The Effect of Steroid Hormones in the Female Brain During Different Reproductive States

ELIN BANNBERS
Women are twice as likely as men to suffer from depression and anxiety disorders and have an increased risk of onset during periods associated with hormonal changes, such as the postpartum period and the menopausal transition. Furthermore, some women seem more sensitive to normal hormone fluctuations across the menstrual cycle, since approximately 3-5% suffers from premenstrual dysphoric disorder (PMDD). Why these disorders are so common in women has not been established but there is a probable involvement of the ovarian hormones.

The aim of this thesis was to investigate the effect of the ovarian hormones on the female brain during different reproductive states using psychological tests known to affect brain activity in different ways.

Paper one examined the effect of the ovarian hormones on prepulse inhibition (PPI) on the acoustic startle response (ASR) and comprised cycling women and postmenopausal women. The cycling women had lower levels of PPI compared to postmenopausal women and postmenopausal women with moderate estradiol levels had lower PPI compared to postmenopausal women with low estradiol levels.

Paper two examined the effect of anticipation and affective modulation on the ASR in women with PMDD and healthy controls. Women with PMDD have an increased modulation during anticipation of affective pictures compared to healthy controls during the luteal phase of the menstrual cycle.

Paper three examined brain activity during response inhibition among women with PMDD and healthy controls by the use of a Go/NoGo task and fMRI. Women with PMDD displayed a decreased activity in the left insula during follicular phase and an increased activity during the luteal phase compared to controls.

Paper four comprised women in the postpartum period and non-pregnant controls to examine brain activity during response inhibition. While this study revealed decreased activity at 4 weeks postpartum compared to 48 hours postpartum we cannot ascertain the role of the ovarian steroids, since none of the significant brain areas correlated with ovarian steroid or neurosteroid serum concentrations.

The results of this thesis demonstrate that the ovarian hormones, or at least various hormonal states, have a probable impact on how the female brain works.

Keywords: Premenstrual dysphoric disorder, Postpartum, Estradiol, Progesterone, Menstrual cycle, Functional magnetic resonance imaging, Response inhibition, Prepulse inhibition, Startle response

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


II Bannbers, E., Kask, K., Wikström, J., Risbrough, V., Sundström-Poromaa, I. (2011) Patients with premenstrual dysphoric disorder have increased startle modulation during anticipation in the late luteal phase period in comparison to control subjects. *Psychoneuroendocrinology, 36*(8):1184-92


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## Abbreviations

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<th>Definition</th>
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<tr>
<td>AAI</td>
<td>Automated anatomical labelling</td>
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<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
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<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>ASR</td>
<td>Acoustic startle response</td>
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<tr>
<td>BA</td>
<td>Brodmann area</td>
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<td>BOLD</td>
<td>Blood oxygen level dependent</td>
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<td>CD</td>
<td>Cyclicity diagnoser</td>
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<td>COC</td>
<td>Combined oral contraceptive</td>
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<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<tr>
<td>dB</td>
<td>Decibel</td>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical manual of Mental Disorders, 4th edition</td>
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<tr>
<td>ER</td>
<td>Estradiol receptor</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
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<td>EPT</td>
<td>Estrogen-progestagen therapy</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<tr>
<td>GABA</td>
<td>Gamma aminobutyric acid</td>
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<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
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<tr>
<td>HAB</td>
<td>Habitation</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
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<tr>
<td>IAPS</td>
<td>International affective picture system</td>
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<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
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<tr>
<td>MADRS-S</td>
<td>Montgomery-Ásberg Depression Rating Scale-Self-rated version</td>
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<tr>
<td>MINI</td>
<td>Mini International Neuropsychiatric Interview</td>
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<tr>
<td>NMDA</td>
<td>N-Methyl-D-Aspartate</td>
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<tr>
<td>MP&lt;sub&gt;A&lt;/sub&gt;</td>
<td>Mean magnitude of pulse alone</td>
</tr>
<tr>
<td>MP&lt;sub&gt;PP&lt;/sub&gt;</td>
<td>Mean magnitude of prepulse-pulse trials</td>
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<tr>
<td>MR</td>
<td>Magnetic resonance</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>PA</td>
<td>Pulse-alone</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<td>PM</td>
<td>Postmenopausal</td>
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</table>
PMD  Premenstrual disorders
PMDD  Premenstrual dysphoric disorder
PMS  Premenstrual syndrome
PPI  Prepulse inhibition
PR  Progesterone receptor
RF  Radiofrequency
RIA  Radioimmunoassay
ROI  Region of interest
SEM  Standard error of mean
SD  Standard deviation
SPM5  Statistical parametric mapping 5
SPSS  Statistical package for the Social Sciences
SSRI  Selective serotonin reuptake inhibitor
VAS  Visual analogue scale
5-HT  5-hydroxytryptamine
Introduction

Ovarian steroid hormones
The ovaries produce all three classes of sex steroids, estrogens, progestins and androgens, through the conversion of cholesterol. Cholesterol is essential for the synthesis of the ovarian hormones and can be synthesised in-situ from acetate or, more importantly, obtained from the blood system. The progestins consist of pregnanolone and progesterone and are produced in both the granulosa and theca cells of the ovarian follicle. Progesterone can in turn be converted into testosterone, the precursor of estrogens. The estrogens, estradiol and estrone, are produced in the granulosa cells of the ovarian follicle [1].

The ovarian steroids are involved in pubertal development and regulation of the menstrual cycle, through the hypothalamic-pituitary axis. They are also required during pregnancy for maintenance of the uterus and embryo, inhibition of myometrial contractions, and the suppression of response towards foetal antigens (progesterone). During pregnancy the ovarian hormones are also involved in processes that regulate the increase in uterine size and blood flow, they are important for the timing of implantation, and enhance foetal organ development (estrogens) [1].

The menstrual cycle, pregnancy and menopause
The standard menstrual cycle consists of 28 days, but may range between 25-35 days, with ovulation usually occurring 14 days prior to menstruation. The follicular phase is characterized by follicular development (in response to increased levels of follicle stimulating hormone) and gradually increasing estradiol serum concentrations. Ten to sixteen days from the onset of menstruation the ovulatory phase start and last about 24-48 hours. Following ovulation the luteal phase begins, characterized by estradiol and progesterone production from corpus luteum. The luteal phase length is fairly consistent and lasts 14 days, why deviating menstrual cycle length is usually caused by prolonged (or shortened) follicular phases. The luteal phase can also be subdivided into early, mid and late luteal phase. If no pregnancy occurs, progesterone levels drop and menstruation begin [1].
The levels of progesterone and estradiol vary substantially between individuals but also within individuals. In an individual woman, estradiol and progesterone serum concentrations may vary from one menstrual cycle to another but also across the fertile period, typically manifested by lower luteal phase progesterone levels in the premenopausal period. Within a single menstrual cycle, serum progesterone levels range from less than 1.6 to 23.6 nmol/L between the follicular and luteal phase, while serum estradiol levels range from 210 to 1700 pmol/L between phases, reaching peak estradiol levels before ovulation [1].

When pregnancy occurs progesterone and estradiol levels increase further, and are at the end of pregnancy as high as 480 nmol/L and 90 nmol/L, respectively. As the placenta is the major source of ovarian steroids, the steroid hormones quickly drop to postmenopausal levels following parturition and remain suppressed until the menstrual cycle restarts. Typically, progesterone levels are reduced by more than half within 12 hours, and by two weeks postpartum, levels have reached subnormal levels. However, besides the drop in estradiol and progesterone levels, the postpartum period is characterized by complex hormonal changes involving many different hormonal pathways. Following the pregnancy-induced increases in placental corticotrophin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and cortisol [2, 3], delivery results in a sharp drop of placental CRH levels [4, 5] and a more gradual decrease in cortisol levels [4-13]. The postpartum period is therefore characterized by CRH withdrawal, resulting in transient suppression of hypothalamic CRH release and dexamethasone non-suppression [14-16], which is not normalized until five weeks postpartum [17]. In addition, the postpartum period is also characterized by increased levels of oxytocin [18], which has been associated with maternal behavior and breast feeding [19, 20].

As the ovaries age and gradually lose activity women will eventually reach menopause. The menopause is defined as that point in time when permanent cessation of menstruation occurs, i.e. the last menstrual bleeding. The ovaries, due to loss of follicular activity, are no longer able to produce enough estrogen for the endometrium to proliferate and thus signal the permanent end of fertility. In normal, non-surgical menopause the definite diagnosis cannot be established until 12 months have passed without any menstrual bleeding. In the industrialized world menopause occurs at approximately 51 years of age [21, 22]. The term “perimenopause” is used to describe the period that commences when the first features of approaching menopause begin until at least one year after the final menstrual bleeding [23]. During menopause the steroid levels decrease and the postmenopausal levels of progesterone is less than 1.6 nmol/L and the estradiol levels range between 3.4-82.7 pmol/L [1, 24].
The distribution of estradiol- and progesterone receptors in the brain

Estradiol and progesterone are both highly lipophilic and can therefore easily pass through the blood-brain barrier to bind to their specific receptors in the brain. The estradiol receptors (ER) and the progesterone receptors (PR) belong to the nuclear receptors and exist in several tissues of the human body, including the brain, for review see [25, 26]. In the brain the receptors are highly expressed in areas associated with reproductive function, such as the hypothalamus and the limbic system. The expression of the estradiol receptors, ERα and ERβ, has been demonstrated in the human amygdala, hippocampus, claustrum, hypothalamus, and the cerebral cortex. Within the cerebral cortex, the most distinct expression of estradiol receptors is found in the temporal cortex [27, 28]. The progesterone receptors, PRA and PRB, are according to animal studies, as well as human post mortem studies, also distributed throughout the amygdala, hippocampus, hypothalamus, thalamus and the frontal cortex [29-33]. This distribution of the receptors suggests that the ovarian hormones modulate areas involved in emotional processing, cognitive function, sensory input, attention, decision making, and motor function (among others).

Ovarian hormones and the effect on mood

During their reproductive years women are twice as likely as men to suffer from major depressive episodes and anxiety disorders [34-38]. Certain periods may even confer an increased risk of depression, such as the postpartum period [39, 40] and the late premenopausal period, for review see [41, 42]. Furthermore, almost 70% suffer from transient and mild depression within the first five days of delivery, often called “the postpartum blues”, which has been attributed to the rapid withdrawal of estradiol and progesterone following parturition [43]. Finally, some women appear sensitive to the normal menstrual cycle changes in ovarian steroid levels (premenstrual syndrome and premenstrual dysphoric disorder), while others respond with mood-related side effects to exogenously administered hormones.

Generally, estradiol has been associated with positive effects on quality of life, well-being and mood. Women of reproductive ages report increased well-being in the estrogen-dominant follicular phase [44], and estrogen depletion induced by gonadotropin-releasing hormone (GnRH) agonists is generally associated with mood worsening in healthy women [45-49]. Estradiol enhance mood in healthy postmenopausal women without climacteric symptoms [50, 51], is effective for the treatment of depressive disorder in perimenopausal women, and possibly increases the responsiveness towards antidepressants [52-55]. However, the effect of estradiol on mood during the
postmenopausal period seem less evident and most studies find no effect of estradiol for the treatment of clinical depression during menopause [56-58] (however, see [59, 60]). On the other hand, extreme estrogen depletion in the postmenopausal period, typified by treatment with third generation aromatase inhibitors has been associated with lowered quality of life [61, 62].

Far less conclusive, estradiol has also been advocated to have a positive effect during the postpartum period, for review see [63]. However, the only placebo-controlled estradiol treatment study (which suggested tremendous effects of transdermal estradiol on postpartum depression) [64] has never been replicated and subsequent open, observational studies have not contributed with relevant information [65].

While estradiol is associated with positive effect on mood during the menopausal transition, pregnancy, and the postpartum period, progesterone is associated with the negative effects in the premenstrual dysphoric disorder (PMDD, further described below) and among combined oral contraceptive (COC) users reporting adverse mood symptoms. Adverse mood symptoms among COC-users are relatively common and the best available evidence suggest that approximately 4-10 % of all users are affected [66]. As the ethinylestradiol dose is relatively stable between preparations, the side-effect profile has been attributed to the progestagen component of the pill. More specifically, women using contraceptives with androgenic progestagen tend to report more depression and irritability than women using contraceptives with anti-androgenic progestagen [67, 68]. In addition, mood effects of COCs are most pronounced during the pill-free interval, supporting a hormone-withdrawal effect [67, 69, 70]. In postmenopausal women progestagen use (or addition) is also associated with mood worsening. Administration of progesterone as well as progestagen during sequential estrogen replacement therapy is related to more pronounced mood symptoms in postmenopausal women [71-74]. Also, Zou and colleagues (2009) revealed a correlation between the rapid changes in estrogen and progesterone levels in the first trimester and postpartum period and depression and anxiety [75].

Why depression and mood changes are so common in women is not yet fully understood. However, before puberty and after menopause no gender-related differences in prevalence rates of depression are found [76], further supporting that ovarian hormones have an effect on mood. At least in some women that may be extra vulnerable to hormone fluctuations or withdrawal-effects.

Premenstrual dysphoric disorder (PMDD)

Of all women in reproductive age, 3-8% suffer from the severe form of premenstrual syndrome (PMS) called premenstrual dysphoric disorder, for review see [77]. Normal and manageable symptoms that emerge prior to menstruation, which most women experience and that have little or no impact on
a woman’s ability to function are; bloating, breast tenderness, food cravings, and pelvic heaviness. PMS, on the other hand, is characterised by both physical and mood-related symptoms in the late luteal phase and women with PMDD suffer from a significant functional impairment and a strong negative impact on the quality of life [77].

Because the symptoms are different between individuals and since no specific endocrine diagnostic test exist, premenstrual disorders are often thought to be under-diagnosed. To help recognise the somatic and emotional changes diagnostic criteria for PMDD has been developed and can be found in the *Diagnostic and Statistical Manual of Mental Disorders, 4th edition* (DMS-IV), Table 1.

More recently, a definition of premenstrual disorders (PMD) was suggested by the International Society for Premenstrual Disorders [78]. The definition comprises core PMD (PMS and PMDD) as well as variant forms including premenstrual exacerbation, progestagen-induced PMD, and PMD with absent menstruation. It is thus far unclear if this new definition will be helpful to clinicians or researchers.

**Table 1. Criteria for premenstrual dysphoric disorder as defined in DSM-IV.**

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A. In most menstrual cycles during the past year, five (or more) of the following symptoms were present for most of the time during the last week of the luteal phase, began to remit within a few days after the onset of the follicular phase and were absent in the week post menses, with at least one of the symptoms being either (1), (2), (3), or (4):

1. markedly depressed mood, feelings of hopelessness, or self-deprecating thoughts
2. marked anxiety, tension, feeling of being “keyed up” or “on edge”
3. marked affective lability (e.g., feeling suddenly sad or tearful or increased sensitivity to rejection)
4. persistent or marked anger or irritability or increased interpersonal conflicts
5. decreased interest in usual activities (e.g. work, school, friends, hobbies)
6. subjective sense of difficulty in concentrating
7. lethargy, easy fatigability, or marked lack of energy
8. marked change in appetite, overeating, or specific food cravings
9. hypersonomnia or insomnia
10. a subjective sense of being overwhelmed or out of control
11. other physical symptoms, such as breast tenderness or swelling, headaches, joint or muscle pain, sensation of “bloating”, weight gain

B. The distribution markedly interferes with work or school or with usual activities and relationship with others (e.g., avoidance of social activities, decreased productivity and efficiency at work or school).

C. The distribution is not merely an exacerbation of the symptoms of another disorder such as major depressive disorder, panic disorder, dysthymic disorder, or a personality disorder (although it may be superimposed on any of these disorders).

D. Criteria A, B, and C must be confirmed by prospective daily ratings during at least two consecutive symptomatic cycles. (The diagnosis may be made provisionally prior to this confirmation.)
The aetiology of PMDD

Despite that menstrual-cycle related symptoms have such an impact on the life of the women affected, knowledge about the aetiology of PMD is relatively limited. Two major lines of evidence, however, suggest that ovarian hormones as well as the serotonin system are involved.

Due to the cyclic pattern of PMDD, with symptom onset in luteal phase and remission in follicular phase, and the fact that the disorder only affect women in their childbearing years [74] much attention has been given to the steroidal hormones. While the majority of studies indicate that PMDD patients display neither excess nor deficiency of progesterone (exemplified by [79]), suppression of the corpus luteum formation will result in significant symptom relief [80]. The usefulness of GnRH agonists with or without add-back hormone replacement therapy (HRT) for the treatment of premenstrual disorders has been evaluated in a meta-analysis, which included seven randomized, placebo-controlled, double blind clinical trials with altogether 71 women [81]. Compared to placebo, GnRH agonist treatment on its own resulted in significant symptom relief in behavioural as well as physical premenstrual symptoms [81]. The result of the meta-analysis is also corroborated by a number of randomized, placebo-controlled studies that were not included because data could not be extracted [82-87]. Response rate to GnRH agonist treatment is reportedly between 60-75 % in these trials [82, 84, 88, 89] although no uniform definition of treatment response has been employed. However, it has been concluded that doses sufficient for inhibiting ovulation are needed for optimal symptom relief [80].

The serotonin system seems to be involved in the PMDD because of the tremendous treatment effect of serotonin reuptake inhibitors. Selective serotonin reuptake inhibitors (SSRI) are, at least in Sweden, advocated as first-line treatment for PMDD and the clinical effects, in particular for psychological symptoms, are well documented [90, 91]. Forty randomized clinical trials were included in the 2009 Cochrane review, which concluded an overall reduction of symptoms for all tested SSRIs, compared to placebo [92]. After the Cochrane review, a placebo-controlled trial on two different doses of luteal phase ecitalopram (10 and 20 mg/day) was published [93]. Cyclic ecitalopram resulted in a 90% decrease in irritability, depressed mood, tension, and affected lability with the higher dose and it had a response rate among participants of 80%.

The precise mechanisms, by which ovarian steroids and the serotonin system interacts is, however, not established. Although menstrual cycle related changes in serotonin function are consistently reported in healthy women [94-99] (however, see [100]) findings in PMDD patients are less conclusive. Some studies identify the expected luteal phase alteration [101-107] while others find no difference from controls [108-111] or differences confined to the follicular phase [112-114]. Jovanovic and colleagues reported a signifi-
cantly smaller increase in the $5\text{-HT}_{1A}$ receptor binding potentials in the dorsal raphe nuclei (an area responsible for a substantial proportion of the serotonin innervations) across the menstrual cycle in women with PMDD compared to healthy controls [115]. Furthermore, brain serotonin precursor trapping has been inversely associated with premenstrual irritability and depressed mood in women with PMDD [116].

The acoustic startle response

Most of what is known about the ovarian steroid effects in the central nervous system (CNS) has been derived from randomized clinical trials or animal research. However, useful information can also be gained from psychophysiological studies, particularly if experimental measures are used where the underlying biological mechanisms have been established in animal studies. The acoustic startle response (ASR) is a withdrawal reflex to sudden or noxious auditory stimuli that can be measured as an eye blink in humans or as a whole body response in laboratory animals. The reflex is coupled to a stimulus and can therefore be measured within a certain time period shortly after the stimulus has been given [117]. In humans startle response is usually measured with electromyography (EMG) of the musculus orbicularis oculi [118] and it may be modulated in several ways, resulting in enhancement or attenuation of the startle magnitude.

Prepulse inhibition

One way to attenuate the ASR is to use prepulse inhibition (PPI). PPI refers to the reduction in response to an intense startling stimulus when it is preceded by a weak, non-startling, acoustic stimulus (the prepulse), Figure 1. It is thought to reflect an individual’s ability to screen or “gate” sensory stimuli and allows the individual to focus on important events [118, 119].

Deficient PPI has been demonstrated in various anxiety disorders [120-122], in patients with PMDD [123] and in patients with schizophrenia [124], emphasizing the clinical relevance of PPI as a psychophysiological measure.

The ovarian hormones and PPI

Of specific relevance for this thesis is the fact that PPI consistently has been shown to be influenced by sex and ovarian hormones. Women, regardless of menstrual cycle phase, have decreased PPI compared to men [125-130]. Although, in women of reproductive age the menstrual cycle phase also influences PPI, with lower levels found during periods of high ovarian steroid levels (such as the mid-luteal phase) [131, 132]. That periods of high ovarian steroid levels influence PPI was confirmed in a study by Kask and colleagues (2009) that examined women during late pregnancy and the
postpartum period [133]. Reduced PPI was found in pregnant women compared to women examined 3-7 days postpartum. The PPI in women examined within 48 hours postpartum, still in a relatively high hormonal state, did not differ from pregnant women. Finally, once women reach menopause and ovarian steroid levels decline, there is no longer any difference in PPI between women and age-matched men [134].

The PPI also appears reduced in women during reproductive events associated with increased vulnerability to mood and anxiety disorders. Patients with PMDD have lower levels of PPI during the late luteal phase in comparison with healthy controls [123]. Furthermore, women with subjective reports of depression and anxiety while using COC have lower levels of PPI compared to healthy COC users [135].

The regulation of PPI

A number of neurotransmitter systems are involved in the regulation of PPI. The gamma aminobutyric acid- (GABA), dopamine-, and N-methyl-D-Aspartate- (NMDA) system exert an inhibitory effect resulting in a reduced PPI in healthy humans [136-140] while serotonin receptor agonists are thought to increase the PPI [141]. However, the results are somewhat inconsistent [142-145] and seem to be dependent on the type of drug administration. Furthermore, since PPI is frequently used as a model for schizophrenia, most human pharmacological studies have been performed in schizophrenia patients or in male populations and the results might therefore not be applicable in women.
The few pharmacological studies examining the effect on PPI in women have found no effect of tryptophan depletion [146] or amphetamine [147], although, Talledo and colleagues (2009) reported that amphetamine reduced the PPI levels in women with a high baseline PPI [147]. In addition, a study by Kask and colleagues (2009) examined the effect of the GABA<sub>A</sub> receptor agonist, alloprognanolone, in healthy women and found no effect on PPI [142].

The lowered PPI observed in women during the luteal phase has been suggested to be a result of the increased estradiol concentrations, which in turn influences a number of neurochemical activities. For instance, estradiol acts on the dopaminergic system, increasing the release of dopamine, for review see [148], that has an inhibitory effect on PPI in neural areas critical for PPI [149, 150]. Also, administration of estradiol to ovariectomized rats induce a decrease in the number of inhibitory synaptic inputs, an increase in the number of excitatory synapses and an enhancement of the frequency of neuronal firing [151]. However, the sex- and menstrual cycle differences can not be entirely explained by estradiol, since no direct relationship between PPI and estradiol levels in healthy women have been detected [134, 147, 152]. The effect of progesterone therefore also has to be taken into consideration. A study by Kumari and colleagues (2010) suggest that progesterone is involved in the regulation of PPI since a decrease in PPI is correlated with a larger increase in progesterone [152]. This and the fact that progesterone, or its metabolites, is also capable of modifying neuroactive receptors implicate its involvement in the regulation of PPI, for review see [153].

Anticipation and affective modulation

Startle reactivity may also be used as an unbiased measure (at least compared to self report) of emotional processing of both appetitive and aversive stimuli [154]. Animal studies as well as human studies show that the ASR is enhanced during arousal and fearful situations, such as during threat of shock or aversive pictures, Figure 2, while it is reduced when presented with rewarding stimuli such as pictures of food or erotica [155, 156]. This modulation is often referred to as affective modulation of the ASR. Studies investigating different anxiety disorders show an enhanced startle magnitude during modulation of the ASR [157, 158]. This enhancement by exposing subjects to aversive situations, suggest that amygdala modulates the startle circuit during threat situations [154, 159]. Importantly, startle reactivity is also dependent upon emotional valence, unlike other physiological measures of arousal (e.g. skin conductance) which are elevated in the presence of either highly rewarding or highly negative stimuli [154].
Figure 2. Schematic picture describing affective modulation of the ASR by an aversive event. In reaction to the 105 dB startle stimulus a startle response reflecting the magnitude of the eye blink is elicited (response without aversive event). If the startle stimulus is accompanied by an aversive event, e.g. a picture with negative content, the startle response will be increased (response with aversive event). The magnitude of the affective modulation can then be calculated using the difference between the two responses, in this figure demonstrated as potentiation of the acoustic startle response.

Prior studies have also investigated the effect of anticipation on startle magnitude by eliciting acoustic stimuli during specific cues prior to pleasant, neutral and unpleasant picture stimuli [160-162]. The results suggest that the expected arousal by the upcoming picture elicits an elevated startle magnitude already during the anticipation phase [160, 162]. Hence, each person is only anticipating what they are told will be an unpleasant or pleasant image, thus the construct of anxious anticipation is probed as opposed to the construct of stimulus-specific fear or aversion. One obvious utility of examining startle responses during instructed anticipation of an image type (either pleasant or unpleasant), is that startle reactivity is not dependent upon the image itself, thus differences in how subjects respond to a particular image based on different life experiences do not confound the interpretation of startle effects. Recent findings in functional magnetic resonance imaging (fMRI) suggest that image anticipation tasks probe important anxiety neural substrates, namely the insular cortex and amygdala [163, 164].

Amygdala and the affective modulation
The neural pathway underlying fear-potentiated startle in rats suggests that amygdala and its projections may have an important modulatory role for the startle response. Lesions of the central nucleus or the lateral and basolateral nuclei of the amygdala block the expression of fear-potentiated startle, and electrical stimulation of the central nucleus increases the startle response amplitude [165, 166]. In humans, lesions of the amygdala result in failure to show the typical startle potentiation induced by negative emotions [167,
Prior positron emission tomography (PET) studies have indicated that startle modulation by negative affect is associated with activation in the left amygdaloid-hippocampal area in subjects with snake and spider phobias [159], and similar results have been obtained in patients with social phobia [169-171].

**Emotional anticipation and the insular cortex**

An area important for the anticipation of an affective event is the insular cortex. Recent fMRI and PET findings suggest that the insula is activated during emotional anticipation of aversive events in healthy individuals [172-176] and animal studies demonstrate that insular cortex activation by aversive stimuli modulates startle reactivity [177]. Furthermore, the insular cortex is suggested to be involved in interoception and the maintenance of physiological and emotional homeostasis, for review see [178], with recent suggestions that disordered activation may underlie some anxiety states [163, 164, 176, 179]. Furthermore, this region is highly sensitive to estrogen effects on neural excitability [180], although progesterone influences have yet to be investigated.

Although the effect of the ovarian hormones and menstrual cycle phases have been extensively studied during PPI of the startle response, the effect of anticipation and affective modulation has not been studied (with the exception of one study examining affective modulation of the ASR in women with PMDD [181]).

**Magnetic resonance imaging**

Magnetic resonance imaging (MRI) is a technique that uses the interaction between radio waves and a strong magnetic field to create images of biological tissue. To create images the scanner uses pulse sequences, which consist of a series of oscillating magnetic fields and radiofrequency (RF) pulses. When a RF pulse of a certain (resonance) frequency is switched on, the energy is absorbed by the hydrogen atoms in the body and changes the direction of the nuclear magnetization. After the RF pulse is switched off the nuclear magnetization will change back to its original state emitting the energy previously absorbed by the hydrogen atom. The energy is released in form of new radio waves that constitutes the basis for the data that is used to create images. Depending on the pulse sequence used, MRI can detect different types of tissues and tissue properties [182].
Functional Magnetic Resonance Imaging

Functional MRI is used to identify different areas in the brain where particular mental processes take place. Functional MRI is a type of MRI that measures the hemodynamic response, i.e. the change in blood flow, in response to neural activity in the brain. In response to neural activity, there is an increase in blood flow that surpasses the increased oxygen consumption. Thus neural activation results in an increase in blood oxygen concentration and since the MR signal from oxyhemoglobin is larger than that from deoxyhemoglobin, this leads to an increase in MR signal. This method is therefore called blood oxygen level dependent (BOLD) imaging.

To measure changes in brain function the subjects are asked to perform a specific experimental task designed to activate the regions of interest [182]. Over the past years, a number of exploratory studies have aimed at describing the brain activity in response to emotional and cognitive tests during various hormonal settings. Studies performed in women of reproductive age and using fMRI are summarized in Table 2 and Table 3. Additional information on the hormonal influence on emotion-induced brain activity (or corresponding activity during various cognitive tests) may also be derived from clinical trials in postmenopausal women [183-199]. Because of lack of space, these studies are not summarized in this thesis.

Response inhibition

Cognition is a term that describes the process of thought. It includes processes like memory, perception, language, conscience, and problem solving. Basic processes can be localized to specific brain areas while more complex cognitive functions demand cooperation between different brain regions. One such complex cognitive function is called executive function.

Executive function is a process crucial for complex cognitive activities like planning and problem solving (goal-directed behaviour) and includes the ability to inhibit or suppress a predefined (unwanted) response, also referred to as response inhibition [200]. Inhibition is thought critical to the successful completion of many everyday tasks, such as stopping at traffic lights, preventing impulse behaviour, resisting eating all the candy in the bag, and waiting in line.

The Go/NoGo task is a test that measures response inhibition. Together with fMRI the Go/NoGo task can be used to investigate areas activated during successful or unsuccessful inhibition. It is often used to examine response inhibition in healthy participants and different psychiatric disorders characterised by impulsive behaviour [201-204] and deficits in response inhibition have been implicated in clinical disorders such obsessive compulsive disorder and posttraumatic stress disorder [205, 206].
The involvement of the prefrontal cortex (inferior, middle and superior frontal gyrus) in response inhibition has been consistently demonstrated [207-211], but other key areas involved in response inhibition include the anterior cingulate cortex (ACC), insula, inferior parietal lobule, superior temporal gyrus and the caudate body [212-216].

The literature examining the effect of ovarian hormones on brain activity during response inhibition using fMRI and the Go/NoGo task is sparse, nevertheless, functional differences are evident between men and women [207] and menstrual cycle effects in healthy women are documented [210, 217, 218]. Furthermore, activation differences between women with PMDD and healthy controls have been demonstrated during the luteal phase of the menstrual cycle [219].

Among the areas common for Go/NoGo tasks, menstrual cycle effects are, however, only documented in the dorsolateral prefrontal cortex, the inferior frontal gyrus, and the ACC [210, 217, 218]. In addition, the only areas thus far related to ovarian steroid levels include the inferior frontal gyrus, inferior parietal lobule and caudate [218]. As all of these areas express estradiol and progesterone receptors, it is possible that some PMDD symptoms may arise from the direct influence of these hormones on inhibitory control [27, 28, 33]. It is also plausible that the abrupt drop in steroid hormones after childbirth and the near absence of those hormones during the postpartum period could affect brain activation during cognitive control.
Table 2. A summary of a number of studies using emotional tasks to examine the brain activity in women of reproductive age.

<table>
<thead>
<tr>
<th>Task</th>
<th>Subjects</th>
<th>Intervention</th>
<th>Results</th>
<th>Positive correlations</th>
<th>Negative correlations</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food images (fed &gt; fasting)</td>
<td>9 HC</td>
<td>Early vs. late FP</td>
<td>Late FP &gt; early FP: Insula, IFG, Fusifrom Gyrus</td>
<td>E2: IFG, precentral gyrus, insula, MTG, cerebellum, occipital gyrus, cuneus.</td>
<td>E2: SFG, MFG, MeFG, insula, posterior cingulate, MTG, STG, precuneus</td>
<td>[220]</td>
</tr>
<tr>
<td>Negative, neutral pictures</td>
<td>12 HC</td>
<td>Early FP vs. Mid-cycle</td>
<td>Early FP &gt; mid-cycle: ACC, posterior cingulate, amygdala, MFG, MeFG, hypothalamus (PVN, VMN), brainstem, hippocampus, pallidum, cuneus, middle occipital gyrus, MTG, fusiform gyrus, cerebellum. Mid-cycle &gt; early FP: supramarginal gyrus, precentral gyrus</td>
<td>NA</td>
<td>NA</td>
<td>[221]</td>
</tr>
<tr>
<td>Negative, neutral pictures</td>
<td>17 HC</td>
<td>Early FP vs. mid-LP</td>
<td>Mid-LP &gt; early FP: Amygdala, IFG, hippocampus, fusiform gyrus, cerebellum, caudate nucleus</td>
<td>None found</td>
<td>E2: hypothalamus</td>
<td>[222]</td>
</tr>
<tr>
<td>Positive, negative, neutral words</td>
<td>14 HC</td>
<td>Mid-FP vs. mid-LP</td>
<td>No phase differences reported. Positive distracters &gt; positive targets: ACC, IFG, putamen</td>
<td>Luteal E2: IFG, IPG</td>
<td>Luteal E2: caudate, inferior parietal gyrus</td>
<td>[218]</td>
</tr>
<tr>
<td>Monetary reward</td>
<td>11 HC</td>
<td>Mid-FP vs. mid-LP</td>
<td>Anticipation: Mid-FP &gt; mid-LP: Amygdala, OFC, ITG, MTG. LP &gt; FP: MFG, SFG, ACC Reward: Mid-FP &gt; mid-LP: Midbrain region, IFG, amygdala, caudate. Mid-LP &gt; mid-FP: cingulate gyrus, MTG</td>
<td>Several reported correlations</td>
<td>Several reported correlations</td>
<td>[223]</td>
</tr>
<tr>
<td>Neutral, angry, happy or fearful faces</td>
<td>26 HC</td>
<td>Late FP vs. late LP</td>
<td>Late LP &gt; late FP neutral faces: Amygdala</td>
<td></td>
<td></td>
<td>[224]</td>
</tr>
<tr>
<td>Task</td>
<td>Subjects</td>
<td>Intervention</td>
<td>Results</td>
<td>Positive correlations</td>
<td>Negative correlations</td>
<td>Author</td>
</tr>
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<td>-------------------------------------------------------------------------</td>
<td>-----------------------</td>
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<td>--------</td>
</tr>
<tr>
<td>Monetary reward</td>
<td>27 HC</td>
<td>Late FP vs. late LP</td>
<td>Late LP &gt; Late FP reward: Ventral striatum</td>
<td>None found</td>
<td>None found</td>
<td>[225]</td>
</tr>
<tr>
<td>Negative, neutral words</td>
<td>12 HC</td>
<td>Mid-FP vs. late LP</td>
<td>Late LP &gt; mid-FP: medial OFC, Mid-FP &gt; late LP: lateral OFC, cingulate, insula.</td>
<td>NA</td>
<td>NA</td>
<td>[217]</td>
</tr>
<tr>
<td>Negative, neutral words</td>
<td>8 PMDD vs. 12 HC</td>
<td>Mid-FP vs. late LP</td>
<td>Not clearly reported: Amygdala, lateral OFC, medial OFC</td>
<td>NA</td>
<td>NA</td>
<td>[219]</td>
</tr>
<tr>
<td>Negative, neutral pictures</td>
<td>10 MDD vs. 10 HC</td>
<td>Mid-cycle</td>
<td>MDD &gt; HC: Hypothalamus, Amygdala, Hippocampus, OFC, ACC, sgACC</td>
<td>PROG HC: Hypothalamus, hippocampus, OFC</td>
<td>PROG MDD: Hypothalamus, hippocampus, OFC</td>
<td>[226]</td>
</tr>
<tr>
<td>Neutral, angry, happy or fearful faces</td>
<td>11 HC</td>
<td>Mid-FP vs. mid-LP</td>
<td>FP &gt; LP: Amygdala increase in response to disgust and happiness</td>
<td>PROG: Amygdala</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Healthy controls (HC), Major depressive disorder (MDD), Premenstrual dysphoric disorder (PMDD), Follicular phase (FP), Luteal phase (LP), Estradiol (E2), Progesterone (PROG), Not applicable (NA) Anterior cingulate cortex (ACC), Subgenual anterior cingulate cortex (sgACC), Inferior frontal gyrus (IFG), Middle frontal gyrus (MFG), Medial frontal gyrus (MeFG), Superior frontal gyrus (SFG), Inferior temporal gyrus (ITG), Middle temporal gyrus (MTG), Superior temporal gyrus (STG), Orbitofrontal cortex (OFC), Paraventricular nucleus (PVN), Ventromedial nucleus (VMN).

The brain areas displayed in the results column in table 2 and 3 have, in some cases, been translated using the reported coordinates to make it easier to compare the areas presented in the tables.
Table 3. A summary of a number of studies using cognitive tasks to examine the brain activity in women of reproductive age.

<table>
<thead>
<tr>
<th>Task</th>
<th>Subjects</th>
<th>Intervention</th>
<th>Results</th>
<th>Positive correlations</th>
<th>Negative</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal memory</td>
<td>30 HC</td>
<td>GnRH vs. HC</td>
<td>HC &gt; GnRH: MeFG, IFG, ACC, precentral gyrus.</td>
<td>NA</td>
<td>NA</td>
<td>[228]</td>
</tr>
<tr>
<td>Visual memory</td>
<td>34 HC</td>
<td>GnRH vs. HC</td>
<td>HC &gt; GnRH: superior parietal cortex, ACC, posterior cingulate, precuneus, parahippocampus, MTG, fusiform gyrus, cerebellum.</td>
<td>NA</td>
<td>NA</td>
<td>[229]</td>
</tr>
<tr>
<td>Verbal memory</td>
<td>26 HC</td>
<td>GnRH vs. placebo</td>
<td>Placebo &gt; GnRH: IFG</td>
<td>NA</td>
<td>NA</td>
<td>[231]</td>
</tr>
<tr>
<td>Verbal memory</td>
<td>16 HC</td>
<td>FP</td>
<td>NA</td>
<td>Follicular E2: IFG</td>
<td>None found</td>
<td>[232]</td>
</tr>
<tr>
<td>Word stem completion, mental rotation</td>
<td>6 HC</td>
<td>Early FP vs. late FP</td>
<td>Word stem completion: IFG, MeFG, postcentral gyrus. Mental rotation: Angular gyrus, superior parietal gyrus.</td>
<td>NA</td>
<td>NA</td>
<td>[233]</td>
</tr>
<tr>
<td>Working memory</td>
<td>8 HC</td>
<td>Early FP vs. late FP</td>
<td>Early FP &gt; late FP: OFC, ACC, fusiform gyrus, hippocampus, cerebellum, midbrain, vermis, pons/medulla, caudate, MTG Late FP &gt; early FP: postcentral gyrus</td>
<td>Follicular E2: postcentral gyrus</td>
<td></td>
<td>[234]</td>
</tr>
<tr>
<td>Verb generation</td>
<td>12 HC and 12 OC-users</td>
<td>Early FP vs. ovulatory phase</td>
<td>Early FP vs. ovulatory phase: no difference. OC-users &gt; early FP: STG. OC-users &gt; ovulatory phase: IFG. HC vs. OC-users: no difference</td>
<td>NA</td>
<td>NA</td>
<td>[235]</td>
</tr>
<tr>
<td>Task</td>
<td>Subjects</td>
<td>Intervention</td>
<td>Results</td>
<td>Positive correlations</td>
<td>Negative</td>
<td>Author</td>
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</tr>
<tr>
<td>Synonym generation</td>
<td>12 HC</td>
<td>Early FP vs. mid-LP</td>
<td>No difference</td>
<td>E2 and PROG both phases: PFC</td>
<td></td>
<td>[236]</td>
</tr>
<tr>
<td>Multiplication</td>
<td>15 HC</td>
<td>Early FP vs. mid-LP</td>
<td>Early FP &gt; mid-LP: medial PFC</td>
<td>NA</td>
<td>NA</td>
<td>[237]</td>
</tr>
<tr>
<td>3D mental rotation</td>
<td>12 HC</td>
<td>Early FP vs. mid-LP</td>
<td>Early FP &gt; mid-LP: STG, MeFG. Mid-LP &gt; early FP: MFG, SFG, ACC, MTG, lentiform nucleus, thalamus, corpus callosum, superior occipital, angular gyrus</td>
<td>Follicular E2: fusiform gyrus, ITG, inferior parietal gyrus, superior parietal lobe, precuneus, IFG, MFG, postcentral gyrus. Luteal E2: superior parietal gyrus, IFG, inferior parietal gyrus, postcentral gyrus, fusiform gyrus.</td>
<td></td>
<td>[238]</td>
</tr>
<tr>
<td>Go No/Go - female/male faces</td>
<td>12 HC</td>
<td>Mid-FP vs. mid-LP</td>
<td>Mid-FP &gt; mid-LP: culmen, SFG Mid-LP &gt; mid-FP: IFG (male stimuli).</td>
<td>NA</td>
<td>NA</td>
<td>[210]</td>
</tr>
</tbody>
</table>

Healthy controls (HC), Oral contraceptive (OC), Gonadotropin-releasing hormone (GnRH), Follicular phase (FP), Luteal phase (LP), Estradiol (E2), Progesterone (PROG), Not applicable (NA), Anterior cingulate cortex (ACC), Inferior frontal gyrus (IFG), Middle frontal gyrus (MFG), Medial frontal gyrus (MeFG), Superior frontal gyrus (SFG), Prefrontal cortex (PFC), Inferior temporal gyrus (ITG), Middle temporal gyrus (MTG), Superior temporal gyrus (STG), Orbitofrontal cortex (OFC)

The brain areas displayed in the results column in table 2 and 3 have, in some cases, been translated using the reported coordinates to make it easier to compare the areas presented in the tables.
Aims

Paper I
To investigate the hypothesis that high ovarian hormone levels compared to low levels would reduce prepulse inhibition. This was examined by comparing healthy postmenopausal women with cycling women in their late luteal phase.

Paper II
To test the hypothesis that patients with premenstrual dysphoric disorder have an enhanced startle response while anticipating pleasant or unpleasant images during the luteal phase of the menstrual cycle.

Paper III
To investigate brain activity during response inhibition across the menstrual cycle in women with premenstrual dysphoric disorder and healthy control subjects.

Paper IV
To investigate brain activity during response inhibition in healthy postpartum women, assessed immediately following delivery and one month postpartum, and comparing them with regularly cycling healthy women.
Materials and methods

Participants and study protocols

Paper I

The first study comprised three groups of healthy women; 1) 43 women between the ages 18 and 48 (cycling women), 2) 20 postmenopausal women without hormone replacement therapy (PM without HRT), and 3) 22 postmenopausal women with ongoing estrogens-only or estrogen and progesteragen (EPT) therapy (PM with HRT). The postmenopausal women were between 45 and 63 years of age.

Postmenopausal women using estrogen or EPT therapy during the last three months were considered as HRT-users, while postmenopausal women who had never used HRT or women without HRT during the last three months were considered as non-users. Cycling women had regular menstrual cycles (between 25-31 days).

Exclusion criteria for all subjects were hearing deficiencies, treatment with psychotropic drugs, and ongoing psychiatric illness. Absence of any psychiatric illness was confirmed with the Swedish version of the Mini International Neuropsychiatric Interview (MINI) [239] and depressive symptoms at the time of the test session were assessed using the self-rating version of the Montgomery-Åsberg Depressive Rating Scale (MADRS-S) [240]. Additional exclusion criteria for cycling women were ongoing pregnancy or breast feeding, use of hormonal contraceptives, and presence of PMDD. For further details about the exclusion criteria, see paper I.

Cycling women were scheduled according to a positive luteinizing hormone (LH) assay to participate during the luteal phase of the menstrual cycle (day 1 to 7 prior to onset of menstruation), while postmenopausal women participated on any arbitrary day. Luteal phase testing was confirmed by progesterone serum concentrations and records on the next menstrual bleeding provided by the Cyclicity diagnoser (CD) scale [80].

Paper II & III

Study II and III both consist of women with premenstrual dysphoric disorder and healthy controls. Study II comprises 22 patients with PMDD and 17 healthy controls, while 18 patients with PMDD and 14 healthy controls participated in study III. Ten of the women with PMDD and 5 of the healthy
controls in study II also took part in study III. However, the test sessions were scheduled on different days in order to prevent that the test methods influenced each other.

All patients and controls were included following advertisement in local news-papers. All included subjects had to be above 18 years old and have a history of regular menstrual cycles (between 25-31 days). Both patients and controls were screened for PMDD using the CD scale [80] during two consecutive months of prospective daily ratings to verify (patient) or exclude (healthy control) the presence of PMDD. Patients were considered to have PMDD if they had a 100 % increase in at least five symptoms during seven premenstrual days compared to seven mid-follicular days, associated with a clinically significant social and occupational impairment. For diagnostic criteria, see page 15. Control subjects displayed no significant cyclicity in mental symptoms between the follicular and luteal phase (< 50 % increase) and had no impact daily life.

Exclusion criteria for all subjects were treatment with hormonal contraceptives, benzodiazepines or other psychotropic drugs; ongoing depression, anxiety or any other psychiatric illness; and ongoing pregnancy or breastfeeding. Ongoing psychiatric illness was evaluated with the MINI. Additional exclusion criteria for study III were related to the fMRI procedures, according to the guidelines from the department of Radiology.

All subjects were scheduled for two visits, once during mid-follicular phase (6 – 12 days after onset of menstruation) and once during late luteal phase (1 – 7 prior to onset of menstruation). Half of the subjects were scheduled to start in the follicular phase and the other half in the luteal phase to avoid test order effects across the menstrual cycle. The time-point of the luteal phase testing was determined as in study I.

Paper IV

The fourth study consists of 26 healthy postpartum women recruited by midwives at local maternal health care centres in Uppsala and at the Maternity ward at the Department of Obstetrics and Gynaecology, Uppsala University Hospital.

Women with a normal pregnancy and delivery (including caesarean section) were included in the study. Women with ongoing neurological disorders, depression, anxiety or other psychiatric illness were excluded, as well as women with ongoing treatment with benzodiazepines or other psychotropic drugs (including SSRI). Diagnosed pregnancy complications, delivery complications, postpartum complications, and women with children at the neonatal department were also excluded. In addition, the 14 healthy controls of study III were used as controls to the postpartum women.
All participants took part in two fMRI sessions. The postpartum women were examined once within 48 hours after delivery (early postpartum) and once 4-6 weeks after delivery (late postpartum). Blood samples were also taken to establish progesterone- and estradiol serum concentrations.

Study I-IV was conducted according to ethical standards for human experimentation and approved by the Independent Research Ethics Committee, Uppsala University. All participating women gave written informed consent prior to inclusion.

Methods

Modulation of the acoustic startle response

Electromyography
In paper I and II the eye blink component of the ASR was assessed using electromyographic (EMG) recording of the right musculus Orbicularis Oculi while the acoustic startle probes were delivered binaurally by telephonic headphones (TDH-39-P, Maico, Minneapolis, MN, USA). To measure the contraction of the eye muscle two miniature silver/silver chloride electrodes (In Vivo Metric, Healdsburg, CA, USA) were positioned underneath the eye on top of the muscle and one ground electrode were placed in the centre of the forehead [241], Figure 3. The delivering of the acoustic startle stimuli and the recording of the eye blink response were controlled by a commercial startle system (SR-LAB, San Diego Instruments, San Diego, CA). For further details see paper I and II.

Figure 3. Localization of the electrodes.
Prepulse inhibition (paper I)

The startle reflex test session began with a five-minute acclimation period with a background 70 decibel (dB) white noise delivered by the headphones, allowing the subjects to acclimate to the test situation. Thereafter, a series of trials were administered and the startle responses recorded. The test session included four trial blocks with the background 70 dB white noise continuing in-between the trials, Figure 4.

The first block consisted of five pulse-alone trials (115 dB, 40 ms broadband white noise) and was used for measurement of baseline startle response. Blocks 2 and 3 were used to measure PPI and consisted of 25 trials each: 5 pulse-alone and 20 prepulse-pulse trials presented in pseudorandom order. The pulse-alone trials within blocks 2 and 3 were used to measure the within-test session startle response for calculation of PPI. The prepulse stimuli within block 2 and 3 consisted of a 115 dB, 40 ms noise burst preceded at a 100 ms interval by prepulses (20 ms noise bursts) that were 2, 4, 8, and 16 dB above the 70 dB background noise (i.e. PP1 = 72 dB; PP2 = 74 dB; PP3 = 78 dB; PP4 = 86 dB). The last block consisted of five pulse-alone trials, which allowed a measure of within-test habituation. The inter-trial interval was variable averaging 30 seconds.

Affective modulation of the acoustic startle response (study II)

With the above electromyographic set-up, the subjects were instructed to watch a 14.1-inch computer monitor during the entire session. Each test session began with a five-minute acclimation period without startle probes or pictures. Following the acclimation period, a ten-minute slide show was displayed on a computer monitor while semi-randomized startle probes (105 dB) were delivered.

Each session consisted of 34 blocks containing three different startle conditions; 1) a black screen during which baseline ASR was measured (control condition), 2) a red or green screen as the negative or positive anticipation stimuli, 3) an unpleasant or pleasant picture stimulus. The red screen always preceded unpleasant pictures and the green screen always preceded pleasant pictures, Figure 5.
Figure 5. Schematic presentation of the 10 minute slide show. The startle conditions (pleasant, unpleasant, green, and red pictures) were semi-randomized across the slide show and the 105 dB startle probes were distributed an equal number of times across each condition. (The pictures used in this figure are from a personal library and are only displayed as representatives for the IAPS pictures).

Across the 34 blocks a total number of 48 startle probes were delivered; 20 during control condition, 7 during positive anticipation stimuli, 7 during negative anticipation stimuli, 7 during positive picture stimuli, and 7 during negative picture stimuli. In addition, 22 trials were recorded during which amplitude was registered but no startle probe was delivered (non-stimulus recordings). The duration of each picture type is specified in paper II.

The pictures were obtained from the International Affective Picture System (IAPS) [242] and were selected to be pleasant (sport activities and romantic content) or unpleasant (threatening and/or disgusting content) with 34 pictures from each category equally divided into two series (A and B). All subjects were presented with image series A on the first visit and series B on the second visit. For the specific picture slide numbers used in this study, see paper II.

**Analyses of startle responses**

Peak startle amplitudes were measured automatically within 20-150 ms following the onset of the startle probes. A zero response score was given if no response was detectable, according to the default criteria provided by the software: (1) the peak startle response occurred outside the 20-150 ms time frame, (2) a baseline shift exceeded 40 arbitrary units, and (3) a startle response was 25 arbitrary amplitude units or less. An arbitrary unit corresponded to 2.12 mV. Patients with negligible startle responses (mean amplitude <10 mV) were considered as non-responders.

In both study I and II startle magnitude was defined as the total amplitude of all trials with response / total number of trials; hence all responses set to zero were included in the statistical analyses.
Paper I
The prepulse inhibition (PPI) from blocks 2 and 3 was computed as the percentage reduction in peak magnitude of startle on pulse-alone (PA) trials using the formula;

\[ PPI = \frac{(M_{PA} - M_{PP})}{M_{PA}} \times 100, \]

where \( M_{PA} \) is the mean magnitude of pulse-alone and \( M_{PP} \) is the mean magnitude of prepulse-pulse trials during block 2 and 3. As there was no difference between blocks 2 and 3 in PPI, data were collapsed across these two blocks and used for comparison in the two-way analysis of variance (ANOVA).

The within-test habituation (HAB) of the startle response was calculated as the reduction in startle amplitude between the first and last block of pulse-alone trials using the formula:

\[ \% \text{HAB} = \frac{\text{Block 1} - \text{Block 4}}{\text{Block 1}} \]

Paper II
In study II the startle magnitude was normalized to z-scores, calculated across all conditions per subject and session, in order to reduce between-subjects variance caused by differences in baseline startle. The z-scores were then analysed using a three-way ANOVA.

Functional Magnetic Resonance Imaging
Image acquisition
To obtain the BOLD images in study III and IV the participants were positioned supine in the MR scanner with their head secured with foam padding. An anatomical reference data set was acquired for each patient with a T1 weighted inversion recovery sequence; 60 slices with no interslice spacing, field of view 230×230 mm², voxel size 0.8×1.0×2.0 mm³, repetition time 5700 msec, echo time 15 msec, and inversion time 400 msec. Functional data sets were acquired using a conventional single shot echo planar imaging sequence; 30 slices with 1.0 mm interslice spacing, field of view 230×230 mm², voxel size 3.0×3.0×3.0 mm³, temporal resolution 3000 msec and echo time 35 msec. The visual stimuli were presented to the participant with a pair of head coil mounted goggles and finger press responses were collected using hand held buttons, both part of a commercial hardware package for fMRI (NordicNeuroLab, Bergen, Norway). All MR imaging was performed with a whole body 3 Tesla scanner equipped with an 8 channel head coil (Achieva 3T X-series, Philips Medical Systems, Best, The Netherlands).
The Go/NoGo task
Inhibitory control in study III and IV was assessed using the Go/NoGo task. The task consists of a series of X and Y presented for 600 msec with an interstimulus interval of 400 msec. The participants were instructed to press a button with the right index finger as quickly as possible whenever the target letters were presented, referred to as Go-stimuli. However, when the same letter was presented two times in a row, the participants were instructed to refrain from pressing the button (i.e. inhibiting the response), referred to as NoGo-stimuli, Figure 6. There were 225 Go-stimuli and 25 (NoGo-stimuli), resulting in a total of 250 stimuli presentations. Correct response to a Go-stimulus was defined as a finger press within 600 msec, while correct performance for a NoGo-stimulus was the withholding of a finger press (i.e. correct inhibition). Incorrect response to a Go-stimulus was defined as no finger press within the 1 sec time-frames, whereas incorrect response to a NoGo stimulus was defined as a finger press on NoGo-stimuli. The paradigm was constructed using the E-prime stimulus presentation program (Psychology Software Tools, Pittsburgh, PA).

Image processing and fMRI analysis
All image processing and fMRI analyses were conducted using the freeware package called Statistical Parametric Mapping (SPM5). Reorientation of the BOLD images was performed using the parameters obtained through manual reorientation of the anatomical image, with the origin set in the anterior commissure. Spatial realignment, to correct for head motion, was performed using the mean image of all BOLD images as a template. Slice timing correction was performed to account for differences in acquisition time for the individual slices within each whole brain volume using the middle slice of the brain as a template. To transform each individual brain into MNI-space, the BOLD images were registered to the anatomical image and then segmented into white matter, grey matter, and cerebrospinal fluid. The normalization parameters obtained from this procedure were used to normalize the BOLD images and finally, spatial smoothing was performed using a kernel of 8 mm.
For each participant BOLD signal changes were regressed against the six movement parameter time-series and time-series contrasts to each condition (correct/incorrect Go and correct/incorrect NoGo) of the Go/NoGo task were created. For the subsequent group and menstrual cycle phase analyses of brain activity during response inhibition, the NoGo to Go stimuli contrast (i.e. correct NoGo > correct Go) was used, while other contrasts were not considered relevant for the purpose of the current study.

**Analysis of brain activity during response inhibition (paper III-IV)**

Whole-brain voxel-wise two-way ANOVA with cycle phase (follicular vs. luteal phase) as within-group variable and group (PMDD patients vs. healthy controls) as between-group variable was performed, using a statistical threshold of $p < 0.001$ (uncorrected) and an extent threshold of ten contiguous voxels. Similar approaches were also used in paper IV when comparing postpartum women with healthy controls, for details see paper IV.

Region of interest (ROI) analyses were performed on hormone-sensitive areas according to the hypotheses of paper III-IV. Using Automated Anatomical Labeling (aal) in the PickAtlas toolbox for SPM masks covering the ROIs were generated and a statistical threshold of $p < 0.05$, with small volume correction and an extent threshold of ten contiguous voxels were used.

**Hormone assay and analysis**

In paper I-II the serum estradiol and progesterone concentrations were analysed using an Immulite 1000 (DPC, Los Angeles, CA, USA). The measure interval was 73-7300 pmol/L for the estradiol assay and 0.64-64.0 for the progesterone assay. Patients with estradiol levels below the limit of detection was set to 73 pmol/L and patients with progesterone levels above the limit of detection was set to 64 nmol/L. The serum concentrations of estradiol and progesterone in study III-IV were analyzed by competitive immunometry electrochemistry luminescence detection at the Department of Medical Sciences, 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 estradiol the measurement interval was 18.4 – 15781 pmol/L and for progesterone it was 0.1-191 nmol/L.

Serum allopregnanolone was measured using radioimmunoassay (RIA) after extraction with diethyl ether and purification by high performance liquid chromatography (HPLC). The antibody used was raised against 3α-hydroxy-20-oxo-5α-pregnan-11-yl carboxymethyl ether coupled with bovine serum albumin as antigen (AgriSera AB, Umeå, Sweden). Because of the cross reactivity of the antibody, separation by HPLC was performed prior to the RIA. All samples were counted in a RackBeta (Wallace, Finland) scintillation counter. The RIA and extraction procedure [243] as well as the HPLC procedure [244] has been described previously.
Summary of results

Paper I

Four subjects (one PM woman with HRT and three subjects in the cycling group) were considered to be non-responders and were removed from the analyses. Consequently, the study group consisted of 40 cycling women, 20 PM women without HRT and 21 PM women with HRT. Demographic data and use of HRT are specified in paper I.

The duration of menopause did not differ between the two groups of postmenopausal women (PM women without HRT 6.1 ± 1.0 years and PM women with ongoing HRT 7.8 ± 1.3 years). The life-time ever duration of HRT use was significantly longer in women with ongoing hormone treatment (5.8 ± 0.8 years) compared to PM women without HRT (0.3 ± 0.3 years), p<0.001.

The two-way ANOVA revealed a significant main effect of prepulse intensity, p < 0.001; a main effect of group, p < 0.001; and a significant group by prepulse intensity interaction, p < 0.05, Figure 7. Post-hoc tests for main effect of group indicated that cycling women had lower PPI in comparison to both postmenopausal groups. Post-hoc tests for differences between groups for all prepulse intensities are indicated in Figure 7. There was no difference in PPI between PM women with or without ongoing HRT (F(1,39) = 1.87; p = 0.18), Figure 7. Also, there was no difference in PPI between estradiol-only users and EPT users (F(1,19) = 0.029; p = 0.87), data not shown.

Estradiol levels varied considerably within each of the two postmenopausal groups, independent of whether they were on HRT or not. When postmenopausal women were grouped according to serum concentrations of estradiol more than 130 pmol/L or less than 130 pmol/L, a significant interaction between estradiol-group (low-estradiol vs. moderate estradiol serum concentrations) and prepulse intensity was found p < 0.05, Figure 8.

Postmenopausal women, as a group, had a significantly lower startle response to the first block of pulse-alone trials than cycling women, p < 0.01. Likewise, when all three groups were compared in the one-way ANOVA, a significant main effect of group was detected for the startle response (F(2, 79) = 3.58; p < 0.05). However, the post-hoc tests only revealed a borderline significant difference between startle response in PM women without HRT and cycling women, p = 0.056, Figure 9.
Figure 7. Mean ± SEM percent prepulse inhibition by trial type and group. Women in cycling ages had lower levels of prepulse inhibition compared to postmenopausal women with and without ongoing HRT (**p < 0.01, *p < 0.05).

Figure 8. Mean ± SEM percent prepulse inhibition by trial type in postmenopausal women with low estradiol serum concentrations and moderate estradiol levels (group by prepulse intensity interaction, p < 0.05).
Figure 9. Mean ± SEM startle magnitude during the first and last block of the test session. Startle magnitude is reduced in postmenopausal women compared to women in cycling ages (p<0.01).

Paper II

One PMDD patient and one control subject were considered to be non-responders and were excluded. Data from three PMDD patients in the follicular phase and two PMDD patients in luteal phase are lacking, either due to technical problems or because patients dropped out of the study. Hence, data from 16 patients and 16 asymptomatic controls are included in the study. For demographic data, see table 2 in paper II.

Startle magnitude during the control condition did not differ between PMDD patients and controls or between cycle phases. A main effect of stimulus was found in the ANOVA (p < 0.001) and post hoc test revealed significant decreases in ASR between the control condition and the pleasant picture stimuli (p < 0.001) and the positive anticipation stimuli (p < 0.001), whereas there was no difference between the control condition and the negative picture stimuli and negative anticipation stimuli, respectively. However, pleasant picture stimuli resulted in lower ASR in comparison with unpleasant picture stimuli (p < 0.05), and similarly, the ASR response to positive anticipation stimuli was lower in comparison with negative anticipation stimuli (p < 0.001). There were no significant effects of order of testing.

The three-way ANOVA indicated no difference in startle magnitude response to pleasant and unpleasant picture stimuli across the menstrual cycle. Also, there was no difference between PMDD patients and controls in startle magnitude response to pleasant and unpleasant picture stimuli, Figure 10.
Figure 10. Startle amplitude during positive and negative picture stimuli, presented as mean ± SEM z-scores. No significant difference in response to pleasant and unpleasant picture stimuli between PMDD patients and control subjects or between cycle phases.

As depicted in Figure 11, a significant group by menstrual cycle phase by stimulus interaction for the positive and negative anticipation stimuli was detected (p < 0.05). This interaction was driven by an increased difference in startle magnitude responses between positive and negative anticipation stimuli in the luteal phase compared to the follicular phase in PMDD patients (p < 0.05), whereas control subjects did not differ in their startle modulation between cycle phases. Also, the interaction was driven by an increased difference in startle magnitude responses between positive and negative anticipation stimuli among PMDD patients compared to control subjects in the luteal phase (p < 0.05), whereas there was no difference between groups in the follicular phase.

Figure 11. Startle amplitude during positive and negative anticipation stimuli, presented as mean ± SEM z-scores. PMDD patients displayed an increased difference in startle response to positive and negative anticipation stimuli in the luteal phase compared with control subjects (p < 0.05) and compared with their own follicular phase responses (p < 0.05).
There were no correlations between estradiol and progesterone serum concentrations and startle response modulation from positive/negative anticipation stimuli in the luteal phase in either group.

Paper III

One patient and one control dropped out of the study and three patients were excluded due to excessive head movement (movement > 3 mm or rotation > 2 degrees). Thus, 14 PMDD patients and 13 controls were included in the analysis.

No differences between groups or cycle phases were found in the number of correct and incorrect responses to Go- and NoGo-stimuli. Similarly, reaction times to correct and incorrect responses did not differ between groups or cycle phases, data not shown.

During both menstrual cycle phases, PMDD patients displayed decreased activity during response inhibition compared to controls in several parietal areas including the bilateral inferior parietal lobule, left precuneus, left supramarginal gyrus, right postcentral gyrus, and right superior parietal lobule. They also displayed decreased activity in the right caudate body, Table 4. No group by phase interaction was found in the whole brain analysis.

Table 4. Significant areas displaying main effects of group during the NoGo trials, presented in Talairach coordinates (mm). For more details see table 1 in paper III.

<table>
<thead>
<tr>
<th>BA</th>
<th>Hemisphere</th>
<th>Cluster size</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls &gt; PMDD</td>
<td>Inferior parietal lobule</td>
<td>40</td>
<td>R</td>
<td>12</td>
<td>56</td>
<td>-33</td>
</tr>
<tr>
<td></td>
<td>Inferior parietal lobule</td>
<td>40</td>
<td>L</td>
<td>12</td>
<td>-53</td>
<td>-30</td>
</tr>
<tr>
<td></td>
<td>Postcentral gyrus</td>
<td>3</td>
<td>R</td>
<td>44</td>
<td>36</td>
<td>-27</td>
</tr>
<tr>
<td></td>
<td>Precuneus</td>
<td>31</td>
<td>L</td>
<td>13</td>
<td>-15</td>
<td>-56</td>
</tr>
<tr>
<td></td>
<td>Superior parietal lobule</td>
<td>7</td>
<td>R</td>
<td>24</td>
<td>27</td>
<td>-53</td>
</tr>
<tr>
<td></td>
<td>Supramarginal gyrus</td>
<td>40</td>
<td>L</td>
<td>16</td>
<td>-42</td>
<td>-39</td>
</tr>
<tr>
<td>PMDD &gt; Controls</td>
<td>Sub-lobar</td>
<td>R</td>
<td>13</td>
<td>18</td>
<td>18</td>
<td>10</td>
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</tbody>
</table>

BA = Brodmann area, L = left, PMDD = premenstrual dysphoric disorder, R = right
In the predefined ROI analyses a significant group by phase interaction was found in the left insula (x = -42, y = -11, z = 6, k = 37), Figure 12. Relative to controls, this interaction was driven by lower insula activity in the follicular phase (p < 0.05), and increased insula activity in the luteal phase among PMDD patients (p < 0.05). In addition, PMDD patients displayed increased left insula activity across menstrual cycles phases (p < 0.001), whereas insula activity in controls was unaltered (p = 0.20). No correlation between left insula activity and ovarian steroid levels were found in either group or cycle phase. No main effects of group or group by phase interactions were found in the right insula or bilateral ACC.

Because of the a priori hypothesis of higher follicular phase activity in prefrontal areas among controls, analyses were performed in each group. In comparison with the follicular phase, controls displayed reduced activity during the luteal phase to correct NoGo trials in the left precentral gyrus and left precuneus. PMDD patients displayed increased left insula activation to NoGo trials during the luteal phase, but did otherwise not display any menstrual cycle differences in brain activation, Table 5.

Figure 12. ROI analysis displaying BOLD activity in the left insula during response inhibition examined across the menstrual cycle. A. Axial (z = 6), coronal (y = -11), and sagital (x = -42) sections displaying the group by phase interaction in BOLD activity in the left insula, with p < 0.05 and small volume correction. B. The graph displays the BOLD activity of the group by phase interaction seen in the left insula in arbitrary units (AU). PMDD patients had a significantly decreased activity during the follicular phase (p < 0.05) and a significantly increased activity in the luteal phase (p < 0.05) compared to healthy controls. In addition, PMDD patients displayed a significant increase in left insula activity from the follicular to luteal phase of the menstrual cycle (p < 0.001).
Table 5. Significant areas displaying menstrual cycle effects during the NoGo trials presented in Talairach coordinates (mm). For more details see table 1 in paper III.

<table>
<thead>
<tr>
<th></th>
<th>BA</th>
<th>Hemisphere</th>
<th>Cluster size</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>p value</th>
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<td><strong>Follicular phase</strong></td>
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<tr>
<td>Controls</td>
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<tr>
<td>Frontal lobe</td>
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<tr>
<td>Precentral gyrus</td>
<td>9</td>
<td>L</td>
<td>12</td>
<td>-33</td>
<td>13</td>
<td>35</td>
<td>&lt;0.001</td>
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<tr>
<td>Parietal lobe</td>
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<tr>
<td>Precuneus</td>
<td>31</td>
<td>L</td>
<td>42</td>
<td>-18</td>
<td>-42</td>
<td>33</td>
<td>&lt;0.001</td>
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<tr>
<td><strong>PMDD</strong></td>
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<td><strong>Luteal phase</strong></td>
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<td>No significant difference</td>
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<tr>
<td><strong>PMDD</strong></td>
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<td>Sub-lobar</td>
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<tr>
<td>Insula</td>
<td>13</td>
<td>L</td>
<td>14</td>
<td>-42</td>
<td>-9</td>
<td>3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BA = Brodmann area, L = left, PMDD = premenstrual dysphoric disorder, R = right

**Paper IV**

Of the twenty-six postpartum women included, eight could not be examined within the 48 hour time-frame due to lacking accessibility to the fMRI equipment. One participant dropped out of the study after the first test session and four additional participants were excluded; two participants because of claustrophobic symptoms while in the camera, one for medical reasons and one participant due to lack of correct NoGo-trials. Hence, 13 postpartum women were included in the final analysis. Of the 13 women included, seven (53.8%) were delivered vaginally and six were delivered with caesarean section. Postpartum women performed their first scanning 28 ± 10 hours after delivery and returned for their second scanning after 34 ± 5 days from delivery. During the period close to the second scanning, all postpartum women were breastfeeding. For demographic data, see table 1 in the manuscript for paper IV.

As expected, estradiol, progesterone and allopregnanolone serum concentrations decreased from the early to the late postpartum assessment (see table 2 in the manuscript for paper IV). Estradiol and progesterone serum concentrations were significantly higher in early postpartum women than in regularly cycling women and significantly lower in late postpartum women than in healthy cycling women.
Postpartum women had a higher number of correct responses to Go-stimuli during the late postpartum assessment than in the early postpartum, (see table 2 in manuscript for paper IV). Otherwise, no differences between test sessions or groups in the number of correct inhibitions to NoGo-stimuli or in reaction times to correct Go- and incorrect NoGo-stimuli were evident.

Because of the a priori hypothesis that the changes in ovarian steroid levels during the post partum period would be associated with brain activity changes in hormone sensitive task-related areas, ROI analyses of the ACC, inferior- and middle frontal gyrus were conducted in the postpartum women. These ROI analyses revealed significantly decreased activations in the right ACC, right inferior frontal gyrus, and left middle frontal gyrus among late postpartum women compared with their early postpartum scanning, Figure 13 and Table 6.

On the other hand, late postpartum women also displayed significantly increased activation in the left inferior frontal gyrus, extending into the pre-central gyrus, in comparison with their early postpartum scanning, Table 6. When delivery mode (vaginal vs. caesarean) was controlled for, the activations discovered with the ROI-analyses were still significant, data not shown.

Figure 13. ROI analysis displaying the BOLD activity in the right ACC, right inferior frontal gyrus, and left middle frontal gyrus during response inhibition among postpartum women. A. Axial (z = 18), coronal (y = 36), and sagital (x = 15) sections displaying the decreased BOLD activity in the right ACC from early to late postpartum. B. Axial (z = 7), coronal (y = 24), and sagital (x = 45) sections displaying the decreased BOLD activity in the right inferior frontal gyrus from early to late postpartum. C. Axial (z = 23), coronal (y = -33), and sagital (x = -33) sections displaying the decreased BOLD activity in the left middle frontal gyrus from early to late postpartum. All activations are small volume corrected and have a p < 0.01.
Table 6. ROIs displaying significant activations in response to correct NoGo trials during the early compared to late postpartum session, presented in Talairach coordinates (mm). For more details see table 3 in paper IV.

<table>
<thead>
<tr>
<th>Talairach coordinates</th>
<th>BA</th>
<th>Hemisphere</th>
<th>Cluster size</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>p value</th>
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<tbody>
<tr>
<td>Early PP &gt; Late PP</td>
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<tr>
<td>Frontal lobe</td>
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</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>32</td>
<td>R</td>
<td>40</td>
<td>15</td>
<td>36</td>
<td>18</td>
<td>0.005</td>
</tr>
<tr>
<td>Inferior frontal gyrus</td>
<td>13</td>
<td>R</td>
<td>64</td>
<td>45</td>
<td>24</td>
<td>7</td>
<td>0.001</td>
</tr>
<tr>
<td>Middle frontal gyrus</td>
<td>9</td>
<td>L</td>
<td>30</td>
<td>-33</td>
<td>33</td>
<td>23</td>
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<tr>
<td>Late PP &gt; Early PP</td>
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<tr>
<td>Frontal lobe</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>9</td>
<td>L</td>
<td>42</td>
<td>-42</td>
<td>4</td>
<td>27</td>
<td>0.000</td>
</tr>
</tbody>
</table>

BA = Brodmann area, L = left, PP = postpartum, R = right

With the exception of a group by session interaction in the locus in the middle frontal gyrus (-33, 34, 34, z = 2.50 k = 11 p < 0.01), no significantly different cluster activations between postpartum women and cycling controls were found in the ROIs (main effects of group or group by session interaction). The group by session interaction in the middle frontal gyrus was driven by attenuated activity in late postpartum women in comparison with follicular phase controls (p < 0.01), whereas no difference between early postpartum women and luteal phase controls was found (p = 0.49). Additionally, the whole brain two-way ANOVA exploring potential differences in brain activity during response inhibition between postpartum women and healthy controls revealed group differences in only three brain areas; the right superior frontal gyrus, left lingual gyrus, and the left cuneus, Table 7.

In all these areas, the postpartum women displayed decreased activity compared to the control women during both the early and late postpartum. No group by test session interaction was revealed in the whole brain analysis.

Table 7. Significant areas displaying whole-brain main effects of group between postpartum women and healthy controls during the NoGo trials, presented in Talairach coordinates (mm). For more details, see table 5 in paper IV.

<table>
<thead>
<tr>
<th>Talairach coordinates</th>
<th>BA</th>
<th>Hemisphere</th>
<th>Cluster size</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls &gt; Postpartum women</td>
<td></td>
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<tr>
<td>Frontal lobe</td>
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<td></td>
</tr>
<tr>
<td>Superior frontal gyrus</td>
<td>10</td>
<td>R</td>
<td>18</td>
<td>18</td>
<td>59</td>
<td>19</td>
<td>0.000</td>
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<tr>
<td>Occipital lobe</td>
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<tr>
<td>Lingual gyrus</td>
<td>18</td>
<td>L</td>
<td>16</td>
<td>-18</td>
<td>41</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>Cuneus</td>
<td>2</td>
<td>R</td>
<td>94</td>
<td>-9</td>
<td>-78</td>
<td>18</td>
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</tbody>
</table>

BA = Brodmann area, L = left, R = right
Discussion

Methodological considerations

This thesis aimed to investigate the effect of the ovarian hormones on the female brain during different reproductive states using psychological tests known to affect brain activity in different ways. The thesis includes four groups of women: healthy women with regular menstrual cycles; women suffering from premenstrual dysphoric disorder; women in the postpartum period; and postmenopausal women in an attempt to further extend the knowledge of how the ovarian hormones can influence brain activity, a somewhat understudied field. This was conducted using three different tasks measuring different effects; the prepulse inhibition of the startle response, the effect of anticipation and affective modulation on the startle response, and response inhibition.

In paper one we discovered that women in postmenopausal age had an increased PPI compared to cycling women and although the most striking age-related change in endocrine function is menopause it may be difficult to separate whether the increased PPI among our postmenopausal subjects is related to declining levels of estradiol or whether it is due to increasing age (albeit that previous studies have suggested no age influence on PPI [134]). In order to properly examine if the change in PPI is exclusively related to the menopausal transition rather than being an effect of age, postmenopausal women need to be compared with age-matched cycling women.

Furthermore, PPI did not differ between postmenopausal women with ongoing HRT and postmenopausal women without HRT, but because of the cross-sectional design of the study this finding cannot rule out the possibility that hormone treatment in postmenopausal women may influence PPI. HRT effects may be concealed by biases such as the participating subjects’ reasons for using or not using hormone therapy during menopause, and by prior HRT use. Preferably, the influence of estradiol on PPI in postmenopausal women should be evaluated in a placebo-controlled, randomized trial, but because of the declining use of HRT and the need to randomize subjects without vasomotor symptoms this was not considered feasible.

The experimental task used for study II had been developed by our San Diego collaborators for use in male marines on their way to Iraq. While the startle experiment worked as expected in males, we encountered a number of problems in women. The most obvious problem was that the startle modulation by positive and negative images was substantially smaller than expected. One reason for this could be that we specifically refrained from using
pornographic pictures, thus rendering positive and negative pictures unmatched in terms of arousal ratings. Secondly, probe times for the acoustic startle responses were in the range of 0.5 – 1.5 seconds, introducing the possibility that the image onset in itself may act as a weak pre-pulse that decreases startle magnitude [245]. Nevertheless, we have learned the lesson and are currently developing a more suitable task for use in women.

Additional limitations include the fact that the control condition used in the task was merely a resting state. Preferably, a neutral picture should have been used as control, by which we could have assessed the direction of the physiological response and more specifically targeted anxious anticipation in our PMDD patients. At present, our results merely suggest an enhanced physiologic response during anticipation of emotionally laden visual stimuli in PMDD patients in the luteal phase.

Using fMRI impose a number of methodological issues that has to be taken into consideration. There is a number of steps from that the participant is placed in the camera until the analysis is finally conducted, which may influence the sensitivity of the analyses. These include, but are not limited to, the quality of the task, preprocessing considerations, choice of noise and signal model, and the amount of smoothing performed. The task in use is of course critical, especially in cognitive science where fMRI is used to map relevant brain areas involved in complicated cognitive functions, such as speech and language. For such studies, the task comparison is of outmost importance for validity of the test. Task subtractions between experimental and unwisely chosen control conditions may otherwise involve greater, or additional, differences than what is claimed [246]. As the present thesis primarily aimed at investigating the role of ovarian steroids, we used a well-known task (Go/NoGo) where the neuroanatomical correlates of the various cognitive aspects (of which we chose only to use activation during correct NoGo trials) had been firmly established previously [207, 208, 247]. The issue of how many participants are needed to obtain reliable results has also been raised in a number of studies [248-250], however, fMRI studies are expensive and the number of participants is usually a trade-off between scientific need and money. It has been estimated that approximately 20-27 subjects are needed to provide reproducible results in cross-sectional studies [248-250] (corresponding number for longitudinal studies was more difficult to obtain from the literature). Our results should therefore be interpreted with caution, as we may have false negative findings, i.e. failed to detect important areas. Similarly, the thresholds used to label areas as activated were relatively liberal. However, as imaging data in women with premenstrual dysphoric disorder and postpartal women are scarce, we did not opt for specificity control in our studies.

Besides the cost of fMRI, the careful timing of the menstrual cycle phase did impose a number of logistic problems when recruiting participants for study three, specifically in the scheduling of the scanning sessions. The
fourth study is, again, limited by the relatively small sample size, but repre-
sents the first attempt to describe the neuroanatomical correlates of cognitive
control in the postpartum period of healthy, non-depressed women. How-
ever, it must be emphasized that generalizations to newly delivered women
may be hampered by the fact that women in this study, for obvious reasons,
were highly motivated and well-educated, with physically and psychologi-
cally uncomplicated deliveries and postpartum periods. Also the study would
have been strengthened by a more uniform group of only vaginally delivered
women, even though the study did not detect any activation differences be-
tween women who had had a vaginal or caesarean delivery. The ideal study
would compare women during the last weeks of the third trimester with
postpartum women although, at the time, this was not possible. In addition,
any study investigating women across the postpartum period is, for obvious
reasons, forced to evaluate women in a given order.

Despite these limitations, the thesis is strengthened by the rigorous moni-
toring of menstrual cycle phase in cycling women in the first three studies,
and by the use of prospective symptom ratings of PMDD for an accurate
diagnosis. Prospective ratings of mood symptoms were also used to ensure
that the women included as controls were symptom free, since PMS/PMDD
in the control group would influence the interpretation of the studies. Sec-
ondly, by counter-balancing the follicular and luteal phase test sessions in
study II, III and IV learning effects were avoided and lastly, depressive dis-
orders and anxiety disorders were carefully excluded.

Cycling women have lower levels of PPI than postmenopausal women

Paper I examined the role of circulating ovarian hormones on sensorimotor
gating by measuring prepulse inhibition of the startle response in cycling
women and postmenopausal women with and without HRT. The main find-
ing was that cycling women, measured in the late luteal phase, had signifi-
cantly lower levels of PPI than postmenopausal women. There was no dif-
fERENCE in PPI between postmenopausal HRT users and non-users, but when
postmenopausal subjects were grouped according to their actual estradiol
serum concentrations our results suggested that postmenopausal women with
estradiol levels in the cycling range had lower levels of PPI in comparison
with postmenopausal women who had low estradiol serum concentrations.

This finding is in accordance with our hypothesis and previous results
suggesting that men have increased PPI compared to women [130, 132] and
that menstrual cycle phase influence PPI [131]. During the mid-luteal phase
of the menstrual cycle and during third trimester pregnancy women display
lower levels of PPI compared to women in conditions characterized by low levels of endogenous ovarian steroids [131-133].

Our results are also partly in line with Kumari and colleagues (2008) who recently published a study demonstrating no difference in PPI between postmenopausal women and similarly aged men, possibly as a result of the decline in steroid hormone production in the postmenopausal women, whereas premenopausal women had lower levels of PPI compared to age matched men. Yet, in the same study, there was no difference in PPI between women in cycling ages and postmenopausal women [134]. The most likely reasons for the discrepancy between studies presumably reside in the larger sample size used in the current study and the more uniform scheduling of cycling women in the late luteal phase.

The possible influence of estradiol and progesterone on PPI

PPI did not differ between women with ongoing HRT and women without HRT. However, because of the cross-sectional design of the study, this finding cannot rule out the possibility that hormone treatment in postmenopausal women may influence PPI. Ongoing HRT users differed considerably in estradiol serum concentrations and, when categorized based on serum concentrations of estradiol, our results suggested that subjects with estradiol levels in the cycling range had lower PPI than postmenopausal women who had low estradiol serum concentrations. This finding further strengthens the possible influence of estradiol on the neuro-circuitry involved in PPI.

The estradiol receptors are present throughout the brain, but predominantly located in the limbic system thus indicating that estradiol receptors are responsible for emotional processing and cognition [251, 252]. Estradiol may influence PPI via the dopamine system as several studies have suggested that estrogen may reduce dopamine activity [253]. High doses of estrogen treatment in ovariectomized female rats have been reported to prevent 8-OH-DPAT-induced disruptions of PPI [254] and similar findings have also been obtained in females where estrogen treatment prevented buspirone-induced PPI deficits [255]. However, treatment with 2 mg estradiol during the early follicular phase did not affect PPI in healthy women [255] and furthermore, Kumari and colleagues (2010) investigated the effect of circulating ovarian hormones on PPI and found no effect of estradiol on PPI across the menstrual cycle [152].

The involvement of progesterone in PPI is less well studied, but may also influence the current results, in particular as cycling women was examined during the luteal phase. Progesterone receptors, similar to the estrogen receptors, are widely distributed in the brain and predominantly in the amygdala, hippocampus, hypothalamus, and thalamus [32, 33]. Although many of the CNS effects of progesterone may be modulated through interaction with the progesterone receptor, progesterone metabolites through interaction via the
gamma aminobutyric acid (GABA<sub>A</sub>) receptor may also influence circuits involved in PPI regulation. As baseline PPI is unchanged following progesterone treatment in rat [256] as well as following a single allopregnanolone injection in women [142] and since low doses of estrogen in ovariectomized rats have an effect on 8-OH-DPAT induced disruption of PPI only in combination with progesterone (progesterone on its’ own had no effect) [254], it is plausible that ovarian steroid influences on PPI are the result of combined estradiol and progestagen effects. However, a large increase in progesterone across the menstrual cycle is associated with a smaller decrease in PPI from the follicular to luteal phase (estradiol had no effect) [152].

As described previously PPI seem reduced specifically during reproductive events associated with increased vulnerability to mood and anxiety disorders, manifested by the low levels of PPI reported in the mid-luteal phase [131, 132] and during third trimester pregnancy [133]. This is further supported by the fact that patients with PMDD seem to have lower levels of PPI during the late luteal phase in comparison with healthy controls [123] and that COC users with subjective reports of depression and anxiety have lower levels of PPI compared to healthy COC users [135]. Whether this ovarian steroid-induced reduction of PPI is an epiphenomenon or a risk marker of women susceptible for development of depression and/or anxiety remains to be elucidated.

The acoustic startle response

We found that cycling women had significantly higher startle response than postmenopausal women. There are only a small number of studies focusing on age effects on startle response in healthy women but our findings are clearly in line with these, although prior studies have measured startle response regardless of gender.

Startle magnitude steadily decrease with age [257, 258]. Elderly participants between 50 and 60 years of age have reduced startle magnitude compared to subjects between 20 and 30 years but do not differ in startle magnitude in comparison to participants at intermediate ages (30 – 39 years and 40 – 49 years) [258]. Furthermore, studies investigating gender-related effects on startle response indicate no difference between men and women of cycling age [127, 130, 258] and no differences across the menstrual cycle [123] or across pregnancy and during the postpartum period [133].
Women with PMDD have an increased startle modulation during anticipation

The main finding of the present study was that PMDD patients displayed an increased startle modulation by positive and negative anticipation stimuli in the luteal phase of the menstrual cycle. This finding was mainly driven by an increased ASR modulation in the luteal phase in comparison with the follicular phase among PMDD patients but also by an increased ASR modulation in PMDD patients compared to control subjects in the luteal phase. However, no differences in startle modulation by positive and negative picture viewings were found between PMDD patients and control subjects.

Based on the inclusion of a larger sample size than the study by Epperson et al (2007), we had hypothesized that PMDD patients would display enhanced startle modulation during image anticipation as well as during picture viewing in comparison with control subjects. However, according to our results, and the previous results by Epperson and co-workers, women with PMDD clearly have an intact affective modulation while viewing positive and negative pictures [181]. Instead, PMDD patients displayed an enhanced startle modulation by positive and negative anticipation stimuli in the luteal phase of the menstrual cycle, suggesting that the neural circuits underlying response to emotional anticipation are more sensitive during this period in these women.

These findings, together with the fact that many anxiety disorders exhibit increased startle responding during ambiguous threat compared to discrete cued threat (e.g. shock), points to other brain areas of interest than the amygdala. Recent imaging findings suggest that the insula is activated during aversive image anticipation compared to a resting state and in comparison to anticipation of positive images [163, 164, 176]. The insular cortex is suggested to be involved in interoception and the maintenance of physiological and emotional homeostasis, for review see [178]. Increased insula activity is demonstrated during anticipation of interoceptive threat, and participants with high fear of unexplained somatic symptoms exhibited higher insula activations during anticipation than participants with low fear [259]. Furthermore, disordered activation may underlie some anxiety states and possibly also PMDD (in light of our findings in paper III). Patients with anxiety disorders exhibit increased insula activation during image anticipation (e.g. women with post traumatic stress disorder due to domestic violence) [164]. Insula activation during image anticipation is also reduced by chronic serotonin reuptake inhibition, suggesting that this circuit is sensitive to anxiety-modulating drugs used for treatment of PMDD [260, 261]. Finally, and animal studies show that insular cortex activation by aversive stimuli modulates startle reactivity [177].
The possible influence of estradiol and progesterone on startle modulation

The effect of ovarian steroid fluctuations across the menstrual cycle on the acoustic startle response also has to be taken into consideration when evaluating our findings of increased startle modulation by positive and negative anticipation stimuli in the luteal phase in women with PMDD. Prior studies indicate that progesterone and allopregnanolone might induce differential changes in the startle response, where allopregnanolone attenuates the CRH-enhanced startle response whereas medroxyprogesterone acetate, acting via the progesterone receptor, instead amplifies the CRH-enhanced startle response [262]. Possibly, the underlying neural circuits regulating the ASR during anticipation are influenced by progesterone rather than by its GABA-active metabolites and women with PMDD may be more susceptible to progesterone influences on these processes than healthy women. This assumption is further substantiated by the fact that no allopregnanolone-induced changes in startle response or prepulse inhibition were detected in women with PMDD following double-blinded allopregnanolone or placebo injections [142].

Response inhibition in women with premenstrual dysphoric disorder and healthy controls

The most striking difference between PMDD patients and controls during response inhibition was the differential left insula response, where PMDD patients displayed decreased activation in the follicular phase and increased activation in the luteal phase in comparison with controls. In addition, women with PMDD displayed enhanced luteal phase insula activity compared to their own follicular phase.

The insula is involved in interoception and maintenance of physiological and emotional homeostasis and, of relevance for the Go/NoGo task, takes part in decision making [263]. Furthermore, this region is also highly sensitive to estrogen effects on neural excitability [180], and is rich in both serotonin and serotonin receptors [264]. Thus, the altered insula activity in women with PMDD suggest increased luteal phase activity in an area involved in interoceptive awareness, affective and cognitive processing. Although the insula activity was not directly correlated to any of the measured hormones it is still plausible that fluctuations in ovarian steroids across the menstrual cycle contribute to the differential activation pattern in women with PMDD since ovarian steroid receptors are ubiquitously expressed throughout the cerebral cortex [27-30, 33]. Furthermore, increased interoceptive awareness may also be related to the increased reporting of physical symptoms during the premenstrual phase [265].
Although no statistical difference in impulsivity was found between the two groups in this study, increased insula activity has been correlated with elevated trait impulsivity [266]. The indirect evidence in paper II also suggests increased luteal phase insula activity in PMDD patients since they display increased startle modulation by anticipation stimuli in the luteal phase [267].

Decreased activation in parietal regions among women with PMDD is independent of menstrual cycle phase

Besides the altered activity in the left insula, our findings suggest that women with PMDD display decreased brain activation compared to controls predominantly in parietal regions during response inhibition, independent of menstrual cycle phase. The diminished responses were seen in regions typically involved in attention and motor function [207, 216, 268].

Our findings are in line with previous reports suggesting lower left frontal electroencephalography activity across both menstrual cycle phases in women with high PMDD symptomatology [269]. While our study failed to demonstrate any difference between groups in the hypothesized prefrontal areas, activations in the inferior parietal lobule and caudate body have previously been correlated with estradiol levels [218]. In addition, the inferior parietal lobule and precuneus display sex-related activation differences during response inhibition tasks, with greater activation in women than in men [207]. This suggests that female ovarian steroids have possible organizational or activational influences on the biological processes involved in brain reactivity during response inhibition tasks. However, our finding in PMDD patients clearly suggest that menstrual cycle fluctuations in ovarian steroids do not affect brain activation in parietal regions during response inhibition in this patient group, further corroborated by the absent correlations with estradiol or progesterone levels. Moreover, as PMDD patients did not differ from controls in performance during the Go/NoGo task, it is difficult to judge if the differences in brain activation reflect reduced effort, increased neural efficiency, or inappropriate recruitment of resources required for the task.

Follicular phase increased activations among healthy controls

In line with previous observations the healthy controls displayed increased activation during the inhibitory task in mid-line areas of the prefrontal and the parietal cortex during the follicular phase [210, 217]. When incorporating attractive male and female stimuli in the inhibitory task, response inhibition is predominantly associated with increased activity in the follicular phase in areas such as the inferior frontal gyrus and culmen [210]. Similarly,
increased activation in prefrontal areas and the cingulate cortex is reported during the follicular phase for a Go/NoGo task with emotional words [217].

The menstrual cycle changes in healthy controls could, in addition to activational changes induced by the ovarian steroids, be associated with alterations in serotonergic neurotransmission, as the parietal cortex, insula, and cingulate cortex are rich in both serotonin and serotonin receptors [264]. For instance, a lowered level of serotonin, as a result of tryptophan depletion, is associated with decreased brain activation in prefrontal areas during response inhibition [270], administration of a 5-HT₂c agonist resulted in increased activations of prefrontal regions in response to a Go/NoGo task [271], and changes in serotonin function related to menstrual cycle is consistently reported in healthy controls [94-96, 99]. Furthermore, some of these changes have been correlated with peripheral ovarian steroid hormone levels [95, 99] and PET studies suggest lower 5-HT₁A receptor binding potentials and higher serotonin transporter binding potentials during the follicular compared to luteal phase in the dorsal raphe nuclei [100, 115].

Prefrontal activity during response inhibition decreases over time in the postpartum period

Across the first postpartum weeks, newly delivered women displayed altered brain activity during response inhibition in the right ACC, right inferior frontal gyrus, and left middle frontal gyrus. As predicted, decreased activation was found during the late postpartum phase when ovarian steroid levels were at sub-normal levels in comparison with the early postpartum assessment when estradiol and progesterone levels were relatively high. However, the opposite pattern was discovered in the precentral gyrus.

The ACC can be subdivided into an affective and cognitive division where the dorsal part of Brodmann are (BA) 32, seen in this study, belongs to the cognitive division, usually activated by cognitively demanding tasks without any emotional content. The cognitive division has been subscribed a number of functions including the modulation of attention and executive function, conflict monitoring, complex motor control, motivation, novelty, error detection, working memory, and anticipation of cognitively demanding tasks [212]. The decreased ACC activation found among late postpartum women compared to early postpartum could thus, besides the role of the hormonal changes, be connected to decreased attention and motivation in the postpartum period. The difference in activation observed in the ACC between the early and late postpartum session is also consistent with previous findings displaying increased activation during the luteal compared to follicular phase of the menstrual cycle [218] and decreased activation during
use of GnRH agonists [228, 229]. Taken together, these findings suggest that the ACC may be affected by different hormonal states.

Apart from the activity changes in the ACC, the ROI analyses revealed a decreased activity in the inferior frontal gyrus, again, during the late postpartum compared to early postpartum assessment. The inferior frontal gyrus is involved in a number of functions important for social interaction, for instance speech processing, verbal language production, empathy, and motor execution [272]. Motor tasks with great difficulty or high demand on selective attention, such as the Go/NoGo task, have also been shown to activate the bilateral inferior frontal gyri [272-275]. Furthermore, our findings are in line with studies examining the menstrual cycle effects during response inhibition, where reduced inferior frontal gyrus activity in the follicular phase in response to male compared to female stimuli [210], and increased luteal phase activity compared to follicular phase in response to emotional distracters [218] and negative words [222] have been reported. Furthermore, studies investigating the effect of GnRH agonists on working memory show decreased activity in the inferior frontal gyrus during the treatment period compared with untreated controls or with placebo [228, 230, 231].

The involvement of the middle frontal gyrus has consistently been demonstrated in regulation of response inhibition. However, the area of decreased activation in the middle frontal gyrus was clearly more anterior than the dorsolateral prefrontal cortex areas previously shown to be influenced by ovarian hormones [193, 276]. While no previous studies examining the effect of ovarian hormones in the prefrontal cortex have been able to show any specific activation in this area [193, 210, 218, 276], activity in the middle frontal gyrus was not only affected by postpartum changes but also differed from non-pregnant controls. More specifically, late postpartum women had significantly reduced activity in the middle frontal cortex in comparison with follicular phase healthy controls, possibly suggesting that this area respond to estradiol serum concentrations in the lower range.

While this study set out to unravel potentially important cognitive aspects of the postpartum period that might be influenced by changes in ovarian steroid levels, it is far from established that the different activation patterns across the postpartum period are caused, or even related to, changes in estradiol and progesterone levels. None of the activated brain areas revealed by this study did, in fact, correlate with ovarian steroid or neurosteroid serum concentrations and adaptive changes to motherhood may be an equally, or more important, contributing factor. Indeed, structural changes in brain regions implicated in maternal motivation and behaviors have been reported, evidenced by increases in gray matter volume of the prefrontal cortex, parietal lobes, and midbrain areas such as hypothalamus and amygdala at 3 - 4 months postpartum [277]. Furthermore, it must be emphasized that the Go/NoGo paradigm is not a representative paradigm for evaluation of human
correlates of maternal behavior, for which other types of paradigms, involving the offspring, appear more relevant [277-279].

Finally, whereas hormonal levels rapidly decline following delivery, compensatory receptor turnover changes in the brain may not yet have taken place when the early postpartum women were evaluated (on average 26 hours after delivery). Following parturition, expression changes in estradiol, progesterone or oxytocin receptors occur within 6 hours to 2 days, depending on receptor type and brain region in female rats [280-283], but corresponding receptor turnover time-course following human delivery is essentially unknown. Other hormone sensitive tasks that reflect brain function, such as prepulse inhibition [125, 132], is reduced in pregnant women and not yet normalized in the early postpartum period [133]. It is thus possible that brain activity in early postpartum women more reflects the pregnant rather than the postpartum brain.

As behavioural performance during correct NoGo trials did not differ between time-points, the clinical significance of the decrease in prefrontal brain activation between early and late postpartum assessment may reflect reduced effort as well as increased neural efficiency during response inhibition. The relevance of our findings in terms of maternal behaviour can also not be established by the present study design.

Comparing postpartum women with regularly menstruating women

The control subjects were included in the study merely to ascertain (to the best of our ability) whether potential differences in brain activations across the postpartum period were within the normal range or not and, for instance, not affected by pain processing of residual symptoms after the delivery [284] or sleep deprivation. The relatively similar pattern of brain activation during response inhibition between regularly cycling healthy controls and postpartum women, in ROI as well as more exploratory whole brain analyses, also points to the fact that the study was conducted in two groups of healthy women. Even though the postpartum period is associated with increased risk of depressive mood [40], none of our participating women fulfilled criteria for major or minor depression. In addition, the inclusion of postpartum women within 48 hours of delivery inevitably resulted in a study population of highly motivated and well-educated women, with physically and psychologically uncomplicated deliveries and postpartum periods.

The subtle activation differences between regularly cycling controls and postpartum women are also important in light of potential adaptive changes in response to hormonal changes throughout the reproductive life of women. Between the early and late postpartum assessments used for this study, estradiol levels decreased by tenfold and progesterone levels by 50-fold. In
addition to these changes, the postpartum period is also characterized by placental CRH withdrawal, resulting in transient suppression of hypothalamic CRH release and dexamethasone non-suppression [14-16], which is not normalized until five weeks postpartum [17]. Animal research has also established that elevated estradiol levels during gestation, together with the lactogenic hormones prolactin and oxytocin, act in concert to stimulate maternal behavior in the female rat [285]. Although early postpartum women had several-fold increased estradiol and progesterone serum concentrations (presumably reflected by increased brain concentrations), task-related differences between postpartum women and healthy controls were only detected in the superior frontal gyrus, middle frontal gyrus, and two occipital foci. This implies that while estradiol levels within the individual may affect cognitive changes via the prefrontal cortex, between-subject differences may also be influenced by adaptive changes during long-term exposure to extremely high pregnancy-induced estradiol levels. Furthermore, while the superior frontal gyrus for instance has been associated with working memory [286, 287], recent studies have failed to demonstrate any cognitive differences between pregnant and non-pregnant women [288].
General conclusions

The involvement of the ovarian steroids in PPI regulation is further strengthened as PPI is increased in postmenopausal women in comparison to regularly menstruating women examined during the late luteal phase. Further studies in age-matched subjects with variable menopausal status are, however, needed to fully separate the effect of menopause and the effect of age on PPI.

Women with PMDD have an increased modulation by positive and negative anticipation stimuli, which is confined to the late luteal phase of the menstrual cycle. This finding points towards the need of exploring amygdala and insular cortex reactivity further in this specific group of female patients.

Women with PMDD differ from healthy controls in their brain activity during response inhibition throughout the menstrual cycle. The altered insula activity in women with PMDD suggests increased luteal phase activity in an area involved in interoceptive awareness, and affective and cognitive processing. Further studies with larger sample sizes are needed to confirm the reported results and future studies could benefit from including a wider range of cognitive tasks or hormonal manipulations to elucidate the specific roles of the ovarian steroids.

Brain activity during response inhibition decreases throughout the course of the first postpartum weeks. While the study cannot ascertain the role of ovarian steroids for our results, we support prior findings across the menstrual cycle of non-pregnant women by demonstrating changes in brain activity across a period influenced by hormonal fluctuations in postpartum women.
Sammanfattning på svenska

Kvinnor har en dubbelt så hög risk som män att utveckla depressions- och ångestsjukdomar och en ökad risk för detta ses i perioder av livet som medför stora hormonförändringar, som till exempel i barnsägsperioden (postpartum-perioden) och i övergången till menopaus. Dessutom finns det kvinnor som anses vara känsliga för de normala hormonfluktuationerna under menstruationscykeln, där uppskattningsvis 3-5 % av kvinnor i barnafödande åldrar lider av premenstruellt dysforiskt syndrom (PMDS). Varför depressions- och ångestsjukdomar är så vanliga hos kvinnor är ännu inte klarlagt men inblandningen av hormoner stöds av det faktum att prevalensen av depressionssjukdomar är den samma mellan könen innan puberteten inträffar.

Syftet med detta avhandlingsarbete var därför att med hjälp av olika psykologiska tester utöka våra kunskaper om hur könshormoner påverkar hjärnan hos kvinnor i olika reproduktiva tillstånd. För att åstadkomma detta inkluderades fyra olika grupper av kvinnor: friska kvinnor med regelbunden menstruationscykel, kvinnor som led av PMDS, kvinnor i den tidiga postpartum-perioden och postmenopausala kvinnor.

I delstudie ett undersöktes vilken påverkan de kvinnliga könshormoner har på sensomotorisk hämning genom att mäta prepulsinhibering (PPI) av den “akustiska startle responsen” (ASR) hos kvinnor i fertile ålder samt postmenopausala kvinnor med eller utan hormonerapi (HRT). Eftersom tidigare studier har visat att det finns skillnad i PPI mellan män och kvinnor, mellan menstruationscykelns faser samt mellan kvinnor i sen graviditet och kvinnor några veckor in i barnsägsperioden var syftet att utöka kunskaperna om hur könshormoner påverkar PPI.

ASR är en fysiologisk reflex, en ryckning i ögonlocket, som uppstår när man utsätts för plötsliga eller obehagliga ljud och kan, hos människa, mätas som blinkamplitud med hjälp av elektromyografi. Genom att utdela ett svagt ljud, en s.k. prepuls, strax innan den kraftiga ljudpulsen kan man åstadkomma en minskning av ASR. Skillnaden mellan en ASR utan prepuls och en ASR med prepuls anges som den procentuella PPI.

Studiens huvudfynd var att kvinnor i fertile ålder hade lägre PPI än postmenopausala kvinnor. Det var ingen skillnad mellan postmenopausala kvinnor med eller utan HRT men postmenopausala kvinnor med högre östrogen-
nivåer hade lägre PPI än postmenopausala kvinnor med låga östrogennivåer. Detta resultat styrker tidigare fynd som föreslår att könshormonerna har en påverkan på det system som styr regleringen av PPI.  

I delstudie två undersöktes hur ASR påverkas av förväntan av bilder med ett emotionellt innehåll samt hur bilderna i sig påverkar ASR hos kvinnor med PMDS jämfört med friska kontroller.  

Mätning av ASR är i högsta grad kliniskt relevant då förändrad ASR har demonstrerats vid ångest- och depressionssjukdomar hos människa och djur samt i en gagnarmodell för PMDS. Dessutom regleras ASR av de faktorer, däribland GABA-systemet, som tros vara kritiska för etiologin vid PMDS. I delstudie två undersöktes därför kvinnor med PMDS och friska kontroller under menstruationscykeln follikelfas och lutealfas. Vid varje testtillfälle tittade försökspersonen på ett bildspel innehållande bilder med behagligt (t.ex. en söt hundvalp) och obehagligt (t.ex. en spindel) innehåll för att mäta emotionell påverkan på ASR. Dessutom visades en grön respektive en röd bild före varje behaglig och obehaglig bild som ett mått på positiv och negativ förväntan.  

Studie två visade att kvinnor med PMDS har en ökad modulering av ASR under menstruationscykeln lutealfas, jämfört med friska kontroller, när de förväntar sig att se behagliga och obehagliga bilder. Resultaten stämmer delvis överens med tidigare studier som sett en ökad ASR under lutealfas men inte funnit någon effekt av exponering för emotionella bilder. Däremot har inga tidigare studier undersökt förväntan av emotionella bilder på denna patientgrupp och fyndet att kvinnor med PMDS reagerar annorlunda vid förväntan visar att det finns ett behov att undersöka denna patientgrupp ytterligare med fokus på områden i hjärnan som är involverade i förväntan (ex. insula) och emotioner (ex. amygdala).  

Ett vanligt symptom vid PMDS är en känsla av kontrollförlust och sett ur ett kliniskt perspektiv klagar patienten ofta över en ökad impulsivitet under den symptomatica fasen, dvs. i lutealfas. Syftet med delstudie tre var därför att undersöka vad som händer i hjärnan över menstruationscykeln hos kvinnor med PMDS och friska kontroller med hjälp av funktionell magnetresonanstomografi (fMRT) och ett test (Go/NoGo) som undersöker förmågan att hämma inlärda reaktioner.  

Fjorton patienter med diagnostiserad PMDS och 15 friska kontroller deltog i två fMRT-undersökningar, en i follikelfas och en i lutealfas. Go/NoGo-testet bestod av en snabb följd av bokstäver (X/Y) som visades på en skärm. Varje gång ett X eller Y visades hade kvinnan instruerats att trycka på en knapp så snabbt som möjligt men om samma bokstav visades två gånger i följd skulle kvinnan avstå från att trycka, dvs. hämma sin reaktion. Hjärnans aktivitet vid korrekt hämning undersöktes sedan genom att jämföra friska kvinnor med kvinnor med PMDS över båda faserna i menstruationscykeln.  

Huvudfyndet i delstudie tre var att patienter med PMDS visade en minskad insula-aktivitet (vänstersidig) i follikelfas och en ökad aktivitet i
lutealfas jämfört med kontroller samt att kvinnor med PMDS visade en ökad aktivitet i vänster insula från follikelfas till lutealfas. Den förändrade insula-aktiviteten hos kvinnor med PMDS tyder på förändringar i ett område involverat i tolkning av kroppsliga signaler samt känslomässig och kognitiv bearbetning. Kvinnor med PMDS visade, förutom förändrad insula-aktivitet, dessutom minskad aktivitet jämfört med friska kontroller i områden som är involverade i uppmärksamhet och motorisk funktion.

Målet med delstudie fyra var att undersöka tänkbara kognitiva aspekter hos kvinnor i postpartum-perioden genom att undersöka aktivitet i hjärnan inom två dygn postpartum samt fyra veckor postpartum och jämföra dessa med friska kvinnor med regelbunden menstruation. Baserat på tidigare studier predicerade vi en lägre aktivitet under den senare delen av postpartum-perioden i områden som påverkas av kvinnliga könshormoner. Aktivitet i hjärnan under postpartum-perioden undersöktes med hjälp av Go/NoGo-testet som är beskrivet ovan.

Delstudie fyra visar att nyförlösta kvinnor har en minskad aktivitet i ”anterior cingulate cortex”, ”inferior frontal gyrus” och ”middle frontal gyrus” från tidig postpartum när hormonnivåerna är relativt höga till sen postpartum när hormonnivåerna är extremt låga.

Trots att syftet med studien var att undersöka potentiellt viktiga kognitiva aspekter under postpartum-perioden som skulle kunna påverkas av förändringar i nivåerna av könshormoner så är det svårt att säga om den minskade aktiviteten verklig är orsakad, eller ens relaterad till förändringar i estradiol- och progesteronnivåer. Inga av de områden som visade aktivitetsskillnader i denna studie korrelerade till de uppmätta nivåerna av könshormoner och omställningen till att ta hand om ett barn är en eventuellt lika stor, om inte större, bidragande faktor till de skillnader vi ser. Sammanfattningsvis har denna studie visat att hjärnans aktivitet vid hämning av inlärda reaktioner minskar under de första veckorna postpartum.
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References


12. Scott EM, McGarrigle HH, and Lachelin GC. The increase in plasma and saliva cortisol levels in pregnancy is not due to the increase in corticosteroid-binding globulin levels. J Clin Endocrinol Metab. 1990 Sep;71(3):639-44.
42. Freeman EW. Associations of depression with the transition to menopause. Menopause. 2010 Jul;17(4):823-7.


123. Kask K, Gulinello M, Backstrom T, Geyer MA, and Sundstrom-Poromaa I. Patients with premenstrual dysphoric disorder have increased startle response across both cycle phases and lower levels of prepulse inhibition during the late luteal phase of the menstrual cycle. Neuropsychopharmacology. 2008 2008 Aug;33(9):2283-90.


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