Studies on Premenstrual Dysphoria

OLLE ERIKSSON
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Abstract

Premenstrual dysphoria, so severe that it affects the lives of the women afflicted, is the condition studied in this thesis. Physiological and pharmacological mechanisms of pathogenetic relevance were investigated.

Women with premenstrual dysphoria showed a stronger and less dampened response of LH to an estradiol challenge than asymptomatic women, indicating an altered neuroendocrine regulation. In women with premenstrual dysphoria, the LH response was correlated to the severity of irritability and bloating, and the early FSH response was correlated to the severity of depressed mood.

The positron-emission study showed strong, consistent correlations between worsening of mood symptoms and a decrease in brain trapping of the immediate serotonin precursor, from the mid-follicular to the late luteal phase in women with premenstrual dysphoria. The strongest correlations were seen for the cardinal mood symptoms of premenstrual dysphoria, and for their opposites. Physical symptoms showed weaker or no correlations with the exception of nociceptive symptoms from erogenous body regions which showed positive correlations to serotonin precursor trapping in the right caudate nucleus. The findings are consistent with the serotonin hypothesis of premenstrual dysphoria, and might possibly explain the observed effects of serotonin-augmenting drugs in this condition.

The partial 5-HT₃ receptor agonist buspirone was superior to placebo in the treatment of premenstrual dysphoria. The weak SRI and 5-HT₆ receptor antagonist nefazodone was not superior to placebo. For women with premenstrual dysphoria in need of medication and who do not tolerate SRIs because of the sexual sideeffects, buspirone may be an alternative drug, since it had no adverse effects on sexual function.

The prevalence of polycystic ovaries and serum levels of androgens were not higher in women with premenstrual dysphoria than in their asymptomatic counterparts. The findings are not consistent with the hypothesis that irritability in women with premenstrual dysphoria is induced by elevated testosterone levels.

Thesis results, which are in line with the serotonin hypothesis of premenstrual dysphoria, may imply that increased brain sensitivity is one of the factors underlying severe premenstrual mood symptoms, thereby further supporting a common serotonergic dysregulation in this condition.

Keywords: premenstrual dysphoria, PMDD, premenstrual syndrome, estrogen challenge test, gonadotropin feedback response, LH, FSH, neuroendocrine regulation, buspirone, nefazodone, PCO, testosterone, irritability, depressed mood, bloating, visual analogue scale, VAS, 11C-5-hydroxytryptophan, positron emission tomography, right caudate nucleus

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To my dear parents and ancestors who through their strivings, sacrifices and hopes enabled me to go to university, and to reach the boundaries of contemporary knowledge in a minute but still important fragment of reality.
Timing is everything!
List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I  Olle Eriksson, Torbjörn Bäckström, Mats Stridsberg, Margareta Hammarlund-Udenaes, Tord Naessén.
Gonadotropin feedback response to Estrogen challenge test differs in women with Premenstrual Dysphoria, and is related to symptom severity
Submitted for publication.

II  Olle Eriksson, Anders Wall, Ina Marteinsdottir, Hans Ågren, Per Hartvig, Gunnar Blomqvist, Bengt Långström, Tord Naessén.
Mood changes correlate to changes in brain serotonin precursor trapping in women with premenstrual dysphoria
Psychiatry Research: Neuroimaging, 2005, accepted for publication.

III  Mikael Landén, Olle Eriksson, Charlotta Sundblad, Björn Andersch, Tord Naessén, Elias Eriksson.
Compounds with affinity for serotonergic receptors in the treatment of premenstrual dysphoria: a comparison of buspirone, nefazodone, and placebo
Psychopharmacology 2001;155:292-298

IV  Olle Eriksson, Mikael Landén, Charlotta Sundblad, Jan Holte, Elias Eriksson, Tord Naessén
Ovarian ultrasound morphology and serum levels of androgen hormones in women with premenstrual dysphoria
Manuscript.

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Abbreviations

AAAD aromatic amino acid decarboxylase
ACTH adrenocorticotropic hormone
AOC area over the curve
AUC area under the curve
BMI body mass index; weight in kg/(height in m)²
¹¹C carbon-11; an unstable positron emitting carbon isotope
CBA computerized brain atlas
CGI clinical global improvement
CT computerized tomography
DSM IV Diagnostic and Statistical Manual of Psychiatric Disorders, 4th Edition
fMRI functional magnetic resonance imaging
FSH follicle-stimulating hormone
GnRH gonadotropin-releasing hormone
HPA hypothalamic-pituitary-adrenal
HPG hypothalamic-pituitary-gonadal
HRT hormone replacement therapy
5-HT 5-hydroxytryptamine; serotonin
5-HT₁A serotonin receptor type 1A
5-HT₂ serotonin receptor type 2
5-HTP 5-hydroxytryptophan
5-HTT 5-hydroxytryptamine transporter; serotonin transporter
5-HIAL 5-hydroxyindole-aldehyde
5-HIAA 5-hydroxyindole-acetic acid
ITT intention to treat
IVF in vitro fertilization
LH luteinizing hormone
LLPDD late luteal-phase dysphoric disorder
LOCF last observation carried forward
MAO-A monoamine oxidase A
MINI Mini International Neuropsychiatric Interview
MADRS Montgomery Åsberg Depression Rating Scale
MHz Megahertz; one million cycles per second
MRI magnetic resonance imaging
NPY neuropeptide Y
¹⁵O oxygen-15; an unstable positron emitting oxygen isotope
<table>
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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>PCO</td>
<td>polycystic ovary</td>
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<tr>
<td>PCOS</td>
<td>polycystic ovary syndrome</td>
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<td>PET</td>
<td>positron emission tomography</td>
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<td>PMD</td>
<td>premenstrual dysphoria</td>
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<td>PMDD</td>
<td>premenstrual dysphoric disorder</td>
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<tr>
<td>PMS</td>
<td>premenstrual syndrome</td>
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<tr>
<td>PRA</td>
<td>progesterone receptor A</td>
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<td>PRB</td>
<td>progesterone receptor B</td>
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<tr>
<td>RIA</td>
<td>radio immuno assay</td>
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<tr>
<td>ROI</td>
<td>region of interest</td>
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<tr>
<td>rpm</td>
<td>revolutions per minute</td>
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<td>rs</td>
<td>regression coefficient by Spearman rank test</td>
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<td>SCID</td>
<td>Structured Clinical Interview for DSM IV personality disorders</td>
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<tr>
<td>SHBG</td>
<td>sex hormone binding globulin</td>
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<tr>
<td>SRI</td>
<td>serotonin reuptake inhibitor</td>
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<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
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<tr>
<td>SUV</td>
<td>standardized uptake value</td>
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<td>V</td>
<td>volume</td>
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<td>VAS</td>
<td>visual analogue scale</td>
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<tr>
<td>VIP</td>
<td>vasoactive intestinal peptide</td>
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<td>VOI</td>
<td>volume of interest</td>
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Introduction

Premenstrual symptoms in women
The majority of women of fertile age experience negative mental or physical symptoms of some degree during the premenstrual phase of the menstrual cycle. Epidemiological surveys have shown that approximately 75% of addressed menstruating women do (Andersch, Wendenstam et al. 1986; Hallman 1986). About 10% of addressed women declare that their negative symptoms are of the kind that they would like professional help to get rid of them (Andersch, Wendenstam et al. 1986; Hallman 1986). Two to eight percent of women of fertile age experience negative symptoms severe enough to disrupt their social and/or professional lives for one to two weeks each month (Rivera-Tovar and Frank 1990; Reid 1991; Ekholm and Backstrom 1994; Cohen, Soares et al. 2002; Halbreich, Borenstein et al. 2003). Thus, this is an important health problem with enormous personal, social, economic and equality consequences for the women affected and for society as a whole (Carney 1981; Merikangas, Foeldenyi et al. 1993; Dean and Borenstein 2004; Borenstein, Chiou et al. 2005). The cardinal premenstrual mood symptoms are irritability, depressed mood, affective lability and impaired impulse control (American Psychiatry Association 1994). Anxiety, feelings of hopelessness, lack of energy, difficulty in concentrating, decreased interest in usual activities, change in appetite and sleep disturbances (American Psychiatry Association 1994) are other common symptoms. The main physical symptoms are swelling, bloating, breast tenderness, headache and pelvic pain (American Psychiatry Association 1994). Moreover, many women experience premenstrual aggravation of ongoing physical (McLelland and Lawrence 1991; Cawood, Bancroft et al. 1993; Tan 2001) and mental conditions (Severino and Yonkers 1993; Yonkers 1997) of various sorts.

Premenstrual disorders - description, definition and delineation
Historical descriptions
There are numerous descriptions from ancient times onwards of severe mood and physical symptoms afflicting fertile women in the premenstruum, one of
the earliest by the Greek writer Semonides in his more than 2600 year-old “Essay on Women”. Hippocrates, Plato, Aristotele and Pliny have all contributed to the description of the condition and have tried to interpret the phenomenon. Trotula of Salerno in 11th–century Italy wrote that “…there are young women who suffer in the same manner who are relieved when the menses are called forth…” In late 19th–century Germany, von Feuchtersleben wrote that “menstruation is always attended, in sensitive individuals, by mental uneasiness, which manifests itself according to the temperament, as irritability or sadness”.

**Premenstrual tension**

Robert Frank, a New York psychiatrist, gave the first modern medical description of what he called “premenstrual tension” in a lecture and in a medical article in 1931 (Frank 1931). He wrote:

> The group of women to whom I refer especially complain of a feeling of indescribable tension from ten to seven days preceding menstruation which, in most instances, continues until the time that menstrual flow begins. The patients complain of unrest, irritability, ‘like jumping out of their skin’ and a desire to find relief by foolish and ill-considered actions. Their personal suffering is intense and manifests itself in many reckless and sometimes reprehensible actions. Not only do they realize their own suffering, but they feel conscience-stricken toward their husbands and families, knowing well that they are unbearable in their attitude and reactions. Within an hour or two after the onset of menstrual flow complete relief from both physical and mental tension occurs.

**Premenstrual syndrome**

In 1953 Green and Dalton, in an article in the British Medical Journal (Greene and Dalton 1953), pointed out that tension was merely one psychological component of a syndrome also characterized by emotional lability, weight gain, edema, lumbar pain, breast tenderness, abdominal pain, nausea and headache. They proposed the name “premenstrual syndrome” for the disorder.

**Late Luteal Phase Dysphoric Disorder (LLPDD) in DSM III-R**

In 1987, the American Psychiatry Association in view of the need to reach a consensus on the definition and delineation of the disorder, defined an operational definition for diagnostic and research purposes of what was called “Late Luteal Phase Dysphoric Disorder” (American Psychiatry Association 1987) in their Diagnostic and Statistical Manual III Revised Edition. The diagnostic entity appeared under the heading “Other conditions worth studying”.

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Premenstrual Dysphoric Disorder (PMDD) in DSM IV

In 1994, the American Psychiatry Association revised and renamed their operational definition of the disorder which was termed Premenstrual Dysphoric Disorder (American Psychiatry Association 1994) and since has been considered the golden standard (Steiner 1997) of work in the field. The definition is as follows:

Research Criteria for Premenstrual Dysphoric Disorder

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 postmenstrual, 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, feelings 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 and 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) hyposomnia 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, a sensation of “bloating”, weight gain

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

C. The disturbance is not merely an exacerbation of the symptoms of another disorder, such as Major Depressive Disorder, Panic Disorder, Dysthymic Disorder or 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).

Premenstrual dysphoria

Although approving of the PMDD diagnostic criteria and trying to adhere to it, several researchers in the field have criticized the arbitrary quantitative five (or more) requirements of criterion A (Eriksson, Andersch et al. 2001). Eriksson et al (Eriksson, Andersch et al. 2001) have advocated that the occurrence of one or more core symptoms, e.g. irritability and/or depressed mood, should be sufficient if the criteria B-D are also met. They have named this unorthodox variant of PMDD premenstrual dysphoria PMD (Eriksson, Sundblad et al. 2000). This is the disorder entity used in the present thesis.

Results of previous research in the field

The common conclusion of more than seventy years of research in the field is that cyclical hormonal secretions from the ovaries elicit the disorder in susceptible women (Backstrom, Sanders et al. 1983; Hammarback and Backstrom 1988; Steiner 1992; Steiner and Pearlstein 2000). How, and why, this takes place is not yet understood (Schmidt, Nieman et al. 1998). No consistent differences in ovarian hormonal levels or patterns of secretion have been found between women suffering from the disorder and those who do not (Rubinow and Schmidt 1995; Schmidt, Nieman et al. 1998; Kessel 2000). The focus of research has thus shifted from ovaries and hormonal levels to the central nervous system, in particular the brain (Halbreich 1995). Hereditary transmission patterns in premenstrual dysphoria have been found (Kendler, Silberg et al. 1992; Condon 1993; Kendler, Karkowski et al. 1998), and women with premenstrual dysphoria as a group have been shown to have an increased comorbidity of anxiety- and depressive disorders (Tobin, Schmidt et al. 1994; Maskall, Lam et al. 1997; Yonkers 1997). As many of the major symptoms of Premenstrual dysphoria/PMDD occur in both general anxiety states and major depression (Yonkers 1997), and knowing that the latter disorders plausibly are caused by deficient signaling within the serotonergic system of the brain (Pollock 2001), this has highlighted deficient brain serotonergic signaling as a possible precipitating factor (Halbreich and Tworek 1993; Eriksson 1999; Parry 2001).
Etiology and pathogenesis

It is beyond doubt that hormonal substances secreted cyclically by the ovaries elicit the disorder (Muse 1989; Casson, Hahn et al. 1990; Schmidt, Nienman et al. 1998; Cronje, Vashisht et al. 2004). Studies have shown that in women suffering from the disorder, menstrual cycles with higher mid-luteal blood estradiol and progesterone concentrations are rated worse than cycles with lower levels (Hammarbäck, Damber et al. 1989). Also, in women suffering from the disorder, spontaneous anovulation during a menstrual cycle reduces symptoms (Hammarback, Ekholm et al. 1991). Ovarian suppression by GnRH-agonist treatment causes the cyclical distracting symptoms to disappear (Muse, Cetel et al. 1984; Muse 1989; Wyatt, Dimmock et al. 2004) and surgical bilateral ovariectomy has been shown to produce a permanent cure (Casper and Hearn 1990; Casson, Hahn et al. 1990; Cronje, Vashisht et al. 2004).

Treatment

Like preeclampsia, the premenstrual syndrome/premenstrual dysphoria has been called “the disorder of theories” (Steiner and Carroll 1977). Almost as frequently as precipitating mechanisms have been suggested, new possible remedies have been proposed and tried. In New York in the 1930’s Frank thought women suffering from premenstrual tension did so due to excessive endogenous estrogen production and deficient estrogen excretion, and sought to cure this by venesection, enemas, and in very severe cases, external X-radiation of the ovaries. Frank observed substantial improvement especially with this latter treatment. A vast number of other treatments have been suggested and tried. These include diuretics (Daniel and Prockl 1966), gestagens (Dalton 1977), oral contraceptives (Andersch and Hahn 1981; Bancroft and Rennie 1993), vitamin B6 (Stokes and Mendels 1972), spironolactone (O’Brien, Craven et al. 1979), clonidine (Nilsson, Eriksson et al. 1985), estradiol (Magos, Brincat et al. 1986), anxiolytics (Freinhar 1984), antidepressants (Harrison, Endicott et al. 1989), high-dose gestagens (Kaunitz 1998), magnesium (Hronek and Kolomaznik 1985), calcium (Alvir and Thys-Jacobs 1991), GnRH-agonists (Muse 1989), SRIs (Eriksson, Lisjo et al. 1990), SSRIs (Stone, Pearlstein et al. 1990) and, in extreme cases, bilateral ovariectomy (Casson, Hahn et al. 1990; Cronje, Vashisht et al. 2004). There are two principal lines of treatment: causal or symptomatic. Causal treatment aims at suppressing or stopping ovarian hormonal secretion. Symptomatic treatment aims at reducing the effects of ongoing spontaneous ovarian secretion.
Premenstrual symptoms in other primates

The first description of perimenstrual behavioral changes with similarities to premenstrual dysphoria in a non-human primate was reported in 1985 by Hausfater and Skoblick (Hausfater and Skoblick 1985), who studied free-living yellow baboons in a national park in Kenya. Female yellow baboons a few days prior to menstrual onset withdrew from social contact and spent more time alone in trees: 30%, instead of the normal approximately 12%, ate on their own flowers, seed pods and sugar gum scrapings. They spent a total of 50% of their daylight hours feeding instead of the normal 35%. As the onset of menstruation approached, female yellow baboons initiated one third less contacts with other individuals than usual. Compared to females around ovulation, perimenstrual yellow baboons showed increased rates of agonistic interaction and decreased rates of sexual interaction with adult males. However, they did not display unusual fatigue or hostility.

Two earlier studies of primates in captivity had found a rise in the number aggressive attacks by females around the time of menstruation (Rapkin, Pollack et al. 1995). However, later studies in other primate species have not shown such clearcut perimenstrual female symptomatology (Loy, Lavelle et al. 1993; Bassoff 1995). The menstrual cycle of baboons and of other primates is very similar to that of humans. Together, this indicates common biological mechanisms behind the occurrence of premenstrual mood and behavioral symptoms (Eriksson, Sundblad et al. 2000).

The role of the menstrual cycle in women

The obvious role of the menstrual cycle is to coordinate ovarian, uterine, vaginal, locomotor and behavioral energy-demanding processes to allow for conception to occur, and thus to enable the propagation of the species. In humans, as in all mammals, the female investment in procreation is far greater and more committing than the male (Zeveloff and Boyce 1986).

Regulation of the menstrual cycle is under complex integrated neuronal and hormonal control, requiring certain minimal constitutional, neurological, emotional and environmental conditions to make conception possible. Neurological maturation admitting adult type GnRH-pulse generation has to prevail (Kapen, Boyar et al. 1974; Ross, Loriaux et al. 1983). Energy deposits in the form of body fat of a certain minimal amount are required (Van der Spuy 1985). Furthermore, for the intricate coordination of menstrual cycle pacing, the woman needs to be on the whole healthy and living under physically and mentally bearable conditions with regard to food intake, temperature, light and dark conditions, and external threats (Bronson 1985).

Compromise to any of these conditions is a potential cause of female infertility, often manifested as anovulation. Subliminal afferent inputs, such as
pheromonal signals and the mere presence of fertile males or females, also affect the regulation of the menstrual cycle in women (Sobel, Prabhakaran et al. 1999; Jacob, Kinnunen et al. 2001).

Ovulation

GnRH-pulses released from the hypothalamus induce the pulsatile secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary, which act predominantly on the ovaries. FSH causes recruitment and growth of early follicles, and the selection of a dominant follicle. LH induces ovulation, and the development and support of the corpus luteum. Both gonadotropins show negative and positive feedback to increasing estrogen secretion. Estrogen in the form mainly of 17-β-estradiol, is secreted in increasing amounts by the follicles as they develop in size and aromatase activity, predominantly in the dominant follicle, with peak estrogen levels at mid-cycle, triggering the LH-surge that induces ovulation about 20-36 hours later.

Ovulation is caused by an inflammatory (Bukulmez and Arici 2000) reaction and shows the *calor, dolor, rodor, tumor et functio laesae* of such a reaction. The ‘lost function’ causes a rapid drop in blood estradiol levels. The ruptured follicle is luteinized to form a corpus luteum, a source of endocrine support for the early product of conception. During the first week after ovulation it produces increasing amounts of estradiol and progesterone. Estrogen levels in the midluteal phase are about half (Guerrero, Aso et al. 1976) to two thirds (Bakos, Lundkvist et al. 1994) of the preovulatory peak values. Progesterone values in the midluteal phase are between twenty and more than one hundred times higher than in early follicular phase (Guerrero, Aso et al. 1976).

In the absence of conception, the corpus luteum declines in function during the second week of the luteal phase and undergoes luteolysis (Davis and Rueda 2002) with a rapid premenstrual drop in both estradiol and progesterone levels. When both hormones fall below a critical threshold value, the late luteal-phase endometrium loses its hormonal support and is shed. This is manifested as a menstrual bleeding.

In a normal fertile woman, the more intense the gonadotropin stimulation of the ovaries, the higher the preovulatory estrogen peak, quality of the ovulation, and mid luteal phase estradiol and progesterone levels. Both weak gonadotropin stimulation and diminished ‘ovarian reserve’, cause lower early follicular phase basal levels and diminished hormonal amplitudes during the menstrual cycle as well as ovulations of lower quality.
The overall role of the brain

According to John Allman, Professor of Biology at California Institute of Technology, “Brains exist because the distribution of resources necessary for survival and the hazards that threaten survival vary in space and time”. Professor Allman in his book “Evolving Brains” gives a current view of the evolution of brains (Allman 2000). He continues:

Brains are informed by the senses about the presence of resources and hazards; they evaluate and store this input and generate adaptive responses executed by the muscles. Thus, when the required resources are rare, when the distribution of these resources is highly variable, when the organism has high energy requirements that must be continuously sustained, and when the organism must survive for a long period of time to reproduce, brains are usually large and complex. In the broadest sense then, brains are buffers against environmental variability.

Human brains are large and complex and could ultimately be considered as evolved adaptive supporters of the gonads to facilitate the procreation and survival of our species.

The brain - from merely degenerating, via plasticity to self-renewing

During the last decades, there have been several major paradigm shifts regarding the cellular status and functioning of the brain. Our understanding has changed from the outdated static view that the brain is an immensely complicated but once and for all wired neural structure only capable of degeneration and successive cellular death. Increasing evidence successively emerged of ongoing dynamic neural plasticity with, for instance the discovery of ebbs and flows of axonal sprouting driven by estrogen during the estrus cycle of rodents (Woolley, Gould et al. 1990; McEwen, Akama et al. 2001). Moreover, in the last decade it has been observed that the adult human brain also is capable of de novo synthesis of neurons in certain locations and under certain circumstances (Eriksson, Perfilieva et al. 1998; Anderson, Aberg et al. 2002; Crespel, Baldy-Moulinier et al. 2004; Lasky and Wu 2005). These new findings have emphasized the dynamic and state-dependent nature of the functioning of mammalian brains.

Brain transmitter systems

More than four hundred different transmitter substances acting in the human brain have been described so far, each with effects on between one and 14
different subtypes of specific receptors. Through complex mechanisms including intracellular cross-talk, the combined action of all these substances and receptors causes changes in the ion flux of four different types of ion channels. These are distributed in the surface membranes of the neurons of the brain and affect the electrophysiological state of the neuron membranes, causing depolarisation or hyperpolarisation of the respective neuron.

The effect of these depolarisations and hyperpolarisations is propagation or inhibition of electrical impulses between neurons. This, as a common denominator, directly or indirectly, steers all our activities from simple motor control to cognition, social interaction and the formation of our specific personal selves.

The serotonin system of the brain

The serotonin system is phylogenetically very old dating back about 500 million years (Allman 2000). The build-up of the system has been remarkably preserved during evolution (Allman 2000). The amphiouxus shows basically the same kind of brain serotonergic organisation as fish, birds and mammals including humans (Allman 2000).

The serotonin system has a dual role of transmitter function and neuromodulator function, regulating the effects of other transmitters in the brain (Rang, Dale et al. 2003). In the human brain there are a few hundred thousand serotonin neurones constituting about one per million of the brain neurons, yet these serotonin neurons via extended axonal rete networks, have contact with the majority of the neurons of the brain (Allman 2000).

In humans, all the cell bodies of brain serotonergic neurons are located in the 10 raphe nucei of the brain stem (Molliver 1987). Axons from these reach almost all parts of the brain through a network of axonal retes (Molliver 1987), one rostral, one caudal. The caudal also sends axons to the spinal cord (Molliver 1987). The serotonergic system of the brain is crucial for the regulation of mood, aggression, sexual function, appetite and feeding, thermoregulation, and sleep and wakefulness (Rang, Dale et al. 2003). It has profound impact on hypothalamic regulation (Leibowitz and Alexander 1998; Neeck 2000; Smith and Jennes 2001) including the secretion of prolactin (Kato, Nakai et al. 1974) and other pituitary hormones (Contesse, Lefebvre et al. 2000). A large number of disorders have been linked to serotonergic dysfunction (Deakin 1998; Clark and Neumaier 2001; Swerdlow 2001; Valentino and Commons 2005). One important aspect of serotonergic dysfunction seems to be that of impaired impulse control (Lucki 1998), probably associated with an inability to disregard noise from signals (Braff, Geyer et al. 2001).
**Serotonin**

In the middle of the nineteenth century it was discovered that blood serum could cause smooth-muscle contractions. In 1933, Vialli and Erspamer isolated an endogenous amine from intestinal enterochromaffin cells, which they named enteramine. In 1948 Rapport, Green and Page isolated a vasoconstrictor substance from serum and called it serotonin (Rapport, Green et al. 1948). Serotonin and enteramine were shown to be the same indolamine substance: 5-hydroxytryptamine (5-HT).

The total amount of serotonin in the body is approximately 10 mg. Of this, approximately 90% is located in the gastrointestinal tract, some 8% in thrombocytes and 1-2% in the brain (Rang, Dale et al. 2003). Serotonin together with dopamine and noradrenaline belong to the family of monoamines. It works as a neurotransmitter and neuromodulator in the brain. Serotonin cannot cross the blood-brain barrier, but has to be synthesized in the brain (Hagberg, Torstensson et al. 2002). Serotonin is synthesized from the essential amino acid tryptophan which is taken up from protein-containing food in the gut and, in competition with other long-chain neutral amino acids, is actively transported across the blood-brain barrier by a long-chain neutral amino acid transporter (Rang, Dale et al. 2003).

In brain neurones, tryptophan is hydroxylated in the 5 position by the rate-limiting enzyme tryptophan-hydroxylase to yield 5-hydroxytryptophan (5-HTP) (Rang, Dale et al. 2003). Tryptophan can also be converted to 5-HTP in several other tissues outside the brain and the resulting 5-HTP can readily cross the blood-brain barrier (Rang, Dale et al. 2003). In neurones, the intermediate substance 5-HTP is quickly decarboxylated into 5-hydroxytryptamine (serotonin) by the unsaturated enzyme aromatic amino acid decarboxylase (AAAD) (Rang, Dale et al. 2003). Serotonin is then stored in reserpine-sensitive granules in nerve terminals and is released into the synaptic cleft on depolarisation of the serotonergic neurone.

Serotonin released into the synaptic cleft binds to, and activates, pre- or postsynaptic serotonin receptors of which there are several types (Rang, Dale et al. 2003). To date, at least 15 different types of serotonin receptors have been identified, 14 of which are present in human brains (Kroeze, Kristiansen et al. 2002). Serotonin-signaling is terminated by the active uptake of serotonin from the synaptic cleft by the serotonin neurone membrane-bound serotonin transporter (5-HTT) (Rang, Dale et al. 2003) Serotonin is either restored in granules or enzymatically deaminated by monoamine oxidase A (MAO-A) to 5-hydroxyindole-acetaldehyde (5-HIAL) in neural mitochondria. 5-HIAL is further oxidized to 5-hydroxyindoleacetic acid (5-HIAA). This end product can be found in the cerebrospinal fluid and is used as a rough measure of brain serotonin metabolism. 5-HIAA is finally excreted in the urine.
Serotonin receptors

Fifteen different serotonin receptors have been identified, of which fourteen are present in humans. The serotonin receptors are classified into seven major subgroups (Hoyer, Clarke et al. 1994). All of these are membrane-bound and all but one is G-protein coupled, the exception being the 5-HT3 receptor, which instead is coupled to a cation channel. In the brain, all fourteen receptors identified in humans are present (Rang, Dale et al. 2003). Of these, five groups of receptors appear to be of greater importance than the others. These are the 5-HT1A, 5-HT1B, 5-HT1D, 5-HT2 and 5-HT3 receptors (Rang, Dale et al. 2003).

5-HT1A receptors are predominantly inhibitory. They appear as autoinhibitory receptors on 5-HT neurones in the raphe nuclei and tend to limit the rate of firing of these cells. They are dispersed throughout the limbic system and are believed to be the main targets of drugs used for the treatment of anxiety and depression (Rang, Dale et al. 2003). 5-HT1B and 5-HT1D receptors act mainly as inhibitory receptors in the basal ganglia (Rang, Dale et al. 2003).

5-HT2 receptors, of which the subtype 5-HT2A are the most common, exert excitatory postsynaptic effects and are abundant in the cortex and in the limbic system (Rang, Dale et al. 2003). 5-HT3 receptors are excitatory ionotopic receptors. They are found chiefly in the medullary vomiting centre, the area postrema, but are also found in the cortex. They may have an anxiolytic effect, but this has not been proved (Rang, Dale et al. 2003).

The dopamine systems of the brain

Dopamine, like serotonin, is a monoamine transmitter and neuromodulator. It is also the intracellular precursor in the formation of noradrenaline. There are three main dopaminergic systems or pathways in the brain (Rang, Dale et al. 2003). The largest is the nigrostriatal pathway (Moore 2003) going from the substantia nigra to the corpus striatum, containing about 75% of the dopaminergic neurones of the brain. It is essential for motor control.

The second largest dopamine system is the mesolimbic/mesocortical system (Melis and Argiolas 1995). The neurones are located in the midbrain and send out axonal projections to the limbic system, especially to the nucleus accumbens, the amygdala, and the cortex, especially the frontal and the prefrontal areas. This system is involved in emotions and drug-induced reward (Robbins and Everitt 2002). It forms the core of the “brain-reward system” further described below.

The third dopamine pathway is the tuberohypophyseal by which the hypothalamus, via inhibitory secretion of dopamine, regulates the secretion of prolactin from the adenohypophysis (Sellix, Egli et al. 2004). Apart from
these “systems” there are dopaminergic interneurones in the olfactory cortex and in the retina (Rang, Dale et al. 2003). There are two classes of receptor families: the D₁ and the D₂ class. The D₁ class causes stimulation and the D₂ class inhibition of intracellular adenylate cyclase (Rang, Dale et al. 2003).

The noradrenaline system of the brain

Noradrenaline is a catecholamine and belongs to the monoamine group of neurotransmitters. All catecholamines are synthesised from the amino acid L-tyrosine, the immediate precursor of noradrenaline being dopamine (Rang, Dale et al. 2003). In the brain, the cell bodies of noradrenergic neurones are located in small clusters in the pons and in the medulla. These clusters send extensively branching axons to most parts of the brain, especially to the cortex, the limbic system, the hypothalamus, the cerebellum and also to the spinal cord (FitzGerald and Folan-Curran 2002).

The most important noradrenergic cluster of the brain is the nucleus ceruleus, located in the gray matter of the pons (FitzGerald and Folan-Curran 2002). It sends axons to the cortex, the hippocampus and the cerebellum. The activity of the nucleus ceruleus regulates arousal and indirectly, mood. Amphetamine-like drugs, in part working through the noradrenergic system, increase wakefulness, alertness and exploratory behavior (Rang, Dale et al. 2003). The noradrenergic system of the brain is thus involved in arousal, blood pressure regulation, mood and the functioning of the meso-cortico-limbic “reward system” of the brain (Rang, Dale et al. 2003)

The glutamate system of the brain

L-glutamate is the most abundant and most important fast excitatory neurotransmitter in the brain (Rang, Dale et al. 2003). It belongs to the group of excitatory amino acids and is part of the intracellular metabolism of almost every cell. It is formed from Krebs cycle metabolism of glucose or from glutamine produced by glial cells. After release from synaptic vesicles it is taken up by a reuptake mechanism that can be reversed by high potassium levels, e.g. during brain ischemia, and induce cell damage due to excitotoxicity (Rang, Dale et al. 2003).

Overdrive of the glutamatergic system causes seizures, and hypofunction respiratory and cardiac arrest. It is important for synaptic plasticity, such as long-term potentiation, short-term potentiation and long-term depression, involved in learning, memory and neural adaptations. It is also involved in the pathogenesis of epilepsy (Rang, Dale et al. 1999).
The GABA system of the brain

Gamma-aminobutyric acid, or GABA, is the most abundant and most important inhibitory neurotransmitter of the brain (Watanabe, Maemura et al. 2002). It is formed from glutamate in GABA-containing neurones of the brain. These are located all over the brain but are particularly abundant in the nigrostriatal system and in grey matter.

GABA works as an inhibitory transmitter in several CNS pathways. GABA is mainly released by short interneurones. The only long GABAergic tracts go to the cerebellum and to the striatum. GABA in the brain works on two types of receptors: the GABA<sub>A</sub> receptor, which is a quick-acting ligand-gated ion channel localized postsynaptically, and GABA<sub>B</sub>, which is a G-coupled receptor located both pre- and postsynaptically (Rang, Dale et al. 2003). Benzodiazepines bind to a specific receptor site on the GABA<sub>A</sub>-receptor and potentiate the effect of GABA on the receptor. Barbiturates and neurosteroids both act as modulators of the GABA<sub>A</sub>-receptor and potentiate the effect of GABA on the receptor (Rang, Dale et al. 2003).

The meso-cortico-limbic system of the brain

This is the rough pseudoanatomical name for what in layman language is also called “the reward, or pleasure, system of the brain”. This ancient, preserved, brain system, integrates afferent conscious and subconscious input to the brain, with information from memories, experiences, emotions, urges, drives and wills, to generate useful, satisfying, directed motor behavior and actions. This is the system of the brain that makes us socialize, feed, communicate, love, hate, groom, procreate, nourish and care for our children, elucidate, research, write theses, want to go to the moon, run marathons (Allain, Bentue-Ferrer et al. 2004), and unfortunately, use all sorts of addictive drugs (Adinoff 2004).

The main transmitter substance of this system and what produces most of the reward sensation is dopamine (Nieoullon and Coquerel 2003), especially when large amounts flood a nucleus crucial to the perception of reward: the nucleus accumbens. In electrophysiological experiments on rodents, it was discovered that electrical stimulation of this nucleus, appeared to be intensely pleasurable for the animals. They are easily taught how to electrically self stimulate themselves, but the activity in itself becomes so meaningful that they can disregard hunger and actually starve to death while stimulating their pleasure-giving nucleus. This obviously is an immensely important brain system, which must have evolved to sustain the propagation of the species. All addictive drugs pharmacologically “hijack” this system and give artificial, for the survival of the species, non-useful and in the long run deleteriously harmful kicks.
Estradiol in females induces extensive effects on this system, directing pleasure-evoking focused behavior (Graziottin 2000). Estradiol also potentiates the effects of other physiological and pharmacological stimulators of this system (Becker and Rudick 1999; Becker 2000; Russo, Festa et al. 2003). Another known potentiator of this system is cortisol (Marinelli and Piazza 2002). The effect of this, is that persons with increased cortisol secretion, for instance due to psychological stress, are more prone to develop addiction of various sorts, than persons with normal cortisol secretion. Thus severe stress in itself increases the risk of developing substance dependence (Goeders 2003).

Estrogen and its effects on the brain and behavior

Estrogens, mainly in the form of estradiol-17β are produced predominantly in the gonads, in small amounts from the adrenal cortex, and also from androgen precursors and estrogen metabolites in adipose tissue. Estrogens are also synthesized in the brain itself (Naftolin, Ryan et al. 1975). Estradiol exerts trophic and neuroprotective effects on brain neurons (Toran-Allerand 2004). Estrogens are important for early brain development, neural differentiation, sexual dimorphism, neuron survival and normal brain function (Toran-Allerand 2004), including cognition, memory, locomotion, affect and motivation (McEwen 2001; McEwen 2002). Estrogens exert excitational effects on neurons, stimulating glutamatergic activity (Woolley and McEwen 1992). Estrogen has trophic and stimulatory effects on most neurotransmitter systems including the monoaminergic and endorphinergic systems (McEwen 2002). Estradiol-17β exerts genomic effects through the binding to its receptors: estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), thereby modulating gene transcription (Ostlund, Keller et al. 2003), but it has also been shown to exert rapid non-genomic effects by binding to membrane receptors (McEwen 2001).

Progesterone and its effects on the brain and behavior

Progesterone is synthesized by the corpus luteum of the ovary and also in small amounts by the adrenal cortex. Progesterone exerts genomic effects by binding to its intranuclear receptors: progesterone receptor A (PRA) and B (PRB) which are induced by estradiol and found in many of the same areas as the estrogen receptors, including the hypothalamus and the limbic system (Sherwin 1999). Through some of its metabolites, it can also exert rapid non-genomic effects on brain neurons, described below. Progesterone decreases brain excitability. Many of the effects of progesterone on the brain counteract the effects of estrogens. Progesterone increases the concentrations of
monoamine oxidase (MAO-A), the enzyme that catabolizes serotonin in the brain, whereas estrogen has the opposite effect (Sherwin 1999). Progesterone has also been shown to counteract the axonal sprouting which is induced by estrogen during the estrus in rodents. However, estrogen and progesterone together elicit estrous behaviour in rodent models (Meyerson 1972).

Estrogen as an endogenous psychostimulant with addictive potential

Estrogen has excitatory effects on the brain (McEwen 2002). Arousal is heightened and afferent input facilitated (McEwen 2002). Grand mal seizures are more frequent during high estrogen states (McEwen 2002). Estrogen increases cognition (Wolf, Kudielka et al. 1999; McEwen 2002). Rapid estrogenic increments can give rise to euphoria (Utian 1972). Estrogen reduces the appetite (Johnson, Corrigan et al. 1994; Geary 1998; Geary 2000) and has anorectic effects (Bonavera, Dube et al. 1994; Rocha, Grueso et al. 2001) at high preovulatory and ovulatory levels. Both in human and rodent models estrogen treatment has been shown to induce weight loss (Cox and King 1980; Asarian and Geary 2002; Geisler, Zawalich et al. 2002). Estrogen potentiates the effects of psychostimulant drugs like amphetamine and cocaine (Peris, Decambre et al. 1991; Becker and Rudick 1999; Justice and de Wit 1999; Becker, Molenda et al. 2001; Russo, Festa et al. 2003). There are reports in the literature of tachyphylactic reactions to exogenous estrogen treatment (Garnett, Studd et al. 1990; Gangar and Whitehead 1991) especially among subcutaneous implant users. In one report about three per cent of the women treated wanted additional implants because of diminishing effects of the treatment administered (Gangar and Whitehead 1991).

Anecdotally, we have in clinical practice encountered several patients who wanted increasing doses of estrogen during hormonal replacement therapy (HRT) but who on laboratory tests showed supraphysiological estradiol levels, at or above preovulatory peak values for fertile women, and sex hormone binding globulin (SHBG) levels three to five times those of normal fertile women.

In the fall of 1997 the Swedish Board of Health and Welfare reported an intriguing medical profession misconduct matter: # 500/95:34 (Hont 1997) in which a 60-year old consultant physician had been reprimanded by the Board because of an extreme overprescription and overuse of 1 mg estriol (Ovesterin ®) tablets by the physician herself. The escalating overuse had been going on for the preceding ten years. Between February 1989 and November 1993 she had prescribed and consumed 40,930 one-mg tablets and 280 two-mg tablets. When reported, she had reached a daily consumption of 213 one-mg tablets. The woman denied addiction and stated that the regimen
only made her feel alert and fit, and made her work well. The Board ordered
dose-reduction therapy, but no disciplinary punishment was issued.

Like psychoactive drugs in general, there seems to be an effect of the
route of administration (Quinn, Wodak et al. 1997) for the reported mental
tonic effects of estrogen with nasal > subdermal > transdermal > oral. All in
all, there is substantial support for the view of estrogen as an endogenous
psychostimulant with innate addictive potential.

Luteal phase progesterone metabolites as potential
endogenous tranquilizers

Progesterone and estradiol are produced in the corpus luteum. One week
after ovulation, in the mid-luteal phase, progesterone levels in nmol per litre
are between twenty and one hundred times higher than in the follicular phase
(Guerrero, Aso et al. 1976). Estradiol levels in pmol per litre are about half
(Guerrero, Aso et al. 1976) to two thirds of preovulatory estradiol levels
(Bakos, Lundkvist et al. 1994). Thus, the hormonal environment is drasti-
cally changed in the luteal phase compared to the follicular phase. Proges-
terone is more lipophilic than estradiol and about 60% of the progesterone
produced by the corpus luteum is retained in the brain. Progesterone exerts
genomic effects by binding to nuclear receptors but can also exert rapid non-
genomic effects through the actions of its metabolites.

The brain contains steroidogenic enzymes and is capable of de novo pro-
duction of gonadal steroids (Naftolin, Ryan et al. 1975; Baulieu 1997). It is
also capable of enzymatic conversion of gonadal steroids and gonadal ster-
oid metabolites, which enter the brain from the circulation (Baulieu 1997).
Steroids produced in the brain are called neurosteroids, whereas steroids
entering the brain from the circulation are called neuroactive steroids.

The progesterone metabolites allopregnanolone and pregnanolone are
both neuroactive steroids as well as neurosteroids (Baulieu 1997). They bind
allosterically to GABA_A receptors and potentiate the effect of GABA
(Majewska, Harrison et al. 1986), thus enhancing hyperpolarization and re-
ducing excitation of brain neurones. Allopregnanolone is the most potent of
the progesterone metabolites in this respect (Reddy 2003). Pharmacological
experiments have shown that allopregnanolone exerts anxiolytic, anti-
aggressive, sedative, anti-epileptic and anesthetic effects in increasing doses.
Like other sedative substances which bind to GABA_A receptors, develop-
ment of tolerance is possible (Backstrom, Andersson et al. 2003). Also, like
other GABA_A potentiators, paradoxic, reverse effects of low concentrations
have been described (Thomas, Mameli et al. 2005). The bimodal effect of
allopregnanolone has also been implicated in the pathophysiology of premen-
strual dysphoria (Backstrom, Andersson et al. 2003).
Premenstrual dysphoria as a possible endogenous withdrawal reaction

The cardinal symptoms of premenstrual dysphoria, i.e. irritability, depressed mood, affective lability, impaired impulse control and fatigue, as well as the common symptoms of carbohydrate craving, overeating, hypersomnia or insomnia (American Psychiatry Association 1994), are quite similar to the cardinal mental symptoms of psychostimulant drug withdrawal which, according to DSM IV, are dysphoric mood, fatigue, psychomotor retardation or agitation, increased appetite, insomnia or hypersomnia and vivid unpleasant dreams (American Psychiatry Association 1994). With many symptoms common to both conditions and the reasoning about estrogen given above (Becker and Rudick 1999), the suggestion that premenstrual dysphoria is elicited by an endogenous estradiol withdrawal reaction is reasonable.
Aims of the thesis

General aim
This thesis aims to investigate physiological and pharmacological mechanisms of pathogenetic relevance to premenstrual dysphoria, through four separate, curiosity driven clinical studies of principal theoretical interest.

Specific aims

Study I
To test whether the sensitivity of the brains of women with premenstrual dysphoria to fluctuations in a gonadal hormone (estradiol-17ß) is increased compared to that of the brains of asymptomatic women.

Study II
To test the serotonin hypothesis of premenstrual dysphoria: that there is an association between premenstrual decline in brain serotonin function, and concomitant exacerbations of cardinal mood symptoms.

Study III
To investigate the efficacy, tolerability and sexual side-effects of buspirone, (a partial 5-HT1A receptor agonist), and nefazodone (a combined serotonin reuptake inhibitor [SRI] and 5-HT2 receptor antagonist), administered intermittently and continuously to women with premenstrual dysphoria, compared to placebo.

Study IV
To investigate the occurrence of polycystic ovaries (PCO), measure ovarian volume, count ovarian follicles and measure levels of androgen hormones in women with premenstrual dysphoria and compare these values with those of age-matched controls.
Methods

Descriptions of study designs

Study I
An estrogen challenge test with blood sampling on 11 occasions over 144 hours was done in 13 cases, self-referred for severe premenstrual dysphoria, and in 12 controls of similar ages, with no premenstrual problems. Medical history was taken. Both groups were evaluated clinically and symptoms were rated according to a 14-item visual analogue scale (VAS). Blood levels of estradiol, FSH and LH were monitored. Gonadotropin responses were analyzed for differences between the groups and for correlation with the VAS ratings.

Study II
Positron emission tomography using \(^{11}\text{C}\)-5-hydroxytryptophan and \(^{15}\text{O}\)-water tracers was done in the mid-follicular and late luteal phases in 8 cases with severe premenstrual dysphoria, seven of the cases recruited from Study III, one self-referred. Evaluation was done by a psychiatrist. Symptoms were rated using a 14-item VAS. Ovulation was verified by urinary LH-test. Post-imaging processing included the drawing of ROIs and calculation of standardized uptake values. Changes in VAS scores and in \(^{11}\text{C}\)-5-hydroxytryptophan trapping were recorded and tested for correlation.

Study III
This was a randomized, double-blind, placebo-controlled, two-center drug treatment trial with parallel groups that tested the effect of buspirone and nefazodone on premenstrual dysphoria. 69 cases with severe premenstrual dysphoria were randomized, and 63 entered the treatment trial. Recruitment was done by newspaper advertisements, followed by telephone interviews and clinical evaluation including MINI and MADRS. Pre-trial VAS rating was done for 3 cycles of which at least two had to be cyclical. VAS rating
was done during the trial: two cycles with luteal-phase treatment and two cycles with continuous treatment. Any adverse effects were recorded.

Study IV

Ovarian ultrasound morphology and serum levels of sexual hormones were analyzed in 26 cases with severe premenstrual dysphoria and 26 age-matched (± 2 years) asymptomatic controls. Cases were recruited from Study III, and controls by invitation from centers for a generalized Pap-smear screening program. Clinical evaluation included MINI and MADRS. Ovulation was verified by urinary LH-test. Serum samples for hormone analyzes were taken at 4 occasions during the menstrual cycle. Transvaginal ultrasoundography was done on day 5 in both groups. Examinations were recorded on videotape and evaluated blind.

VAS-instruments and assessments (I, II, III, IV)

The essence of premenstrual dysphoria/PMDD is the cyclicity of distracting mood symptoms, coinciding with the late luteal phase, in combination with the absence of such symptoms during the week after menstrual bleeding. This symptom pattern has to appear in at least two consecutive menstrual cycles and has to fulfil the cyclicity criteria D of the PMDD. It is generally accepted that the optimal way to document this pattern is by daily, prospective self-ratings using a visual analogue scale (VAS). All the cases in the studies were evaluated in this way and, in addition, had to meet quantitative operant criteria of cyclicity. All the controls did ratings in the same way either for inclusion/exclusion or for later comparison. Two different visual analogue scales were used in the studies.

A 14 item VAS instrument developed by Bäckström et al. incorporating two more variables than that of their first description (Hammarbäck, Bäckström et al. 1989), was used for daily prospective self-ratings in Study I for the cyclicity diagnosis in the 13 cases; in Study II in one of the eight cases; in Study I for the diagnosis of absence of such cyclicity in the 12 controls, and in Study II for the evaluation of changes in symptoms during the study in all of the eight subjects. The variables assessed were four mood symptoms: irritability, depressed mood, fatigue and tension; four positive mood variables: happiness, energy, relaxation and friendliness; and six somatic manifestations: headache, bloating, breast tenderness, pelvic pain, craving for sweets and sexual desire.

In Study III all the 69 subjects recruited and, out of these, the seven of the eight subjects in Study II, the 26 cases in Study IV as well as the 26 controls in Study IV, were assessed using a 7 item VAS comprising the following symptoms: irritability, depressed mood, tension, affective lability, food crav-
ing, breast tenderness and bloating. Prior to the start of the ratings, subjects were thoroughly instructed how to do accurate daily VAS self-ratings before going to bed. All the subjects were instructed to score the total absence of a symptom as 0 mm on the VAS scale, and the most intense form of the variable ever experienced as 100 mm.

For inclusion as a case, an increase of at least 100% in the symptoms irritability and/or depressed mood, from follicular (mean of days 6 to 10 from menstrual start) to luteal phase (mean of days 5 to 1 before menstrual start) as well as a luteal phase mean-value (of days 5 to 1 before menstrual start) of the symptoms irritability and/or depressed mood exceeding 30 mm on the 0-100 mm VAS scale, had to be present in two of three rated cycles in Studies II, III and IV, and in one or both of two rated cycles in Study I. The 14 item instrument was manually read using a transparent mm-graded ruler, whereas the 7 item instrument was optically read and calculations done on a computer.

The MINI (III, IV)
The Mini International Neuropsychiatric Interview is a structured psychiatric evaluation instrument, developed by Sheehan and Lecrubier, based on DSM IV diagnostic criteria of axis I disorders, and used clinically and in research for screening of the occurrence of axis I conditions (Sheehan, Lecrubier et al. 1998). The interview instrument is designed to cover all major axis I disorders and takes about 15 minutes to go through with the subject being evaluated. This instrument was used in Studies III and IV, in which all presumptive subjects displaying a current axis I condition were excluded.

MADRS (III, IV)
MADRS stands for the Montgomery, Åsberg Depression Rating Scale, which is a depression rating instrument developed by Montgomery and Åsberg, for use as a quick and reliable tool in clinical and research subject evaluation, and designed to be sensitive to changes in affective state (Montgomery and Åsberg 1979). The instrument was designed to measure the degree of depression, once a diagnosis of depression had been made. The instrument consists of 10 questions, the first posed to the evaluator, the other nine to the subject being tested. The answer to each question is given between 0 and 6 points on the depression rating scale, so the entire instrument can result in total sums from 0 to 60. Scores close to, but below, 20 have been considered to correspond to a low degree of depression, scores from 20 to 30 to a depression of medium degree, and scores of 30 and above to a severe depression. In Studies III and IV, this instrument was used to evaluate
presumptive subjects, who for inclusion had to display a total MADRS score of $\leq 14$.

Control recruitment (I, IV)

In Study I, 12 controls of a similar age distribution to that of the cases, were recruited by local advertisements among hospital employees. Inclusion criteria for controls were: self-reported absence of luteal-phase mood symptoms and prominent physical symptoms, and absence of the VAS-rated cyclic mood changes required for cases. Remaining inclusion and exclusion criteria were the same as those for cases: current physical and mental health as assessed by clinical evaluation, including normal findings at gynecological examination, and spontaneous regular menstrual cycles of 21-35 days’ duration. Exclusion criteria were: pregnancy, breast-feeding, steroid hormonal treatment (other than thyroxin substitution in one woman with well managed congenital hypothyroidism, who was accepted for participation), current medication that might interfere with the study results, a history of chronic mental illness or of substance abuse, a family history of thromboembolic disorder, and allergy to benzoate.

In Study IV, 26 women, age-matched to $\pm 2$ years of the respective cases, were recruited through the centers for cervical Papanicolau (Pap) smear screening in the city of Uppsala, Sweden. The overall rate of acceptance of the invitation to participate in the automated triennial screening program call is around 30% (Eaker 2003). Midwives engaged in Pap smear sampling handed out information sheets about the study to women of the ages required for controls. Women interested in participating in the study underwent a structured telephone interview and a clinical evaluation identical to that of the cases. Inclusion criteria for controls were: absence of premenstrual mood symptoms and associated physical symptoms. Other inclusion and exclusion criteria were the same as those for the cases: age 18-45 years, regular menstrual cycles of 22-35 days’ duration, current physical and mental health, including the MINI without criteria of any axis I disorder, and an MADRS score $\leq 14$. Moreover, women who were taking any potentially interfering medication or who had previously received serotonin reuptake inhibitor (SRI) or selective SRI (SSRI) treatment were not eligible for inclusion. Exclusion criteria were: pregnancy, breast-feeding or intended pregnancy within the following 7 months.

The Estrogen challenge test (I)

The estrogen challenge test was developed and introduced by Shaw and coworkers in the 1970’s as a method of assessing the status of the hypothat-
lamic-pituitary axis in women with amenorrhea (Shaw, Butt et al. 1975). The underlying principle is that by introducing an exogenous sudden increase in the blood levels of estradiol, the status of the investigated woman’s hypothalamic/pituitary feedback response, both negative and positive, can be registered and assessed.

In their original description Shaw and co-workers used an intramuscular estradiolbenzoate dose of 1 mg. However, after performing a pilot test with a similar dose of 0.02 mg/kg body weight, where the response registered was considered suboptimal, we decided to use a challenge dose of 0.04 mg/kg body weight for this study.

The estrogen challenge test was performed in cases and controls as follows: a zero-hour baseline blood-sample of 30 ml was drawn from an antecubital vein between 7.00 a.m. and 10.00 a.m. of day three or four of the menstrual cycle, counted from the first day of the menstrual bleeding. The baseline blood sample was immediately followed by a gluteal i.m. injection of 0.04 mg/kg body weight of estradiolbenzoate in arachid oil (estradiolbenzoate 1 mg/ml: estradiolbenzoas 1 mg, alcoholi benzylicus 50 mg, arachis oleum a.u.p. 1 ml, Apoteksbolaget, Umeå, Sweden). Then, 20 ml blood-samples were taken from an antecubital vein at 0.6, 6.5, 24, 32, 48, 56, 72, 96, 120, and 144 h, using sterile unprepared 10 ml Vacutainer® test tubes. Samples were stored in a refrigerator for at least one hour, and then centrifuged at 3,000 rpm for 10 min, and the serum was separated into standard plastic tubes, which were plugged and stored at -20°C until analyzed.

Positron emission tomography (II)

Positron emission tomography is a non-invasive, quantitative radionuclide imaging technique with which the distribution and relative, as well as absolute, tissue concentration of positron emitting tracers introduced into the body, can be calculated and visualized as images.

The underlying principle behind the technique is registration with a radiosensitive ring collimator of the simultaneously emitted mutually perpendicular photons released when a positron from a tracer, after traversing an infinitesimally small distance in the body being imaged, reacts with an electron causing annihilation of both particles. By means of advanced mathematical algorithms and powerful computers, a compiled tissue volume; pixel information on the relative or absolute content of the positron emitting substance can be achieved.

In this thesis, PET scans were done with a GEMS PC2048-15B scanner (General Electric Medical Systems, Milwaukee, WI, USA) with an axial field of view of 10 cm. This produces 15 slices spaced 6.5 mm apart and has an in-plane resolution of 6 by 6 mm (Holte, Eriksson et al. 1989). Each subject was scanned in the follicular phase (8-11 days from menstrual onset).
and in the luteal phase (1-4 days before menstrual onset) at about 9.00 a.m. after overnight fasting. Ovulatory cycles were confirmed by daily urinary LH self tests from the mid-follicular phase until a positive test was obtained (Clear Plan, Unipath Ltd, Bedford, UK)(Behre, Kuhlage et al. 2000). The scanning order of the menstrual phases was reversed in half of the patients to avoid scanning order bias.

The subjects were scanned in the supine position using an individually molded foam head holder. The head position was centered 10 mm caudal to the orbital-meatal line. A standardized calm atmosphere with dimmed light, recorded melodious music and room temperature prevailed. An antecubital vein cannula was used to administer the tracers: Two injections of radio-labeled water ($^{15}$O-H$_2$O) in a dose of 15 MBq/kg body weight were given 10 min apart to enable the identification of brain regions. Ten minutes later, $^{11}$C-5-HTP (Bjurling, Watanabe et al. 1989) was injected in a dose of 6 MBq/kg body weight. One $^{15}$O-H$_2$O scan was recorded in 15 frames of 10 s and the other in one frame of 300 s. $^{11}$C-5-HTP scans were recorded in 5 frames of 60 s, 5 frames of 120 s, 5 frames of 180 s and 6 frames of 300 s. Correction for attenuation was made with a transmission scan preceding tracer injections.

**Brain regions of interest (II)**

Irritability, depressed mood, affective lability and impaired impulse control, the major mood symptoms displayed in premenstrual dysphoria, are all emotional and affective symptoms. From previous functional PET and functional MRI studies, mood and affective states are known to be reflected in the activity of prefrontal, mediofrontal and striatal regions of the brain (Davidson 2002), and there is an apparent laterality of different emotions, with positive, “approaching” emotions more often displayed in frontal regions of the dominant hemisphere – in the overwhelming majority on the left side – and negative, “withdrawing” emotions more often reflected in the activity of the right hemisphere.

The brain regions of interest chosen in Study II, have all been used in previous studies of major depression (Agren and Reibring 1994) and in other psychiatric conditions currently considered to belong to the group of serotonergic dysregulation disorders.

In Study II, the following nine ROIs were delineated: dorsolateral prefrontal cortex, medial prefrontal cortex, putamen and caudate nucleus, each on the right and left side, and a single whole brain ROI. The eight forebrain regions were chosen on the basis of their known serotonergic innervation and because of their documented involvement in affective disorders (Agren and Reibring 1994; Brody, Barsom et al. 2001; Davidson 2002).
The ROIs were drawn on $^{15}$O-H$_2$O summation images, which allowed fair identification of anatomical landmarks, and then copied to $^{11}$C-5-HTP scans to optimize the anatomical precision. The ROIs were delineated in the following way: starting in the two slices with the most intense striatal radioactivity, right and left putamen ROIs of about 2.5 cm$^2$ were drawn by hand on each slice after topographical comparison with a Computerized Brain Atlas (CBA) (Greitz, Bohm et al. 1991). Circular ROIs of 0.9 cm in diameter were drawn in the caudate area on both sides of the same slices and, in the rostral of these slices, ROIs were drawn in the right and left dorsolateral prefrontal cortex and right and left medial prefrontal cortex. The cortical ROIs were drawn using an automated cortex-ROI algorithm in the Image Display and Directory computer program (Scanditronix/General Electric, Uppsala, Sweden). Dorsolateral prefrontal cortex ROIs were drawn 40 mm long and median prefrontal cortex ROIs were drawn 12 mm long. On the two following rostral slices, 40 mm long identical dorsolateral prefrontal cortex ROIs were drawn, as well as medial prefrontal cortex ROIs 18 mm and 20 mm long on both sides, respectively. Ten mm wide cortical ROIs were drawn throughout. A whole brain ROI was drawn on the second slice below the striatal starting slice. All dorsolateral prefrontal cortex ROIs on each side were coupled to a volume of interest. The same was done for the medial prefrontal cortex ROIs, the putamen ROIs and the caudate nucleus ROIs, on both sides.

**SUV calculations (II)**

The regional radioactivity trapping (radioactivity of $^{11}$C-5-HTP and its metabolic products) in the brain was measured as a dimensionless standardized uptake value (SUV), which is the radioactivity measured by the scanner divided by the given tracer dose per kg body weight. SUV curves, standardized time-radioactivity uptake curves, from the whole brain ROI after injection of $^{11}$C-5-HTP are shown in Figure II:2. The radioactivity was corrected for physical decay from the time of injection. Total radiotracer accumulation of an ROI was calculated as the area under the curve (AUC) from 9 to 60 minutes after radiotracer injection (frames 8-21), of the standardized uptake curve. AUC values were calculated for each ROI for the two menstrual cycle phases in every subject.
Drug trial (III)

Subjects were randomized to buspirone ($n = 19$), nefazodone ($n = 22$) or placebo ($n = 22$). During the two first treatment cycles, subjects only took medication during the luteal phase of the menstrual cycle (intermittent administration). Drug intake started at the estimated day of ovulation with a daily dose of 10 mg buspirone or 100 mg nefazodone (or placebo). After 3 days, the daily dose was increased to 20 mg buspirone or 200 mg nefazodone (or placebo), and this was the dose the subjects were recommended to take until the first day of menstruation, when drug intake should stop.

In the second treatment cycle, the medication regimen was the same as in the first cycle. The daily dose was always given as two capsules, one to be taken in the morning, the other in the evening. If subjects were bothered by side-effects they were allowed to reduce the dose to one capsule a day (that is 10 mg buspirone or 100 mg nefazodone). If the effect of the medication was unsatisfactory, patients were allowed to increase the dose to three capsules a day (that is 30 mg buspirone or 300 mg nefazodone).

During the third and fourth treatment cycles, the medication was taken continuously throughout the menstrual cycle. The recommended dose was higher when the drug was given continuously compared to when it was given intermittently. During the third and fourth treatment cycles, the participants were recommended a daily dose of four capsules: two in the morn-
ing and two in the evening (equal to 40 mg buspirone or 400 mg nefazodone). If bothered by side-effects they were allowed to reduce the dose to three, two or one capsule daily.

Blood sampling (IV)

Blood samples were drawn from all study participants between 7 and 10 a.m. after a night’s fast on four occasions: days 3 and 8 after the start of menstruation, the day of ovulation (approximated by the results of the urine LH tests), and the fourth day preceding menstruation (approximated by menstrual history). For practical reasons, the blood samples were sometimes taken one or more days outside these parameters. It was originally planned that the blood samples would be drawn during the course of one menstrual cycle but they were taken over two separate cycles in five cases and seven controls, and over three cycles in one control subject, due to circumstances beyond our control.

All blood samples were drawn and prepared by the same study nurse. Antecubital venous blood, 30 ml, was drawn into unprepared Vacutainer® tubes using Vacutainer® syringes and holders. Day 3 samples were sent for immediate analysis to the Laboratory of Clinical Chemistry, University Hospital, Uppsala, Sweden. Samples from the other three sampling days were stored in a refrigerator for at least one hour and then centrifuged at 3,000 rpm for 10 min. The serum was separated into plastic tubes, which were plugged and stored at -20°C. These serum tubes, packed with dry ice into a frigolite box were delivered express, in less than three hours, from Uppsala to the Institute of Physiology and Pharmacology, University of Göteborg, for further storage at -70°C until analyzed.

The urine LH test (II, III, IV)

In Studies II, III and IV, the day of ovulation was estimated using an immunological urine LH self test, the Clearplan® test (Unipath Limited, Bedford, UK) (Behre, Kuhlage et al. 2000). This test consists of a pack of seven sticks impregnated with monoclonal antibodies against LH including a color reagent. The stick is dipped into freshly voided urine and, after two minutes, shows a blue transverse stripe for a correctly performed negative test and two blue stripes for a correctly performed positive test. Subjects were instructed to test the first voided urine in the morning, starting three to four days before the expected day of ovulation. The test identifies the LH surge, which precedes ovulation by 20 to 36 hours.
Transvaginal ultrasonography (IV)

Transvaginal ultrasonography is a minimally invasive imaging technique for visualizing the pelvic organs and assessing their sonographic morphology. The principle behind the ultrasonographic technique is the difference in acoustic impedance between different tissues of the body, i.e. the speed of spreading sound waves varies with the physical properties of the tissues, such as the water content. A transducer emitting soundwaves in the range 5-7 MHz from an array of piezo-electrical crystals alternates between sending pulses of sound and receiving reflections of these pulses from the boundaries of the tissue. By means of mathematical algorithms and continuous computation, time and amplitude differences in output and input pulses of sound generate a real-time image of the structures under examination. Since the resolution of the ultrasound images is directly proportional to the frequency utilized, and the tissue penetrance is inversely proportional to the frequency squared, natural body openings produce pictures of optimal quality of internal organs.

In Study IV, cases and controls underwent systematic transvaginal ultrasonography of the uterus, the ovaries and the adnexa, on the fifth day from menstrual start, or as close to that day as possible. The uterine corpus diameters were measured in three mutually perpendicular planes to calculate the approximate corporal volume. The occurrence of any intrauterine device (IUD) was noted, as was that of myometrial thickening, fibroids, myometrial calcifications and other deviations from normal uterine sonographic anatomy. The ovaries were measured in three mutually perpendicular diameters; maximal length, height and width, in order to calculate their volume. Each ovary was scanned from pole to pole and recorded on video tape so the number of follicles could be calculated later.

An Acuson 128 XP Ultrasonograph with an Acuson EC 7 vaginal probe of 7 MHz was used. Real-time examinations were recorded on Super VHS tape (Fuji Pro, Fuji Magnetics GmbH, Germany) using a Panasonic AG-7350 (Panasonic Corp., Japan) video tape recorder. Paper-printer pictures of the uterus, the right and left ovary in the longitudinal and transverse planes, including the respective caliper-marked measurements, were taken using a Sony Video-Graphic Printer UP-850 (Sony Corporation, Japan) with thermal paper for Mitsubishi Video Copy processor K 65 HM (Mitsubishi Corporation, Japan), and the pictures were stored for documentation together with the videotapes.

Blinded evaluation of ultrasonographic recordings (IV)

An unbiased external expert on the assessment of sonographic ovarian morphology (J.H.), with expertise in the field of gynecological ultrasonography
and specifically of PCO (Holte, Bergh et al. 1994; Holte, Gennarelli et al. 1998), was contracted to scrutinize and evaluate the recorded sonograms, in a blinded manner. Blinding was achieved by coded numbers. The assessor set ovarian measurements, counted the total number of follicles in each ovary, assessed the stroma of the ovary, and classified the ovary. Each ovary was classified into one of the four categories: oligofollicular- (< 5 follicles), normofollicular- (5-9 follicles), multifollicular- (≥ 10 follicles, without stromal thickening), and polycystic-ovary (PCO; ≥ 10 follicles, with stromal thickening) (Holte 2002). A Panasonic Super VHS 625 Video Cassette Recorder AG-7330 with a Finlux Type 2714 monitor was used for the evaluations.

Hormone analyses (I, II)

Hormones were analyzed at two different laboratories: the laboratory of Clinical Chemistry, University Hospital, Uppsala, Sweden, and the laboratory of the Institute of Physiology and Pharmacology, University of Göteborg, using different techniques.

In Study I, serum estradiol, FSH and LH were measured in three batches by means of an automated fluorescence detection system (Autodelfia, Wallac Oy, Turku, Finland) at the ISO 15189/17025-accredited (by Swedac, Sweden) routine laboratory of Clinical Chemistry, University Hospital, Uppsala. Total assay variations for estradiol were 5.3% (range: 3.4% - 7.1%), for FSH 3.8% (range: 3.0% - 4.5%) and for LH 2.6% (range: 2.2% - 2.9%).

In Study IV, serum estradiol, FSH, LH and sexual hormone binding globulin (SHBG) from menstrual cycle day 3 were measured consecutively at the Uppsala laboratory as described above. Serum samples from cycle day 8, expected day of ovulation, and expected day -4, were analyzed at the Göteborg laboratory, in the following manner: total testosterone, SHBG, and dehydroepiandrosterone sulfate (DHEA-S) were measured using a chemiluminiscent enzyme immunoassay; free testosterone and androstenedione were measured by radio-immuno-assay (RIA). All assays were performed in accordance with the instructions provided by the manufacturer (Diagnostic Products Corporation, Los Angeles, CA, USA).

Statistical methods (I, II, III, IV)

Two-sided non-parametric statistical tests were used throughout. A p-value < 0.05 was considered significant. The Spearman rank correlation test was used for correlation analyses. The Lagrange method was used for AUC calculations in Studies I and II. The trapezoidal method was used for AOC calculations in Study I. Linear interpolation was used for the imputation of two
missing values (out of 275) and to calculate intersecting points in the calculations of total AOC in Study I. The Wilcoxon test for two random samples was used for comparison of AOC values and an exact Wilcoxon test was used to compare the AUC of the different groups in Study I. Adjustment for multiple tests of correlations was performed in Study II using an exact permutation test as described by Churchill and Doerge (Churchill and Doerge 1994). The Wilcoxon matched pairs signed rank test was used for significance testing of menstrual cycle phase changes in VAS ratings of symptoms in Study II.

Linear regression analysis was used to test interaction between study groups and the change in LH from baseline level to maximum negative feedback (LH32h - LH0h), for luteal phase irritability mean VAS scores (days 10 to 1 before menstrual onset) in Study I and, in the cases, multivariate regression analyses were used to calculate the explanatory value of the combined negative feed-back response of both LH and FSH for each of the VAS rated luteal phase symptom scores and for three composite symptom variables, respectively. An F-test was used to test difference in variability of gonadotropin responses between the groups in Study I. This test was applied on the residual variances from linear models for each group. In the models, the change from baseline was the dependent variable, and subject was used as a categorical predictor as well as a linear time term. Fishers’ exact test was used to analyze differences in LH surges and surge-like reactions between groups in Study I. An LH surge was defined as an LH concentration ≥ 3µgram/L, and an LH surge-like reaction as an LH concentration ≥ 2.5µgram/L and < 3µgram/L. The Kruskal-Wallis ANOVA by ranks test, followed by the Mann-Whitney U-test was used to compare changes in symptom ratings in the groups in Study III. The chi-square two-tailed test was used for between-group comparisons of categorical variables in Study III.

The analyses were performed with the use of SAS® software (SAS Institute, Cary, NC, USA). A p-value < 0.05 was considered significant. Special methodological considerations concerning the statistical analyses are given for each study below.

Study I
Analyses including and excluding the subject with the presumed intravasation of the estradiol injection yielded essentially similar results, so this subject was included in calculations throughout.

Study II
Changes in 11C-5-HTP trapping were calculated as AUC for the luteal phase minus AUC for the follicular phase of the standardized uptake curves.
Changes in symptom scores were calculated as luteal phase score (in mm) minus follicular phase score (in mm).

Study III
Throughout the four treatment cycles, symptoms were assessed with the same VAS instrument as during the pretreatment reference cycles. The change in symptom score in per cent for each treatment cycle was calculated according to the formula: (baseline score minus treatment score) x 100/baseline score. The baseline score was defined as the mean rating of the 5 days prior to menstruation during all three reference cycles. The score for each treatment cycle was defined as the mean rating of the 5 days prior to menstruation of that cycle. After the last treatment cycle, or immediately after dropout, the patients were asked to assess the global efficacy of the drug using a form corresponding to the clinical global improvement (CGI) scale (Guy 1976). Assessment of efficacy was based on intention-to-treat (ITT) analysis, using the last observation carried forward (LOCF) strategy. This included all randomized patients who had completed at least one treatment cycle. For the LOCF procedure, the intermittent treatment phase of the trial and the continuous treatment phase were regarded as separate trials. If a patient had dropped out after having completed the first treatment cycle, the scores obtained in cycle 1 were carried forward to cycle 2, but not to cycle 4. If a patient had dropped out after treatment cycle 3, her scores from that cycle were carried forward to cycle 4.

Study IV
The ovarian volume was calculated using the formula of a prolate ellipsoid: $V = 0.523 \times (\text{length} \times \text{height} \times \text{width})$ (DePriest, van Nagell et al. 1993).
Results

Study I

Subject characteristics and VAS ratings
The study groups did not differ significantly with regard to baseline characteristics. There were, however, highly significant differences in the VAS ratings for the core symptoms of Premenstrual Dysphoric Disorder (PMDD), "irritability" and "depressed mood", during the last 10 days of the menstrual cycle between the study groups (p < 0.0001, both symptoms).

Estradiol
Serum estradiol levels did not differ significantly between the study groups at baseline; the mean peak estradiol levels reached (at 6.5 h for both groups), the estradiol levels at the point of maximum negative feedback effect on gonadotropins (32 h for both groups) and the AUCs for estradiol (0 h – 144 h) were also similar. Women with premenstrual dysphoria, however, had significantly higher estradiol serum levels at the final sampling point (144 h) than the control group (p = 0.034).

LH
The negative feedback effect of estradiol on serum LH levels occurred promptly in both groups from the time of the estradiol injection, reaching a plateau between 6.5 h and 32 h after the injection. This was followed by the positive feedback phase, with mean peak LH values occurring at 72 h for both groups. Baseline LH levels did not differ significantly between the groups. Women with premenstrual dysphoria displayed significantly higher serum LH levels at the time of maximum negative feedback (nadir levels; 32 h) than did controls (p = 0.01). There was no significant difference in serum LH levels at the time of maximum positive feedback (72 h) between the groups, but the number of positive feedback responses classified as either overt LH surges or LH surge-like reactions was higher in women with pre-
menstrual dysphoria (9/13 versus 3/12; p = 0.047). As with estradiol, serum LH levels were significantly higher in women with premenstrual dysphoria at the end of the challenge (144 h; p = 0.03). The total variance around the mean of serum LH for the entire challenge was significantly greater in women with premenstrual dysphoria than in the asymptomatic controls (0.51 and 0.22, respectively; p < 0.0001). The AOC values for the negative increment in LH levels during the negative feedback phase from 0 h to 32 h and from 0 h to 48 h, adjusted for baseline LH levels, were significantly larger in the premenstrual dysphoria group than in the controls (p = 0.014 and 0.034, respectively). The AUC value for the entire challenge (0 h to 144 h) was 50% larger (p = 0.03) in women with premenstrual dysphoria than in controls; 80% larger in the negative feedback phase (0 h to 32 h; p = 0.002) and 49% larger in the positive feedback phase (32 h to 144 h; p = 0.03).

Association between symptoms and serum LH concentration dynamics
For the premenstrual dysphoria group, the mean VAS score for premenstrual irritability (days 10 to 1 before menstrual onset) in the pre-challenge cycle correlated with the AUC for LH in the negative feedback phase (0 h to 32 h; r_s = 0.58; p = 0.040), and with the AUC for LH during the negative feedback plateau (6.5 h to 32 h; r_s = 0.64; p = 0.022). For the controls, no significant correlations were seen, and both correlation coefficients were negative: r_s = -0.44 (p = 0.154) and r_s = -0.29 (p = 0.366). These opposing correlations in the two study groups yielded a highly significant interaction term between study group and changes in LH levels during the negative feedback phase for LH (32 h - 0 h), with regard to the premenstrual score for irritability, in the pre-challenge cycle (test for interaction p = 0.005).

For the premenstrual dysphoria group, the mean premenstrual VAS score for the symptom bloating in the pre-challenge cycle displayed a strong association with the negative increment (AOC) for LH from 0 h to 48 h (r_s = 0.73; p = 0.0069) and with the AOC for LH over the entire negative feedback phase (r_s = 0.58; p = 0.049, both adjusted for baseline LH values). For the control group, the corresponding associations were positive but nonsignificant.

Within the premenstrual dysphoria group, the explanatory values of the joint FSH and LH changes during the negative feedback phase (0h to 32h) were highest for the VAS-rated symptoms irritability, bloating and depressed mood. The explanatory values of the three composite variables: the accumulated negative mood symptom scores, the accumulated positive mood variable scores, and the accumulated physical manifestation scores, were R^2 = 0.57; R^2 = 0.16 and R^2 = 0.14, respectively.
FSH

The negative feedback effect of estradiol on serum FSH levels was similar in both groups from the time of the estradiol injection, reaching a negative feedback plateau between 32 h and 48 h. This was followed by a positive feedback phase, with a mean peak value for both groups at 120 h after the injection. Serum FSH levels did not differ significantly between the groups at baseline, at the point of maximum negative feedback (32 h for both groups), or during the positive feedback phase. There were no differences in AOCs during the negative feedback phase, or in AUCs throughout the challenge (0 h to 144 h), between the groups.

Association between symptoms and serum FSH concentration dynamics

For the premenstrual dysphoria group, but not the controls, the mean premenstrual score for depressed mood correlated with the baseline FSH concentration \( r_s = 0.60; p = 0.034 \), with the AUC from baseline to the point of maximum negative feedback on FSH (0 h to 32 h; \( r_s = 0.58; p = 0.043 \)), and with the AUC for FSH during the entire FSH negative feedback phase (0 h to 48 h; \( r_s = 0.58; p = 0.043 \)).

Study II

Associations of changes in VAS ratings of mood symptoms with changes in serotonin precursor trapping (11C-5-HTP) between menstrual cycle phases showed a consistent pattern, with several very strong correlations. For negative mood symptoms, negative correlations were found for all ROIs, and for positive mood variables the corresponding correlations were all positive. The core symptoms of premenstrual dysphoria showed strong negative correlations to changes in serotonin precursor trapping in the ‘whole brain’ region; for the symptom irritable \( r_s = -0.83 \) (\( p = 0.010 \); after adjustment for multiple tests \( p = 0.030 \)), and for depressed mood \( r_s = -0.81 \) (\( p = 0.015 \); after adjustment for multiple tests \( p = 0.042 \)).
Figure 2. Association between change in the VAS-rated symptom "depressed mood" and change in trapping of $^{11}$C-labelled 5-hydroxytryptophan ($^{11}$C-5-HTP) in left dorsolateral prefrontal cortex (LPFC).

$r_s = -0.93; p < 0.001$

Figure 3. Changes in VAS-rated variables in relation to changes in trapping of $^{11}$C-labelled 5-hydroxytryptophan for the “whole brain” region of interest. Ranking by strength of association (correlation coefficients).
Changes in serotonin precursor trapping in the eight forebrain ROIs showed significant correlations for depressed mood. In the left dorsolateral prefrontal cortex \( r_s = -0.93 \) (\( p < 0.001 \); adjusted \( p = 0.018 \)), Figure 2. The strongest positive correlations were seen for the nearly opposite mood variables: for happy \( (r_s = 0.95; p < 0.001) \) in the left medial prefrontal cortex and for energetic \( (r_s = 0.92; p = 0.001) \) in the right dorsolateral prefrontal cortex.

The left and right dorsolateral prefrontal cortex ROIs, the left medial prefrontal cortex ROI and the left caudate nucleus ROI, in order, showed the strongest correlations to mood symptoms. The right caudate nucleus ROI showed the weakest correlation to mood symptoms. Changes in the VAS ratings of somatic symptoms generally had weaker or no correlations with changes in serotonin precursor trapping, with the exception of the right caudate nucleus ROI, which showed strong positive correlations with the symptoms pelvic pain \( (r_s = 0.88; p = 0.004) \) and breast tenderness \( (r_s = 0.81; p = 0.014) \). No other significant correlation with somatic variables was seen for this ROI. The correlations between changes in VAS ratings of the 14 variables evaluated and changes in serotonin precursor trapping in the "whole brain" ROI are ranked in order of their strength in Figure 3.

In summary, the results indicate significant correlations between changes in perceived irritability and depression of mood and changes in brain serotonin precursor trapping \( (^{11}C-5-HTP) \).

**Study III**

The 5HT\(_{1A}\) partial agonist buspirone was significantly superior to placebo in the treatment of premenstrual dysphoria as assessed by self-rated global improvement. Buspirone appeared to reduce VAS-self-rated irritability better than placebo.

The combined SRI and 5HT\(_2\) antagonist nefazodone was not shown to be better than placebo for the treatment of premenstrual dysphoria.

Sexual dysfunction was not more common in women treated with buspirone or nefazodone than in those treated with placebo.

**Study IV**

**Subject characteristics**

Cases and controls were almost identical with regard to age, and there were no statistically significant differences in descriptive characteristics. However, a tendency for control women to be somewhat heavier was noted, with 5 of the 26 women weighing more than 75 kg, the heaviest 117.5 kg, com-
pared to 3 of the 26 women in the premenstrual dysphoria group, the heaviest 80.6 kg. Accordingly, a BMI score above 25 was seen in 11 controls and in 10 cases, and a BMI score above 30 was recorded in 4 controls but in none of the cases.

For cases, mean VAS ratings of the five last days of the three cycles rated, compared to the mean of the two cycles rated in controls were for the symptom “irritability”: 66.4 ± 23.5 mm, compared to 4.1 ± 6.2 mm (p < 0.0001), and for “depressed mood”: 49.2 ± 28.6 mm for cases, compared to 3.1 ± 5.5 mm for controls (p < 0.0001).

Ovarian sonographic morphology

Of the 52 women participating in the study, both ovaries were identified in 51 (only the right ovary was identified in one control). The occurrence of PCO morphology did not differ significantly between the groups. In four of the 26 women with premenstrual dysphoria and 7 of the 26 control women, both ovaries showed polycystic morphology. One woman with premenstrual dysphoria had unilateral PCO. The total frequency of PCO was therefore 19.2% in the cases and 26.9% in the controls. Of the 4 controls with a BMI above 30, one showed PCO morphology. The overall distributions of ovarian morphology type, total number of ovarian follicles, and total ovarian volume did not differ significantly between the groups. There was a similar, highly significant correlation between total number of follicles and total ovarian volume in both groups: rs = 0.78, p < 0.0001 in cases; rs = 0.87, p < 0.0001 in controls.

Serum hormone levels

Serum levels of free testosterone, total testosterone, androstenedione, dehydroepiandrosterone sulfate and the ratios of testosterone/SHBG did not differ significantly between the groups. Nor were serum levels of FSH, LH, estradiol, SHBG or LH/FSH or estradiol/SHBG ratios on menstrual day 3 significantly different between the groups.
General discussion

This thesis has shown that the brain response to a gonadal steroid hormone provocation is stronger and less dampened in women with severe premenstrual dysphoria than in asymptomatic women of similar age (I). Thus, it was revealed that neuroendocrine regulation in women with severe premenstrual dysphoria was different compared to that of the asymptomatic control women (I). This finding strongly supports the hypothesis of increased brain sensitivity to gonadal hormones in women with premenstrual dysphoria (Schmidt, Nieman et al. 1998). Indirectly, it also supports the commonly held belief that the fluctuations in gonadal hormones during the menstrual cycle elicit the mood and physical symptoms of premenstrual dysphoric women, because of their increased cerebral sensitivity to such fluctuations (Schmidt, Nieman et al. 1998; Steiner and Pearlstein 2000).

Our results also imply a constitutively increased GnRH activity in women with premenstrual dysphoria (I). According to the current understanding of GnRH regulation (Smith and Jennes 2001; Petersen, Ottem et al. 2003; Han, Todman et al. 2004), this, together with the diminished dampening of the entire feedback response of LH to the estradiol provocation, all point to reduced GABAergic and serotonergic activity in this group of women (I).

Our findings of diminished irritability and significantly improved well-being with the intake of the partial serotonin receptor agonist buspirone, compared to placebo (III), also imply serotonergic underactivity at least during the symptomatic phase in premenstrual dysphoric women.

The serotonin hypothesis of the premenstrual dysphoric disorder (PMDD) (Steiner and Pearlstein 2000; Parry 2001; Eriksson, Andersch et al. 2002) suggests that there is a causal connection between diminished serotonergic activity in the brain and the development of premenstrual mood symptoms. Our findings of strong correlations between decline in brain presynaptic serotonin precursor trapping and exacerbation of the core mood symptoms of premenstrual dysphoria (II) are consistent with the serotonin hypothesis of this disorder. Brain trapping of $^{11}$C-5-hydroxytryptophan ($^{11}$C-5-HTP) has been shown to strongly correlate to brain serotonin production (Hagberg, Torstensson et al. 2002), so this tracer might be considered a marker of presynaptic serotonin synthesis (Hagberg, Torstensson et al. 2002). However, such synthesis does not only take place in serotonin neurones (Lloyd and Hornykiewicz 1972). The formation of $^{11}$C-serotonin from $^{11}$C-5-hydroxytryptophan in the brain is dependent upon the activity of the enzyme
aromatic amino acid decarboxylase (Hartvig, Lindner et al. 1992; Hartvig, Tedroff et al. 1993; Lindner, Hartvig et al. 1997), which is present in monoaminergic neurones (Lloyd and Hornykiewicz 1972; Hokfelt, Fuxe et al. 1973; Eaton, Gudehithlu et al. 1993) as well as in so called D-cells (Nagatsu, Yamamoto et al. 1979; Jaeger, Teitelman et al. 1983). Since our PET-study findings are only correlative and do not reveal causes, they do not by themselves directly support the serotonin hypothesis of premenstrual dysphoria (II). However, the findings of our pharmacological study do (III).

Irritability, the most important of the core symptoms of premenstrual dysphoria (Eriksson 1999), is a major symptom in states of reduced serotonergic activity (Young and Leyton 2002).

Irritability in our challenge study showed the strongest explanatory value of the joint LH and FSH negative feedback responses (I). In the PET study, changes in the irritability scores showed the strongest negative correlation to the changes in serotonin precursor brain trapping (II). In the treatment trial, irritability was the only symptom that improved significantly with the partial serotonin receptor agonist treatment (III). The results of the ovarian morphology study (IV) did not support the hypothesis that luteal phase irritability is induced by testosterone (Eriksson, Sundblad et al. 1992).

Irritability is a primary symptom in states of diminished prefrontal-cortex inhibition of negative affect of limbic origin (Davidson, Putnam et al. 2000; Bufkin and Luttrell 2005). Apart from during pure hypo-serotonergic states (Menkes, Coates et al. 1994; Bond, Wingrove et al. 2001), severe irritability is often seen in post-lesional conditions such as after head injury (Ryan 2000) and in dementia of various origins (Ryan 2000). Irritability is also frequently seen in states of substance withdrawal (Hoaken and Stewart 2003).

The temporally restricted exhibition of severe irritability typically seen during the luteal and especially the late-luteal phase in women with premenstrual dysphoria, with the absence of these symptoms for at least a week after menstruation (American Psychiatry Association 1994), indicates that it is self-evident that there are state-dependent underlying mechanisms. There is strong evidence that these mechanisms at least partly are serotonin-mediated (Menkes, Coates et al. 1994; Bond, Wingrove et al. 2001). However, pathogenetic mechanisms related to “substance” withdrawal in premenstrual dysphoria are not only possible, but also plausible (Casper, Graves et al. 1987; Giannini, Martin et al. 1990; Smith, Gong et al. 1998; Backstrom, Andersson et al. 2003).

Our buspirone findings are directly supportive of a serotonin-mediated effect on irritability in this condition (III). Although our study findings do not directly support underlying “substance” withdrawal mechanisms, they are all consistent with the existence of such mechanisms (I, II, III, IV).

To be speculative, such a “substance” or “coctail of substances” that causes a withdrawal-like reaction, is most probably estradiol, possibly in
combination with the progesterone/progesterone metabolite/neurosteroid central nervous system “tranquilizers”, which are currently in the focus of almost anything to do with brain function. In other words, a repetitive steroid hormone withdrawal reaction, superimposed on a brain that is more vulnerable to afferent input, possibly because of constitutional, or acquired serotonin dysregulation, is currently the most plausible pathogenetic mechanism behind severe premenstrual dysphoria.

In agreement with our findings of increased brain sensitivity to gonadal hormones in women with premenstrual dysphoria (I), there is one earlier study that has convincingly shown this (Schmidt, Nieman et al. 1998). In an ingeniously designed study, Schmidt and co-workers recorded the induction of negative mood by either estradiol, or progesterone, alone, as well as in combination (Schmidt, Nieman et al. 1998).

Also, in line with our findings of altered neuroendocrine regulation in women with premenstrual dysphoria, other groups have reported differences in HPG-axis regulation in this condition. Facchinetti and co-workers found changes in the pulsatile pattern of LH secretion, comprising increased frequency but reduced amplitude of plasma LH pulses (Facchinetti, Genazzani et al. 1990; Facchinetti, Genazzani et al. 1993). However, other groups have not found such differences (Reame, Marshall et al. 1992). Alterations in the time lag between LH and progesterone pulses in women with premenstrual dysphoria have been reported (Lewis, Greenblatt et al. 1995). Furthermore, defects in hypothalamic opioid activity have been claimed by some (Facchinetti, Martignoni et al. 1988; Facchinetti, Fioroni et al. 1994; Rapkin, Shoupe et al. 1996), but refuted by others (Chuong and Hsi 1994).

Alterations in HPA-axis regulation in women with premenstrual dysphoria have been reported in several studies (Rabin, Schmidt et al. 1990; Facchinetti, Fioroni et al. 1994; Odber, Cawood et al. 1998), but disputed by others (Endicott, Amsterdam et al. 1999). Aberrations in autonomic tone (Landen, Wennnerblom et al. 2004) as well as in cardiovascular and emotional responses to stress (Van Goozen, Frijda et al. 1996) have been reported. Morofushi and co-workers showed abnormal functioning of the internal circadian clock in women with the premenstrual syndrome (Morofushi, Shinohara et al. 2001).

To conclude, there are substantial indications in the literature of central regulatory disturbances in premenstrual dysphoria, lending further support to our findings.

To date, there is no study that convincingly shows that brain serotonin activity is lower in women with premenstrual dysphoria than in asymptomatic women, something we hope we will be able to report on in the near future. The very strong support for the commonly held view of a lower serotonergic brain activity in women with premenstrual dysphoria thus rests on indirect evidence. The strongest evidence is that serotonin reuptake inhibitors effectively relieve symptoms of premenstrual dysphoria in the majority of sub-
jects (Steiner, Steinberg et al. 1995; Yonkers, Halbreich et al. 1997; Dimmock, Wyatt et al. 2000; Wyatt, Dimmock et al. 2002), that serotonin-releasing drugs (Brzezinski, Wurtman et al. 1990; Su, Schmidt et al. 1997) and the serotonin precursor tryptophan (Steinberg, Annable et al. 1994) alleviate such symptoms and that tryptophan depletion aggravates premenstrual dysphoria (Menkes, Coates et al. 1994; Bond, Wingrove et al. 2001).

In the studies by Menkes et al. and by Bond et al., acute lowering of dietary tryptophan intake in women with premenstrual dysphoria, with the inferred subsequent decrease in brain serotonin production, drastically aggravated the negative mood symptoms, especially irritability and aggression (Menkes, Coates et al. 1994; Bond, Wingrove et al. 2001). These findings are in line with the reduction in irritability we recorded in women treated with the partial 5-HT$_{1A}$ receptor agonist buspirone.

Methodological considerations

The small number of samples recruited, with the subsequent issue of power, and the risk of type I, and particularly type II, errors is a common shortcoming of all the studies in this thesis. This is highly regrettable but reflects the current clinical reality with respect to the time and money available for such research. However, the strict operant criteria for inclusion, also contributed to the small size of the samples recruited.

In Study I, the asymptomatic controls were recruited in-house, among hospital employees, which might have introduced a selection bias, possibly influencing the psychological reactions to venous puncture between groups. One other shortcoming of the study was the long interval between blood samples, which might have interfered with the interpretation of actual time differences in negative or positive feedback responses between the groups, and were not detectable with the protocol used. Furthermore, we have not been able to check or measure differences between the groups in endogenous estradiol production throughout the experiment, other than at baseline.

In Study II the calendar timing of the PET scans was not always optimal, but reflects the realities in the clinical research setting. Theoretically the counterbalanced study design was a strength of the study, but it did introduce unphysiological “split cycles” which, however, seemed to reduce cycle phase differences, thus reducing the strengths of correlations.

Our $^{11}$C-5-hydroxytryptophan trapping outcome measure was unspecific, albeit of topographically varying degree, depending on the brain region registered. This is because the enzymatic conversion of $^{11}$C-5-hydroxytryptophan to $^{11}$C-serotonin can take place in any cell of the brain containing aromatic amino acid decarboxylase (AAAD), and the relative content of serotonin neurones to others containing AAAD, varies with brain region. Arterial blood sampling during the PET-recordings was not done, so we
were not able to calculate exact brain levels of the $^{11}$C-5-hydroxytryptophan trapped. Another methodological weakness of Study II was that magnetic resonance imaging (MRI) and computerized tomography (CT) were not available for anatomical determination of the ROIs. Instead we used summation images of $^{15}$O-H$_2$O-flow scans, aided by the simultaneous comparison of ROI configurations in the Computerized Brain Atlas (CBA) (Greitz, Bohm et al. 1991). Compared to MRI-aided drawing, the precision of the ROIs was thus negatively affected, which has to be taken into consideration when interpreting our data.

In Study III, the small size of the study groups and the subsequent low statistical power of the trial were the fundamental weaknesses of the study. This was accentuated by the drop-outs from the study groups.

In Study IV, as stated above, the number of participants is a shortcoming of the study. Because of the small groups, the lack of significant differences in the prevalence of PCO, overall ovarian classification, ovarian volume, number of follicles, and serum levels of androgens between the groups could be the result of a type II error. However, the point estimate indicating a lower prevalence of PCO in women with premenstrual dysphoria, against the study hypothesis, given an unbiased control recruitment, suggests that a type II error regarding differences in PCO prevalence is unlikely. The possibility of subtle group differences in the timing of sample taking, together with the well-known variability in hormone levels during the menstrual cycle, also calls for caution in the interpretation of the results of Study IV.

One of the strengths of Study I was the fact that all the women in the premenstrual dysphoria group were consecutively self-referred, seeking help for severe, intractable mood symptoms, and did not just respond to an advertisement. Further strengths of the study included the similarity in age, body-composition and parity between the study groups. All participants adhered meticulously to the study protocol, manifested in the collection of 274 of the intended 275 blood samples.

A strength of Study II was the thorough standardization of the study conditions. Each participant was her own “control”. PET-registrations were done by 9 a.m., in the fasting state, in a standardized supine position and with standardized afferent input as far as light, sound and temperature. Since it was important not to have subjects fall asleep during PET scans, we used a standardized sound input in the form of a recorded tape with melodiou, rhythmic but, in our opinion, rather neutral jazz music (Jan Johansson, “Folkvisor”, Heptagon Records 1994, Heptagon Records, Scandinavia).

One strength of Study III was the randomized, double-blind, placebo-controlled design and the strict operant criteria for the inclusion of the study participants.

In Study IV, the striking similarity in age between the groups, the clearly defined inclusion requirements, the uniformity in blood sampling and sample
Future study plans

Using exactly the same experimental design as in Study II, we have after renewed approval from the Medical Ethics committee, extended the PET study with four more premenstrual dysphoria subjects and eight asymptomatic control women, of whom all but two are already PET-registered. Thus, in the near future we hope to be able to report on comparisons of $^{11}$C-5-hydroxytryptophan trapping between these groups. We will also analyse correlation of $^{11}$C-5-hydroxytryptophan trapping with chronological age within and between the groups. These comparisons we hope will give new important insight within this field.

Also, we are about to analyze correlations between relative blood-flow data and data on $^{11}$C-5-hydroxytryptophan trapping, already collected, from Study II.

We have also started collaboration with Professor Medvedev at the Department for Mathematics and Computer science/Systems and Control, initiated by our gonadotropin response findings of Study I.

Ideas about and plans for further research within the extentions of the topics of this thesis are abundant.
Conclusions

I. Women with premenstrual dysphoria showed a stronger and less dampened response of luteinizing hormone (LH) to an estradiol challenge than asymptomatic women, indicating an altered neuroendocrine regulation.

In women with premenstrual dysphoria, the LH response was correlated to the severity of irritability and bloating, and the early follicle-stimulating hormone (FSH) response was correlated to the severity of depressed mood.

II. In women with premenstrual dysphoria, strong, consistent correlations were found between changes in mood symptoms and changes in brain trapping of the immediate serotonin precursor, from the mid-follicular to the late luteal phase.

The strongest correlations were seen for the cardinal mood symptoms of premenstrual dysphoria, and for their opposites. Physical symptoms showed weaker or no correlations, with the exception of nociceptive symptoms from erogenous body regions which showed strong positive correlations to serotonin precursor trapping in the right caudate nucleus.

The findings are consistent with the serotonin hypothesis of premenstrual dysphoria, and might possibly explain the observed effects of serotonin-augmenting drugs in this condition.

III. The partial 5-HT$_{1A}$ receptor agonist buspirone was superior to placebo, in the treatment of premenstrual dysphoria.

The weak SRI and 5-HT$_{2}$ receptor antagonist nefazodone was not superior to placebo.

For women with premenstrual dysphoria in need of medication, and who do not tolerate SRIs because of the sexual side-effects, buspirone may be an alternative drug, since it had no adverse effects on sexual function.
IV. The prevalence of polycystic ovaries and serum levels of androgens were not higher in women with premenstrual dysphoria than in their asymptomatic counterparts.

The findings are not consistent with the hypothesis that irritability in women with premenstrual dysphoria is induced by elevated testosterone levels.
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