

Platelet serotonin function and personality traits in affective disorder

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To Leonard, Michael and Olga

“And how can we talk of order overall
when the very placement of the stars
leaves us doubting just what shines for whom?”

W.Szyborska

ABSTRACT

Serotonin (5-HT, 5-hydroxytryptamine) is a neurotransmitter in the central nervous system which has been implicated in the aetiology and pathogenesis of affective disorders like depression and anxiety disorders. The serotonergic system has been shown to be involved in the modulation of mood, sleep, appetite, libido, energy and cognition and memory functions.

The aim of the present study was to investigate and compare hypothesized divergences in central serotonergic function in major depression and panic anxiety and moreover to investigate the relationship between serotonergic function and personality traits in panic anxiety and healthy individuals. The influence of the light and dark seasons on serotonin function in healthy individuals was also investigated.

On the basis of similarities in serotonin uptake, storage and release, and morphological, biochemical and pharmacological properties, we used platelets as peripheral models of serotonergic nerve terminals.

To assess the serotonin function we investigated serotonin uptake kinetics, the serotonin transporter density and 5-HT_{2A} receptor density. Using the Temperament and Character Inventory (TCI) and Karolinska Scales of Personality (KSP) we studied the personality traits in untreated panic patients, in comparison with healthy controls, and during 6 months of citalopram treatment.

Platelet serotonin uptake was significantly lowered in depression, particularly in women and there was a significant increase in the density of serotonin transporters in both sexes. There was no difference in 5-HT_{2A} receptor density between patients and controls. The lowered serotonin uptake in combination with increased density of serotonin transporters may be an indication of malfunctioning transporters and reflect a specific vulnerability in depression.

In panic anxiety the serotonergic dysfunction was illustrated by a reduced density of serotonin transporters, while there was no difference in serotonin uptake kinetics or 5-HT_{2A} receptor density between patients and controls.

We observed 75 % reduction in anxiety and depression scales (BAI, BDI) after 6 months citalopram treatment and 12 % changes in the direction to normalization in all KSP *anxiety* related items, *aggression* and *hostility* related items and the item of *Socialisation*. Citalopram treatment caused a significant inhibition of serotonin uptake and a reduction in 5-HT_{2A} receptor density but did not influence the density of serotonin transporters. Thus a reduced density of serotonin transporters may constitute a trait marker in panic disorder. Modulation of serotonergic functions in terms of a reduction in serotonin uptake and downregulation of 5-HT_{2A} receptors appears to have an anxiolytic as well as an antidepressive effect.

We observed significant seasonal changes in serotonergic variables and what seems to be a compensating relationship between these changes. The seasonal pattern for the serotonin uptake with peaks during dark period and lowest values during light period of the year was the opposite to what was described by the density of serotonin transporters and 5-HT_{2A} receptors. This dynamics of the serotonin transporter function may explain our findings of a stable platelet serotonin content over the year. Compensating relationships in seasonal changes between serotonergic variables in healthy controls may be essential for mental health. Conversely, it seems reasonable to assume that disturbances in the annual rhythm of one serotonergic function could unsettle the balance between interrelated serotonergic mechanism and contribute to mental illness.

The work presented here indicates that the serotonergic dysfunction in major depression and panic disorder has distinctive and separate features. The serotonin transporter density correlated negatively with *Harm Avoidance* scores and positively with *Self-Directedness* scores. These correlations corroborate each other and give tentative evidence for that one link between serotonergic function and behaviour implicates the serotonin transporter. Furthermore, the findings indicate that both the temperament and character dimensions may be serotonergically modulated.

Key words: serotonin, depression, panic disorder, platelets, personality, citalopram

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LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following publications, which will be referred to with the roman numerals I – V:

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ABBREVIATIONS

ANOVA	analysis of variance
B_{\max} [^3H]LSD	density of serotonin receptors
B_{\max} [^3H]paroxetine	density of serotonin transporters
Cl^-	chlorine ion
CNS	central nervous system
CSF	cerebrospinal fluid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders
G-protein	guanine-nucleotide binding protein
K^+	potassium ion
K_d	dissociation constant
K_m	Michaelis-Menten constant
LNAAs	large neutral amino acid
LSD	lysergic acid diethylamide
MAO	monoamine oxidase
Na^+	sodium ion
PAS	para-amino salicylic acid
SMHI	Swedish Meteorological and Hydrological Institute
SSRI	selective serotonin reuptake inhibitor
TCI	tricyclic antidepressant
V_{\max}	maximum velocity of serotonin uptake
5-HT	5-hydroxytryptamine (serotonin)
5-HTP	5-hydroxytryptophan
5-HIAA	5-hydroxyindoleacetic acid
5-HTT	serotonin transporter

INTRODUCTION

MAJOR DEPRESSIVE DISORDER

Descriptions of depression and notions of a biochemical cause dates back to at least 2500 years. Hippocrates (460 – 357 BC) and Greek physicians believed that a melancholic temperament, associated with black bile controlled by the planet Saturn, predisposed to pathological melancholia through its influence on the brain. Autumn was considered as the season most inclined to melancholy. Aristotle (384 – 322 BC) divided the temperaments into one choleric variant (hostile and irritable) associated with yellow bile and one phlegmatic (indolent, irresolute and shy) that was associated with phlegm. In Arabic texts such as those of Avicenna (980 –1037), temperament was believed to represent a mixture of yellow and black bile. Avicenna observed that the appearance of anger, violence and restlessness caused a transition of melancholia into mania. In the sixteenth century, Paracelsus (1493 – 1541) proposed that chemical substances were involved in mental disorders and could affect the state of mental health. In “Anatomy of Melancholy” (published in 1621) Robert Burton described melancholic individuals to be “moody, cold, bitter ironic, eccentric, misanthropic and suicidal”. Contemporary European physicians referred to melancholia as the “English sickness”, a state of mind “very likely to be connected with climate”.

In modern biological psychiatry this old concept about harmony and balance between the “humours” plays the same heuristic role as the imbalance and dysfunction of biogenic amines and their association with personality changes.

To emphasize a biological influence in melancholia Adolf Meyer (1866 –1950) brought about a terminological revision and coined the term depression (pressed down). (Lyons and Petrucelli, 1978, McGrew, 1985, Himmelhoch et al., 2001).

In DSM-IV, major depression is referred to as “major depressive disorder” to emphasize that the syndrome includes both psychological and somatic symptoms (see American Psychiatric Association, DSM-IV, 1994). The diagnostic criteria can be grouped into four categories: 1. Disturbances in mood: the central feature of depression is a subjective experience of marked sadness, (depressed mood) nearly every day. 2. Disturbance in cognition: loss of interest and pleasure in individual activities (anhedonia), feelings of worthlessness or excessive or inappropriate guilt, difficulty in concentration (slowed thinking, poor memory). 3. Behavioural disturbances: social withdrawal, changes in psychomotor activity (agitation or psychomotor retardation). 4. Somatic disturbances: disturbances in sleep and appetite, fatigue, loss of energy and tiredness. To meet the diagnosis of major depressive disorder according to DSM-IV a patient must experience five of the symptoms for at least 2 weeks and at least one symptom must be depressed mood or anhedonia (see American Psychiatric Association, DSM-IV, 1994).

The combination of hopelessness, pessimism, low self-estimation and guilt may lead to suicidal thoughts and suicide.

It has been estimated that 42 % of adult women and 22,5 % of men are at risk to develop major depressive disorder during their lifetime. Up to 15 % of patients with major depressive disorder die by suicide (Lundby study: Hagnel et al., 1994).

PANIC DISORDER

Anxiety has been recognized as a symptom of mental disturbance as long as melancholia. For more than 100 years the conceptualization of panic disorder has developed on two different axes, one medical and one psychological. In medical reports, terms such as “soldier’s heart” or “hyperventilation syndrome” date back to the French Revolution. In psychological medicine, panic attacks were thought to be caused by strong emotions. In 1895, Freud created the concept of anxiety neurosis. In 1980, panic disorder was recognized as a distinct diagnostic entity by the DSM-III (American Psychiatric Association, 1980). Taylor and Koch (1996) described anxiety sensitivity as a “fear of anxiety-related bodily sensations, which arises from beliefs that the sensations have harmful somatic, psychological, or social consequences”. Panic disorders are included in anxiety disorders. Patients with panic disorder experience unexpected panic attacks that vary in severity and frequency, followed by worry about the implications of the attack, anticipatory anxiety and disorganisation of personality (changes in behaviour e.g. avoidance). Panic attacks are manifested by: rapid heart rate, palpitation, sweating, hot flashes or chills, shaking or trembling, shortness of breath, hyperventilation, gastrointestinal distress or nausea, derealization or depersonalisation, fears of losing control, of going crazy or fear of dying. To be diagnosed as suffering from panic anxiety, patients must experience one or more panic attacks with four or more symptoms per week during the previous four weeks (see American Psychiatric Association, DSM-IV, 1994).

Panic disorder has been found to occur in approximately 3 – 6 % of the population (Kessler et al., 1994, Lydiard et al., 1996) and is associated with significant morbidity and an increased risk of suicide (Taylor and Koch, 1996).

Syndrome overlap between depression and panic, DSM IV:

Major depressive disorder

Panic

Emotional:

sadness
anhedonia

anxiety

Cognitive:

impaired concentration and memory

uncontrollable worry

poor concentration

guilt
worthlessness
suicidal ideas

Endocrine manifestation:

change in sleep and appetite
diurnal variation

sleep disturbance

Somatic expressions:

preoccupation with
physical symptoms and health

autonomic symptoms

muscle tension
fatigue

Behavioural

irritability
restlessness

SEROTONIN

In 1937, Erspamer and Vialli found an indole-like substance in the enterochromaffin cells of the gut that caused smooth muscle contraction, and they named the substance *enteramine*. Rapport and Page in 1948 isolated and identified a substance with vasoactive properties in bovine blood serum that they called serotonin. Enteramine and serotonin were found to have identical chemical structure and to be the indolealkylamine 5- hydroxytryptamine. After that, in 1953 serotonin was found in brain tissue by Twarog and Page (Whitaker-Azmitta, 1999) and was considered to act as a neurotransmitter (Bogdanski et al., 1956). The observation at the same time (Wooley and Shaw, 1954) of structural similarities between serotonin and the hallucinogenic drug lysergic acid diethylamide (LSD) gave birth to the idea that serotonin may have important behavioural effects (Bogdanski et al., 1956, Pineyro and Blier, 1999, Aghajanian and Marek, 1999).

Serotonin, 5-hydroxytryptamine (5-HT) is synthesized from the essential amino acid l-tryptophan and is provided to the body by protein-rich food. After intestinal, enzymatic protein digestion, serotonin synthesis takes place in the enterocromaffine cells of the gastrointestinal tract. About 95 % of total body serotonin (10 mg) are localized in the enterochromaffin cells of the gastrointestinal mucosa (Lambert et al., 1995).

In blood 80 – 90 % of tryptophan will bind to plasma albumin where it competes with free fatty acids at the binding site (McMenamy and Oncley, 1958). The overflow of serotonin from the enterocromaffine cells is rapidly taken up by the platelets

through their serotonin transporters, and stored in specific granules called the dense bodies (Stahl, 1985).

The serotonin levels in the central nervous system (CNS) represent only a small fraction of that found in the body. Serotonin is a hydrophilic substance and does not cross the blood-brain barrier; it must be synthesized from tryptophan locally. Tryptophan, enters the brain through a specific carrier in competition with other large neutral amino acids (LNAA) and is taken up into the serotonergic neurons by a plasma membrane transporter and hydroxylated in a reaction catalyzed by the enzyme tryptophan-5-hydroxylase to 5-hydroxytryptophan (5-HTP). 5-HTP is converted into 5-HT by the enzyme aromatic amino acid decarboxylase. Serotonin is stored in synaptic vesicles where it stays until it has been released out in the synaptic cleft by a neuronal impulse. The rate of serotonin release is dependent on the firing rate of the serotonergic soma.

Following release and activation of postsynaptic receptors, serotonin is inactivated by reuptake into the presynaptic nerve terminals by specific serotonin transporters (5-HTT). Serotonin is metabolized by the enzyme monoamine oxidase (MAO-A) to an inactive metabolite 5-hydroxyindoleacetic acid (5-HIAA).

The majority of serotonin-containing cell bodies are concentrated in the raphe nuclei located along the midline of the brainstem and their axons innervate each area of the brain. Five important serotonergic pathways are established in the CNS, from raphe nuclei to: prefrontal cortex, basal ganglia, hippocampus, hypothalamus and spinal cord (Stahl, 1998). The organisation of serotonergic cells in the brain provides insight into the functions of this neurotransmitter

as well as possible roles in mental processes and psychiatric disorders. Serotonin is involved in a large number of CNS processes, including the regulation of mood, feeding behaviour, sleep, libido, pain, energy, fatigue, cognition and memory function (Roth, 1994).

In the periphery, serotonin plays a number of important roles. Serotonin is important in the regulation of vascular smooth muscle contraction, uterine smooth muscle growth (Ramamoorthy et al., 1993), pulmonary endothelium (Lee and Fanburg, 1986), gastrointestinal functioning (Woodman et al., 1998) and platelet shape change and aggregation (Lesch et al., 1993).

5-HT_{2A} receptor

Recognition of 5-HT receptor subtypes began over 40 years ago when Gaddum and Picarelli (1957) discovered two physiological actions of serotonin, which were blocked by different antagonist. Today the structural identification of receptor subtypes using the cloning technique has resulted in recognition of 14 structurally distinct 5-HT receptors grouped into seven families.

In the brain a high density of 5-HT_{2A} receptors is found in many areas of the cortex, in the claustrum, a region that is connected to the visual cortex, in parts of the limbic system and in the basal ganglia and the olfactory nuclei (Hoyer et al., 1994, Barnes and Sharp, 1999). The 5-HT_{2A} receptor has been reported to be involved in control of transmitter release, control of sexual activity, regulation of sleep and in psychiatric disorders like depression, anxiety, migraine and schizophrenia.

The serotonin transporter

Serotonin transporters are localised on presynaptic axon terminals as well as on the cell bodies of serotonergic neurons. Reuptake of serotonin is an active transport energized by inwardly directed Na^+ and Cl^- gradients and an outwardly directed K^+ gradient (Masson et al., 1999).

As a modulator of extracellular serotonin levels and the site of action of many antidepressant drugs, including the SSRIs, the serotonin transporter has been in focus of intense studies for many years.

PLATELETS AS MODELS FOR SEROTONERGIC NERVE ENDINGS

It is difficult to study details of serotonergic mechanism in the brain directly, and easily available peripheral models are therefore of great importance. Human platelets have for a long time been recognized to resemble serotonergic nerve endings in several aspects (Stahl, 1977, Wirz-Justice, 1988, Lingjærde, 1990). Blood platelets, the function of which is mandatory in the primary hemostasis, are disk-shaped structures of 1,5 to 3,0 μm in diameter which circulate in the blood for 8 – 10 days. The significant difference between platelets and cells in general is that platelets are cell fragments (of megakaryocytic origin) and as such they lack a cell nucleus and DNA, and thus the prerequisites for protein synthesis. Platelet also lack the necessary enzymes to synthesize serotonin from tryptophan, but serotonin metabolism by MAO-B

to 5-hydroxyindoleacetic acid (5-HIAA) takes place in these cells (Stahl, 1985). In other aspects they exhibit the properties common to secretory cells including a large number of secretory granules, mitochondria, microtubules and a dense tubular system (Parise et al., 2001).

Serotonin is taken up by platelets, and stored, and released through processes similar to that in brain synapses (Marcusson and Ross, 1990). The pharmacological profile in terms of the responses of the serotonin transporter and serotonin receptor to agonists and antagonists are also alike. The cloned platelet serotonin transporter has been shown to have amino acid sequences identical to the serotonin reuptake transporter in the human brain. Furthermore, platelets and neuronal serotonin transporters are encoded by the same gene (SLC6A4), located on the human chromosome 17q11.2 (Lesch et al., 1993). Platelets and human brain serotonin transporters react similarly to serotonin uptake inhibition by SSRIs (Rausch et al., 1995). Moreover, the platelet serotonin receptor has its counterpart in the postsynaptic 5-HT_{2A} receptor in the brain (Elliot and Kent, 1989, Leonard, 1991). 5-HT_{2A} receptors are G protein-coupled receptor subtypes. Stimulation of the 5-HT_{2A} receptor has been demonstrated to activate phospholipase C and lead to accumulation of inositol phosphates and intracellular Ca²⁺ in both platelets and brain tissue (Peroutka and Howell, 1994, Roth et al., 1998).

SEROTONIN IN AFFECTIVE DISORDER

Monoamine hypothesis of depression

The neurobiological basis of depression came from two separate observations. Patients given reserpine for high blood pressure often became depressed. Reserpine depletes the storage vesicles of their monoamine content of serotonin, norepinephrine and dopamine (Shore et al., 1955, Alpers and Shore, 1969). This led to the hypothesis that monoamines might be reduced in depression. The other observation was that patients with tuberculosis experienced a mood-lifting sensation while being treated with the antitubercular drug para-amino salicylic acid (PAS). The finding that PAS blocked the enzyme monoamine oxidase that metabolizes serotonin gave further support for the monoamine hypothesis of depression. Åsberg and colleagues coined the term “serotonin depression” on the basis of observations of decreased levels of the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA) in cerebrospinal fluid, indicating a reduced turnover of brain serotonin in depressed patients (Åsberg et al., 1976).

The development in the last decades of therapeutically effective antidepressants that have specific actions on serotonin including tricyclic antidepressants (TCIs), monoamine oxidase inhibitors (MAO) and SSRIs has further confirmed the hypothesis that depression is associated with disturbances in brain serotonergic mechanisms.

Serotonin and behaviour

Serotonergic neurons are present in all species that have a nervous system from the most primitive organism to humans. In most of them, serotonin modulates adaptive behaviours, including feeding, sexual and aggressive performance. In the nematode *Caenorhabditis elegans*, serotonin enhances feeding and the release of eggs and in the mollusc *Aplysia californica*, serotonin modulates feeding behaviour and enhances a defensive reflex (Weiger, 1997, Sze et al., 2000). The serotonergic system has been associated with control of aggressive or dominant behaviour and with changing of social status in lobsters, fish, rodents and other primates, and in humans (Olivier et al., 1995, Coccaro et al., 1996, Higley et al., 1996, Yeh et al., 1997, Huber and Delago, 1998, Sneddon et al., 2000, Fairbanks et al., 2001).

Serotonin in aggression

Animal and human studies suggest that serotonin is a modulator of aggressive behaviour. Several investigators have reported that CSF levels of 5-HIAA are decreased in patients with attempted suicide and in violent criminal offenders. Furthermore, platelet 5-HT_{2A} receptor density has been shown to be increased in patients with suicide ideation and post-mortem studies have revealed enhanced 5-HT_{2A} receptor binding in the frontal cortex in patients who had committed violent suicide (Stanley and Mann, 1983, Owen et al., 1983, Arora and Meltzer, 1989).

Cognitive factors and personality in depression and panic anxiety

Personality traits are assumed to contribute or predispose to the development of mood disorders. Using different validated questionnaires to characterize the dimensions of personality, the association between personality traits and affective disorders has been explored in several studies (Cloninger, 1986, Fava et al., 1993, Tanaka et al., 1998, Stracevic et al., 1996). In depression the cognitive process involves a negative interpretation of life events and a pessimistic view of the future. The feeling of hopelessness and helplessness is central in depression. Self-criticism and self-oriented perfectionism are specific for major depression (Bagby et al., 1992, Hewitt and Flett, 1991, Shea and Hirschfeld, 1996).

Anxiety sensitivity, that is, fear of anxiety, based on the belief that anxiety symptoms may have harmful consequences acts as a cognitive predisposition for the development of panic disorder (Schmidt et al., 1995). Panic anxiety is characterised by unexpected, uncontrollable, inexplicable sensations of horror and consequently the victims of these sensations have an apprehensive anticipation of fear for the future.

There is also evidence that several constellations of personality traits are associated across a wide variety of mood and anxiety disorders characterized by maladaptive personality traits including high levels of neuroticism, high levels of avoidance, dependence and traits such as reactivity and impulsivity, introversion and social withdrawal (Hirschfeld et al., 1989, Noyes et al., 1995, Nicholi, 1999, Bienvenu et al., 2001).

CITALOPRAM

Citalopram belongs to the group of selective serotonin reuptake inhibitors (SSRIs) that has a validated efficacy in mental disorders which in some way are related to serotonergic dysfunction, including depression, anxiety and panic disorder. Citalopram is metabolized by the hepatic cytochrome P450 system. Biotransformation of citalopram to the major metabolite demethylcitalopram occurs via the isoenzymes CYP2C19, CYP3A4 and CYP2D6. The plasma half-life is 33 – 36 hours allowing for one daily and 6 – 10 days administration to reach steady state (Hiemke and Härtter, 2000). The recommended initial dosage of 20 mg/day may be increased to a maximum of 60 mg / daily depending on the severity of illness and clinical response.

AIMS OF THE STUDY

The aim of the present study was to investigate and compare hypothesized divergences in central serotonergic function in major depression and panic anxiety and moreover to investigate the relationship between serotonergic function and personality traits in panic anxiety and healthy individuals.

This was performed by investigating platelet serotonergic function:

I. in major depression patients compared with healthy subjects

II. in patients with panic disorder before and during citalopram treatment in comparison with healthy controls

III. in panic patients before and during citalopram treatment in relationship to personality traits as defined by Karolinska Scales of Personality

IV. in panic patients compared with healthy controls in relationship to Cloninger's Temperament and Character Inventory

V. in healthy women in relation to season and climactic variables

MATERIAL AND METHODS

PATIENTS

Patients with major depression were in- or outpatients at the Psychiatric Clinic, S:t Göran's Hospital. Patients were diagnosed as having a major depressive disorder according to DSM-IV during the present episode. Patients with panic anxiety recruited for the studies were outpatients at the Psychiatric Clinic, Danderyd Hospital, who fulfilled the DSM IV-R criteria for panic anxiety. The patients had had one or more panic attacks with 4 or more symptoms per week during the previous 4 weeks. The patients, both in the depression and panic anxiety group should not have received any psychotropic drug (with the exception of benzodiazepines) during the two months preceding the study. Physical illness and signs and symptoms of drug abuse or organic mental disorder were other exclusion criteria.

CONTROL SUBJECTS

The control group consisted of healthy volunteers recruited from the hospital staff and their relatives and friends. The control group was matched for age, gender and the month of blood sampling. Physical examination and psychiatric review by a physician assessed the physical and mental health of the volunteers. Exclusion criteria were somatic illness, own psychiatric disorder and a family history of mental disorders.

ETHICAL CONSIDERATION

The patients and the controls received information about the studies both orally and in writing, each of whom gave their informed consent before participating in the studies. The Ethic committee at Karolinska Hospital approved the studies, diary numbers 94 – 195, 94 – 290, 95 – 131, 96 – 257, 96 – 395, 97 – 080.

DETAILED DESIGN

In paper I, thirty untreated major depression patients were compared with thirty healthy volunteers.

In paper II, thirty-three panic patients were compared with thirty-three controls and the patients were studied before, after 6 – 8 weeks and after 6 months of citalopram treatment.

In paper III, thirty of the panic patients, who participated in study II before and after 6 months of citalopram treatment, were studied in relation to personality traits as assessed by the KSP personality inventory.

In paper IV, the serotonergic functions and personality traits according to Cloninger's Temperament and Character Inventory were investigated in a new group of twenty-eight untreated panic patients in comparison with twenty-eight controls.

In paper V, twenty-two healthy female volunteers were blood sampled twice, once during the light period and once during the dark period of the year.

SYMPTOM AND PERSONALITY ASSESSMENT

In study II, III and IV the degree of anxiety and depression was assessed using the self-rating scales Beck Anxiety Inventory (BAI) (Beck et al., 1988) and Beck Depression Inventory (BDI) (Beck et al., 1979).

The Clinical Anxiety Scale (CAS) (Snaith et al., 1982) was used in study II, III.

Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) was used for clinical evaluation in study I, II and III.

The Karolinska Scale of Personality (KSP) (Schalling et al., 1987) was used in study III and the Temperament and Character Inventory (TCI) (Cloninger et al., 1993) in study IV.

BLOOD SAMPLING (paper I – V)

About 100 ml of fasting blood was drawn (between 8.00 and 9.00 A.M.) from an antecubital vein. Approximately 30 ml of blood was used for determination of platelet serotonin uptake, 60 ml was used for determination of [³H]paroxetine- and [³H]LSD-binding to platelet membranes. Briefly, blood was collected in 10 ml vacutainer tubes, containing 1 ml ACD for the uptake assays and 1 ml 4.5 % EDTA for binding assays. Platelet- rich plasma (PRP) was prepared by low-speed centrifugation.

MEASUREMENT OF SEROTONIN UPTAKE VELOCITY (paper I – V)

The kinetics of platelet [^{14}C]5-HT uptake was studied in undiluted PRP as previously described by Malmgren (1984), using 5 evenly distributed concentrations of [^{14}C]5-HT between 0.1 and 5 μM . The uptake time was 60 seconds. After subtraction of unsaturable uptake, determined after inhibition of saturable uptake with imipramine, the kinetic parameters K_m and V_{\max} were calculated according to the method described in detail by Eadie - Hofstee (Zivin and Waud, 1982).

MEASUREMENT OF TRANSPORTER AND 5-HT_{2A} RECEPTOR DENSITY (paper I – V)

[^3H]paroxetine-binding to platelet membranes was used to determine the number of serotonin transporters, while [^3H]LSD-binding was used to determine the number of 5-HT_{2A} receptors.

The incubation mixtures consisted of 50 μl [^3H]paroxetine or [^3H]LSD, respectively. The concentrations were between 0.035 and 1.8 nM, 50 μl incubation buffer or incubation buffer containing 1.0 μM clomipramine or spiperone, respectively and 400 μl of membrane suspension. After incubation for 2 hours at room temperature for [^3H]paroxetine and 4 hours at 37 °C for [^3H]LSD, the samples were rapidly filtered through Whatman GF/C filters presoaked in 0.3 % polyethylenimine (Sigma Chemicals, Munich, Germany), as described in detail in paper I. Each determination was performed in duplicate. Protein was determined for all analyses (Smith et al., 1985). The kinetic parameters B_{\max} and K_d were

derived from computerised curve fitting to a one site binding model.

MEASUREMENT OF SEROTONIN CONCENTRATION IN WHOLE BLOOD (paper V)

Measurement of serotonin in whole blood was performed using HPLC with fluorometric detection (ex. 270 nm/em. 330 nm). The blood specimen was mixed with α -methyl-5-HT as internal standard. After deproteinization and centrifugation, 20 μ l was injected into the HPLC system. Quantification of serotonin in unknown samples was accomplished by comparing the peak height ratio with reference to the calibration curve ($r^2 = 0.99$). The method has previously been described by Xiao et al., (1998).

CLIMATIC VARIABLES (paper V)

Data on climatic variables was obtained from Swedish Meteorological and Hydrological Institute (SMHI) in Norrköping, Sweden. Seasons were defined by their respective solstices and equinoxes, i.e. dark period: 24 September – 20 Mars, light period 21 Mars – 23 September. The day and night length was determined by the time of sunrise and sunset respectively. The cloudiness was calculated by counting percentage of clouds covering the sky.

STATISTICAL ANALYSES

Calculations were performed with the StatView software in study I, II (SAS Institute Inc., Cary, NC, USA) and Statistica software in study III, IV and V ('99 edition, StatSoft, Inc, Tulsa, USA). The significance of group differences was tested with two-way analysis of variance (ANOVA) with independent groups for diagnosis (patients, control subjects), sex (women, men), groups for hospitalisation (in- and out-patients) in paper I. The Mann-Whitney non-parametric test was used to determine statistical significance between groups (patients versus controls) in paper II and IV. Student's two-tailed, paired t-test was used to determine changes between baseline and after weeks or months of treatment, in paper II and III, changes between light and dark period, paper V. Correlations were determined by regression analysis or Spearman rank correlation, paper I – V.

All results are expressed as means \pm SD and in all statistical analyses a p value < 0.05 was considered statistically significant.

Summary of serotonergic parameters in presented paper:

Study	Uptake velocity (V _{max}) [pmol/10 ⁶ platelets/min]	Uptake affinity (K _m) [μM]	Density of 5-HT transporters [fmol/mg protein]	Density of 5-HT _{2A} receptors [fmol/mg protein]
Controls				
Paper I	0.79 (0.17)	0.79 (0.29)	944 (223)	33 (7)
Paper II	0.77 (0.30)	0.80 (0.15)	977 (194)	37 (8)
Paper IV	0.74 (0.22)	0.70 (0.32)	862 (120)	30 (10)
Paper V Women				
Dark period	0.90 (0.18) ^d	0.67 (0.18)	701 (171) ^d	27 (7) ^d
Light period	0.70 (0.28)	0.65 (0.26)	766 (183)	33 (11)
I. Untreated depression n = 30	0.64 (0.28) ^a	0.83 (0.27)	1035 (323) ^a	35 (11)
II. Untreated panic n = 33	0.78 (0.24)	0.75 (0.31)	785 (268) ^b	35 (15)
II. Panic after 6-8 weeks citalopram treatment	0.34 (0.24) ^c	4.70 (3.14) ^c	790 (367)	20 (7) ^c
II. Panic after 6 months citalopram treatment	0.40 (0.41) ^c	7.29 (7.25) ^c	757 (213)	24 (6) ^c
III. Untreated panic n = 28	0.81 (0.22)	0.80 (0.28)	775 (273)	34 (15)
III. Panic after 6 months citalopram treatment (KSP before/after treat)	0.34 (0.25) ^c	7.46 (7.64) ^c	770 (211)	24 (6) ^c
IV. Untreated panic (TCI/controls) n = 28	0.73 (0.12)	0.67 (0.19)	716 (214) ^b	30 (12)

Mean values and (SD) are presented, p value < 0.05 is considered statistically significant

^a difference between untreated depressed patients and controls

^b difference between untreated panic patients and controls

^c difference before and under treatment, in panic patients

^d difference between season in healthy women

Changes in serotonergic parameters:

Serotonergic parameter	Untreated depression patients /controls n = 30	Untreated panic patients /control n = 29	Panic patients during citalopram treatment n = 29
Velocity of serotonin uptake [pmol/10 ⁶ platelets/min]	↓	ns	↓
5-HT transporters density [fmol/mg protein]	↑	↓	ns
5-HT_{2A} receptors density [fmol/mg protein]	ns	ns	↓

Data obtained from the present studies, ↓ indicate a statistically significant decreased of value, ↑ indicate a statistically significant increase of value, **ns** indicate no significant changes

RESULTS AND DISCUSSION

SEROTONIN FUNCTIONS IN MAJOR DEPRESSION (paper I)

The serotonin hypothezis suggests that depression is associated with a deficiency of serotonergic activity in the brain (Maes and Maltzer, 1995, Mann 1999). We found a lowered velocity of serotonin transport (V_{max}) and an increased number of platelet serotonin transporters in untreated depressed patients in comparison with corresponding variables in healthy controls (paper I). There are numerous reports of a lowered platelet serotonin uptake in major depression (Coppen et al., 1978, Faludi et al., 1988, Stain-Malmgren et al., 2001). The presynaptic reuptake of serotonin appears to be functionally linked to the firing rate of serotonergic neurons. Thus, extrapolating the findings in platelets to the brain, a lowered serotonin uptake may be interpreted as a reflection of a reduced serotonergic activity in depression. Recognizing the

cautional attitude one has to maintain regarding the interpretation of a scenario presented by an experimental model, the repeated observations of a lowered platelet serotonin uptake in major depression may indeed genuinely reflect a neuronal dysfunction. Our study (paper I) suggests that this dysfunction may be more markedly pronounced in women than in men. The influence of sex hormones may be an important issue in this aspect. In men, a lowered serotonin uptake was only observed in those who were hospitalized and the decisive criterion for hospitalisation was a high suicidal ideation.

Investigations concerning platelet [³H]paroxetine binding as a measure of platelet serotonin transporters in depression are considerably fewer than serotonin uptake studies and the results are controversial. Our result of enhanced density of serotonin transporters is in conformity with the findings of Mellerup et al. (1993) but is in conflict with those who have reported unchanged (D'Hond et al., 1994, Hrdina et al., 1995) or decreased density (Nemeroff et al., 1994, Sheline et al., 1995). It is possible that the increased density of serotonin transporters is an indication of malfunctioning transporters and a compensatory countermeasure as a consequence of serotonin overflow in synaptic cleft.

However, we found no evidence for abnormalities in serotonin receptor 5-HT_{2A} density in major depression. Our finding of a close positive correlation between 5-HT_{2A} receptor density and MADRS subscale for suicide in major depression confirms the suggested association between 5-HT_{2A} receptors and aggression and suicidal ideation (Hrdina et al., 1993, Pandey et al., 1995).

SEROTONIN FUNCTIONS IN PANIC DISORDER (paper II, III, IV)

In contrast to what we found in depression, platelet serotonin uptake was comparable to controls in panic anxiety and the serotonergic dysfunction was displayed by a reduced density of serotonin transporters. This result was confirmed in our two different groups of panic patients (paper II, IV). Our finding of a reduced platelet density of serotonin transporters is in agreement with studies in panic anxiety by other investigators as well as anxiety disorders such as generalised anxiety disorder and post-traumatic stress disorder (Arora et al., 1993, Faludi et al., 1994, Iny et al. 1994, Fichtner et al., 1995, Marazziti et al., 1999).

In panic patients we did not find any association between 5-HT_{2A} receptor density and aggression or suicidal behaviour. The risk for suicide attempts in panic disorder patients appears to occur when the patients suffers from comorbid diagnoses, especially comorbid depression has been shown to predict suicidality. However, none of our patients had a past history of suicide attempts and no suicide risk was observed during the treatment.

Platelet studies in anxiety disorders are considerably fewer and have yielded more inconsistent results than in depression (Meltzer and Lowy, 1987, Faludi et al., 1988, Stain-Malmgren et al., 2001). In contrast to the lowered serotonergic function believed to be inherent in depression, animal studies have suggested that anxiety may be associated with a serotonergic overactivity since inhibition of serotonin synthesis and reduction of serotonergic activity apparently produces anxiolytic effects (Briley et al., 1990). The initial finding of an enhanced serotonin uptake in panic anxiety

seemed to support this assumption (Norman et al., 1989) but attempts to confirm this finding have met with failure (paper II, III, Pecknold et al., 1988, Den Boer and Westenberg, 1990). Furthermore, the hypothezis of serotonergic overactivity in anxiety is difficult to incorporate with the fact that SSRIs presumably enhance the serotonergic activity in the brain, has been shown to be effective in the treatment of anxiety disorders (paper II, III, Delgado et al., 1990, Eriksson and Humble, 1990, Pollock 2001).

The exact role of serotonin in panic disorders is still disputed (Eison, 1990, Humble and Wistedt, 1992).

MAJOR DEPRESSION AND PANIC DISORDER – SEPARATE CONDITIONS ? (paper I, II, III, IV)

Our results suggest that major depression and panic anxiety may be separate conditions with different underlying serotonergic dysfunction. However, they frequently appear together with overlapping symptoms and it is very likely that ratio of anxious symptoms to depressive symptoms may vary over time. This interpretation implies that the underlying serotonergic dysfunctions may transform over time and explains, at least in part, the inconsistency in serotonergic aberrations found in depression and anxiety disorders. In other words, alterations in transporter density in major depression and panic anxiety may be associated with the degree of anxiety across diagnoses (paper IV, Iny et al., 1994, Fichtner et al., 1995). In the present studies we took the precaution to evaluate the comorbidity of anxiety and depression in the panic patients by using both anxiety and depression rating scales. Thus we could establish that in our panic patients the anxiety symptoms dominated over depressive symptoms by a factor of two. We are

therefore inclined to believe that the found serotonergic aberrations reflect an anxiety state rather than a depressive state. Nevertheless, future studies of panic anxiety without a comorbid diagnosis of depression may clarify the serotonergic dysfunction more distinctly.

Depressive illness appears to be secondary to anxiety disorder. Over 20 % of patients who fall ill with anxiety disorder will end up with depression while the converse is rare (Kendell, 1974). Moreover, anxiety disorders are more common than depression in children, the prevalence of anxiety disorders is above 10 – 15 %, while the prevalence of depression is between 3 – 6 % (Costello et al., 1988, Cohen et al., 1993). A history of anxiety disorder in childhood entails an increased risk for developing depression in adolescence (Breslau et al., 1995, Lewinsohn et al., 1995).

The investigation of serotonergic function in major depressive patients as two separate groups, with or without a past history of anxiety disorders would be of large interest.

ANXIOLYTIC EFFECTS OF CITALOPRAM (paper II, III)

We found that six months of citalopram treatment did not alter the serotonin transporter density. However, treatment caused a reduction in serotonin uptake as expected, both in terms of uptake velocity and in the affinity of the carrier for serotonin, but moreover, we found that treatment caused a downregulation of the 5-HT_{2A} receptor density and a reduction in sensitivity of the receptors for their substrate. Despite more than a decade of

intensive studies, the mechanisms behind the therapeutic effect of serotonin uptake inhibitors in affective disorders remain elusive. Recognizing the time lag between uptake inhibition, which is immediate, and the weeks it takes to achieve amelioration of symptoms and recovery, it is generally assumed that recovery is achieved because inhibition of presynaptic reuptake of serotonin leads to secondary changes in subservient structures later on. Our finding of downregulated 5-HT_{2A} receptors may therefore be viewed as an expected outcome following serotonin reuptake inhibition.

The finding that citalopram treatment did not alter the serotonin transporter density suggests that a reduced number of serotonin transporters may be a trait marker of panic anxiety.

The changes in serotonin function coincided with a reduction of 75 % in anxiety and depression scores in two-thirds of the panic patients and 12 % change in the direction to normalization in all KSP anxiety items, the aggression and hostility related items and the item of *Socialisation*. This indicates that the observed changes in personality traits could be attributed to the improvement in anxiety and depressive symptoms rather than changes in personality.

In the case that antidepressants may influence additional features in the depressive state, such as social functioning, the changes in KSP may reflect changes in self-perception rather than true changes in behavioural patterns.

Modulation of serotonergic functions apparently has an anxiolytic effect as well as an influence on mood and personality. Perhaps a

plasticity in the serotonergic system is prerequisite in order to respond to treatment. However, it would be wrong to assume that the neurological dysfunction in depression and anxiety disorders only encompasses the serotonergic system. The serotonergic disturbance may be due to an imbalance in serotonergic activity in relation to that of other neurotransmitters. For example, anomalies in noradrenergic functions and in the hypothalamic-pituitary-adrenergic axis have been reported in both conditions (Heninger et al., 1988, Ressler and Nemeroff, 2000, Schatzberg, 2000, Blackburn-Munro and Blackburn-Munro, 2001, Versiani et al., 2002). Thus restoration of balance between serotonergic mechanisms and other neurotransmitter systems could be an essential requirement in effective antidepressant and anxiolytic treatment. One may also discuss if not the mechanism to re-create balance may differ in depression and anxiety disorders since the underlying serotonergic dysfunctions apparently are different (paper I, II, III).

Our results provide evidence about changes in peripheral markers of serotonergic activity, which presumably are paralleled by similar changes in the brain, although it is not known in which area of the brain these changes occur.

**SEROTONIN TRANSPORTER DENSITY AND
PERSONALITY TRAITS
DIFFERENCES IN PERSONALITY SCORES
BETWEEN PANIC PATIENTS AND CONTROLS
(paper I, III, IV)**

The novelty in our studies is the correlations between measures of serotonergic functions and the personality dimensions *Harm Avoidance*, *Self-Directedness*, and *Reward Dependence* that we found in panic anxiety and healthy controls (paper IV).

Our findings of a reduced serotonin transporter density and its inverse correlation with state anxiety scores in panic patients are in agreement with our previous study (paper II) and the studies of other investigators (Arora et al., 1993, Fichtner et al., 1995).

Also, our findings of increased scores in *Harm Avoidance* and decreased scores in *Self-Directedness* and *Cooperativeness* in panic patients are in agreement with the results of others (Stracevic et al., 1996, Ampollini et al., 1997, Richter et al., 2000). Of novel interest is that we found that the serotonin transporter density was inversely correlated with *Harm Avoidance*. The serotonin transporter has a pivotal role in brain serotonin homeostasis and the fine-tuning of serotonergic neurotransmission. Given the role of serotonin being central in emotional regulation, it is not farfetched to assume that one link between serotonergic function and behaviour would implicate that serotonin transporter. Several polymorphisms in genes involved in serotonergic neurotransmission have been suggested to influence endogenous serotonin function. A 5-HTT-linked promoter region insertion/deletion polymorphism with a long (*l*) and a short (*s*) variant has been demonstrated (Greenberg et al.,

1999). The observation that the short variant of the polymorphism reduces the efficacy of the 5-HTT-gene promoter resulting in decreased serotonin transporter expression (Heils et al., 1996, Greeneberg et al., 1999) and is associated with anxiety-related traits support our present finding (Lesch et al., 1996, Katsuragi et al., 1999).

We also found a correlation between the density of serotonin transporters and *Self-Directedness* although in the opposite direction. According to Cloninger (1994), high scores in *Harm Avoidance* describe someone who is nearly always fearful, anticipates harm, and has pessimistic worry about of the future. These personality traits fit well together with the traits described by low scores in *Self-Directedness* which indicate poorly developed concepts about self and the external world, with a tendency to be immature, revengeful, irrational, reactive, irresponsible, undisciplined and having a poor ability to control impulse. Thus the findings of opposite correlations between serotonin transporter density and *Harm Avoidance* and *Self-Directedness*, respectively, corroborate each other and seem intuitively to be valid. If a decreased expression of serotonin transporters is associated with anxiety traits and increased scores in *Harm Avoidance*, as consistent findings seem to imply, it seems reasonable to find that higher density of serotonin transporters is related to higher scores in *Self-Directedness*.

We also found that panic patients in comparison to controls had lower scores in the character dimension *Cooperativeness*. The observation is in conformity with the finding of Hamar et al., (1999) who found that the *Cooperativeness* trait was lowest in the serotonin transporter (5-HTT) short variant (*s*)-genotype group.

The fact that *Harm Avoidance* was reduced during SSRIs treatment, while the number of serotonin transporter remained unchanged may be an illustration of the plasticity of the serotonergic system and indicative of that recovery was achieved by secondary alterations within the serotonergic machinery, such as the downregulation of 5-HT_{2A} receptors and/or repercussions affecting connecting neurotransmitter circuits. On the other hand, since we studied the serotonergic variables in patients in recovery, but still under treatment, we do not know how the serotonin function is expressed in drug- and symptom- free panic patients.

Our finding of a correlation between *Self-Directedness* and serotonin transporter density suggests that *Self-Directedness*, at last in part, is serotonergic modulated. This finding seems to be in conflict with Cloninger's hypothesis that neurobiological factors are less influential on the character dimensions which are mostly socioculturally determined. Cloninger's biosocial model give emphasis to a difference between emotional reactions which form the basis for temperament as compared to intellectual concepts of the self which are the origin of character (Cloninger, 1994). On the other hand, the emotions may influence self-concept and conversely self-concepts modify the emotional reactions. The overlap between thoughts and feelings may make it impossible to identify any personality trait that is purely genetic, purely learned and purely connected with one neurotransmitter. Especially, that each of the dimensions of temperament and character (except *Persistence*) is not a single factor but a sum of scores on subscales measuring more specific traits.

SEROTONIN UPTAKE VELOCITY AND PERSONALITY (paper III, IV)

We found a rather strong positive correlation between serotonin uptake (V_{\max}) and *Inhibition of Aggression* (KSP) in panic patients before treatment. There is no easily found explanation for this correlation. *Inhibition of Aggression* is one of four scales related to anxiety, and high scores refer to a lack of ability to speak up and to be self-assertive in social situations rather than describing unexpressed anger (Gustavsson, 1997). However, outspoken anger and aggression has repeatedly been shown to be connected with reduced serotonergic activity. It is therefore tentative to relate the correlation between enhanced serotonin uptake and enhanced scores of *Inhibition of Aggression* to the hypothesized serotonergic overactivity in anxiety disorders. After 6 months of citalopram treatment a significant decrease was observed in *Inhibition of Aggression* and all anxiety-related scales, and the correlation with V_{\max} was eliminated.

The temperament dimension *Reward Dependence* in the TCI scale correlated negatively with serotonin uptake. High scores of *Reward Dependence* describe individuals with high dependence on emotional supports, sentimentality, social attachment and dependence on approval of others. We found no differences in *Reward Dependence* scores between panic patients and controls. *Reward Dependence* traits have been suggested to be linked to norepinephrine (Cloninger, 1987) and to alpha-adrenergic function (Garvey et al., 1996, Ruegg et al., 1997, Gerra et al., 2000) and dopaminergic function (Kuhn et al., 1999). Our finding of serotonergic involvement in *Reward Dependence* demonstrates that

the link between *Reward Dependence* and the monoaminergic systems is still unclear.

ANNUAL VARIATION OF SEROTONIN FUNCTION IN HEALTHY WOMEN (paper V)

The platelet serotonin uptake in healthy individuals is subject to both circadian (Modai et al., 1986) and seasonal changes (Arora et al., 1984). In this work we studied the impact of the dark and light season on not only platelet serotonin uptake but also on the density of serotonin transporters, the density of 5-HT_{2A} receptors and the platelet serotonin content.

We observed significant seasonal changes in serotonergic variables and what seemed to be a compensating relationship between these changes. The seasonal pattern for the serotonin uptake with peaks during dark period and lowest values during light period of the year was the opposite to what was described by the density of serotonin transporters and 5-HT_{2A} receptors. This dynamics of the serotonin transporter function may explain our and the others' findings (Verkes et al., 1998, Jernej et al., 2000) of a stable platelet serotonin content over the year.

Compensating relationships in seasonal changes between serotonergic variables in healthy controls may be essential for mental health. Conversely, it seems reasonable to assume that disturbances in the annual rhythm of one serotonergic function could unsettle the balance between interrelated serotonergic mechanism and contribute to mental illness. Furthermore, it seems as if the largest demand of a co-ordinated flexibility of various

functions in the serotonergic system took place during spring and autumn, seasons during which the onset of major depression occurs most frequently in the northern latitudes (D'Mello and Flanagan, 1996, Näyhä et al., 1994).

CONCLUSION

1. The serotonergic dysfunction in major depression and panic disorders appears to have distinctive and separate features.

In depression the disturbance is illustrated by a decreased uptake velocity in combination with an increased density of serotonin transporters. The scenario may be an indication of malfunctioning transporters and reflect a specific dysfunction in depression (paper I).

In panic, the serotonergic dysfunction is manifested by a decreased number of serotonin transporters, the density of which is not normalized after citalopram treatment and thus may constitute a trait marker in panic disorder (paper II, III, IV).

2. The serotonin transporter density is correlated negatively with *Harm Avoidance* scores and positively with the scores of *Self-Directedness*. The correlations corroborate each other and give tentative evidence for that one link between serotonergic function and behaviour implicates the serotonin transporter. Both the temperament dimension *Harm Avoidance* and the character dimension *Self-Directedness* appear to be serotonergic modulated (paper IV).

3. The reduction of serotonin uptake and downregulation of 5-HT_{2A} receptors may have bearings on the anxiolytic effect of citalopram treatment in panic (paper II, III) as shown by the parallel reduction in anxiety scores. The treatment is followed by improvement in all KSP anxiety-, aggression- and hostility- related items, and in

Socialisation. The observed changes in personality traits could be explained by the improvement in anxiety and depressive symptoms.

4. Compensatory, rhythmic variations in the serotonergic uptake function and serotonin receptors in pace with seasonal changes appear to be one inherent ability in the plasticity of the serotonergic system, which might contribute to mental health (paper V).

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