From THE DEPARTMENT OF CLINICAL NEUROSCIENCE
Karolinska Institutet, Stockholm, Sweden

PET EVALUATION OF CENTRAL SEROTONERGIC NEUROTRANSMISSION IN WOMEN

Hristina Jovanovic

Karolinska Institutet

Stockholm 2008

Cover picture: 3-D volume rendering of [^{11}C]WAY100635 and [^{11}C]MADAM binding in human brain by Zsolt Cselenyi

ISBN 978-91-7357-510-2

Printed by

REPROPRINT AB
Stockholm 2008
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

To my son Mihailo

ABSTRACT

The serotonin (5-HT) system is of central interest in the patophysiology and treatment of several psychiatric disorders including depression, anxiety and suicide. In women, functioning of the 5-HT system is of particular importance since they have been found to suffer more often from 5-HT-associated disorders compared to men. The aim of the present thesis was to further explore the central serotonergic system in women by examining 5-HT_{1A} receptors and 5-HTT binding in two psychiatric disorders, borderline personality disorder (BPD) and premenstrual dysphoric disorder (PMDD), during different phases of the menstrual cycle and in relation to gender.

In the first study positron emission tomography (PET) and [^{11}C]WAY100635 were used to examine 5-HT_{1A} receptors in control group of women and in women with PMDD. Two PET examinations were performed in each subject, one before (follicular phase) and one after ovulation (luteal phase). The 5-HT_{1A} binding potential (BP) was measured in six regions of interest and calculated according to the simplified reference tissue model (SRTM). For the region of dorsal raphe nuclei, there was a significant difference between the groups in the change of 5-HT_{1A} receptor binding. The study provides principally new support *in vivo*, for a serotonergic dysregulation in women with PMDD.

In the second study, PET and selective radioligands [^{11}C]WAY100635 and [^{11}C]MADAM were used to study differences in 5-HT_{1A} receptors and 5-HTT BPs between healthy women and men. The BPs were estimated both on the level of anatomical regions and voxel wise, derived by the SRTM and wavelet/Logan plot parametric image techniques respectively. The volume of interest (VOI)-based analysis revealed higher mean 5-HT_{1A} BP and lower mean 5-HTT BP values in women compared to men. The parametric analysis of [^{11}C]WAY100635 and [^{11}C]MADAM images showed similar results to those obtained with VOI analysis. In women, a positive correlation between 5-HT_{1A} receptor and 5-HTT BPs for the region of hippocampus was found. Sex differences in 5-HT_{1A} receptor and 5-HTT binding may reflect biological distinctions in the 5-HT system contributing to sex differences in the prevalence of psychiatric disorders such as depression and anxiety. The result may help understanding sex differences in drug treatment responses to drugs affecting the 5-HT system.

In the third study, healthy women were investigated in the follicular and luteal phase of the menstrual cycle with radioligands [^{11}C]WAY100635 and [^{11}C]MADAM to study 5-HT_{1A} and 5-HTT BPs. The BPs values were quantified using the SRTM. The phases of the menstrual cycle were characterized by transvaginal ultrasound (TVS) and plasma levels of hormones estradiol (E_2), progesterone (P_4), follicle stimulating hormone (FSH) and luteinising hormone (LH). The 5-HT_{1A} receptor and 5-HTT BPs did not significantly differ between follicular and luteal phases in any of the investigated regions. There were no significant correlations between hormones E_2 or P_4 and 5-HT_{1A} receptors BP or 5-HTT BP in any of the regions, neither did the change in plasma E_2 or P_4 correlate with the change in 5-HT_{1A} BP or 5-HTT BP values in brain regions. The results provide principally new *in vivo* evidence on human female biology, suggesting no difference in 5-HT_{1A} receptors and 5-HTT binding between the phases of the menstrual cycle in healthy women that can be revealed with the present methodology.

In the fourth study, 5-HT_{1A} receptor BP in female patients with BPD and controls were examined. Out of two hundred female patients with BPD, seven met inclusion criteria (i.e. drug naïve including, no previous or present alcohol or drug abuse/dependency). Eight age and sex matched controls were recruited. PET and selective radioligand [^{11}C]WAY100635 were used to study brain 5-HT_{1A} receptor BP in dorsolateral prefrontal cortex, anterior cingulate, orbitofrontal cortex, amygdala, hippocampus, insula, temporal cortex and dorsal raphe nuclei. BP was estimated using the SRTM. Compared to controls, women with BPD had a significantly lower 5-HT_{1A} receptor BP in the brain regions examined. The results suggest a lower 5-HT_{1A} receptor BP in drug naïve patients with BPD. The finding corroborates previous studies suggesting the impairment of the 5-HT system in patients with BPD.

In conclusion, the present thesis provides new evidence for the implication of the serotonin system in psychiatric disorders in women, effects of gonadal hormones and sex differences in serotonergic neurotransmission.

LIST OF PUBLICATIONS

I. Jovanovic H, Cerin Å, Karlsson P, Lundberg J, Halldin C, Nordström AL. A PET study of 5-HT_{1A} receptors at different phases of the menstrual cycle in women with premenstrual dysphoria. *Psychiatry Research: Neuroimaging* 2006 Dec; 1;148(2-3):185-93.

II. Jovanovic H, Lundberg J, Karlsson P, Cerin A, Saijo T, Varrone A, Halldin C, Nordström AL. Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. *NeuroImage* 2007 Oct 25; [Ahead of print].

III. Jovanovic H, Karlsson P, Cerin Å, Halldin C, Nordström AL. 5-HT_{1A} receptor and 5-HTT binding during the menstrual cycle in healthy women examined with [^{11}C] WAY100635 and [^{11}C] MADAM PET. *Submitted to PsychiatryResearch: Neuroimaging.*

IV. Andersson EE, Jovanovic H, Karlsson P, Halldin C, Nordström AL, Åsberg M, Farde L Lower Serotonin-1A Receptor Binding in Drug Naïve Patients with Borderline Personality Disorder: A PET Study Using [^{11}C]WAY100635.*Submitted to Biological Psychiatry.*

CONTENTS

LIST OF ABBREVIATIONS

AADC	aromatic amino acid decarboxylase
α-[^{11}C]MTrp	α-[^{11}C]methyl-L-tryptophan
BBB	blood brain barrier
BDNF	brain derived neurotropic factor
BP	binding potential
BPD	borderline personality disorder
CL	corpus luteum
CNS	central nervous system
CSF	cerebrospinal fluid
DAT	dopamine transporter
DASB	diphenyl sulfide derivative 3-amino-4-(2-dimethylaminomethylphenylsulfanyl)- benzonitrile
E_2	estradiol
EB	estradiol benzoate
8-OH- DPAT	8- hydroxyl-2-(di-n-propylamino)tetralin
FSH	follicle stimulating hormone
FWHM	full width at half maximum
GH	growth hormone
5-HT	5-hydroxytryptamine, serotonin
5-HIAA	5-hydroxyindoleacetic acid
5-HTP	5-hydroxytryptophan
5-HTT	serotonin transporter
HBA	human brain atlas
[^{123}I]β-CIT	iodine-123-2 beta-carbomethoxy-3beta-(4-iodophenyltropane) ([^{123}I]β-CIT)
McN 5652	isoquinoline derivativetrans-1,2,3,5,6,10-beta-hexahydro-6-[4-(methylthio) phenyl[pyrrolo-[2,1-a]isoquinoline
LH	luteinising hormone
MAO	monoamine oxidase
MNI	Montreal Neurological Institute
mRNA	messenger ribonucleic acid
MRI	magnetic resonance imaging
WAY100635	N-(2-(4-(2-meathoxyphenyl)-l-piperazinyl)ethyl-N-(2- pyridyl) cyclohexanecarboxamide
MADAM	N,N-Dimethyl-2-(2-amino-4-methylphenylthio) benzyl amine
PET	positron emission tomography
PMDD	premenstrual dysphoric disorder
P_4	progesterone
ROI	regions of interest
SCID-I	Structural Clinical Interview for Disorders on Axis I
SCID-II	Structural Clinical Interview for Disorders on Axis II
SPECT	single-photon emission computed tomography
SPM	statistical parametric mapping
SRTM	simplified reference tissue model
SSRIs	selective serotonin reuptake inhibitors
TAC	time activity curve
TRP	tryptophan
TRH	tryptophan hydroxylase
VOI	volume of interest

INTRODUCTION

The serotonin (5-HT) system was one of the first neurotransmitter systems generated during mammalian development. Phylogenetic comparisons between three major classes of 5-HT receptors in various species suggested that the primordial 5-HT receptor was developed several hundreds million years ago (Peroutka 1994). The phylogenesis and its wide distribution within the central nervous system (CNS) render this system a variety of physiological and behavioral functions, including regulation of sleep, temperature, sexuality, perception, emotion, and cognition. Dysfunctions of 5-HT neurotransmission have been associated with several psychiatric disorders in humans, such as depression (Deakin et al 1990), anxiety disorders (Graeff et al 1996; Miller et al 2000), borderline personality disorder (Leyton et al 2001), and suicidal behavior (Asberg et al 1976). Moreover, this system is an important target for the pharmacological action of selective serotonin reuptake inhibitors (SSRIs), which are drugs widely prescribed in the treatment of depression and anxiety (Nutt et al 1999).

In women, functioning of the 5-HT system is of particular importance since they have been found to suffer more often from 5-HT-associated disorders than men. Several epidemiological studies reported rates two times higher for depression in women compared to men (Kessler et al 1993, Kessler et al 1994). Similarly, it has been shown that women more often than men suffer from anxiety disorders (Pigott 1999) and that the sexes differ in suicidal behavior (Lewinsohn et al 2001). The pathophysiological mechanisms underlying these differences are, however, unknown. There is considerable preclinical and clinical evidence supporting a sex difference in serotonergic neurotransmission, which might underlie differences in the prevalence of 5-HT-related disorders. These differences could be, to some degree, modulated by the action of gonadal hormones. In support for this hypothesis, it has been observed in women that the highest prevalence of depression occurs during the reproductive years when the actions of gonadal hormones are most apparent (Pearlstein et al 1997). Additionally, the reproductive-related mood disorders in women, such as premenstrual dysphoric disorder, postpartum depression, and perimenopausal depression, have all been associated with fluctuations in the levels of ovarian hormones (Steiner et al 2003).

The development of positron emission tomography (PET), as a highly sensitive imaging technique, and selective radioligands has made it feasible to study wide numbers of biomarkers within the central nervous system. Using this technique direct measurement of serotonin biomarkers in the normal human brain can be obtained and related to molecular changes within the central nervous system that are associated with different psychiatric disorders in humans.

The overall aim of the present thesis was to further explore the central serotonergic system in women by examining 5-HT_{1A} receptors and 5-HTT binding in two psychiatric disorders, borderline personality disorder (BPD) and premenstrual dysphoric disorder (PMDD), during different phases of the menstrual cycle and in relation to gender.

The Serotonin System

Serotonin

Serotonin was first isolated in 1884, when it was characterized as a vasoactive substance in serum that appeared during the moment of blood clotting. In the 1940s, Rapport crystallized the same substance from the bovine serum and showed that chemically it was 5-hydroxytryptamin (Rapport et al 1948). In Italy, Erspamer et al., independently studied the pharmacology of substances associated with enterochromaffin cells in mammalian gut (Erspamer et al 1940), and extracted a smooth muscle contractile substance called enteramine. When synthetic 5-hydroxytryptamine became available, it was shown that enteramine was probably 5-hydroxytryptamine, which was then initially given the name 'serotonin' (Erspamer et al 1952). The name indicated its origin from the blood serum and its effects on the vascular muscle tone.

Serotonin is found in many cells of body tissues, such as platelets, mast cells, enterohromaphin cells, and neurons. However, the highest concentrations of 5-HT are located in enterohromaphin cells of the gut (90%) and platelets (8-10%), whereas only about 1-2% of the total 5-HT content is present in the central nervous system (CNS). Since 5-HT cannot readily pass the blood-brain barrier, the serotonergic neurons in the brain synthesize their own transmitter.

Serotonergic pathways in the human brain

In 1964, Dalhström and Fuxe were the first to describe anatomical localization of the serotonergic pathways in the CNS (Dahlstroem and Fuxe 1964). Using histochemical fluorescence techniques they mapped the serotonergic cell bodies within the raphe nuclei in the brainstem in rodent. Later studies have shown a similar distribution of the serotonergic pathways in human brain (Tork 1990).

Serotonergic neurons originate from the clusters of neuronal cell bodies situated in the raphe nuclei, which are structures localized close to the midline in the mesencephalon and rostral pons. Projections arising from these nuclei form two principal serotonergic pathways. The ascending pathway originates from the dorsal and median raphe nuclei, and innervates the limbic system, hypothalamus, striatum, thalamus and cerebral cortex (Steinbusch 1981). The descending pathway arises from the raphe magnus, the obscure nuclei and serotonergic cells in the ventrolateral medulla, and project mainly to the gray matter of the spinal cord (Figure 1).

Brain serotonin synthesis, reuptake and metabolism

In the brain, 5-HT is formed in a two-step reaction from the essential amino acid tryptophan, which originates from diet. The first step in this pathway involves hydroxylation of tryptophan to form 5-hydroxytryptophan (5-HTP) the presence of which was demonstrated in the brain tissue by Grahame-Smith (Grahame - Smith 1964). This reaction is catalyzed by the enzyme tryptophan hydroxylaze. The second step is dexarboxylation of the 5-HTP into 5-HT

by aromatic amino acid decarboxylase (AADC). AADC was found in 5-HT and catecholamine-containing neurons in the CNS (Hokfelt et al 1973).

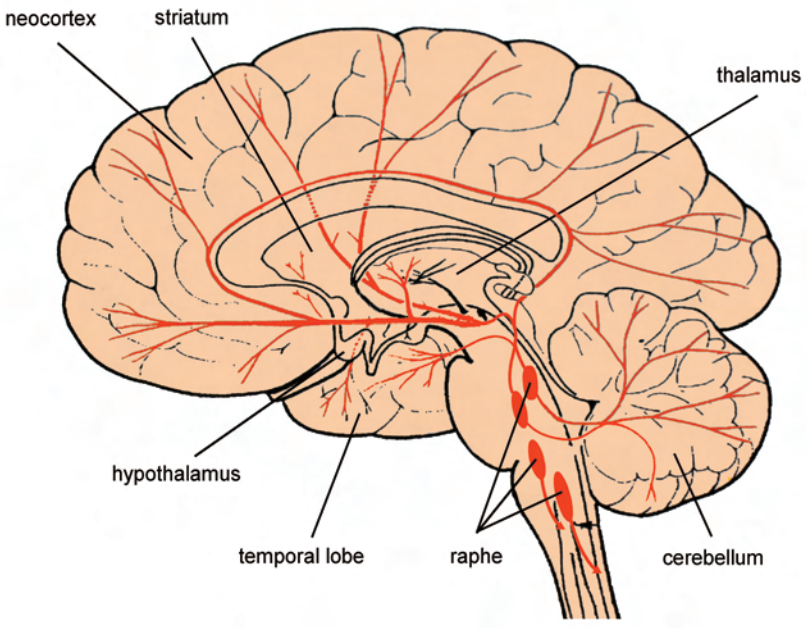


Illustration: Emil Rangden

Figure 1. *Serotonergic pathways in the human brain. Mediosagittal view showing the raphe nuclei localized close to the midline in the mesencephalon and rostral pons containing the 5-HT cell bodies. Projections arising from raphe nuclei form two principal serotonergic pathways: ascending and descending.*

The newly synthesized 5-HT is stored in vesicles of the nerve terminal. Following neural stimulation, 5-HT is released from the vesicles via exocytosis into the synaptic cleft. The activity of the 5-HT system is then mediated by binding to several distinct receptor proteins. Most of the released 5-HT is transported back into the nerve terminal by a reuptake mechanism of the 5-HT transporter (5-HTT). 5-HTT is a plasma membrane protein predominantly located in presynaptic membrane of the nerve terminal, but also on the 5-HT cell bodies and dendrites in the midbrain raphe. The 5-HTT is the primary mechanism for regulation of synaptic 5-HT concentrations.

The major route of 5-HT metabolism in the brain is through oxidative deamination. The 5-HT molecules that are transported back into the neuron are either bound to storage vesicles or exposed to mitochondrial enzyme monoamine oxidase (MAO) in the nerve terminal. The final product of this process is 5-hydroxyindoleacetic acid (5-HIAA), which then diffuses out of the neuron and, to some degree, enters the cerebrospinal fluid (CSF). The pathway for the

5-HT synthesis and degradation in the brain is shown in Figure 2.

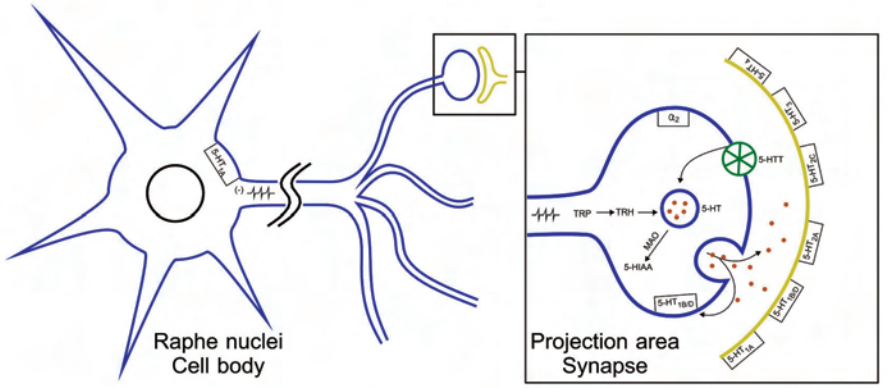


Illustration: Emil Rangden

Figure 2. *Illustration showing a serotonergic neuron with the cell body in the raphe nuclei (left) and a synaptic region with postsynaptic receptors (right, blow up). Major steps in the synthesis, release, reuptake and metabolism of serotonin are indicated. 5-HT, serotonin; 5-HTT, serotonin transporter; 5-HIAA, 5-hydroxyindoleacetic acid; MAO, monoamine oxidase; TRP, tryptophan; TRH, tryptophan hydroxylase. After Blier and de Montigny, 1999.*

Serotonin receptors

The diverse pharmacological actions of 5-HT have been intensely studied since its identification in the 1930s. These studies have led to the discovery and characterization of a multitude of 5-HT receptors.

In 1957, Gaddum and Picarelli demonstrated the presence of two different classes of 5-HT receptors in the ileum of guinea pigs (Gaddum and Picarelli 1957). These receptors were referred to as 'type D' and blocked by dibenzyline, and 'type M', blocked by morphine. In the 1970s, the development of the radioligand binding techniques with utilization of the [^{3}H]5-HT, [^{3}H]spiroperidol and [^{3}H] lysergic diethylamide acid ([^{3}H] LSD), helped identification of two distinct types of 5-HTreceptors (5-HT_1 and 5-HT_2) (Peroutka and Snyder 1979). Since then, several classification systems have been proposed for 5-HT receptors, based on operational (function, antagonism, location), transductional (G-protein, ion channel), and structural (gene sequence, chromosome location) criteria (Hoyer et al 2002).

To date, seven 5-HT receptor families have been identified i.e., 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 receptors (Hoyer et al 2002). These families have further been subdivided into 14 different 5-HT receptor subtypes. Of the 14 subtypes, 5-HT_3 receptor is the only 5-HT receptor coupled to an ion channel.

All other 5-HT receptors interact with regulatory G-proteins, a common feature of which is their ability to shift between high and low affinity states with regard to agonist binding. The 5-HT_1 receptor family is negatively coupled to adenylyl cyclase, and 5-HT_2 receptors mediate activation of protein kinase C via increased phosphoinositide metabolism, while the 5-HT_4, 5-HT_6 and 5-HT_7 receptor subtypes are positively coupled to adenylyl cyclase (Hoyer et al 2002).

The 5-HT_{1A} receptor

The most widely investigated serotonin receptor is the 5-HT_{1A} receptor subtype, the functional properties of which were characterized approximately 20 years ago using the selective 5-HT_{1A} receptor agonist, 8- hydroxyl-2-(di-n-propylamino)tetralin (8-OH- DPAT), in experiments with animals (Arvidsson et al 1981; Hjorth et al 1982). Since then, the 5-HT_{1A} receptors has been the subject of extensive investigation.

5-HT_{1A} receptors are largely distributed throughout the central nervous system. The highest density of 5-HT_{1A} receptors in human brain is found in the hippocampus, the superficial layers of the cortex and the raphe nuclei (Hall et al 1997; Pazos et al 1987). In forebrain regions, 5-HT_{1A} receptors are expressed postsynaptically on the target neurons. At the cellular level they are localized on dendrites of the pyramidal cells of the hippocampus and granule cells of the dentate gyrus (Burnet et al 1995). In dorsal raphe, 5-HT_{1A} receptors are localized on 5-HT cell bodies and dendrites of 5-HT neurons, and are, hence, called somatodendritic autoreceptors (Sotelo et al 1990). Stimulation of 5-HT_{1A} autoreceptors inhibits the firing of serotonin neurons and reduces 5-HT release and neurotransmission in cortical regions (Corradetti et al 1998).

The 5-HT_{1A} receptor has been linked to a variety of behaviors, such as sexual behavior, stress reaction, and feeding behavior, as demonstrated in animal studies. Activation of 5-HT_{1A} receptors in the ventromedial hypothalamic nucleus by 8-OH- DPAT inhibits lordosis, the effect of which is attenuated by estrogen replacement in ovarectomized rats (Uphouse et al 1996). In male rats, androgen increases the affinity of hippocampal 5-HT_{1A} receptors and counteracts the receptor regulation induced by chronic stress in the hippocampus (Flugge et al 1998). These examples indicate significant effects of action of gonadal hormones on the 5-HT_{1A} receptors system.

Involvement of 5-HT_{1A} receptors has been demonstrated in several major psychiatric disorders, such as depression, anxiety, and schizophrenia. Several postmortem studies of human brain from suicide victims and schizophrenia patients have reported an elevated 5-HT_{1A} receptor binding in the neocortex (Hashimoto et al 1993; Joyce et al 1993; Sumiyoshi et al 1996). In vivo investigations with PET and the selective radioligand [^{11}C]WAY100635 have suggested alterations in 5-HT_{1A} receptor binding in depressed patients (Drevets et al 1999; Parsey et al 2006a; Parsey et al 2006b; Sargent et al 2000). PET studies on sex-specific effects on 5-HT_{1A} receptor binding have found higher 5-HT_{1A} receptor binding potentials in women compared to men (Parsey et al 2002), and no age effect on 5-HT_{1A} receptors in women (Cidis Meltzer et al 2001).

Finally, there is substantial evidence from experimental animal and human studies supporting involvement of 5-HT_{1A} receptors in pharmacological drug therapy. Effectiveness was demonstrated for the selective partial 5-HT_{1A} receptor agonist, buspirone, in clinical treatment

for generalized anxiety disorder and PMDD (Skolnick et al 1984; Tunnicliff 1991). In addition, several clinical trials in men reported that pindolol, which is a 5-HT_{1A} receptor antagonist, enhances the therapeutic effects of SSRIs (Artigas et al 1994; Berman et al 1999; Bordet et al 1998).

The 5-HTT

The 5-HTT is a key protein in the regulation of synaptic serotonin concentrations.
The 5-HTT protein is composed of 630 amino acids arranged in twelve transmembrane domains. The single gene encoding the human 5-HTT is localized on chromosome 17, centered at 17q11.2 (Ramamoorthy et al 1993).

Most 5-HTT is situated in presynaptic membranes of the serotonin nerve terminals but also in serotonin cell bodies and dendrites in the midbrain and brain stem raphe nuclei. In the human brain, the density of 5-HTT varies by region. The highest concentrations of 5-HTT have been found in the raphe nuclei, hypothalamus, thalamus, putamen, caudate, hippocampus, insula, and frontal cortex (Backstrom et al 1989; Cortes et al 1988; Ginovart et al 2001; Lundberg et al 2005).

The 5-HTT is of central interest in the pathopysiology and treatment of several psychiatric disorders including depression, anxiety and suicide (Arango et al 2002; Artigas et al 1996; Austin et al 2002). In depression lower 5-HTT densities have been reported by postmortem and *in vivo* human studies (Arora and Meltzer 1989; Parsey et al 2006). Some imaging studies have demonstrated the effect of sex on 5-HTT binding in depression. A single-photon emission computed tomography (SPECT) study using iodine-123-2 beta-carbomethoxy-3beta-(4-iodophenyltropane) ([^{123}I]β-CIT) showed a more prominent decrease of [^{123}I]β-CIT uptake in diencephalon in depressed women compared to depressed men (Staley et al 2006). Finally, 5-HTT is the primary target for the SSRI, which are psychotropic drugs widely prescribed in the treatment of depression and anxiety (Nutt et al 1999).

The 5-HTT has moreover also attracted a significant interest in studies exploring the effects of gonadal hormones on the 5-HT system. In ovarectomized rats, the injection of estradiol benzoate (EB) increased the number of cells expressing the 5-HTT mRNA in the dorsal raphe nuclei (McQueen et al 1999). In addition, in rhesus macaque, the administration of estrogen and progesterone reduced the expression levels of 5-HTT mRNA in dorsal raphe (Pecins-Thompson et al 1998).

Gender differences in the prevalence of serotonin associated psychiatric disorders

Women are more likely than men to suffer from depression and anxiety disorders, which are associated with abnormalities in the 5-HT system. The lifetime prevalence of major depression is approximately 8% in women and 4% in men. Similarly, women are twice as likely than men to suffer from anxiety disorders (Pigott 1999) and the sexes differ in suicidal behavior (Lewinsohn et al 2001). The underlying causality of these gender differences is not clear. Evidence from epidemiological studies indicate that rates of depression are similar in girls and boys and that the gender differences shifts to 2:1 female to male ratio with the onset of puberty and the influences of gonadal hormones (Kessler and Walters 1998). Another

interesting observation is that in women, the highest prevalence of depression occurs during the reproductive years when the actions of gonadal hormones are most apparent (Pearlstein et al 1997). Moreover, the reproductive related mood disorders in women such as PMDD, postpartum depression or perimenopausal depression, have all been related to fluctuations in the levels of gonadal hormones (Steiner et al 2003). These lines of evidence suggest that gonadal hormones might be of particular importance in women contributing to the higher prevalence of depression and anxiety disorders compared to men.

Serotonin and gonadal hormones

Early studies in animals have shown that brain 5-HT concentrations and levels of the 5-HT metabolite, 5-HIAA, vary during the estrous cycle in rats (Fludder and Tonge 1975; Kueng et al 1976). Later, an effect of gonadal hormones has been demonstrated on the 5-HT receptor system. It has been shown that ovariectomy reduces the number of $5HT_{1A}$ receptor binding sites in the ventromedial hypothalamic nucleus and that estradiol replacement counteractes this effect during the oestrous phase in the female rat (Flugge et al 1999). Further, in spayed monkeys the expression of the levels of $5\text{-}HT_{1A}$ mRNA and 5-HTT mRNA changes in the dorsal nuclei raphe with administration of hormones estrogen and progesterone (Pecins-Thompson and Bethea 1999; Pecins-Thompson et al 1998).

In humans, 5-HT has well been recognised for its implication in depression and the ovarian steroids, estrogen and progesterone have been suggested to play an important role in the modulation of mood and affect (Rubinow et al 1998; Steiner et al 2003). Several reports have supported the role of gonadal hormones for reproductive endocrine-associated mood syndromes in women, such as perimenopausal, postpartum depression and PMDD. Hormone replacement therapy beneficially affected mood in hypogonadal women (Montgomery et al 1987; Sherwin and Gelfand 1985). Additionally, an antidepressant effect of estradiol (E2) has been reported in perimenopausal women meeting standardized criteria for depression (Schmidt et al 2000; Soares et al 2001). Interestingly, numerous studies failed to demonstrate low levels of E2 in depressed peri-or postmenopausal women (Ballinger et al 1987; Saletu et al 1996). In women with postpartum depression, fluctuations in the levels of estrogen and progesterone rather than the low levels of these hormones per se have been suggested as a major trigger for depressive symptoms. Finally, in women with PMDD cyclic variations in estrogen and progesterone have been hypothesized to cause PMDD related premenstrual complaints (Schmidt et al 1998).

It has been suggested that depression that is associated with changes in the levels of gonadal hormones is related to the 5-HT system function. This view is primarily driven by observations coming from clinical studies in which drugs affecting the serotonergic neurotransmission such as SSRIs were found to be beneficial in the treatment of PMDD and postmenopausal depression (Eriksson 1999; Kalay et al 2007). SSRIs have been reported highly effective in the treatment of PMDD, as compared to other non – SSRI drugs (Eriksson et al 1995). In addition, some preliminary studies have shown beneficial effects of combining estrogen and SSRIs in the treatment of postmenopausal depression (Schneider et al 1997). A further association between the 5-HT system and reproductive endocrine-associated mood syndromes has been highlighted by the results of the pharmaconeuroendocrine challenges studies. In women with PMDD significant differences in the 5-HT related responses to pharmacological drug challenges have been reported between the follicular and luteal phases of the menstrual cycle compared to control women (FitzGerald et al 1997; Su et al 1997).

Sex differences in the serotonin system

Sexual dimorphisms in the 5-HT system were first suggested in the early ´60 by studies using animal models (Kato 1960). Central 5-HT levels, as well as CSF concentrations of the main 5-HT metabolite 5-HIAA were found to be higher in female compared to male rats (Rosecrans 1970). These observations in animals have later been supported in studies of sex differences in the 5-HT measurements in humans. Higher blood 5-HT levels have been found in women compared to men (Ortiz et al 1988). In addition, studies exploring the CSF concentrations of the 5-HIAA have suggested higher levels of 5-HIAA in women than in men in brain tissue (Gottfries et al 1974). The evidence from these studies raised the possibility that some of these differences may be related to sex differences in brain 5-HT synthesis. This hypothesis was supported in a PET study using α-[^{11}C]methyl-L-tryptophan (α-[^{11}C]MTrp) as an index of the 5-HT synthesis showing lower trapping of α-[^{11}C]MTrp in women compared to men (Sakai et al 2006).

Several studies have indicated sex- differences in expression levels of the 5-HTT and 5-HT$_{1A}$ receptors in healthy subjects and in patients with depression. Postmortem human studies suggested greater 5-HT$_{1A}$ receptor binding in women compared to men (Arango et al 1995) and decreased cortical 5-HTT binding in women (Arora and Meltzer 1989). In vivo measurements using SPECT and [^{123}I]β-CIT has shown that in healthy women there is a higher 5-HTT availability compared to healthy men (Staley et al 2001). In depression, a more prominent decrease in [^{123}I]β-CIT uptake in diencephalon was found in depressed women (22%) compared to depressed men (1%) (Staley et al 2006). However, the limitations of these studies were the use of non-selective radioligand [^{123}I]β-CIT that binds to both 5-HTT and dopamine transporter (DAT) and no control for the phase of the menstrual cycle in women. Variations in female sex hormones during the menstrual cycle have been suggested to influence brain neurochemistry and metabolism (Epperson et al 2002; Reiman et al 1996) highlighting the importance to control for the phase of the menstrual cycle in women.

Another line of mainly clinical research has suggested that women and men differ in treatment response to antidepressant medication. The first study on possible sex differences in response to imipramine was published in 1970s (Raskin 1974). Later a meta-analysis of 35 clinical trials evaluating the imipramine responses rates separately by gender, reported that men responded more favorable to imipramine than did the women (Hamilton et al 1996). A recent study suggested significant differences between genders in response and tolerability to sertraline (a SSRI drug) relative to imipramine (a tricyclic antidepressant) in subjects with chronic depression (Kornstein et al 2000). In women more favorable responses and higher tolerability to sertraline were found compared to imipramine and men showed more favorable responses to imipramine compared to sertraline.

Involvement of the serotonin system in psychiatric disorders of women

Premenstrual dysphoric disorder

PMDD is a cyclic mood disorder characterized by affective, behavioural and somatic symptoms appearing during the late luteal phase of the menstrual cycle. In women with

PMDD the most important symptoms described is irritability or anger with a prevalence rate of 46.2 %, which is considerably higher as compared to depressed mood (30.8 %), tension (27.1%) or anxiety (4.9%) (Angst et al 2001). PMDD is diagnosed in approximately 3 to 8% of women of reproductive age.

The cause of PMDD is largely unknown. Several lines of evidence support the role of 5-HT system in pathophysiological mechanisms underlying the PMDD. Abnormal serotonergic activity has been associated with depression and anxiety disorders with which PMDD share significant features (Halbreich 1995; Landen and Eriksson 2003). In addition, disturbed 5-HT function has been associated with aggressive behavior (Coccaro et al 1990; Coccaro et al 1995; Cleare and Bond 2000; Parsey et al 2002) and in women with PMDD the irritability and anger were described as the most prominent symptoms. The strong link between 5-HT function and aggression coupled with the observation that PMDD suffers are often depressed, prompted the suggestion that PMDD was a disorder of the 5-HT system. The theory was later supported by pharmacological trial showing that the SSRIs were more effective in the treatment of PMDD, than the noradrenergic antidepressant maprotiline (Eriksson et al 1995).

The involvement of 5-HT in the mechanisms underlying premenstrual dysphoria has also been supported by research on pharmacological challenges of the 5-HT system (Bancroft et al., 1991; Yatham, 1993; FitzGerald et al., 1997). In these studies the pharmacological drugs have been used to increase the serotonergic activity at postsynaptic receptors by mediating hormone responses. Thus, L-tryptophan has been used as a challenge test for growth hormone (GH) a response of which is supposed to be mediated by 5-HT_{1A} receptors (Smith et al 1991). In women with premenstrual symptoms blunted GH responses have been found in both menstrual cycle phases compared to controls (Bancroft et al 1991) suggesting the abnormalities in the 5-HT_{1A} -receptor function.

Since the symptoms of PMDD are related to the menstrual cycle there have been assumptions that premenstrual complaints could be triggered by reductions in the serum levels of progesterone and /or estradiol in the late luteal phase. This hypothesis was later challenged by the observation that the administration of a progesterone antagonist during the luteal phase neither reduced nor aggravated the symptoms (Schmidt et al 1991; Chan et al 1994). Similarly, luteal administration of estrogen or progesterone has not been found to be an effective treatment for PMDD (Dhar and Murphy 1990). Some authors have suggested differences between women with PMDD and controls with regard to serum levels of estradiol, progesterone and gonadotropine hormones. However, other groups failed to replicate these findings (Rubinow et al 1988) suggesting that women with PMDD differ from women with no complaints not with respect to ovarian activity but rather with respect to brain reactivity to normal variations in serum levels of gonadal hormones. Although many hypotheses have been put forward the current consensus is that normal ovarian function, rather than hormone imbalance, represent a cyclic trigger for PMDD related biochemical events within the central nervous system (Schmidt et al 1998).

Borderline personality disorder

BPD is a common and severe mental disorder characterized by impulsive-aggressive behavior, repeated self-injury, affective lability and instable interpersonal relationships. The prevalence of BPD in the general population has been reported to be 1-2 % (Torgersen et al 2001) with overweight for women and younger people. The suicide rate among psychiatric

patients with BPD is as high as 10% (Paris 2002). There is evidence to suggest that BPD is associated with early childhood neglect, sexual abuse and physical violence (Goldman et al 1992). Although, these risk factors were not reported for all patients, the percentage is high and varies from 20% to 75%. In addition, it has been shown that the presence of a history of sexual abuse correlates with a high degree of auto-aggression in patients with BPD such as automutilation and suicidality (Silk et al 1995).

Across the diagnostic categories, the most prominent features of BPD such as impulsive-aggressive and suicidal behavior have been associated with indices of abnormal 5-HT function. Already in 1976, Åsberg and collaborates published a study suggesting low levels of the serotonin metabolite, 5-HIAA in cerebrospinal fluid in suicide attempters (Asberg et al 1976). The relationship between levels of CSF 5-HIAA and behavioral aspects have since then been widely investigated in subjects with personality disorders, violent offenders, alcoholics and low 5-HIAA levels in CSF has been related to impulsive and aggressive behavior (Brown et al 1982; Limson et al 1991; Linnoila et al 1983).

Studies looking at the endocrinological response (e.g. prolactine, cortisol, growth hormone) to increased 5-HT concentrations (e.g. by fenfluramine or metha-chlorophenylpiperazine(m-CPP)) have found differences between patients with personality disorders and control subjects. In some of these studies patients with BPD were investigated and blunted hormone (Martial et al 1997) and diminished metabolic responses to serotonergic system stimulation (Soloff et al 2000) have been found in BPD patients compared to controls. A more close examination of the function of 5-HT_{1A}-receptors using the highly selective serotonin receptor agonists flesinoxan has suggested lower 5-HT_{1A}-receptor sensitivity in BPD (Hansenne et al 2002). Studies pointing to reduced serotonergic activity in borderline patients have led to studies of serotonergic candidate genes. Recent investigations have linked a variant of the tryptophan hydroxilase1gene (Zaboli et al 2006), gene for monoamine oxidase A (Ni et al 2007) and serotonin transporter gene (Ni et al 2006), which are important for the 5-HT synthesis, metabolism and 5-HT concentrations, with borderline personality disorder.

The biological correlates of reduced serotonergic activity in patients with BPD converge with treatment response data suggesting that impulsive-aggression as well as depressive symptoms improve with the treatment with SSRIs (Coccaro and Kavoussi 1997; Cornelius et al 1990). Reduced serotonergic activity seems, however, to require longer duration (New et al 1997) or higher dose intervention for treatment to be successful, consistent with results of higher dose trials of SSRIs in BPD (Markovitz et al 1991).

AIMS

On the gonadal hormones and the serotonin system

- To examine the impact of gonadal hormones on the 5-HT_{1A} and 5-HTT binding before and after ovulation in the menstrual cycle of healthy women (Study III)

- To examine differences in gonadal hormones between women with PMDD and control women (Study I)

On the sex differences in the serotonin system

- To study differences in the 5-HT_{1A} receptor and 5-HTT binding between healthy women and men *in vivo* (Study II)

On the serotonin system in psychiatric disorders of women

- To examine the 5-HT_{1A} receptor binding in women with PMDD in relation to control women at different phases of the menstrual cycle (Study I)

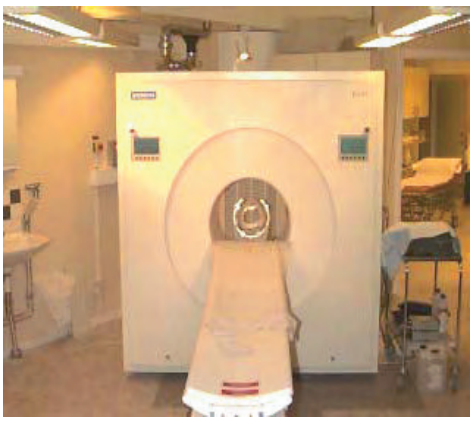
- To examine the 5-HT_{1A} receptor binding in women with BPD in relation to control women (Study IV)

MATERIALS AND METHODS

Positron emission tomography

Principles of PET

PET is a nuclear medicine imaging technique, which allows for accurate non-invasive *in vivo* measurements of a wide range of regional tissue functions in man. Using PET, pre and postsynaptic receptor density, affinity, neurotransmitter release, enzyme activity and drug delivery and uptake are possible to quantify with high selectivity and sensitivity of a pico- to nano-molar range. Basic principles of the PET method are given in Figure 3.



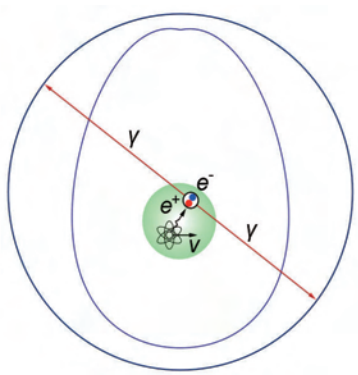


Figure 3. *The PET technique is used to acquire 3-dimensional information regarding the biological distribution of radiopharmaceuticals. The PET system at the Karolinska used for the present thesis (left). The principles of PET are as follows (right):*

1. *The radioactive tracer e.i., a small quantity of a ligand labeled with positron emitter, e.g. [^{11}C], is injected into the body*
2. *The positron-emitting radionuclide ejects a positron (+ß) from the nucleus as it decays*
3. *The positron will combine with an electron in the tissue and annihilate*
4. *The annihilation releases energy and results in conversion of the electron and positron into a pair of 511keV gamma emitted in opposite directions*
5. *Two gamma rays, traveling 180^{o} apart are detected in coincidence*
6. *The pair of photons produced from a single annihilation will register on opposing pairs of scintillation detectors as a " coincidence event"*
7. *Tomographic technique analyzes this detection to yield images of the distribution of the administrated positron-emitting radiotracers.*

Selective radioligands for the serotonin system

The development of PET and selective radioligands has markedly facilitated research on the expression of 5-HT proteins in the human brain *in vivo*. Over the years several high-affinity radioligands has been developed for the examination of 5-HT_{1A} (Pike et al 1996), and 5-HT_{2A} (Ito et al 1998) receptors as well as the 5-HTT (Houle et al 2000; Lundberg et al 2005). The methodological developments in this field have paved the way for studies exploring the status and distribution of 5-HT receptors in healthy human subjects and in patients with psychiatric disorders.

[^{11}C]WAY100635

[^{11}C]WAY100635[N-(2-(4-(2-meathoxyphenyl)-l-piperazinyl)ethyl-N-(2-pyridyl cyclohexanecarboxamide] was the first suitable radioligand developed for labeling of 5-HT_{1A} receptors in vitro and in vivo using PET (Pike et al 1996). The distribution of the 5-HT_{1A} receptors has, however, been mapped extensively and over the years different radioligands including [^{3}H]-5HT, [^{3}H]-8-OH-DPAT were developed for the quantification of 5-HT_{1A} receptors (Gozlan et al 1983). An antagonist with intrinsic activity was preferred and the first 5-HT_{1A} receptor antagonist radioligand used was [^{3}H]WAY100635 (Hall et al 1997). [^{11}C]WAY100635 is the current PET tracer of choice for visualization of 5-HT_{1A} receptors and was demonstrated as a potent and selective antagonist with subnanomolar affinity to 5-HT_{1A} receptors (Hall et al 1997) (Figure 4).

In the studies of the present thesis, [^{11}C]WAY100635 was used to study 5-HT_{1A} receptor binding potentials (BP). The sterile phosphate buffer (pH 7.4) solution of [^{11}C]WAY100635 was administrated intravenously at the start of PET experiments. In the study I, the radioactivity injected varied between 220 and 310 MBq. In study II, the mean radioactivity of [^{11}C]WAY100635 injected was 254.7 (SD=38.4) MBq in women and 252.4 (SD=57.5) MBq in men. In study III, the mean sterile phosphate buffer solution of 251 MBq (SD=37.5) (follicular) and 259 MBq (SD=29.3) (luteal) were administrated. In study IV, the radioactivity of [^{11}C]WAY100635 injected ranged between 231.5 and 302 MBq.

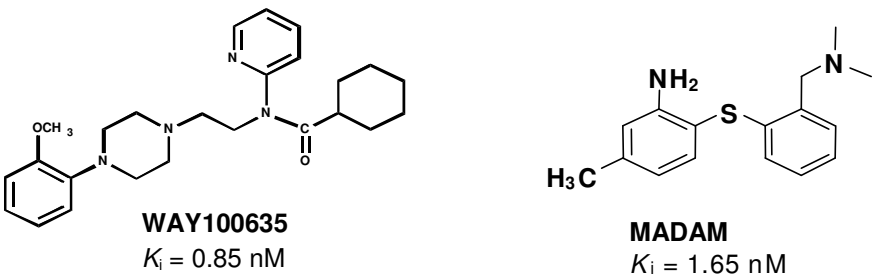


Figure 4. *The chemical structure and affinity to 5-HT_{1A} receptor and 5-HTT of WAY100635 and MADAM, respectively.*

[^{11}C]MADAM

[^{11}C]MADAM [N,N-Dimethyl-2-(2-amino-4-methylphenylthio) benzylamine] is a recently developed radioligand at our laboratory with high specificity and selectivity for the 5-HTT (Halldin et al., 2005) (Figure 4). Of the radioligands developed earlier for 5-HTT, the isoquinoline derivative ^{11}C –(+)-6β-(4-methylthiophenyl)- 1,2,3,5,6 α, 10β – hexahydropyrrolo[[2,1-a]isoquinoline ([^{11}C]McN 5652) has been widely used to image 5-HTT in non-human primates and humans (Suehiro et al 1993; Szabo et al 1995). Another often used radioligand for 5-HTT is diphenyl sulfide derivative ^{11}C-3-amino-4-(2-dimethylaminomethylphenylthio)-benzonitrile (^{11}C-DASB) which has shown a higher signal-to noise ratio compared to [^{11}C]McN 5652 (Frankle et al 2004). However, recent comparison data from study of Lundberg and collaborators (Lundberg et al 2006) with those of Frankle et al (Frankle et al 2004) indicated an even higher signal-to noise ratio for [^{11}C]MADAM (2-3 times higher $BP_{indirect}$ in most regions) when compared with [^{11}C]-DASB. In addition, test-retest measurement of [^{11}C]MADAM binding to the 5-HTT has shown good to excellent reliability by using the simplified reference tissue model (SRTM) (Lundberg et al 2006) suggesting [^{11}C]MADAM as highly suitable for clinical studies, such as those in the present thesis.

In study I-IV, serotonin transporter was examined with radioligand [^{11}C]MADAM. The sterile phosphate buffer (pH 7.4) solution of [^{11}C]MADAM was administrated intravenously at the start of PET experiments. In the study II, the mean amount of [^{11}C]MADAM injected was 258.2 MBq (SD=35.4) in women and 294.1 MBq (SD=10.8) in men. In the study III, mean sterile phosphate buffer solution of 251 MBq (SD=33.4) (follicular) and 267 MBq (SD=18.9) (luteal) of [^{11}C]MADAM were injected intravenously at the start of PET experiments.

Subjects

Control subjects

A total of 14 healthy women, aged 23-39 years old and 17 healthy men aged, 21-37 years, were examined. The healthy subjects participated after giving informed consent. They were all healthy according to medical history, physical examination, routine blood tests, liver, kidney, thyroid function test, urine analysis and toxicology tests and magnetic resonance imaging (MRI) of the brain. Exclusion criteria were: 1) presence of DSM IV Axis I disorder as examined by semistructured psychiatric interview; 2) personal history of psychiatric disorder; 3) history reports of mood or psychotic disorder in the first-degree relatives; 4) presence of a significant current medical condition, including history of seizure disorder and closed head trauma; 5) alcohol and illicit drug abuse and dependency; 6) treatment in the last 6 months with psychotropic drugs, glucocorticoids and hormonal therapy; 7) use of any prescribed medication during the last 4 weeks; 8) current smoking; and 9) women using oral contraceptives, women with irregular menstrual cycles, and pregnant women.

All women were investigated in the follicular and luteal phases of the menstrual cycle with PET. In study II, only PET scans from the follicular phases were included. In study IV, the control subjects were randomized with regard to the phase of the menstrual cycle to serve as controls for women with BPD for which the menstrual phase was not controlled.

Patients

Two patient categories were recruited: women with PMDD and women with BPD. The patients participated after giving informed consent. The women were healthy according to medical history, physical examination, routine blood tests, liver, kidney, thyroid function test, urine analysis and toxicology tests and MRI of the brain.

Patients with PMDD (Study I)

Five outpatients with PMDD, age range 26-39 years, were recruited. Inclusion criteria were history of regular menstrual cycles, physical health confirmed by medical history, physical examination, and routine laboratory tests, negative urine pregnancy test, and no use of psychotropic or hormonal drugs (including oral contraceptives) for the past six months. The exclusion criteria for women with PMDD were the presence of any other Axis I or an Axis II disorder.

Patients with BPD (Study IV)

Approximately 200 female patients were considered as candidates for participation. Inclusion criteria for subjects were sex (female), age (18-45) and fulfillment of the DSM-IV criteria for BPD (American Psychiatric Association 1994). Exclusion criteria were current or lifetime history of Alcohol Abuse or Alcohol Dependence or any use of street drugs, current or lifetime use of SSRI or antipsychotic drugs. In addition, patients with schizophrenia (DSM IV) or any significant neurological disorder were excluded. If patients had been admitted to, or visited, other psychiatric clinics (including Child Psychiatric units), their records were scrutinized to make sure that they had not been prescribed any of the drugs mentioned above.
Of nearly two hundred patients diagnosed, seven satisfied the inclusion criteria. The most common single cause for exclusion was current or lifetime history of Alcohol Abuse/Dependency or drug use. Six of the seven included patients were referred from in-patient units in Stockholm and one was referred from an outpatient unit.

Diagnosis and clinical ratings

In study I, the diagnosis of PMDD was based on the DSM-IV research criteria (American Psychiatric Association 1994), and was confirmed through prospective daily symptom ratings on a 100 mm Visual Analogue Scale (VAS) for at least two consecutive menstrual cycles. PMDD women had to have a more than a 50% increase in symptom severity of irritability, depression, or anxiety, in the luteal phase of the cycle compared with the follicular phase on the VAS.

In study IV, the instruments used for diagnostic evaluation of mental disorders according to DSM IV in patients with BPD were the Structural Clinical Interview for Disorders on Axis I (SCID I) (Zanarini et al 1998a) and the Structural Clinical Interview for Disorders on Axis II (SCID II) (Zanarini et al 1998b). Of the 9 DSM IV criteria for BPD, the number of criteria fulfilled ranged from 5 to 9. Co-morbid Disorders on Axis I and II were frequent (Zimmerman and Mattia 1999).
Five of the seven patients reported a history of childhood physical or sexual abuse. Six

patients had made one or several suicide attempts. One patient died from hanging shortly after participating in the study.

MRI and PET

MRI scans were performed on a 1.5 T GE Signa system (Milwaukee, WI) using a 3-dimensional (3D) spoiled gradient recalled (SPGR) sequence (a standard spin-echo sequence with a 256x256 matrix; repetition time of 4 sec). Two acquisitions were made in one session during 15 minutes. The first was T2-weighted for clinical evaluation regarding pathology. The second was T1-weighted for delineation of regions of interest (ROI).

The PET images were acquired using an ECAT Exact HR 47 scanner (CTI/ Siemens, Knoxville, TN) run in 3D mode (Wienhard et al 1994). The transaxial resolution is 3.8 mm full width at half maximum (FWHM) at the center of the field of view and 4.5 mm FWHM tangentially and 7.4 mm radially at 20 cm from the center. Axial resolution is 4 mm FWHM at the center and 6.8 mm at 20 cm from the center. Prior to the emission scan a transmission scan of 10 min was performed using a three rotating 68G/68Ga source to correct for attenuation.

A head fixation system with an individual plaster helmet was used both in the MRI and PET measurements to allow the same head positioning in the two imaging modalities and between scans (Bergstrom et al 1981).

PET examination procedure

Each subject was placed recumbent with his head in the PET system. The radioligand was injected into the right antecubital vein during 2s and the cannula was immediately flushed with 10 ml saline.

Radioactivity in brain was measured in a series of consecutive frames. The duration time of the scan for [^{11}C]WAY100635 lasted 69 min and consisted of 16 frames (frame sequence: 3x1, 4x3, 9x6 min) The duration of the scan for the radioligand [^{11}C]MADAM lasted 93 min and consisted of 20 frames (frame sequence: 3x1, 4x3, 13x6 min). The emission data were scatter corrected and reconstructed using filter back projection (Hanning filter with cutoff frequency of 2mm). The reconstructed volume was displayed in 47 horizontal sections with center-to center distance of 3.125 mm and a pixel size of 2.02 x 2.02 mm.

After acquisition, the MRI and PET images were transferred to the Statistical Parametric mapping (SPM), version 2 software for spatial normalization and coregistration in 3D space to control for any spatial mismatch between the modalities and allow for standardized regions of interest. The T1-images were aligned so that the horizontal plane was parallel to the line defined by the anterior and posterior commissures (ac-pc line) and the inter-hemispheric plane parallel to the sagittal plane. The reoriented T1 images were resliced to 1mm voxels in a matrix of 256x256x144. This MR was used in the image analysis software Human Brain Atlas (HBA) for manual delineation of regions of interest in any of the three orthogonal projections. In all studies (I-IV) PET images were coregistrated to MR images.

Regions and Volumes of interest (ROI, VOI)

Regions of interest were manually delineated on the MRI images and transferred to the corresponding PET images. The ROIs were defined according to anatomical margins guided by published reports (Bremner et al 1998; Crespo-Facorro et al 2000; Crespo-Facorro et al 1999) and using the Human Brain Atlas.

In study I, the regions of interest included dorsolateral prefrontal cortex, orbito-frontal cortex, anterior cingulate cortex, amygdale, hippocampus and dorsal raphe nuclei. In study III, ROIs were drawn for the anterior cingulate, frontal cortex, temporal cortex, insular cortex, hippocampus, dorsal raphe, nucleus caudate, putamen and thalamus.
In study II and IV, the VOIs were sampled and the total volumes of the structures were calculated in mm^3. In study II, the VOIs were sampled for the anterior cingulate, frontal cortex, temporal cortex, insular cortex, hippocampus, dorsal raphe, nucleus caudate, putamen and thalamus. In study IV, the volumes of interest included dorsolateral prefrontal cortex, anterior cingulate, orbitofrontal cortex, temporal cortex, insular cortex, hippocampus, amygdale and dorsal raphe.

The dorsal raphe does not have discernible borders on MR images and was therefore delineated on an integrated PET emission activity image. The region of raphe was easily detected due to high radioactivity in contrast to surrounding mesencephalic and cerebellar tissues. In studies I-IV, the ROI/VOI included the area of highest uptake, approximately corresponding to the specific tracer uptake in the dorsal raphe nuclei. The 6 mm-diameter circular ROI/VOI size was used according to previous studies to reduce possible underestimation of binding potentials due to partial volume effects (Rousset et al 1998). The approach of ROI/VOI definition used could theoretically introduce a bias of BP toward high values and hence overlook possible differences. To reduce the possibility of such bias, in all studies a standard ROI/VOI of fixed volume overlaid on the PET image was used. In studies I and III, the size and the shape of the ROI for the region of dorsal raphe was kept constant for the PET I and PET II images.

The cerebellar cortex was drawn as a left and right cortical gray matter excluding the central vermis. The ROI/VOI for the cerebellum was localized at approximately 1cm inside the cerebellar surfaces to avoid surrounding radioactivity spill-in (Drevets et al 1999) (Study I-IV).

PET data analysis

The Simplified Reference Tissue Model

In all studies (I-IV), the SRTM was used to obtain BP values. SRTM is a noninvasive modeling approach that uses cerebellar time activity curve (TAC) as an indirect input function for the calculations of BP and has been validated for both [^{11}C]WAY100635 and [^{11}C]MADAM (Gunn et al 1998; Lundberg et al 2005). The utilization of cerebellum as a reference tissue in SRTM was based on assumption of the virtual absence of radioligand binding in this region. For the 5-HT_{1A} receptor and 5-HTT in vitro studies have demonstrated negligible densities of both the receptor and transporter in the cerebellum, which should not

account for specific binding (Cortes et al 1988; Plenge et al 1990). However, the possibility of a few percent activity in the cerebellum can not be excluded and a previous PET study in humans have reported higher uptake of [^{11}C]WAY100635 in the cerebellar vermis (Parsey et al 2005). The cerebellar vermis was accordingly excluded from the cerebellar ROI analysis.

The SRTM accounts for regional differences in the influx rate constant (K_1) between the ROI and the cerebellum as well as regional differences in the time course of free and nonspecifically bound radioligand. The approach provides with the parameter referred to as BP and was calculated according to the formula: $BP\ (k_3/k_4) = B_{max}\ f_2/(K_d\ [1 + \Sigma_i\ F_i/K_{di}])$
where k_3 and k_4 refer to the exchange of tracer between the free and a specifically bound compartment, B_{max} the density of receptor, f_2 is the "free fraction" of unbound radioligand in the tissue, K_d the dissociation constant for the radioligand, and F_i and K_{di} are the free concentration and dissociation constant of the competing endogenous ligand.

Voxel based analysis of binding potential

In study II, a voxel-based approach was used as a complementary method to the VOI based method to investigate differences in 5-HT_{1A} receptors and 5-HTT binding independent of VOI definition. The parametric mapping analysis was done according to previous literature using the same design model in similar sample sizes (Sargent et al 2000; Turner et al 2005).
Recent developments in parametric imaging techniques have used wavelet space to reduce noise and linearize the data to calculate the BP by the Logan method (Cselenyi et al 2006). This method was preferred to the Gunn´s basis function method (Gunn et al 1997) because it has been shown to provide more reliable estimates across regions with a wide range of receptor density (Cselenyi et al 2006).

Thus, in study II, the PET images were converted to parametric images by Logan analysis in wavelet space. Since this template does not completely match the Talairach brain, it was necessary to correct the SPM{t} coordinates. This was achieved using the subroutine implemented by Matthew Brett (http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html), which gives the correspondence between SPM coordinates and Talairach coordinates. After importing the corrected coordinates, anatomical regions and Brodmann areas were identified by the Talairach Daemon Database
(http://www.ric.uthscsa.edu/projects/talairachdaemon.html).
The binding potential images were then normalized to the Montreal Neurological Institute (MNI) template in SPM2 and smoothed using a Gaussian filter (FMHM 12 mm) before statistical comparison between the groups, voxel by voxel using the SPM 2.

Hormone Assays and Gynecological Assessment

All healthy women who participated were scanned with PET in the follicular and luteal phase of the menstrual cycle. In study II, only PET scans from the follicular phases were included. In study IV, the healthy women were randomized for the phases of the menstrual cycle to serve as controls for women with BPD for which the menstrual phase was not controlled. In study I, women with PMDD were examined with PET in the follicular and luteal phase of the menstrual cycle.

The menstrual cycle phase was determined using ultrasound of the ovaries, plasma estradiol (E_2) and progesterone (P_4), concentrations, and blood and urine levels of the luteinising hormone (LH) levels to detect the midcycle LH surge and ovulation. Plasma levels of E_2 , P_4, follicle stimulating hormone (FSH) and LH, were examined at the time of PET examinations. Blood was drawn and immediately sent to the Chemistry Laboratory at Karolinska Hospital for routine analyses of E_2, P_4, FSH and LH. The reference values of the Karolinska Laboratory were used to evaluate hormonal levels. In study I, plasma values of gonadal hormones E_2 and P_4 as well as FSH and LH were compared between patients with PMDD and controls and in study III, the relationship between hormones E_2 and P_4, and 5-HT_{1A} and 5-HTT BPs were estimated at different phases of the menstrual cycle in healthy women (Study III).

Routine gynecological assessment and transvaginal sonographic (TVS) examination were carried out on the first visit, and at least once in the follicular and once in the luteal phase of the menstrual cycle in connection with the PET examinations. TVS examinations were performed using Volusoson 730 Expert (GE Medical Systems with access to 3D) and/or Acuson Sequoia 512 (Acuson Corporation). The ultrasound equipment had a high-resolution vaginal probe. Power Doppler examination was added in the luteal phase, since the corpus luteum (CL) is one of the most vascularized structures in the human body. To assess the day of LH surge and ovulation, Clear Plane (Unipath Limited) was used detecting urinary LH levels above 30IU/l, thus identifying at least 96% of ovulatory cycles compared to TVS used as golden standard (Filicori et al 1984; Filicori et al 1986; Rossmanith et al 1990). Blood was also drawn and levels of LH in blood were measured. To confirm a functioning CL, P_4 levels were measured in blood in the midluteal phase. TVS was also performed looking for a CL with its characteristic blood vessels in the ovary where a growing follicle had been seen in the follicular phase.

RESULTS

Study I: A PET study of 5-HT$_{1A}$ receptors at different phases of the menstrual cycle in women with premenstrual dysphoria

The cause of PMDD is largely unknown. It has been hypothesized that normal ovarian function trigger PMDD related biochemical events within the brain and that 5-HT plays an important role. In the present study PET and [^{11}C]WAY100635 were used to examine 5-HT$_{1A}$ receptors in control group of women and in women with PMDD. Two PET examinations were performed in each subject, one before (follicular phase) and one after ovulation (luteal phase)(Figure 5). Each subject's menstrual cycle was confirmed by ultrasonography of the ovaries as well as with hormone levels in blood and urine. The 5-HT$_{1A}$ binding potential was measured in six regions of interest and calculated according to the simplified reference tissue model.

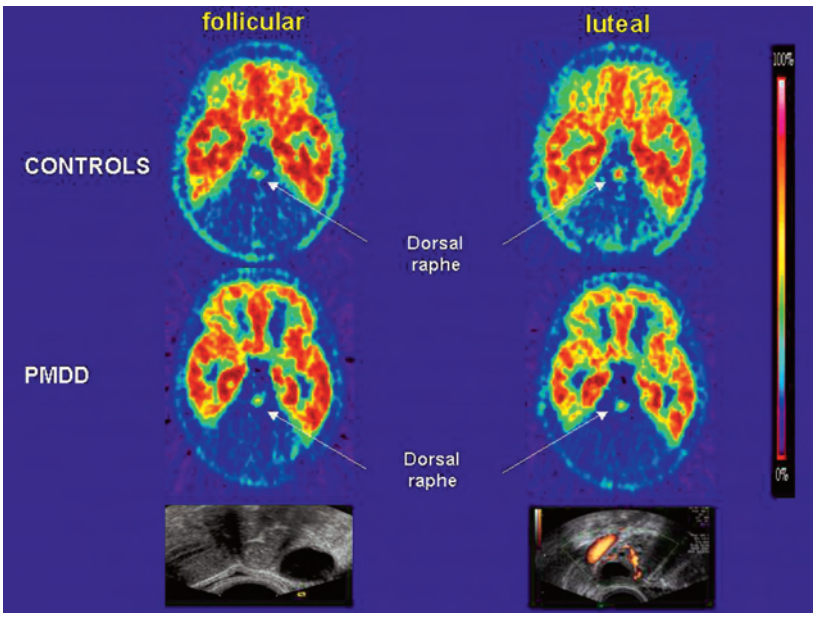


Figure 5. *PET and ultrasound images obtained in the follicular (left) and luteal (right) phases of the menstrual cycle. Horizontal sections through the midbrain visualize differences in [^{11}C]WAY100635 uptake in the dorsal raphe nuclei in a control woman and a woman with PMDD. The ultrasound image obtained in the follicular phase shows a growing follicle (homogenous shadow- lower-right of the US image), while in the luteal phase the corpus luteum is visible (central yellow-orange area of the US image).*

Mean baseline plasma concentrations for E_2, P_4, FSH and LH hormones during the follicular and luteal phases in both PMDD women and asymptomatic controls were within normal reference interval ranges. There were no significant between-group differences for the phases

of the menstrual cycle in basal hormone concentrations of E_2 , P_4, FSH and LH. For the region of dorsal raphe nuclei, there was a significant difference between the groups in the change of 5-HT_{1A} receptor binding (Mann-Whitney U test, z=1.98, p<0.05). In controls, 5-HT_{1A} receptor BP changed from the follicular to the luteal phase whereas this change was much smaller in PMDD patients. No significant differences in 5-HT_{1A} BP values were observed between the groups for other regions investigated.
The study provides principally new support *in vivo*, for a serotonergic dysregulation in women with PMDD.

Study II: Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET

Women and men differ in 5-HT associated psychiatric conditions, such as depression, anxiety and suicide. Despite this, very few studies focus on sex differences in the 5-HT system. Of the biomarkers in the 5-HT system, 5-HT_{1A} receptor is implicated in depression and anxiety, and 5-HTT is a target for SSRIs, psychotropic drugs used in the treatment of these disorders. The aim of the present study was to study sex related differences in the 5-HT_{1A} receptor and 5-HTT BPs in healthy humans, *in vivo*. PET and selective radioligands [^{11}C]WAY100635 and [^{11}C]MADAM were used to evaluate BPs for 5-HT_{1A} receptors (14 women and 14 men) and 5-HTT (8 women and 10 men). The BPs were estimated both on the level of anatomical regions and voxel wise, derived by the SRTM and wavelet/Logan plot parametric image techniques respectively.

The VOI-based analysis revealed higher mean 5-HT_{1A} BP values in women compared to men (Figure 6). A statistically significant mean difference of 1.37 in 5-HT_{1A} BP between women and men were found (p=0.0063) and there was no sex by region effect (p=0.25). Compared to men, women had in general, 39% higher 5-HT_{1A} BP. For the 5-HTT, a statistically significant lower mean 5-HTT BP was measured in women compared to men (p=0.0035) with a difference of 0.40. There was no significant sex by region interaction (p=0.47). A 55% higher 5-HTT BP was observed in men compared to women (Figure 6).

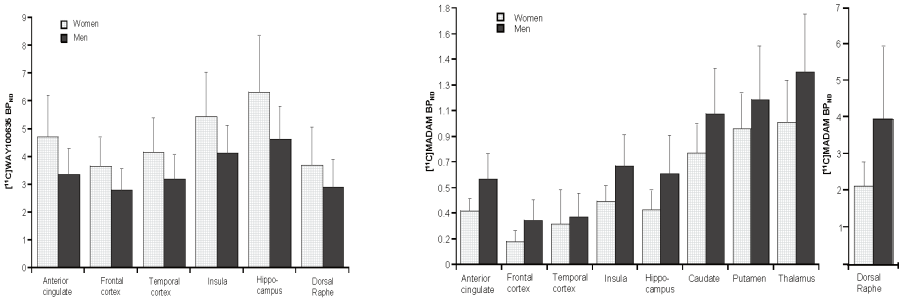


Figure 6. *Column-plots showing higher [^{11}C]WAY100635 BP (left) and lower [^{11}C]MADAM BP (right) in healthy women compared to healthy men.*

The parametric analysis of [^{11}C]WAY100635 images showed similar results to those obtained with VOI analysis. Significantly higher BP values were found in women compared to men in a wide number of brain regions. The parametric analysis of [^{11}C]MADAM showed significantly higher BP values in men compared to women in several brain regions i.e., right caudate, putamen, ventral striatum and left inferior frontal gyrus.

In women, a positive correlation between 5-HT$_{1A}$ receptor and 5-HTT BPs for the region of hippocampus was found (p=0.0009). No significant correlation was observed between regional 5-HT$_{1A}$ receptor and 5-HTT binding in men.

Sex differences in 5-HT$_{1A}$ receptor and 5-HTT binding may reflect biological distinctions in the 5-HT system contributing to sex differences in the prevalence of psychiatric disorders such as depression and anxiety. The result may help understanding sex differences in drug treatment responses to drugs affecting the 5-HT system.

Study III: 5-HT$_{1A}$ receptor and 5-HTT binding during the menstrual cycle in healthy women examined with [^{11}C] WAY100635 and [^{11}C] MADAM PET

As discussed above, a growing body of research indicates that central 5-HT neurotransmission may be modulated by gonadal hormones. Previous studies in animals have reported variations in the central 5-HT levels during the rat oestrous cycle and suggested that the physiological fluctuations of gonadal hormones, estrogen and progesterone might be responsible for that. The aim of the present study was to explore the effects of the menstrual cycle phases on 5-HT$_{1A}$ receptor and 5-HTT BP in healthy women by using PET.
Women were investigated in the follicular and luteal phase of the menstrual cycle with radioligands [^{11}C]WAY100635 (n=13) and [^{11}C]MADAM (n=8) to study 5-HT$_{1A}$ and 5-HTT BPs. The BPs values were quantified using the SRTM. The phases of the menstrual cycle were characterized by TVS and plasma levels of hormones E_2, P_4, FSH and LH hormone.

The 5-HT$_{1A}$ receptor and 5-HTT BPs did not significantly differ between follicular and luteal phases in any of the investigated regions (Figure 7). The only notable difference in 5-HT$_{1A}$ receptors and 5-HTT BPs, which was not statistically significant, was between follicular and luteal phases in the dorsal nuclei raphe. The mean 5-HT$_{1A}$ receptor BP was lower and 5-HTT BP was higher in the follicular compared to the luteal phase in dorsal raphe.

There were no significant correlations between hormones E_2 or P_4 and 5-HT$_{1A}$ receptors BP or 5-HTT BP in any of the regions. Neither did the change in plasma E_2 or P_4 correlated with the change in 5-HT$_{1A}$ BP or 5-HTT BP values in brain regions.

The results provide principally new *in vivo* evidence on human female biology, suggesting no difference in 5-HT$_{1A}$ receptors and 5-HTT binding between the phases of the menstrual cycle in healthy women that can be revealed with the present methodology. The non-significant observation of a possible change in 5-HT$_{1A}$ receptors and 5-HTT BP in dorsal nuclei raphe needs to be further explored in a larger sample of healthy women.

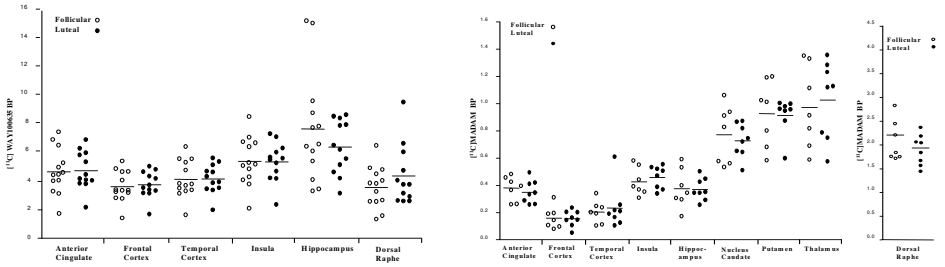


Figure 7. *Scatter-plots demonstrating individual [^{11}C]WAY100635 (left) and [^{11}C]MADAM BPs (right) in regions of interest in healthy women examined during the follicular and luteal phases of the menstrual cycle.*

Study IV: Lower serotonin-1A receptor binding in drug naïve patients with Borderline personality disorder: A PET study using [^{11}C]WAY100635

BPD is a common and severe mental disorder characterized by impulsive-aggressive behavior, repeated self-injury, affective lability and instable interpersonal relationships. Across the diagnostic categories, these behaviors have been associated with indices of abnormal 5-HT function, such as the low concentrations of the main 5-HT metabolite, 5-HIAA in the CSF and low 5-HT platelet content. The aim of the present study was to examine 5-HT$_{1A}$ receptor BP in female patients with BPD. Out of two hundred female patients with BPD, seven met inclusion criteria (i.e. drug naïve including, no previous or present alcohol or drug abuse/dependency). Eight age and sex matched controls were recruited. PET and selective radioligand [^{11}C]WAY100635 were used to study brain 5-HT$_{1A}$ receptor BP in dorsolateral prefrontal cortex, anterior cingulate, orbitofrontal cortex, amygdale, hippocampus, insula, temporal cortex and dorsal raphe nuclei. BP was estimated using the SRTM.

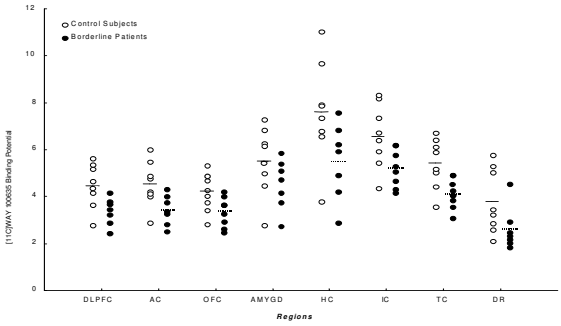


Figure 8. *Scatter graph showing 5-HT$_{1A}$ receptor BP in dorsolateral prefrontal cortex (DLPFC), anterior cingulate (AC), orbitofrontal cortex (OFC), amygdale (AMYGD), hippocampus (HC), insular cortex (IC), temporal cortex (TC) and dorsal raphe (DR) in control subjects and borderline patients. Horizontal lines indicate means.*

Compared to controls, women with BPD had a significantly lower 5-HT_{1A} receptor BP in the brain regions examined (Figure 8). A statistically significant overall group effect was found ($p=0.032$) with no support for significant effect of region ($p=0.265$).
The results suggest a lower 5-HT_{1A} receptor BP in drug naïve patients with BPD. The finding corroborates previous studies suggesting the impairment of the 5-HT system in patients with BPD.

SUMMARY OF FINDINGS AND COMMENTS

On the gonadal hormones and the serotonin system

In study III, we did not find differences in the 5-HT_{1A} receptors and the 5-HTT binding between phases of the menstrual cycle in healthy women. The result is consistent with previous *in vivo* imaging studies of Nordstrom et al (Nordstrom et al 1998) and Best et al (Best et al 2005), who did not find menstrual cycle dependent variation in dopamine-2 (D_2) receptor or in the 5-HTT and DAT binding in healthy women. The fluctuating levels of gonadal hormones during the menstrual cycle have, however, been suggested to affect the 5-HT system. Fludder and Tonge (Fludder and Tonge 1975) reported variations in the concentrations of central 5-HT levels during the estrous cycle of rat. In women, fluctuations in the levels of gonadal hormones have been suggested to play a major role in reproductive endocrine-associated mood syndromes such as PMDD. A significant difference in 5-HT related responses to pharmacological drug challenges has been found between the follicular and luteal phases in women with PMDD compared to controls, supporting the association between cyclic variation in gonadal hormones and the 5-HT system (FitzGerald et al 1997; Su et al 1997).
The results of study III does not, however, preclude that gonadal hormones exert influences on the 5-HT system. We did find a non-significantly lower 5-HT_{1A} receptor BP in the follicular compared to the luteal phase and higher 5-HTT BP in the follicular compared to the luteal phase in women in dorsal raphe nuclei. In rhesus macaque the administration of gonadal hormones, estrogen and progesterone changed the expression levels of the 5-HT_{1A} receptor and 5-HTT mRNA in dorsal raphe (Pecins-Thompson and Bethea 1999; Pecins-Thompson et al 1998). Although non-significant, the observation of a possible change in 5-HT_{1A} receptors and 5-HTT BPs in dorsal raphe suggests potential influences of gonadal hormones on 5-HT receptors, the finding that needs to be further explored in a larger sample of healthy women.

In study I, we explored differences in gonadal hormones between women with PMDD and control women. No differences in mean plasma E_2 and P_4 hormone levels were observed between women with PMDD and controls. Redei and Freeman (Redei and Freeman 1995) reported higher mid cycle estrogen levels in women with PMDD compared to controls. The differences in basal levels of hormones between women with PMDD and control women were, however, not replicated and Backstrom et al (Backstrom et al 1983) found normal basal hormone values of progesterone, estradiol, testosterone and androstenedione in women with PMDD. These results suggested that women with PMDD differ from women with no complaints not with respect to ovarian activity but rather with respect to brain reactivity to normal variations in serum levels of gonadal hormones.
The results of the study I are, thus, consistent with the former findings of no diagnosis-related differences in basal plasma reproductive hormone levels between the two groups (Backstrom et al 1983; Rubinow et al 1988) suggesting normal ovarian function in dysphoric women. Normal ovarian function, rather than hormone imbalance, could represent a cyclic trigger for PMDD related biochemical events within the central nervous system (Schmidt et al 1998).

On the sex differences in the serotonin system

Consistent with evidence from preclinical and clinical studies suggesting sex-differences in 5-HT system, we found significant differences in the 5-HT_{1A} receptor and 5-HTT binding between healthy women and men in study II. Our results replicate that from the study by Parsey and collaborators (Parsey et al 2002) who used similar methods and found higher 5-HT_{1A} receptor binding in women than in men. Furthermore, our results corroborate previous postmortem human data of greater 5-HT_{1A} receptor binding in women compared to men (Arango et al 1995) and decreased cortical 5-HTT binding in women (Arora and Meltzer 1989). However, our results of higher 5-HTT binding in men are discrepant from a study by Staley et al (Staley et al 2001) who reported higher 5-HTT availability in healthy women compared to men. The divergence in findings on 5-HTT may be due to differences in selectivity of radioligands used in the two studies. In the present study a highly selective radioligand [^{11}C]MADAM was used to estimate 5-HTT binding, while in the study of Stanley and collaborators, radioligand [^{123}I]β-CIT with non-selective properties binding to both 5-HTT and DAT was applied.

Viewing that 5-HT_{1A} receptor and 5-HTT are important markers for serotonergic neurotransmission, the results of study II support the hypothesis that differences in the 5-HT function between women and men may underline the known gender differences in the prevalence in depression as well as sex-differences in pharmacological treatment that target serotonergic neurotransmission. Although pathophysiological mechanisms underlying depression in women are still largely unknown, the results of study II suggest that in women there might be lower 5-HT activity designating the trait sensibility to sustain a higher tendency to develop depression.

In summary, we found differences in 5-HT_{1A} receptor and 5-HTT binding between healthy women and men. The findings may help understanding mechanisms underlying sex differences in the prevalence of psychiatric disorders as well as pharmacological treatment responses.

On the serotonin system in psychiatric disorders of women

It could be shown that 5-HT_{1A} receptors are involved in PMDD and BPD of women. In women with PMDD, the 5-HT_{1A} receptor binding significantly differ from asymptomatic controls in relation to menstrual cycle phase in the region of dorsal raphe nuclei. The finding of 5-HT_{1A} receptors abnormality in women with PMDD is consistent with previously reported challenge studies of 5-HT_{1A} receptors mediated effects indicating different serotonergic responses between women with PMDD and controls (FitzGerald et al 1997; Yatham 1993). It has been hypothesized that women with PMDD may be behaviorally or biochemically sub-or supersensitive to biological challenges of the seorotnergic system (Halbreich and Tworek 1993; Leibenluft et al 1994). There have also been suggestions that normal ovarian function, rather than hormone imbalance, could be a cyclic trigger for PMDD related biochemical events within the central nervous system (Schmidt et al 1998). The results of study I further support these views by demonstrating abnormalities in 5-HT_{1A} receptors function but normal basal plasma reproductive hormone levels in women with PMDD.

The results of study IV show abnormalities in 5-HT_{1A} receptors in BPD. Global reduction in 5-HT_{1A} receptors binding was found in drug naïve patients with BPD compared to control women. The finding is consistent with the literature on 5-HT challenges (Martial et al 1997; Rinne et al 2000), which indicated lower sensitivity of 5-HT_{1A}-receptors in patients with BPD (Hansenne et al 2002; Martial et al 1997). The global effect may tentatively be in line with the recently demonstrated role of brain-derived neurotrophic factor (BDNF) in the development and maintenance of the 5-HT system in man.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all members of the Karolinska PET group, colleagues and friends who has contributed to the work presented in this thesis. I particularly would like to thank:

My supervisor, Associate Professor Anna-Lena Nordström, for your high ambition and generous support that made an outstanding atmosphere for my scientific development, for teaching me the scientific thinking and writing including both high sensitivity and sharp criticism, for encouragement and trust through all the years

Professor Lars Farde, for letting me experience and enjoy the beauty of your brilliant mind, for support, encouragement and trust throughout all these years, for deep understanding and for your friendship

Professor Christer Halldin, for devoted work to develop better and new radiolignads

Research nurse Kjerstin Lind, for excellent nursing skills and particular care for PhD students

Dr. Per Karlsson, for kindness and endless patience while teaching me/us the PET method

Associate Professor Balazs Gulyas, for everlasting scientific optimism

Project assistant Karin Zahir for being very helpful in administrative matters and for relaxing chats in Swedish

My colleagues and collaborators in the PET group: Psychologist Jacqueline Borg for being my close friend in life and more than stimulating colleague in science, Dr. Johan Lundberg for warm company and fruitful discussions on the serotonin system, Dr. Simon Cervenka for relaxing chats on different matters and for nice companionship, Dr. Aurelija Jucaite and Dr. Judit Sovago for loyal friendship and for sharing the experiences in life and science, Nina Erixon Lindroth for your warmth and your kind friendship, Dr. Andrea Varrone for deep scientific knowledge and valuable contributions on my manuscripts, Dr. Tomoyuki Saijo, for all image analysis and interesting discussions about psychiatry and philosophy, Dr. Patrik Mattsson for shared interest and inspiring chats on the serotonin system and Dr. Zsolt Cselenyi for your valuable contributions in image analysis matters

Dr Åsta Cerin and other colleagues at the Department of Women and Child Health for their excellent collaboration

Dr Eva Andersson for invaluable contribution on recruiting the patients with borderline personality disorder

Dr. Mirjam-Talvik for friendly support at the beginning of my Ph.D. training, and Dr. Bengt Andree for inspiring discussions on the serotonin system

Other colleagues for shared interest in PET and for being nice company:
Katarina Varnäs, Akihiro Takano, Hans Olsson, Sari Karlsson, Sjoerd Finnema, Nick Seneca

Nils Sjöholm, Stefan Pauli and Julio Gabriel for providing with good care of the PET camera and helped the image reconstruction

Research nurses, Johan Molin, Monica Hellberg, Gudrun Nylen, and Jan Everhov for their valuable contributions in overall research activities

Urban Hansson, for your high level of expertise in computer matters

Present and former members of the radiochemistry group for their impressive work on radioligand synthesis and for their technical assistance: Anu Airaksinen, Arsalan Amir, Jan Andersson, Magnus Schou, Guennadi Jogolev, Phong Truong, Raisa Krasikova, Sean Donohue, Jari Tarkainen

Company in the basement floor: Marita Signarsson, for assisting in administrative matters, Raffaella Björck for supporting me in my attempts to start clinical training in psychiatry, Tove Gunnarsson for lively temperament and nice Christmas presents, Erik Jönsson for friendly chats, Alexandra Tylec for contributing to a dynamic environment in the basement floor, Marianne Youssefi for kind support and helpful assistance in work on PhD documents.

Dr. Björn Mårtensson and Dr. Peter Nordström for many friendly talks

Professor Mats Fredrikson for friendship and for encouraging me to study “emotions” with PET

Dr. Predrag Petrovic for good friendship

All my closest friends from outside the PET world for enriching my life: Drs. Jovan and Aleksandra Antovic, Gordan and Jelena Zdravkovic, Dr. Veselin Pantovic, Mihailo and Aleksandra Trifunovic, Petra and Jimmy Bergqvist, Aleksandra and Dragan Havelka and Olga and Patrik Björklund.

Nada, my mother, for your love, support and believe in me, and Srba, my father, for teaching me the values and skills in life

Braslav, my dearest husband for his unlimited love, endless support, and understanding me working overtime

My beauty, my only son Mihailo, for being the greatest happiness in my life

Finally, I would like to thank to all patients and control subjects for their volunteering participation in the studies, without whom this work won’t be possible

REFERENCES

American Psychiatric Association A (1994): *Diagnostic and statistical manual of mental disorders*, 4th ed: American Psychiatric Association.

Angst J, Sellaro R, Merikangas KR, Endicott J (2001): The epidemiology of perimenstrual psychological symptoms. *Acta Psychiatr Scand* 104:110-6.

Arango V, Underwood MD, Gubbi AV, Mann JJ (1995): Localized alterations in pre- and postsynaptic serotonin binding sites in the ventrolateral prefrontal cortex of suicide victims. *Brain Res* 688:121-33.

Arango V, Underwood MD, Mann JJ (2002): Serotonin brain circuits involved in major depression and suicide. *Prog Brain Res* 136:443-53.

Arora RC, Meltzer HY (1989): 3H-imipramine binding in the frontal cortex of suicides. *Psychiatry Res* 30:125-35.

Artigas F, Perez V, Alvarez E (1994): Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. *Arch Gen Psychiatry* 51:248-51.

Artigas F, Romero L, de Montigny C, Blier P (1996): Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. *Trends Neurosci* 19:378-83.

Arvidsson LE, Hacksell U, Nilsson JL, et al (1981): 8-Hydroxy-2-(di-n-propylamino)tetralin, a new centrally acting 5-hydroxytryptamine receptor agonist. *J Med Chem* 24:921-3.

Asberg M, Traskman L, Thoren P (1976): 5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor? *Arch Gen Psychiatry* 33:1193-7.

Austin MC, Whitehead RE, Edgar CL, Janosky JE, Lewis DA (2002): Localized decrease in serotonin transporter-immunoreactive axons in the prefrontal cortex of depressed subjects committing suicide. *Neuroscience* 114:807-15.

Backstrom I, Bergstrom M, Marcusson J (1989): High affinity [3H]paroxetine binding to serotonin uptake sites in human brain tissue. *Brain Res* 486:261-8.

Backstrom T, Sanders D, Leask R, Davidson D, Warner P, Bancroft J (1983): Mood, sexuality, hormones, and the menstrual cycle. II. Hormone levels and their relationship to the premenstrual syndrome. *Psychosom Med* 45:503-7.

Ballinger CB, Browning MC, Smith AH (1987): Hormone profiles and psychological symptoms in peri-menopausal women. *Maturitas* 9:235-51.

Bancroft J, Cook A, Davidson D, Bennie J, Goodwin G (1991): Blunting of neuroendocrine responses to infusion of L-tryptophan in women with perimenstrual mood change. *Psychol Med* 21:305-12.

Blier P, de Montigny C (1999): Serotonin and drug-induced therapeutic responses in major depression, obsessive-compulsive and panic disorders. *Neuropsychopharmacology* 21:91S-98S.

Bergstrom M, Boethius J, Eriksson L, Greitz T, Ribbe T, Widen L (1981): Head fixation device for reproducible position alignment in transmission CT and positron emission tomography. *J Comput Assist Tomogr* 5:136-41.

Berman RM, Anand A, Cappiello A, et al (1999): The use of pindolol with fluoxetine in the treatment of major depression: final results from a double-blind, placebo-controlled trial. *Biol Psychiatry* 45:1170-7.

Best SE, Sarrel PM, Malison RT, et al (2005): Striatal dopamine transporter availability with [123I]beta-CIT SPECT is unrelated to gender or menstrual cycle. *Psychopharmacology (Berl)* 183:181-9.

Bordet R, Thomas P, Dupuis B (1998): Effect of pindolol on onset of action of paroxetine in the treatment of major depression: intermediate analysis of a double-blind, placebo- controlled trial. Reseau de Recherche et d'Experimentation Psychopharmacologique. *Am J Psychiatry* 155:1346-51.

Bremner JD, Bronen RA, De Erasquin G, et al (1998): Development and Reliability of a Method for Using Magnetic Resonance Imaging for the Definition of Regions of Interest for Positron Emission Tomography. *Clin Positron Imaging* 1:145-159.

Brown GL, Ebert MH, Goyer PF, et al (1982): Aggression, suicide, and serotonin: relationships to CSF amine metabolites. *Am J Psychiatry* 139:741-6.

Burnet PW, Eastwood SL, Lacey K, Harrison PJ (1995): The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. *Brain Res* 676:157-68.

Chan AF, Mortola JF, Wood SH, Yen SS (1994): Persistence of premenstrual syndrome during low-dose administration of the progesterone antagonist RU 486. *Obstet Gynecol* 84:1001-5.

Cidis Meltzer C, Drevets WC, Price JC, et al (2001): Gender-specific aging effects on the serotonin 1A receptor. *Brain Res* 895:9-17.

Cleare AJ, Bond AJ (2000): Ipsapirone challenge in aggressive men shows an inverse correlation between 5-HT1A receptor function and aggression. *Psychopharmacology (Berl)* 148:344-9.

Coccaro EF, Gabriel S, Siever LJ (1990): Buspirone challenge: preliminary evidence for a role for central 5-HT1a receptor function in impulsive aggressive behavior in humans. *Psychopharmacol Bull* 26:393-405

Coccaro EF, Kavoussi RJ (1997): Fluoxetine and impulsive aggressive behavior in personality-disordered subjects. *Arch Gen Psychiatry* 54:1081-8.

Coccaro EF, Kavoussi RJ, Hauger RL (1995): Physiological responses to d-fenfluramine and ipsapirone challenge correlate with indices of aggression in males with personality disorder. *Int Clin Psychopharmacol* 10:177-9.

Cornelius JR, Soloff PH, Perel JM, Ulrich RF (1990): Fluoxetine trial in borderline personality disorder. *Psychopharmacol Bull* 26:151-4.

Corradetti R, Laaris N, Hanoun N, et al (1998): Antagonist properties of (-)-pindolol and WAY 100635 at somatodendritic and postsynaptic 5-HT1A receptors in the rat brain. *Br J Pharmacol* 123:449-62.

Cortes R, Soriano E, Pazos A, Probst A, Palacios JM (1988): Autoradiography of antidepressant binding sites in the human brain: localization using [3H]imipramine and [3H]paroxetine. *Neuroscience* 27:473-96.

Crespo-Facorro B, Kim J, Andreasen NC, et al (2000): Cerebral cortex: a topographic segmentation method using magnetic resonance imaging. *Psychiatry Res* 100:97-126.

Crespo-Facorro B, Kim JJ, Andreasen NC, et al (1999): Human frontal cortex: an MRI-based parcellation method. *Neuroimage* 10:500-19.

Cselenyi Z, Olsson H, Halldin C, Gulyas B, Farde L (2006): A comparison of recent parametric neuroreceptor mapping approaches based on measurements with the high affinity PET radioligands [11C]FLB 457 and [11C]WAY 100635. *Neuroimage* 32:1690-708.

Dahlstroem A, Fuxe K (1964): Evidence for the Existence of Monoamine-Containing Neurons in the Central Nervous System. I. Demonstration of Monoamines in the Cell Bodies of Brain Stem Neurons. *Acta Physiol Scand Suppl*:SUPPL 232:1-55.

Deakin JF, Pennell I, Upadhyaya AJ, Lofthouse R (1990): A neuroendocrine study of 5HT function in depression: evidence for biological mechanisms of endogenous and psychosocial causation. *Psychopharmacology (Berl)* 101:85-92.

Dhar V, Murphy BE (1990): Double-blind randomized crossover trial of luteal phase estrogens (Premarin) in the premenstrual syndrome (PMS). *Psychoneuroendocrinology* 15:489-93.

Drevets WC, Frank E, Price JC, et al (1999): PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry* 46:1375-87.

Epperson CN, Haga K, Mason GF, et al (2002): Cortical gamma-aminobutyric acid levels across the menstrual cycle in healthy women and those with premenstrual dysphoric disorder: a proton magnetic resonance spectroscopy study. *Arch Gen Psychiatry* 59:851-8.

Eriksson E (1999): Serotonin reuptake inhibitors for the treatment of premenstrual dysphoria. *Int Clin Psychopharmacol* 14 Suppl 2:S27-33.

Eriksson E, Hedberg MA, Andersch B, Sundblad C (1995): The serotonin reuptake inhibitor paroxetin is superior to the noradrenaline reuptake inhibitor maprotiline in the treatment of premenstrual syndrome. *Neuropsychopharmacology* 12:167-76.

Erspamer V, (1940): Pharmacology of enteramine. I. Action of acetone extract of rabbit stomach mucosa on blood pressure and on surviving isolated organs. *Naunyn Schmiedebergs Arch of Exper Pathol Pharmacology.* 196: 343-365.

Erspamer V, Boretti G (1952): Identification of Enteramine, the Specific Hormone of the Enterochromaffin Cell System, as 5-Hydroxytryptamine. *Nature.* 1952 169: 800-801.

Filicori M, Butler JP, Crowley WF, Jr. (1984): Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion. *J Clin Invest* 73:1638-47.

Filicori M, Santoro N, Merriam GR, Crowley WF, Jr. (1986): Characterization of the physiological pattern of episodic gonadotropin secretion throughout the human menstrual cycle. *J Clin Endocrinol Metab* 62:1136-44.

FitzGerald M, Malone KM, Li S, et al (1997): Blunted serotonin response to fenfluramine challenge in premenstrual dysphoric disorder. *Am J Psychiatry* 154:556-8.

Fludder JM, Tonge SR (1975): Proceedings: Variations in the concentrations of monamines and their metabolites in eight regions of rat brain during the oestrous cycle: a basis for interactions between hormones and psychotropic drugs. *J Pharm Pharmacol* 27 Suppl?-2:39P.

Flugge G, Kramer M, Rensing S, Fuchs E (1998): 5HT1A-receptors and behaviour under chronic stress: selective counteraction by testosterone. *Eur J Neurosci* 10:2685-93.

Flugge G, Pfender D, Rudolph S, Jarry H, Fuchs E (1999): 5HT1A-receptor binding in the brain of cyclic and ovariectomized female rats. *J Neuroendocrinol* 11:243-9.

Frankle WG, Huang Y, Hwang DR, et al (2004): Comparative evaluation of serotonin transporter radioligands 11C-DASB and 11C-McN 5652 in healthy humans. *J Nucl Med* 45:682-94.

Gaddum JH, Picarelli ZP (1957): Two kinds of tryptamine receptor. *Br J Pharmacol Chemother* 12:323-8.

Ginovart N, Wilson AA, Meyer JH, Hussey D, Houle S (2001): Positron emission tomography quantification of [(11)C]-DASB binding to the human serotonin transporter: modeling strategies. *J Cereb Blood Flow Metab* 21:1342-53.

Goldman SJ, D'Angelo EJ, DeMaso DR, Mezzacappa E (1992): Physical and sexual abuse histories among children with borderline personality disorder. *Am J Psychiatry* 149:1723-6.

Gottfries CG, Roos BE, Winblad B (1974): Determination of 5-hydroxytryptamine, 5-hydroxyindoleacetic acid and homovanillic acid in brain tissue from an autopsy material. *Acta Psychiatr Scand* 50:496-507.

Gozlan H, El Mestikawy S, Pichat L, Glowinski J, Hamon M (1983): Identification of presynaptic serotonin autoreceptors using a new ligand: 3H-PAT. *Nature* 305:140-2.

Grahame –Smith (1964) Tryptophan hydroxylation in brain. *Biochem Biophys Res Commun.* 11;16(6):586-92.

Graeff FG, Guimaraes FS, De Andrade TG, Deakin JF (1996): Role of 5-HT in stress, anxiety, and depression. *Pharmacol Biochem Behav* 54:129-41.

Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ (1997): Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage* 6:279-87.

Gunn RN, Sargent PA, Bench CJ, et al (1998): Tracer kinetic modeling of the 5-HT1A receptor ligand [carbonyl-11C]WAY-100635 for PET. *Neuroimage* 8:426-40.

Halbreich U (1995): Premenstrual dysphoric disorders, anxiety, and depressions: vulnerability traits or comorbidity. *Arch Gen Psychiatry* 52:606.

Halbreich U, Tworek H (1993): Altered serotonergic activity in women with dysphoric premenstrual syndromes. *Int J Psychiatry Med* 23:1-27.

Hall H, Lundkvist C, Halldin C, et al (1997): Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]way-100635. *Brain Res* 745:96-108.

Halldin, C., Lundberg, J., Sovago, J., Gulyas, B., Guilloteau, D., Vercouillie, J., Edmond, P., Chalon, S., Tarkianen, J., Hiltunen, J., Farde, L., 2005. [(11)C]MADAM, a new serotonin transporter radioligand characterized in the monkey brain by PET. Synapse 58, 173-183.

Hamilton JA, Grant M, Jensvold FM (1996): Sex and treatment of depression, in psychopharmacology and women. *Edited by Jensvold MJ, Halbreich U, Hamilton JA. Washington, DC, American Psychiatric Association Press* 241-260

Hansenne M, Pitchot W, Pinto E, et al (2002): 5-HT1A dysfunction in borderline personality disorder. *Psychol Med* 32:935-41.

Hashimoto T, Kitamura N, Kajimoto Y, et al (1993): Differential changes in serotonin 5-HT1A and 5-HT2 receptor binding in patients with chronic schizophrenia. *Psychopharmacology (Berl)* 112:S35-9.

Hjorth S, Carlsson A, Lindberg P, et al (1982): 8- hydroxyl-2-(di-n-propylamino)tetralin, 8-OH- DPAT, a potent and selective simplified ergot congener with central 5.HT receptor stimulating activity. *J of Neural Transm* 55:169-188.

Hokfelt T, Fuxe K, Goldstein M (1973): Immunohistochemical localization of aromatic L-amino acid decarboxylase (DOPA decarboxylase) in central dopamine and 5-hydroxytryptamine nerve cell bodies of the rat. *Brain Res* 53:175-80.

Houle S, Ginovart N, Hussey D, Meyer JH, Wilson AA (2000): Imaging the serotonin transporter with positron emission tomography: initial human studies with [11C]DAPP and [11C]DASB. *Eur J Nucl Med* 27:1719-22.

Hoyer D, Hannon JP, Martin GR (2002): Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 71:533-54.

Ito H, Nyberg S, Halldin C, Lundkvist C, Farde L (1998): PET imaging of central 5-HT2A receptors with carbon-11-MDL 100,907. *J Nucl Med* 39:208-14.

Joyce JN, Shane A, Lexow N, Winokur A, Casanova MF, Kleinman JE (1993): Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics. *Neuropsychopharmacology* 8:315-36.

Kalay AE, Demir B, Haberal A, Kalay M, Kandemir O (2007): Efficacy of citalopram on climacteric symptoms. *Menopause* 14:223-9.

Kato R (1960): Serotonin content of rat brain in relation to sex and age. *J Neurochem* 5:202.

Kessler RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB (1993): Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. *J Affect Disord* 29:85-96.

Kessler RC, McGonagle KA, Zhao S, et al. (1994): Lifetime and 12-month prevalence of DSM-III-R Psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry*. 51:8-19.

Kessler RC, Walters EE (1998): Epidemiology of DSM-III-R major depression and minor depression among adolescents and young adults in the National Comorbidity Survey. *Depress Anxiety* 7:3-14.

Kornstein SG, Schatzberg AF, Thase ME, et al (2000): Gender differences in treatment response to sertraline versus imipramine in chronic depression. *Am J Psychiatry* 157:1445-52.

Kueng W, Wirz-Justice A, Menzi R, Chappuis-Arndt E (1976): Regional brain variations of tryptophan, monoamines, monoamine oxidase activity, plasma free and total tryptophan during the estrous cycle of the rat. *Neuroendocrinology* 21:289-96.

Landen M, Eriksson E (2003): How does premenstrual dysphoric disorder relate to depression and anxiety disorders? *Depress Anxiety* 17:122-9.

Leibenluft E, Fiero PL, Rubinow DR (1994): Effects of the menstrual cycle on dependent variables in mood disorder research. *Arch Gen Psychiatry* 51:761-81.

Lewinsohn PM, Rohde P, Seeley JR, Baldwin CL (2001): Gender differences in suicide attempts from adolescence to young adulthood. *J Am Acad Child Adolesc Psychiatry* 40:427-34.

Leyton M, Okazawa H, Diksic M, et al (2001): Brain Regional alpha-[11C]methyl-L-tryptophan trapping in impulsive subjects with borderline personality disorder. *Am J Psychiatry* 158:775-82.

Limson R, Goldman D, Roy A, et al (1991): Personality and cerebrospinal fluid monoamine metabolites in alcoholics and controls. *Arch Gen Psychiatry* 48:437-41.

Linnoila M, Virkkunen M, Scheinin M, Nuutila A, Rimon R, Goodwin FK (1983): Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior. *Life Sci* 33:2609-14.

Lundberg J, Halldin C, Farde L (2006): Measurement of serotonin transporter binding with PET and [11C]MADAM: a test-retest reproducibility study. *Synapse* 60:256-63.

Lundberg J, Odano I, Olsson H, Halldin C, Farde L (2005): Quantification of 11C-MADAM binding to the serotonin transporter in the human brain. *J Nucl Med* 46:1505-15.

Markovitz PJ, Calabrese JR, Schulz SC, Meltzer HY (1991): Fluoxetine in the treatment of borderline and schizotypal personality disorders. *Am J Psychiatry* 148:1064-7.

Martial J, Paris J, Leyton M, et al (1997): Neuroendocrine study of serotonin function in female borderline personality disorder patients: a pilot study. *Biol Psychiatry* 42:737-9.

McQueen JK, Wilson H, Sumner BE, Fink G (1999): Serotonin transporter (SERT) mRNA and binding site densities in male rat brain affected by sex steroids. *Brain Res Mol Brain Res* 63:241-7.

Miller HE, Deakin JF, Anderson IM (2000): Effect of acute tryptophan depletion on CO2-induced anxiety in patients with panic disorder and normal volunteers. *Br J Psychiatry* 176:182-8.

Montgomery JC, Appleby L, Brincat M, et al (1987): Effect of oestrogen and testosterone implants on psychological disorders in the climacteric. *Lancet* 1:297-9.

New AS, Trestman RL, Mitropoulou V, et al (1997): Serotonergic function and self-injurious behavior in personality disorder patients. *Psychiatry Res* 69:17-26.

Ni X, Chan K, Bulgin N, et al (2006): Association between serotonin transporter gene and borderline personality disorder. *J Psychiatr Res* 40:448-53.

Ni X, Sicard T, Bulgin N, et al (2007): Monoamine oxidase a gene is associated with borderline personality disorder. *Psychiatr Genet* 17:153-7.

Nordstrom AL, Olsson H, Halldin C (1998): A PET study of D2 dopamine receptor density at different phases of the menstrual cycle. *Psychiatry Res* 83:1-6.

Nutt DJ, Forshall S, Bell C, et al (1999): Mechanisms of action of selective serotonin reuptake inhibitors in the treatment of psychiatric disorders. *Eur Neuropsychopharmacol* 9 Suppl 3:S81-6.

Ortiz J, Artigas F, Gelpi E (1988): Serotonergic status in human blood. *Life Sci* 43:983-90.

Paris J (2002): Chronic suicidality among patients with borderline personality disorder. *Psychiatr Serv* 53:738-42.

Parsey RV, Oquendo MA, Simpson NR, et al (2002): Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. *Brain Res* 954:173-82.

Parsey, R.V., Hastings, R.S., Oquendo, M.A., Huang, Y.Y., Simpson, N., Arcement, J., Huang, Y., Ogden, R.T., Van Heertum, R.L., Arango, V., Mann, J.J., (2006). Lower serotonin transporter binding potential in the human brain during major depressive episodes. *Am. J. Psychiatry* 163, 52-58.

Parsey RV, Arango V, Olvet DM, Oquendo MA, Van Heertum RL, John Mann J (2005): Regional heterogeneity of 5-HT1A receptors in human cerebellum as assessed by positron emission tomography. *J Cereb Blood Flow Metab* 25:785-93.

Parsey RV, Olvet DM, Oquendo MA, Huang YY, Ogden RT, Mann JJ (2006a): Higher 5-HT1A receptor binding potential during a major depressive episode predicts poor treatment response: preliminary data from a naturalistic study. *Neuropsychopharmacology* 31:1745-9.

Parsey RV, Oquendo MA, Ogden RT, et al (2006b): Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. *Biol Psychiatry* 59:106-13.

Pazos A, Probst A, Palacios JM (1987): Serotonin receptors in the human brain--III. Autoradiographic mapping of serotonin-1 receptors. *Neuroscience* 21:97-122.

Pearlstein T, Rosen K, Stone AB (1997): Mood disorders and menopause. *Endocrinol Metab Clin North Am* 26:279-94.

Pecins-Thompson M, Bethea CL (1999): Ovarian steroid regulation of serotonin-1A autoreceptor messenger RNA expression in the dorsal raphe of rhesus macaques. *Neuroscience* 89:267-77.

Pecins-Thompson M, Brown NA, Bethea CL (1998): Regulation of serotonin re-uptake transporter mRNA expression by ovarian steroids in rhesus macaques. *Brain Res Mol Brain Res* 53:120-9.

Peroutka SJ (1994): 5-Hydroxytryptamine receptors in vertebrates and invertebrates: why are there so many? *Neurochem Int* 25:533-6.

Peroutka SJ, Snyder SH (1979): Multiple serotonin receptors: differential binding of [3H]5-hydroxytryptamine, [3H]lysergic acid diethylamide and [3H]spiroperidol. *Mol Pharmacol* 16:687-99.

Pigott TA (1999): Gender differences in the epidemiology and treatment of anxiety disorders. *J Clin Psychiatry* 60 Suppl 18:4-15.

Pike VW, McCarron JA, Lammertsma AA, et al (1996): Exquisite delineation of 5-HT1A receptors in human brain with PET and [carbonyl-11 C]WAY-100635. *Eur J Pharmacol* 301:R5-7.

Plenge P, Mellerup ET, Laursen H (1990): Regional distribution of the serotonin transport complex in human brain, identified with 3H-paroxetine, 3H-citalopram and 3H-imipramine. *Prog Neuropsychopharmacol Biol Psychiatry* 14:61-72.

Ramamoorthy S, Bauman AL, Moore KR, et al (1993): Antidepressant- and cocaine-sensitive human serotonin transporter: molecular cloning, expression, and chromosomal localization. *Proc Natl Acad Sci U S A* 90:2542-6.

Rapport MM, Green AA, Page IH (1948): Crystalline Serotonin. *Science* 108:329-330.

Raskin A (1974): Age-sex differences in response to antidepressant drugs. *J Nerv Ment Dis* 159:120-30.

Redei E, Freeman EW (1995): Daily plasma estradiol and progesterone levels over the menstrual cycle and their relation to premenstrual symptoms. *Psychoneuroendocrinology* 20:259-67.

Reiman EM, Armstrong SM, Matt KS, Mattox JH (1996): The application of positron emission tomography to the study of the normal menstrual cycle. *Hum Reprod* 11:2799-805.

Rinne T, Westenberg HG, den Boer JA, van den Brink W (2000): Serotonergic blunting to meta-chlorophenylpiperazine (m-CPP) highly correlates with sustained childhood abuse in impulsive and autoaggressive female borderline patients. *Biol Psychiatry* 47:548-56.

Rosecrans JA (1970): Differences in brain area 5-hydroxytryptamine turnover and rearing behavior in rats and mice of both sexes. *Eur J Pharmacol* 9:379-82.

Rossmanith WG, Laughlin GA, Mortola JF, Johnson ML, Veldhuis JD, Yen SS (1990): Pulsatile cosecretion of estradiol and progesterone by the midluteal phase corpus luteum: temporal link to luteinizing hormone pulses. *J Clin Endocrinol Metab* 70:990-5.

Rousset OG, Ma Y, Evans AC (1998): Correction for partial volume effects in PET: principle and validation. *J Nucl Med* 39:904-11.

Rubinow DR, Hoban MC, Grover GN, et al (1988): Changes in plasma hormones across the menstrual cycle in patients with menstrually related mood disorder and in control subjects. *Am J Obstet Gynecol* 158:5-11.

Rubinow DR, Schmidt PJ, Roca CA (1998): Estrogen-serotonin interactions: implications for affective regulation. *Biol Psychiatry* 44:839-50.

Sakai Y, Nishikawa M, Leyton M, Benkelfat C, Young SN, Diksic M (2006): Cortical trapping of alpha-[(11)C]methyl-l-tryptophan, an index of serotonin synthesis, is lower in females than males. *Neuroimage* 33:815-24.

Saletu B, Brandstatter N, Metka M, et al (1996): Hormonal, syndromal and EEG mapping studies in menopausal syndrome patients with and without depression as compared with controls. *Maturitas* 23:91-105.

Sargent PA, Kjaer KH, Bench CJ, et al (2000): Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. *Arch Gen Psychiatry* 57:174-80.

Schmidt PJ, Nieman L, Danaceau MA, et al (2000): Estrogen replacement in perimenopause-related depression: a preliminary report. *Am J Obstet Gynecol* 183:414-20.

Schmidt PJ, Nieman LK, Danaceau MA, Adams LF, Rubinow DR (1998): Differential behavioral effects of gonadal steroids in women with and in those without premenstrual syndrome. *N Engl J Med* 338:209-16.

Schmidt PJ, Nieman LK, Grover GN, Muller KL, Merriam GR, Rubinow DR (1991): Lack of effect of induced menses on symptoms in women with premenstrual syndrome. *N Engl J Med* 324:1174-9.

Schneider LS, Small GW, Hamilton SH, Bystritsky A, Nemeroff CB, Meyers BS (1997): Estrogen replacement and response to fluoxetine in a multicenter geriatric depression trial. Fluoxetine Collaborative Study Group. *Am J Geriatr Psychiatry* 5:97-106.

Sherwin BB, Gelfand MM (1985): Differential symptom response to parenteral estrogen and/or androgen administration in the surgical menopause. *Am J Obstet Gynecol* 151:153-60.

Silk KR, Lee S, Hill EM, Lohr NE (1995): Borderline personality disorder symptoms and severity of sexual abuse. *Am J Psychiatry* 152:1059-64.

Skolnick P, Paul SM, Weissman BA (1984): Preclinical pharmacology of buspirone hydrochloride. *Pharmacotherapy* 4:308-14.

Smith CE, Ware CJ, Cowen PJ (1991): Pindolol decreases prolactin and growth hormone responses to intravenous L-tryptophan. *Psychopharmacology (Berl)* 103:140-2.

Soares CN, Almeida OP, Joffe H, Cohen LS (2001): Efficacy of estradiol for the treatment of depressive disorders in perimenopausal women: a double-blind, randomized, placebo-controlled trial. *Arch Gen Psychiatry* 58:529-34.

Soloff PH, Meltzer CC, Greer PJ, Constantine D, Kelly TM (2000): A fenfluramine-activated FDG-PET study of borderline personality disorder. *Biol Psychiatry* 47:540-7.

Sotelo C, Cholley B, El Mestikawy S, Gozlan H, Hamon M (1990): Direct Immunohistochemical Evidence of the Existence of 5-HT1A Autoreceptors on Serotoninergic Neurons in the Midbrain Raphe Nuclei. *Eur J Neurosci* 2:1144-1154.

Staley JK, Krishnan-Sarin S, Zoghbi S, et al (2001): Sex differences in [123I]beta-CIT SPECT measures of dopamine and serotonin transporter availability in healthy smokers and nonsmokers. *Synapse* 41:275-84.

Staley JK, Sanacora G, Tamagnan G, et al (2006): Sex differences in diencephalon serotonin transporter availability in major depression. *Biol Psychiatry* 59:40-7.

Steinbusch HW (1981): Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* 6:557-618.

Steiner M, Dunn E, Born L (2003): Hormones and mood: from menarche to menopause and beyond. *J Affect Disord* 74:67-83.

Su TP, Schmidt PJ, Danaceau M, Murphy DL, Rubinow DR (1997): Effect of menstrual cycle phase on neuroendocrine and behavioral responses to the serotonin agonist m-chlorophenylpiperazine in women with premenstrual syndrome and controls. *J Clin Endocrinol Metab* 82:1220-8.

Suehiro M, Scheffel U, Ravert HT, Dannals RF, Wagner HN, Jr. (1993): [11C](+)McN5652 as a radiotracer for imaging serotonin uptake sites with PET. *Life Sci* 53:883-92.

Sumiyoshi T, Stockmeier CA, Overholser JC, Dilley GE, Meltzer HY (1996): Serotonin1A receptors are increased in postmortem prefrontal cortex in schizophrenia. *Brain Res* 708:209-14.

Szabo Z, Kao PF, Scheffel U, et al (1995): Positron emission tomography imaging of serotonin transporters in the human brain using [11C](+)McN5652. *Synapse* 20:37-43.

Torgersen S, Kringlen E, Cramer V (2001): The prevalence of personality disorders in a community sample. *Arch Gen Psychiatry* 58:590-6.

Tork I (1990): Anatomy of the serotonergic system. *Ann N Y Acad Sci* 600:9-34; discussion 34-5.

Tunnicliff G (1991): Molecular basis of buspirone's anxiolytic action. *Pharmacol Toxicol* 69:149-56.

Turner MR, Rabiner EA, Hammers A, et al (2005): [11C]-WAY100635 PET demonstrates marked 5-HT1A receptor changes in sporadic ALS. *Brain* 128:896-905.

Uphouse L, Andrade M, Caldarola-Pastuszka M, Jackson A (1996): 5-HT1A receptor antagonists and lordosis behavior. *Neuropharmacology* 35:489-95.

Wienhard K, Dahlbom M, Eriksson L, et al (1994): The ECAT EXACT HR: performance of a new high resolution positron scanner. *J Comput Assist Tomogr* 18:110-8.

Yatham LN (1993): Is 5HT1A receptor subsensitivity a trait marker for late luteal phase dysphoric disorder? A pilot study. *Can J Psychiatry* 38:662-4.

Zaboli G, Gizatullin R, Nilsonne A, et al (2006): Tryptophan hydroxylase-1 gene variants associate with a group of suicidal borderline women. *Neuropsychopharmacology* 31:1982-90.

Zanarini MC, Frankenburg FR, Dubo ED, et al (1998a): Axis I comorbidity of borderline personality disorder. *Am J Psychiatry* 155:1733-9.

Zanarini MC, Frankenburg FR, Dubo ED, et al (1998b): Axis II comorbidity of borderline personality disorder. *Compr Psychiatry* 39:296-302.

Zimmerman M, Mattia JI (1999): Axis I diagnostic comorbidity and borderline personality disorder. *Compr Psychiatry* 40:245-52.