no differences between groups or phases in any of those areas the interpretation of these correlations must be done with caution, but they may be indicative of an influence of progesterone on regulatory brain reactivity in dIPFC in women with PMDD.

In conclusion the results of study III indicate that women with PMDD have altered anticipatory brain reactivity, which may be influenced by estradiol as well as progesterone levels.

Induction of mood-related symptoms by COC

Scores of depressed mood, mood swings, and fatigue during treatment were higher in women on COC than in women on placebo, and increased significantly as a function of treatment. The correlation between pre-treatment depressive scores and scores during treatment may indicate that pre-treatment depressive symptoms predispose for emotional side effects on COC. This is in line with previous reports stating that prior depression is a risk factor for the development of mood symptoms during COC use (Segebladh et al., 2009; Joffe et al., 2003). As mood symptomatology in general is associated with increased reactivity in emotion processing areas (Etkin and Wager, 2007; Ressler and Mayberg, 2007; Freitas-Ferrari et al., 2010; Del Casale et al., 2012; Hayes et al., 2012; Linares et al., 2012; Patel et al., 2012; Fredrikson and Faria, in press) it is conceivable that treatment with COC in a sub-sample of women with previous mood-related side effects would be associated with increased reactivity in the corticolimbic system. However the effect of COC administration on subjective measures and corticolimbic reactivity were dissociated with increased aversive experiences accompanied by reduced reactivity in the insular cortex. COC exposure was also associated with a lower reactivity in the dIPFC (IFG and MFG). The decrease in insula reactivity was even accentuated in women with a clear-cut mood deterioration which is contrary to previous reports of increased insula activation in anxiety (Simmons et al., 2006; Stein et al., 2007; Klump et al., 2013) and also to reports of increased anticipatory insula reactivity in anxiety prone individuals (Simmons et al., 2006; Simmons et al., 2011). However, even if there was a significant increase in mood symptomatology, the increase in mood symptom scores during treatment is relatively modest and might thus not be comparable with anxiety patients.

The reduced amygdala reactivity in the placebo group may reflect habituation, as time was a confounder, but might also be affected by the normal menstrual cycle as women on placebo are subdued to a normal menstrual
cycle. Even if the exact timing according to the menstrual cycle was not assessed in the placebo group, the majority of the placebo group presumably performed their second scan in the luteal phase. According to both prior reports (Andreano and Cahill, 2010) and the results in study I increased amygdala reactivity in the second session (i.e. the presumed luteal phase) would have been expected to be increased. However, as study II was not possible to counterbalance, the amygdala decrease in the placebo group is most likely the result of repeated exposure to stimuli.

In conclusion, re-exposure to COC induced mood symptoms in women with previous experiences of mood-related side effects to COC, but the increase was relatively modest and brain reactivity was disassociated with reduced reactivity in emotion processing areas during treatment.

Comparisons of PMDD and emotional side effects on COC

Some researchers have speculated that certain women, throughout their fertile life, will display particular types of mood disorders where hormonal change is the common denominator (Studd and Panay, 2009). These women would thus be at increased risk of not only PMDD but also postpartum depression and later on perimenopausal depression. In line with this reasoning, it might be hypothesized that COC-induced mood deterioration is a prequel to PMDD or vice versa. Although the findings of this thesis may not solve the question, some light may be shed on the issue.

As both PMDD and emotional side effects on COC are characterized by negative mood in the presence of increased levels of ovarian steroid hormones, a common pathophysiology might be hypothesized. Cullberg (1972) suggested that women with retrospective reports of premenstrual syndrome (PMS) (and high scores of neuroticism) were at increased risk of experiencing mood symptoms while on COC. However, although under-powered, a later study was not able to confirm this finding when prospective PMDD criteria were applied (Segebladh et al., 2009). Yet, other common factors for both conditions include previous depressive episodes as a predisposition (Pearlstein et al., 1990; Cohen et al., 2002; Segebladh et al., 2009; Joffe et al., 2003) and an increased prevalence of anxiety-related personality traits (Critchlow et al., 2001; Freeman et al., 1995; Gingnell et al., 2010; Borgström et al., 2008). The results from study I and II is also in line with the hypothesis of shared biological mechanisms, as neither women with PMDD nor women exposed to COC reacted with an increased amygdala reactivity to increased levels of ovarian steroid hormones (i.e. in the luteal phase and during treatment).
However, there are also differences between the conditions. First, while many women with PMDD report prior COC-induced mood deterioration, drospirenone-containing COCs have been reported to alleviate both psychological and physiological symptoms of PMDD (Pearlstein et al., 2005; Yonkers et al., 2005). Also the positive correlation observed between pre-treatment depression scores and depression scores during treatment, suggest that pre-treatment depression may be indicative of the COC response, while the luteal phase increase in symptoms is typically uncorrelated with follicular phase ratings in PMDD (O’Brien et al., 2011).

Although it can be argued that no head-to-head comparison was made and that the analytical approaches were different (hypothesis-driven vs. exploratory), data suggest that PMDD and emotional side effects on COC share common features but are, at least partly, induced by different mechanisms. It should, however, be acknowledged that the inclusion criteria of subjects differed between studies. Women with PMDD were subdued to rigorous pre-testing with prospective rating scales to ascertain a menstrual cycle related variability in mood symptoms, while women in the COC-study were included based on retrospective self-reports of previous side effects. It has repeatedly been shown that retrospective report of premenstrual symptoms correctly identifies approximately half of PMDD patients (Halbreich et al., 2007), and the same may also be true in our COC users. However, more direct analytical approaches are possible in the future and if amygdala reactivity in women with COC-induced mood-related side effects are related to social stimuli in a way similar to PMDD remains to be elucidated.

Methodological considerations

Participants
The participants included in the present studies are not a random sample of the general population. Women who applied for participation in the studies had to take the initiative to contact the research unit. This means that included participants will be more dedicated to participating than the general population and perhaps also more compliant. A limitation for generalizability is that study I, III and IV are performed with largely the same participants. A limitation for the COC-study is that no data exists on whether the included subjects had a history of major depression.

Ovarian steroid hormone levels
The use of the menstrual cycle provides a model of variation in ovarian steroids with low and high levels that is highly representative for the natural
variability in ovarian steroid levels. However, the smooth curves used in schematic representations of the variation in ovarian steroid levels over the menstrual cycle (e.g. Figure 2 p. 16) is representative for the mean change in ovarian steroid levels, but might be slightly misleading. As for almost all physiological reactions, the levels of ovarian steroids are subdued to constant homeostatic changes. It should also be acknowledged that blood samples of ovarian steroid levels not fully reflect the concentrations in brain tissue. In addition, concentrations of ovarian steroid levels vary across brain regions and accumulation in certain brain areas have been reported (Bixo et al., 1986; Bixo et al., 1997).

To use exogenous administration of ovarian steroid hormones might improve control over the experimental manipulation, but as reported by van Wingen et al., (2007 and 2008), even the administration of a similar amount of progesterone results in relatively high inter-individual variability in blood concentrations.

Timepoints for fMRI

In study I, III and IV, time points for scanning sessions were chosen to correspond with the period in the luteal phase when women with PMDD in general report most intense symptomatology and the part of the follicular phase where symptomatology not is present (Hartlage et al., 2012). As a result of the chosen interval, the maximum levels of progesterone and estradiol were probably present outside the window for scanning, which limits comparability to studies addressing merely the menstrual cycle variation in brain reactivity.

Paradigms used for fMRI

The type of facial task used in study I and II is one of the most commonly used paradigm in emotional fMRI (Fusar-Poli et al., 2009; Sabatellini et al., 2011) and could therefore be seen as a relevant starting point for understudied fields such as emotionality in PMDD or the effects of COC on mood-related side effects. However, it might be argued that the contrast between faces and geometrical shapes differs not only in emotional content, but also in complexity. This implicates that the reactivity observed when contrasting facial vs. geometrical stimuli also will reflect other processes such as complex recognition and attention.

Finally several of the used paradigms could have benefited from the inclusion of registration of eye movement to assess how differences in attention to stimuli affected especially amygdala reactivity, as well as registration of sympathetic reactivity (e.g. skin conductance or heart rate) as a more objective measure of elicited distress than subjective ratings.
Future directions

The neuroimaging field of emotional effects by ovarian steroid hormones in women in fertile ages is still rather juvenile, and previous research is sparse (see summary of previous studies p. 24). Several lines of research could thus be carried out in order to make valuable contributions:

Regarding menstrual cycle there is a need for well-designed longitudinal studies that include ovulation control as well as repeated sampling of ovarian steroid levels. Ideally scanning should be performed with the same participants across the menstrual cycle and cross-balanced for phase of entrance. Time points might be chosen to correspond with low hormonal levels, high estradiol levels and combined high progesterone and estradiol levels. Inclusion of paradigms involving emotion regulation might further disentangle how variations in ovarian steroid levels affect amygdala reactivity. As the findings of this thesis, as well as several other studies (Amin et al., 2006; Protopopeseu et al., 2005; Rupp et al., 2009; Derntl et al., 2008; Derntl et al., 2010; Goldstein et al., 2005; Andreano and Cahill, 2010) report variability in emotional brain reactivity across the menstrual cycle, future studies should consider routine assessment of menstrual cycle phase in participating women.

PMDD studies designed to include both social and non-social stimuli might further elucidate the hypothesis that changes in affective brain regions in PMDD is restricted to symptom relevant stimuli. In addition, paradigms including emotion regulation might further disentangle the role of dIPFC in PMDD. Other approaches include the treatment effects of the two most commonly used regimens, cyclic SSRI treatment (Brown et al., 2009) or GnRH-agonists (Wyatt et al., 2004).

Regarding COC, further clinical trials should be performed including both other brands as well as longer treatment. Placebo-controlled fMRI studies in COC sensitive women could be of relevance for future testing of adverse mood effects of new oral contraceptives. Controlled re-insertion of COC in combination with a GnRH-agonist might be a way to further study the effects of COC during a more prolonged time period.
Table 10. An overview over aims, hypothesis and main findings of the included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Aim</th>
<th>Hypothesis</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>To study amygdala reactivity to emotional faces across the menstrual cycle in women with PMDD and healthy controls.</td>
<td>Amygdala reactivity higher in the luteal phase than in the follicular phase. Larger increase in women with PMDD than in healthy controls. Higher amygdala reactivity in women with PMDD and high trait anxiety.</td>
<td>Increased amygdala reactivity in the luteal phase in healthy controls. No differences between phases in women with PMDD. Higher amygdala reactivity in the luteal phase for women with PMDD and high trait anxiety. Follicular phase correlation between amygdala reactivity and progesterone in PMDD and luteal phase correlation between amygdala reactivity and depressive scores in the luteal phase.</td>
</tr>
<tr>
<td>Study II</td>
<td>To study the emotional side effects and brain reactivity to emotional faces during re-insertion of COC to women who previously had discontinued COC due to mood-related side effects.</td>
<td>Re-insertion of COC would increase reporting of mood symptoms and increase reactivity in the amygdala, insula and ACC. Exploratory analyses of IFG and MFG reactivity.</td>
<td>Increased mood symptoms on COC, with decreased reactivity in the insula, IFG and MFG.</td>
</tr>
<tr>
<td>Study III</td>
<td>To study anticipation of, and exposure to, emotional images, in women with PMDD and healthy controls over the menstrual cycle.</td>
<td>Increased reactivity in the amygdala, insula, ACC, BA 6, BA 8, BA 9 and BA 10 in the luteal phase in women with PMDD during anticipation. No directional hypothesis for differences between groups or phases for exposure to images.</td>
<td>Increased anticipatory reactivity in mPFC (BA 6 and 9) in women with PMDD in the luteal phase. No differences between groups or phases during exposure to emotional stimuli.</td>
</tr>
<tr>
<td>Study IV</td>
<td>To test the hypothesis that amygdala hyper-reactivity in PMDD not is generalized, but symptom specific and linked to socially relevant, rather than non-social stimulation.</td>
<td>Increased reactivity in the amygdala and insula, decreased reactivity in the ACC in women with PMDD as compared to healthy controls. Altered connectivity in women with PMDD in the luteal phase.</td>
<td>Increased amygdala and insula reactivity in the luteal phase in women with PMDD. Decreased reactivity in regulatory parts of the ACC. Positive connectivity between ACC and amygdala/insula in women with PMDD in the luteal phase.</td>
</tr>
</tbody>
</table>
Sammanfattning på svenska

Avhandlingens titel är "Ovarian Steroid Hormones, Emotion Processing and Mood", i ungefärlig översättning: "Kvinnliga könshormoner, emotionell bearbetning och humör". Avhandlingen syftar till att studera hur emotionell bearbetning påverkas av variation i kvinnliga könshormoner.

Två patientpopulationer studeras: a) kvinnor med svåra humörrelaterade symptom som varierar med menscykeln (premenstrual dysphoric disorder (PMDD)) och b) kvinnor som tidigare reagerat med emotionella biverkningar på p-piller. För att åskådliggöra psykiskt mående används olika typer av skattningsskalor (Montgomery-Åsberg Depression Rating Scale (MADRS), State-Trait Anxiety Inventory (STAI) och Cyclicity-Diagnoser Scale (CD scale)). I studierna av PMDD utnyttjas menscykeln naturliga variation i kvinnliga könshormoner och en jämförelsegrupp av friska kontrolleer ingår. Studien av kvinnor som reagerat med emotionella biverkningar på p-piller är en randomiserad, dubbelblind klinisk prövning där p-piller återinsätts. Emotionell bearbetning studeras med hjälp av funktionell magnetkamera (fMRI) under det att deltagarna utför olika uppgifter av emotionell karaktär.

Emotionell bearbetning sker till stor del i evolutionärt sett gamla delar av hjärnan, i det sk. limbiska systemet: hypothalamus, amygdala, insula, fornix, septum och anteriore cingulum (ACC) (Shin and Liberzon, 2010; Davidsson et al., 2000). Amygdala och insula aktiveras i första hand av inkommande stimuli medan ACC är mer involverat i automatiserad och kognitiv emotionsreglering (Shin and Liberzon, 2010; Bush et al., 2000; Ray and Zald, 2012). Vid emotionell bearbetning involveras även prefrontalkortex (PFC) (Fusar-poli et al., 2009; Pessoa and Adolphs, 2010, Etkin et al., 2011). PFC är bl.a. aktivt under förväntan inför emotionella händelser. Förväntan påverkar hur en efterföljande emotionell händelse uppfattas (Onoda et al., 2008; Sarinopoulos et al., 2010; Denny et al., 2013) och ökad negativ förväntan kan kopplas till ängest och depression.

Vid flertalet ängest- och depressionssjukdomar har ökad reaktivitet påvisats i områden som är involverade i emotionell bearbetning, framför allt amygdala och insula (Etkin and Wager, 2007; Ressler and Mayberg 2007; Freitas-Ferrari et al., 2010; Del Casale et al., 2012; Hayes et al., 2012; Linares et al., 2012; Patel et al., 2012; Fredrikson and Faria, in press). En del ångestsjukdomar, t.ex. posttraumatisk stress (PTSD) kännetecknas av överreaktivitet vid många typer av emotionella stimuli (Rauch et al., 2000; Rauch et al., 2006; Shin et al., 2001; Shin et al., 2005) medan andra, som t.ex. spe-
cifika fobier, är begränsade till bara vissa typer av situationer eller stimuli (Wright et al., 2003). En av de grundläggande hypoteserna för den här avhandlingen är att emotionella symptom vid t.ex. PMDD ska kunna kopplas till en överaktivitet i emotionella områden, generellt eller vid mer specifika stimuli.

Tidigare forskning kring hur emotionell bearbetning påverkas av kvinnliga könshormoner är relativt begränsad. Studier av PMDD begränsar sig till en studie som möjlichen visar på en tendens till ökad amygdalareaktivitet i lutealfas (senare hälften av menscykeln) hos kvinnor med PMDD jämfört med friska kontroller (Protopopesceu et al., 2008). Det är dock dessvärre en studie med flera metodologiska svagheter. Rörande humörbiverkningar av p-piller finns inga tidigare fMRI-studier utförda och överhuvudtaget endast tre dubbelblinda placebokontrollerade studier av emotionella biverkningar (Lee- ton et al., 1978; Graham et al., 1995; O’Connell et al., 2007). Två av dessa studier utfördes dock med steriliserade kvinnor och den tredje med kvinnor som led av dysmenorré, vilket gör att resultaten är svåra att generalisera. Det finns något fler studier av hur emotionell bearbetning påverkas av hormonvariation över menseykeln hos friska kontroller (Amin et al., 2006; Protopo- pesceu et al., 2005; Rupp et al., 2009; Derntl et al., 2008; Derntl et al., 2010; Goldstein et al., 2005; Andreano and Cahill, 2010). Kvalitéen på dessa studier avseende hormonella mätningar, val av emotionella stimuli etc. varierar kraftigt, men det verkar sannantaget som om en ökning av progesteron (så som sker i lutealfasen av menscykeln) skulle orsaka en ökad amygdalaaktiv- vering hos friska kontroller (Andreano et al., 2010; van Wingen et al., 2008).

Studie I i denna avhandling fokuserar på amygdala och använder en uppgift med emotionella ansiktssuttryck för att visa (i linje med resultaten från Andreano and Cahill, 2010) att amygdalareaktivitet hos friska kontroller är ökad i luteal jämfört med follikelfas (den första hälften av menscykeln). Kvinnor med PMDD som grupp uppväsende dock inte denna ökning utan hade istället en ökad amygdalareaktivitet i follikelfas som korrelerade med de (förhållandevis låga) progesteronnivåer som då förelåg. Studie tre och fyra fortsätter att studera PMDD. I studie tre påvisas i lutealfas en ökad reaktivitet i PFC vid förväntan hos kvinnor med PMDD jämfört med friska kontroller. Studie fyra visar slutligen att den ökade lutealfasreaktiviteten i det limbiska systemet hos kvinnor med PMDD (som inte sågs i studie ett) kan utlösas av mer symptomnära stimuli med social prägel. Studie två visar att kvinnor som tidigare reagerat med emotionella sidoeffekter på p-piller ånyo kommer att erfara ökad nedstämdhet och humörsvängningar då de insätts på p-piller. Vid användandet av samma ansiktsstimuli som i studie ett sågs en minskning i insulareaktivitet i gruppen som exponerades för p-piller jämfört med placebo.

Att studera effekten av kvinnliga könshormoner vid emotionell bearbetning är viktigt en när det finns en överrepresentation av ångest och depressionssjukdomar hos kvinnor i fertile ålder (Noble, 2005; Kuehner 2005) jäm-
fört med män. Även om också andra faktorer som social belastning, underdiagnosticering etc. förmodligen också bidrar till dessa siffror så är det anmärkningsvärt att skillnaderna i prevalens inte existerar före puberteten respektive efter menopaus (Weissman and Olfson, 1995). De i avhandlingen ingående studierna har fört fram flera viktiga pusselbitar i det relativt understudera området emotionell bearbetning och kvinnliga könshormoner:

• Tidigare fynd av ökad amygdala reaktivitet under perioder av menscyklens som karakteriseras av höga hormonnivåer hos friska kvinnor (Andreano och Cahill, 2010) har bekräftats (studie I).

• Kvinnor med PMDD har visat sig ha en positiv korrelation mellan amygdalareaktivitet och låga nivåer av progesteron, vilket öppnar upp för en möjlig förklaring av tidigare rapporter om en ökad emotionell känslighet för progesteron vid PMDD (Scmidt et al., 1998).

• Det har för första gången visats i en klinisk prövning att återsättande av p-piller framkallar emotionella symptom hos kvinnor som tidigare slutat med p-piller pga. humörbiverkningar. En minskad aktivitet i insula har också setts vid p-pillerbehandling (studie II).

• Hypotesen att PMDD har en neurobiologi som mer liknar situations- eller stimulibundna ångest- och depressionssjukdomar har formulerats, testats och visat sig stämma (studie I, III och framför allt IV).
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