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PET STUDIES OF THE SEROTONIN SYSTEM IN MAJOR DEPRESSION AND ITS TREATMENT

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ABSTRACT

The serotonin system has been implicated in major depression since the 1960s, mainly based on the serotonin enhancing properties of antidepressants. Positron emission tomography, PET, is the in vivo molecular imaging method with the best spatial resolution. There has been a gradual development of suitable radioligands for serotonergic targets since the mid-1990s, widening the scope of PET studies of the serotonin system. [^{11}C]MADAM is an established radioligand selective for the serotonin transporter, and [^{11}C]AZ10419369 is selective for the 5-HT$_{1B}$ receptor.

The aim of this thesis was to study the serotonin system in major depressive disorder and the serotonergic effects of treatment with antidepressive medication or with psychotherapy. In order to understand the results with [^{11}C]AZ10419369 better we examined the sensitivity of this radioligand to baseline serotonin levels.

In study I we examined serotonin transporter occupancy with PET and [^{11}C]MADAM in responders to treatment with seven different antidepressants in different doses. Two tricyclic antidepressants (TCAs) and four selective serotonin reuptake inhibitors (SSRIs) were examined. Mirtazapine was included as a serotonin transporter "dummy". Serotonin transporter occupancy could be confirmed in vivo for all TCAs and SSRIs. There was no significant difference in serotonin transporter occupancy between the old antidepressants, TCAs, and the new, SSRIs. Mirtazapine did not occupy the serotonin transporter. The average serotonin transporter occupancy in SSRIs and TCAs was 67 %, which was significantly lower than the 80 % serotonin transporter occupancy previously postulated important for SSRI effect.

In study II we investigated the effect of internet-delivered cognitive behavioural therapy (CBT) for recurrent major depressive disorder on [^{11}C]AZ10419369 binding. Ten patients with an ongoing and untreated major depressive episode finished the study according to protocol and were examined with PET and [^{11}C]AZ10419369 before and after CBT. All patients responded to treatment. The binding potential, BP_{ND}, was reduced by 33 % in the dorsal brain stem, which included the raphe nuclei, from which the serotonergic neurons project. Since the 5-HT$_{1B}$ receptor acts inhibitory, a reduction of 5-HT$_{1B}$ receptor density in the raphe nuclei would in theory result in a general stimulation of the serotonin system. There were no other significant changes in radioligand binding in the brain with CBT.

In study III we wanted to compare [^{11}C]AZ10419369 binding in patients with an ongoing and untreated major depressive episode within recurrent major depressive disorder with age- and sex matched controls. Ten patients and ten controls were examined with PET and [^{11}C]AZ10419369. [^{11}C]AZ10419369 binding was lower in the anterior cingulate cortex (25 % lower) and associated regions (20 % lower in the subgenual prefrontal cortex and 45 % lower in the hippocampus). The difference in the anterior cingulate cortex survived Bonferroni correction for multiple comparisons. The anterior cingulate cortex is an established part of the neurocircuitry of depression. There were no significant differences in the other examined brain regions.

In study IV we correlated [^{11}C]AZ10419369 binding with concentrations of serotonin and its metabolite 5-Hydroxyindoleacetic Acid (5-HIAA) in the cerebrospinal fluid (CSF) at baseline in healthy subjects. Twelve healthy subjects without psychiatric history were first examined with PET and [^{11}C]AZ10419369 and then with lumbar puncture for CSF analysis. The CSF concentrations of serotonin and 5-HIAA were determined with high performance liquid chromatography. There were no significant correlations between levels of serotonin and 5-HIAA in the CSF and [^{11}C]AZ10419369 binding in the whole brain, in the caudate nucleus or in the occipital cortex. Since correlations between CSF and brain concentrations of serotonin and 5-HIAA have been demonstrated, [^{11}C]AZ10419369 binding at baseline likely reflects 5-HT$_{1B}$ receptor density. This has bearing for the interpretation of study II and III.

Utan tvivel är man
inte riktigt klok.

Tankar från roten
Tage Danielsson
1974

LIST OF PUBLICATIONS

I.   Johan Lundberg, Mikael Tiger, Mikael Landén, Christer Halldin, and Lars Farde. Serotonin transporter occupancy with TCAs and SSRIs: a PET study in patients with major depressive disorder. The International Journal of Neuropsychopharmacology 2012 Sep;15(8):1167-72.

II.   Mikael Tiger, Christian Rück, Anton Forsberg, Andrea Varrone, Nils Lindefors, Christer Halldin, Lars Farde, and Johan Lundberg. Reduced 5-HT$_{1B}$ receptor binding in the dorsal brain stem after cognitive behavioural therapy of major depressive disorder. Psychiatry Research: Neuroimaging, 2014 Aug 30, 223(2):164-70.

III.   Mikael Tiger, Christian Rück, Andrea Varrone, Nils Lindefors, Christer Halldin, Lars Farde, and Johan Lundberg. Lower serotonin$_{1B}$ receptor binding potential in the anterior cingulate cortex in major depressive disorder. Manuscript.

IV.   Mikael Tiger, Per Svenningsson, Magdalena Nord, Sandra Jabre, Christer Halldin, and Johan Lundberg. No correlation between serotonin and its metabolite 5-HIAA in the cerebrospinal fluid and [^{11}C]AZ10419369 binding measured with PET in healthy volunteers. Synapse, 2014, Oct;68(10):480-3.

The papers will be referred to in the text by their roman numerals I-IV.

CONTENTS

LIST OF ABBREVIATIONS

5-HIAA	5-Hydroxyindoleacetic Acid
5-HT	5-Hydroxytryptamine (serotonin)
5-HTT	5-Hydroxytryptamine Transporter
BBB	Blood Brain Barrier
B_{max}	Maximal number of specific binding sites
BP_{ND}	Binding Potential
CBT	Cognitive Behavioural Therapy
CGI-S	Clinical Global Impression – Severity
CSF	Cerebrospinal Fluid
DASB	3-amino-4-(2-diethylamino-methyl-phenylsulfanyl)-benzonitrile
DBS	Dorsal Brain Stem
DSM	Diagnostic and Statistical Manual of Mental Disorders
ECT	Electroconvulsive Therapy
GABA	Gamma-aminobutyric Acid
HPA	Hypothalamic-Pituitary-Adrenal
LP	Lumbar Puncture
LSD	Lysergic Acid Diethylamide
MADAM	N,N-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine
MADRS	Montgomery-Åsberg Depression Rating Scale
MAOI	Monoamine Oxidase Inhibitor
MDD	Major Depressive Disorder
MRI	Magnetic Resonance Imaging
NET	Norepinephrine Transporter
NMDA	N-methyl-d-aspartate
NRI	Noradrenaline Reuptake Inhibitor
PET	Positron Emission Tomography
RNA	Ribonucleic Acid
ROI	Region Of Interest
SGPFC	Subgenual Prefrontal Cortex
SNRI	Serotonin Noradrenaline Reuptake Inhibitor
SRTM	Simplified Reference Tissue Model
SSRI	Selective Serotonin Reuptake Inhibitor
TCA	Tricyclic Antidepressant
WHO	World Health Organization

1 INTRODUCTION

1.1 RATIONALE FOR THIS THESIS

Major depression is a significant contributor to the global burden of disease, and likely the leading cause of disability in the industrialized world[1]. In most cases major depressive episodes are highly treatable, although merely half of the patients respond to a selective serotonin reuptake inhibitor (SSRI), the standard first line of treatment[2].

Despite accumulating knowledge, the pathophysiology of major depression remains largely elusive[3]. Secondary depressions following neurological diseases, such as stroke, multiple sclerosis, and Parkinson's disease underscore the importance of the brain also in primary depression, the subject of this thesis. The serotonin system has been implicated in the treatment of depression since the 1960s, when it was discovered that tricyclic antidepressants blocked serotonin reuptake[4].

The advent of molecular imaging in the 1980s constituted a leap in the development of methods to explore the chemistry of the living brain[5]. The gradual evolution of radioligands binding to serotonergic targets has paved the way for imaging of the serotonin system in the brain[6, 7].

The general aim of this thesis was to apply these neuroimaging tools for clinical studies of the serotonin system in major depression and its treatment. Paper I describes a $[^{11}C]$MADAM PET study of 5-HTT occupancy in responders to treatment with first and second generation antidepressants. In paper II-III the $5-HT_{1B}$ receptor selective radioligand $[^{11}C]$AZ10419369 was used to measure $5-HT_{1B}$ receptors in recurrent major depressive disorder, in relation to cognitive behavioural therapy (II) and in comparison with controls (III). Since sensitivity to pharmacologically induced endogenous serotonin release has been reported for $[^{11}C]$AZ10419369, we assessed this radioligand's sensitivity to basal serotonin levels, correlating binding potential with concentrations of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) (paper IV), in order to better understand the results from study II-III.

1.2 MAJOR DEPRESSIVE DISORDER

A debilitating condition of lowered mood with congruent symptoms was well described as early as in ancient Greece. The disorder was termed melancholia according to the prevailing humoral pathology paradigm of the time [8]. In addition to the symptoms related to depressed mood and anhedonia, vital symptoms, such as typically disturbed sleep or loss of appetite, are commonly a part of depression. In a group of depressed patients there are instead atypical symptoms such as hypersomnia and increase in appetite[9].

Depression can be characterized as chronic or episodic according to the lapse of time of depressive symptoms. Depressive episodes can occur within different forms of bipolar disorder, with alternating periods of abnormally reduced and elevated mood, or within

major depressive disorder[8]. This thesis focuses entirely on major depressive disorder, a unipolar form of mood disorder.

Major depression is partly a familial disorder, with estimated average heritability in the range of 31-42%[10]. The risk of developing depression is determined by interactions between genetic liability and life stress, elegantly demonstrated in a longitudinal study of different polymorphisms for the serotonin transporter gene and vulnerability for depression in relation to stressful life events[11]. Furthermore, in at least some subgroups of depressed patients there appears to be a kindling effect, where previous depressive episodes induce an increased vulnerability of relapsing into future depressions[12].

Depression may be a life-threatening condition. A dose-response relationship between depressive symptoms and risk of developing coronary heart disease has been reported[13]. Severe depression is one of the disorders associated with the highest risk of suicide[14].

1.2.1 Defining depression

Emil Kraepelin, one of the pioneers in modern psychiatry, emphasized the episodic nature of depression and in particular its risk of recurrence[8]. These features have been captured in the latest versions of the Diagnostic and Statistical Manual of Mental Disorders (DSM). The fourth edition of this manual, DSM-IV, was used for diagnosing depression throughout this thesis, as it was the most validated diagnostic instrument at the time[9].

The third version of DSM (DSM-III) revolutionized psychiatry, providing criteria-based and thereby more reliable diagnoses. The DSM-III definition of major depression is a slightly modified version of the so called Feighner criteria[15]. A major depressive episode is defined by a set of symptoms, requiring at least either depressed mood or loss of interest [16], during at least two weeks. The minimal duration is drastically shorter than the one month that Feighner suggested. The decreased demand of time with symptoms required for fulfillment of the depression diagnosis has been criticized for leading to a risk of false positives, erroneously labeling distressed people as depressed [17].

Horwitz and Wakefield have also voiced what one of the most prominent collaborators in the DSM task force, Robert Spitzer, deemed as the most important criticism of the major depression criteria in DSM-III and IV: the loss of context, except for bereavement. Disproportionate lowering of mood is a key feature in major depression[3] and disregarding stressful life situations can result in mislabeling people who are sad during difficult times as depressed. A later version, DSM-5, has recently been released, omitting context entirely from the diagnostic criteria[18].

Another issue with the DSM definition is its broadness. The criteria for a major depressive episode can be fulfilled in 1497 ways[19]. With this heterogeneity different kinds of conditions are likely included in the definition. DSM critics like Carrol have argued that the broad and inclusive DSM criteria for depression eclipses more

endogenous forms of depression, implying that the DSM definition of depression is more reliable, but less valid[20, 21]. To partially compensate for this there are separate diagnosis codes in DSM for melancholic and psychotic depressions, variants where medical treatment is strongly indicated. The above difficulties in defining depression have hampered the pathophysiological research in depression, and ignited a call for endophenotypic markers beyond the established diagnostic criteria[22, 23].

1.2.2 Epidemiology

As late as in the end of the 1960s depression was considered a rare disorder[24], probably since only the more severe cases, the tip of the iceberg, were brought into attention. The introduction of antidepressive medications spurred WHO-conducted population surveys, where prevalence rates for depression surged, revealing more of the iceberg[25]. In the longitudinal Lundby study, the inhabitants of an entire community were interrogated and psychiatrically assessed, yielding estimated life time incidence rates for depression of 22.5% for men and 30.7 % for women[26]. Based on data from structured interviews in the National Comorbidity Survey Replication in the United States, the life-time risk for major depressive disorder was estimated to 16.2 %, while the 12-month prevalence was 6.6%[27]. Two later WHO surveys of the global burden of disease have ranked major depression as the fourth leading cause of disease burden in the world[28, 29]. Based on the magnitude of the contribution of mental disorders, in particular depressive disorders, to public health according to these surveys, Whiteford et al. argued that prevention and treatment of mental disorders should be a priority[1].

1.2.3 Pathophysiology

There are several challenges in the study of the pathophysiology of major depressive disorder, the main being the heterogeneity in the depressive phenotype and its etiology, confounding effects of medication and large variabilities in potential biomarkers[3, 30, 31]. The contribution of neuroimaging to the understanding of the neurocircuitry of depression will be dealt with in separate sections of this thesis. The two major current hypotheses are the monoamine theory and the stress-related model, revolving around the hypothalamic-pituitary-adrenal(HPA)-axis. The monoamine theory has been credited to Schildkraut, who, based on the noradrenergic actions of currently available antidepressants (monoamine oxidase inhibitors and imipramine) hypothesized that depression might be due to deficiencies of monoamines, especially noradrenaline[32]. This emphasis was soon questioned, since adrenergic stimulants such as amphetamine lacked proper antidepressant effect[33]. Lapin and Oxenkrug instead formulated the serotonin hypothesis of depression[33], which remains the pathophysiological theory with the most supporting evidence[3], and which is described in more detail below. This thesis focuses on the role of the monoamine serotonin in depression and its treatment. In their article Lapin and Oxenkrug bridged the theory of serotonin deficiency in the brain with involvement also of the HPA-axis, in terms of increased blood-corticosteroid levels in depression resulting in reduced serotonin levels in the brain[33].

There is a clear association between stressful life events and depression[34]. The HPA-axis is activated by stressful stimuli[35]. This stress response has been implicated in major depression in a number of ways. Firstly, higher cortisol levels have been reported

in depressed subjects compared with controls[36]. This is in line with the increased risk of depression for patients with Cushing's syndrome, in essence a hypercortisolistic state[37]. Furthermore, lower corticotrophin-releasing receptor messenger RNA has been found in the frontal lobes in a post mortem-study of depressed suicide victims[38]. Finally, there is a vast literature regarding a blunted plasma cortisol response to corticosteroid administration, commonly referred to as an abnormal dexamethasone suppression test[39]. The sensitivity of the dexamethasone test in major depression is modest, albeit higher in severe forms of depression, with psychotic as well as melancholic features[40]. This has led Fink and Taylor to include an abnormal response to dexamethasone as a diagnostic criterion in melancholia, a condition corresponding to what was formerly referred to as endogenous depression[41]. The American Psychiatric Association task force concluded that an abnormal dexamethasone suppression test strongly suggests melancholia, if appropriate exclusion criteria have been applied. A negative dexamethasone suppression test is not very informative[39].

1.2.3.1   The serotonin hypothesis

Allegedly, Gaddum was the first scientist to report on the importance of serotonin for mental health 1954, based on its ability to antagonize the effects of the hallucinogenic compound Lysergic Acid Diethylamide (LSD)[42]. During this early era of psychopharmacology, the effects of the first tricyclic antidepressant imipramine was discovered[43], inducing a paradigm shift in the treatment of major depression. Carlsson and coworkers could show that the examined tricyclic antidepressants blocked serotonin reuptake in the brain[4]. This discovery, together with a potential antidepressive effect of the serotonin precursor tryptophan[44], was fundamental for the serotonin hypothesis of depression[33]. Clomipramine was one of the most popular tricyclic antidepressants and it stood out as the most serotonergic[4]. This spurred Arvid Carlsson into developing the first selective serotonin reuptake inhibitor, zimelidine, indeed showing antidepressive properties[45]. The development of the SSRIs based on the importance of serotonin is to be counted as one of the major success stories of rational drug development, in the otherwise serendipitous trail of psychopharmacological progress.

The antihypertensive reserpine, derived from the root *Rauwolfia serpentina,* and used as a psychotrophic plant for centuries in Indian herblore, was soon after its introduction as a drug reported to have depression as a possible side effect[46]. The monoamine-depleting effects of this compound were later revealed, giving support for the theory that lack of monoamines, including serotonin contributed to depression. Although the depressogenic effect of reserpine has been questioned[47], the depletion model has contributed to the research on depression and its treatment. Most noteworthy is depletion of the serotonin precursor tryptophan, in experiments where subjects are deprived of dietary tryptophan. Tryptophan depletion has induced depressive symptoms in patients with a history of major depressive episodes and has been shown to predict the risk of relapse[48]. However, tryptophan depletion does not affect symptoms in subjects with an ongoing depression[49]. In a meta-analysis, Ruhé and coworkers concluded that decreased mood with tryptophan depletion demonstrates a vulnerability trait becoming depressed, although the depletion studies fail to show a causal relation between serotonin and depression[50]. There is, however, a distinct depressive effect of

tryptophan depletion in recently remitted patients treated with serotonergic medications[49].

The concept of a serotonergic depression has also been postulated based on low levels of the serotonin metabolite 5-HIAA in a subgroup of depressed patients[51]. Low 5-HIAA in CSF was found in suicide attempters[52], and was later corroborated as a biomarker for suicidal behaviour[53, 54]. The low 5-HIAA levels initially found in depression later turned out to be an effect of confounding antidepressant medication, which reduce CSF concentrations of 5-HIAA[53].

Previously, serotonergic function could be assessed through the stimulation of prolactin release with the serotonin enhancing drug fenfluramine. With this neuroendocrine challenge test depressed patients have shown diminished fenfluramine-induced prolactin release compared to controls[55, 56]. The serotonergic function was not normalized with recovery from major depression[57], suggesting that the blunted prolactin response to fenfluramine is a vulnerability trait for depression, rather than a result of the depressive episode in itself[58].

To conclude, the hypothesis that depression is caused by a deficiency of brain serotonin has not been corroborated. However, there is abundant evidence for an involvement of serotonin in the pathophysiology of depression[59]. The crude version of the serotonin hypothesis still has had a major impact in explaining the phenomenon from which it was derived, namely the serotonin enhancement mediating the thymoleptic effect[33] of a majority of the current antidepressants[60-62].

1.2.4 Treatment

There are three treatment modalities for major depression in common clinical practice: antidepressant medications, psychotherapy, and electroconvulsive therapy (ECT), the first two of which are described in more detail below. ECT is the most effective treatment option in daily routine care for major depressive episodes[63], with the relatively unique feature of better effect the more severe the depression[64]. The high remission rate with ECT for depression is to be weighed against the risks associated with general anesthesia and the risk of retrograde amnesia[65]. Therefore ECT is primarily reserved for the treatment of severe and/or treatment refractory major depression[66].

1.2.4.1 Antidepressants

Medications with antidepressive effects can be divided into five different categories based on the known mechanism of action: selective serotonin reuptake inhibitors (SSRI), selective noradrenaline reuptake inhibitors (NRI), nonselective monoamine reuptake inhibitors, monoamine oxidase inhibitors (MAOI) and other mechanisms of action.

An SSRI is generally the first line of treatment, since it in most cases will be well tolerated[67]. The SSRI are generally considered effective for the treatment of depression, with insignificant differences in efficacy within the group[68]. The number

needed to treat has been reported to be 5, with the large placebo effect in mild to moderate depression potentially obscuring antidepressant treatment effetcts[69].

Reboxetine has been the NRI most frequently used in recent times. Its antidepressant effects has been questioned[70], which casts a shadow over inhibition of noradrenalin reuptake as a mechanism of action for antidepressants. Based on the combined noradrenergic and serotonergic effects of venlafaxine[71] and duloxetine [72], these drugs have been referred to as serotonin noradrenaline reuptake inhibitors (SNRI).This dual monoaminergic action is also shared by some of the tricyclic antidepressants, such as amitriptyline and clomipramine[71, 73]. Amitriptyline and clomipramine were reported to have greater efficacies than SSRIs in a meta-analysis of the treatment of hospitalized patients with depression[74]. These tricyclic antidepressants are not selectively targeting reuptake of serotonin and noradrenaline, their anticholinergic effects are also well known[75].

The monoamine oxidase inhibitors (MAOI) were contemporary with the tricyclic antidepressants. The first-generation MAOIs inhibited monoamine oxidase irreversibly and had a convincing antidepressive effect[76]. Their use has been reduced to a third or fourth line treatment for depression[77] due to the risk of life-threatening hypertensive crisis when interacting with dietary tyramine[78]. Moclobemide, a reversible and selective monoamineoxidase-A inhibitor with an improved safety profile[79], has been developed and shown similar antidepressive efficacy as SSRI[80]

Mirtazpine has a receptor-mediated monoaminergic action[81], and does not block serotonin reuptake[82], illustrating other mechanisms of action. The perhaps most intriguing finding recently in the progress of antidepressant development is the potent and fast antidepressive effect of the N-methyl-d-aspartate (NMDA) receptor antagonist ketamine, paving the way for new modes of action in the treatment of depression.

1.2.4.2   Psychotherapy

Several forms of psychotherapy have been found effective in the treatment of depression, although cognitive-behavioural therapy (CBT), interpersonal therapy and problem-solving therapy have the most robust evidence[83]. Cognitive behavioural therapy might have almost as good antidepressive effect as SSRIs[84]. However, the effects may have been overestimated due to publication bias[85]. Furthermore, many randomized controlled trials study the effect of CBT for the treatment of depression with waiting lists as control condition, for which there is virtually no anticipated positive effect [86]. To more accurately address the specific efficacy of CBT, psychotherapy should be compared with pharmacological treatment and a proper placebo group.

In CBT for depression, the focus on modifying dysfunctional thoughts may vary in different schools and according to the therapist's and patient's preferences. In all forms of CBT the main goal with the treatment is to promote functional behaviours in the patient's life context[87, 88]. With this rationale, in-session behaviour becomes secondary, and alternative treatment administration routes have been developed.

Internet-based CBT for depression has shown similar effects as clinician-administered treatment[89] and it has also been found effective in routine psychiatric care[90].

1.3   THE SEROTONIN SYSTEM

Serotonin is an ancient transmitter substance, evolving from tryptophan possibly 3 billion years ago[91]. Based on phylogenetical comparisons, the primordial 5-HT (5-Hydroxytryptamine, chemical name for serotonin) receptors appeared in the world 700-800 million years ago, which is before the dopaminergic and adrenergic receptor systems[91, 92]. The fundamental importance of serotonin is obvious from its widespread distribution, present in nearly all living organisms, and in humans in all the organs of the body[91]. Tryptophan can be synthesized in chloroplasts in plants. In animal cells this ability is lost, making animals dependent on dietary tryptophan for the synthesis of serotonin[93]. The resulting sparsity of serotonin in animals is believed to be one of the reasons for the development of a plethora of 5-HT receptors, in order to make the most out of the available serotonin[91]. The synthesis of serotonin is limited to certain cell types, neurons and mast cells in particular[91].

Serotonin was initially characterized as a vasoconstrictor substance in serum[94], hence its name. Twarog and Page 1953 reported the presence of serotonin in the mammalian brain[95]. Soon after this discovery, serotonin was suggested as a transmitter substance in the brain[96]. Serotonin signaling is mediated through 14 structurally and pharmacologically distinct receptor subtypes[97]. With one exception, the ligand-gated ion channel termed $5-HT_3$ receptor, the 5-HT receptors couple to G-proteins, which transmit the signal into the cell upon serotonin binding to the receptors[97]. Serotonin levels are regulated extracellularly by reuptake via the serotonin transporter and intracellularly by degrading enzymes such as monoamine oxidase.

Serotonin is involved in a variety of functions in the body, such as appetite regulation[98], learning and memory[99, 100], sleep[101], thermoregulation[102], pain control[103] and modulation of sexual behaviour[104]. There is a role for serotonin also in complex behaviours such as social positioning[105]. Improved mood and self-confidence, even in the absence of a distinct major depressive episode, has been described in an early report from patients treated with SSRIs[106].

The serotonin system is one of the main targets for psychotropic medications, with indications ranging from anxiety disorders and depression to premenstrual dysphoria. The importance of serotonin in major depression and its treatment is reviewed in 1.2.3.1.

1.3.1   Serotonergic pathways

The anatomy of the serotonin system was initially mapped in the rat brain with histochemical fluorescence methods by Dahlström and Fuxe[107], who demonstrated that the cell bodies of serotonergic neurons were concentrated in the raphe nuclei in the brain stem. This general architecture, with serotonergic neurons projecting from raphe nuclei, is also found in humans[108, 109].

The serotonin system in mammalian brains can be divided into two distinct subdivisions: a rostral division, projecting from the midbrain and rostral pons to the forebrain, and a caudal division, with cell bodies in the medulla oblongata, with spinal projections[110]. The cell bodies of the rostral serotonergic neurons are mainly grouped into a dorsal and a median nucleus, with ascending projections. The dorsal raphe nucleus is located in the ventral part of the periaqueductal gray matter of the midbrain and contains the largest number of serotonergic neurons, around 165,000 in humans. The median raphe is the second largest cluster of serotonergic neurons in the brainstem, consisting of around 65,000 neurons, and it is mainly found in the rostral ponsis[110].

The ascending serotonergic nerve fibers project diffusely to the brain, mainly from the dorsal and median raphe nuclei, with particularly dense innervations of layer IV in the visual cortex, the striatum, the hypothalamus, and parts of the thalamus[110] (figure 1). Morphologically the ascending serotonergic projections form a dual system, with either fine varicose axons originating from the dorsal raphe nucleus (D-fibers) or beaded axons arising from the median raphe nucleus (M-fibers). The two systems seem to coexist in most parts of the brain, with the cerebral cortex as an example of dual contribution to serotonergic innervation. However, the striatum is almost exclusively innervated by D-fibers and the hippocampus receives serotonergic input largely through M-fibers[110]. The functional importance of the dual system of ascending projections in the serotonin system is not yet fully elucidated, although the M-fiber system from the median raphe nucleus has been suggested to be of particular importance for depression, based on its innervation of the hippocampus and the limbic cortex[111].
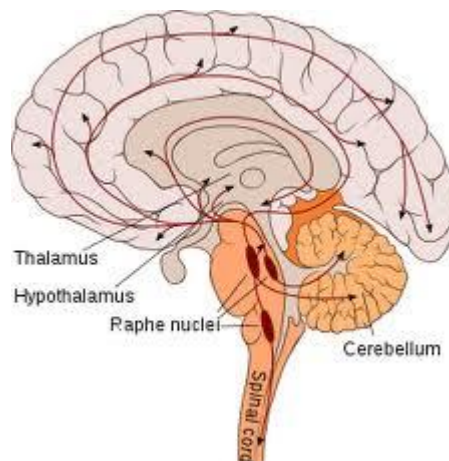

Figure 1. Midline sagittal section of the brain, illustrating the raphe nuclei and their projections.

1.3.2 5-HIAA and serotonin in cerebrospinal fluid

Serotonin is metabolized by monoamineoxidase A and B into 5-HIAA, which can be measured in the CSF. CSF 5-HIAA is considered a good index of serotonin turnover in the central nervous system[112]. Serotonin is generally present in CSF in lower concentrations than 5-HIAA and therefore more difficult to detect[113]. Hence, its metabolite 5-HIAA has been more frequently used in studies of serotonergic activity, especially in the field of psychiatry[52, 53, 114]. With improved quantification

methods, measurement of serotonin concentrations in the CSF has resurfaced as a method to estimate extracellular serotonin in the brain[115, 116].

A positive correlation between CSF and brain levels of serotonin has been shown in rodents[117]. In non-human primates a two-fold increase in serotonin concentration was demonstrated in the CSF after SSRI administration, and CSF serotonin was proposed as a useful index of central serotonin release[118]. In humans, a correlation between serotonin levels in the CSF and in the caudate nucleus in the brain was shown by Wester et al. [119] in a post mortem material. Likewise, correlations have been found between 5-HIAA and serotonin concentrations in the brain[120] and between 5-HIAA in the brain and cerebrospinal fluid in the same subjects[119, 121].

The most non-invasive method for collection of samples from the CSF is by lumbar puncture (LP). With LP, contamination of the brain-derived CSF with serotonin and 5-HIAA from the spinal cord is possible[122]. However, for 5-HIAA a rostral-caudal concentration gradient is well described[119, 121, 123-125], implying that the brain is the major contributor of CSF 5-HIAA.

1.3.3 The serotonin transporter

Extracellular serotonin levels are regulated by a selective Na^+/Cl^- ATP-dependent transport protein, the 5-hydroxytryptamine transporter (5-HTT)[126]. 5-HTT is encoded by a single gene localized to chromosome 17q11.1-17q12 and is expressed on human neuronal, platelet, placental, and pulmonary membranes[127]. In neurons, the active serotonin transporters are located in the plasma membrane presynaptically[128].

Autoradiographical studies of the human brain have demonstrated the highest 5-HTT densities in the raphe nuclei[129, 130], medium densities in putamen, and lower levels in the frontal cortex post mortem[129-132]. Negligible 5-HTT binding was detected in the cerebellum[129-132]. This rank order of 5-HTT densities has been confirmed with PET and the serotonin transporter-specific radioligands [11C]DASB, [11C]MADAM and [11C](+)McN5652[133-135].

1.3.4 Serotonin receptor subtypes

Serotonin binds to a wide repertoire of proteins, with 14 known 5-HT receptors[97] and 20 5-HT receptor transcripts[91]. The 5-HT receptors can be structured into a dendrogram according to their amino acid sequences ([97], figure 2). All 5-HT receptors, except the $5-HT_3$ receptor, are seven-transmembrane-spanning, G protein-coupled receptors[97].

Seven 5-HT receptor families have been identified[97] (figure 2). The $5-HT_1$ receptor family inhibits adenylate cyclase via G_i-proteins[97, 136]. All members of the $5-HT_2$ receptor family mobilize intracellular calcium via phospholipase C. The $5-HT_3$ receptor is a ligand-gated ion channel. The $5-HT_4$, $5-HT_5$, $5-HT_6$, and $5-HT_7$ receptors all stimulate adenylate cyclase via G_s-proteins[137].

The 5-HT$_1$ receptor family stands out among serotonin receptors for its distinctly inhibitory signal transduction properties. The regulatory function of 5-HT$_1$ receptors in the serotonin system makes them especially interesting for studies of generalized conditions such as major depression.

The 5-HT$_{1A}$ receptor is more studied, see 1.6. The 5-HT$_{1A}$ receptor is more frequent in rodents. However, based on the current molecular imaging database, 5-HT$_{1B}$ receptors are four times more frequent in the human brain[138].
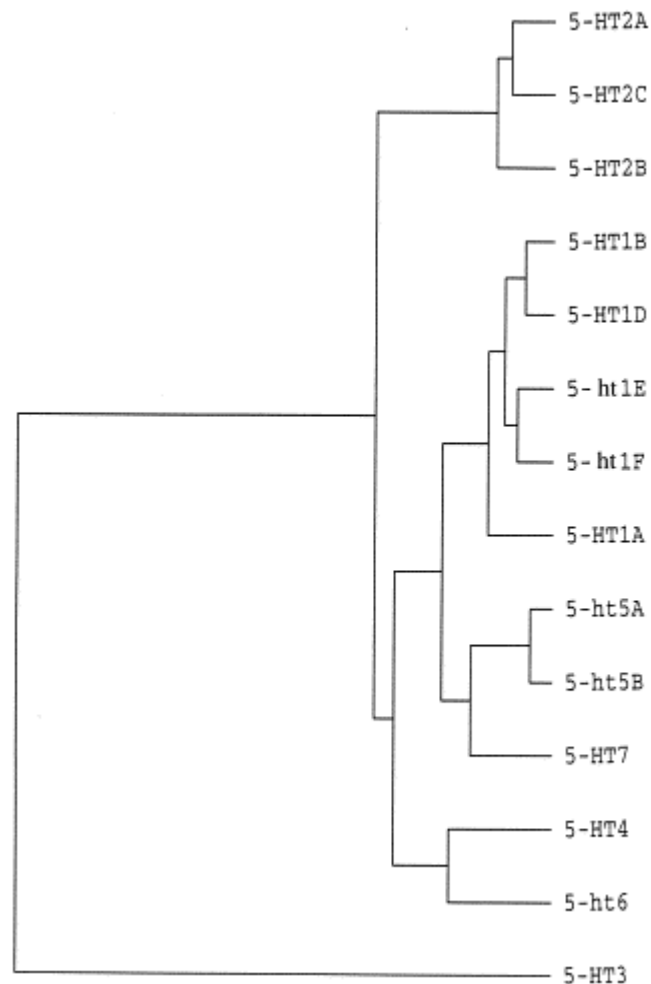

Figure 2. Dendrogram showing the evolutionary relationship between human 5-HT receptor protein sequences. The length of each branch corresponds to the evolutionary distance between the receptor subtypes. Reproduced from: Neuropharmacology, volume 38, N.M. Barnes and T. Sharp: A review of central 5-HT receptors and their function, pages 1083-1152, © 1999, with permission from Elsevier.

1.3.4.1   The 5-HT$_{1B}$ receptor

The 5-HT$_{1B}$ receptor was initially believed to be present only in rodents[139] and the 5-HT$_{1D}$ receptor was thought to be the human counterpart[97]. This fallacy confused the results of early 5-HT$_{1B}$ receptor studies. With the tools of molecular biology it has been made clear that 5-HT$_{1B}$ receptors are expressed in rodents[140] as well as

humans[141]. The receptor initially termed 5-HT$_{1D\beta}$ has hence been recognized as the human 5-HT$_{1B}$ receptor[97].

The gene encoding the 5-HT$_{1B}$ receptor in humans is located on chromosome 6[142]. The 5-HT$_{1B}$ receptor is a G protein-coupled seven-transmembrane-spanning receptor, negatively linked to adenylate cyclase [137]. In the brain, the 5-HT$_{1B}$ receptors act either as presynaptic autoreceptors or as postsynaptic heteroreceptors. Based on the reduced 5-HT levels with 5-HT$_{1B}$ receptor agonists, and the increased serotonin release with 5-HT$_{1B}$ receptor antagonists, the 5-HT$_{1B}$ autoreceptor seems to control the release of serotonin from the presynaptic bulb through inhibition[143]. The heteroreceptor function mediated via 5-HT$_{1B}$ receptors is serotonergic inhibition of non-serotonergic neurons. 5-HT$_{1B}$ receptors have been found postsynaptically mainly on neurons in the major pathways in the brain, GABA and glutamate, as well as on modulatory systems such as acetylcholine [144].

The distribution of 5-HT$_{1B}$ receptors has been mapped with autoradiography[145].The highest 5-HT$_{1B}$ receptor densities were found in the substantia nigra and the globus pallidus. Intermediate 5-HT$_{1B}$ receptor binding was found in the striatum and in the neocortex, although there was a subregion of the medial occipital cortex with denser labeling. The 5-HT$_{1B}$ receptor levels were lower in the amygdala and in the hippocampus. In the cerebellar cortex the 5-HT$_{1B}$ receptor density was negligible. This rank order of 5-HT$_{1B}$ receptor distribution has been confirmed with PET and the 5-HT$_{1B}$ receptor selective radioligand [^{11}C]AZ10419369[146-148].

The functions of the 5-HT$_{1B}$ receptors can be extrapolated from studies with agonists and antagonists and animal models. 5-HT$_{1B}$ receptor mice are partially resilient to the appetite reducing effects of fenfluramine[144]. A 5-HT$_{1B}$ receptor agonist administered to rats decreased immobility time in the forced swimming test, corresponding to antidepressive effect in this animal model[149]. Impaired working memory has been reported in 5-HT$_{1B}$ receptor knockout mice [150]. The absence of 5-HT$_{1B}$ receptors has been reported to have behavioural consequences, with increased aggression [151] and exploratory behaviour [152], impulsivity, and addictive behaviour [153, 154]. Conversely, with overexpression of 5-HT$_{1B}$ receptors in the dorsal raphe nucleus animals became more vulnerable to stress [155].

In humans there are implications of an involvement of 5-HT$_{1B}$ receptors in the pathophysiology of depression. Differences of 5-HT$_{1B}$ mRNA expression between people who committed suicide and controls have been found, in a post-mortem material[156]. An association between a 5-HT$_{1B}$ receptor gene polymorphism and major depression has been found[157]. In the previous PET study on 5-HT$_{1B}$ receptors in MDD a lower binding in the ventral striatum/ventral pallidum in depressed subjects compared to controls was reported[158]. Treatment with 5-HT$_{1B}$ receptor antagonists as adjunctive to SSRIs has been suggested to increase efficacy and reduce time of onset of antidepressant action [159].

1.4 POSITRON EMISSION TOMOGRAPHY

The living brain was formerly thought inaccessible for examination due to the bony fortress of the skull. Ionizing radiation that passes through the cranium provides a means to study the brain. The term positron emission tomography describes the basic principle of the technique: sliced measurement of the emission of positrons. Positron emission is detected and projected into 2-D images (slices) through image reconstruction algorithms.

PET radioligands are molecules with known binding sites, and which are labeled with positron(β^+) emitting isotopes. The radioligand applied determines what can be measured in each PET experiment. The radionuclides most commonly used are ^{11}C, ^{13}N, ^{15}O and ^{18}F[160]. In this thesis carbon-11 radioligands are used throughout. ^{11}C has a half life of approximately 20 minutes. The positron emitting radionuclides in general share a shortage of neutrons, which makes them unstable due to reduced ability to buffer the repelling forces of the nuclear protons. A proton is then converted to a neutron in the nucleus, while releasing a positron and an electron neutrino. The positron is the antiparticle of the electron.

At PET the radioligand is typically injected intravenously. In brain PET, lipophilic radioligands are used to enable passage through the blood brain barrier (BBB). A small portion of the injected dose of radioligand enters the brain and binds to the target protein (figure 3). The radioligand emits a positron through β^+ decay. After a short distance (the β^+ range, typically 1 mm, depending on the isotope) the β^+ interacts with its antiparticle the electron (β^-). This encounter results in annihilation of the particle pair, transforming the conserved energy (1.02 MeV) into a photon pair (γ-particles). The photons are emitted in opposite directions at approximately 180°±0.25° and travel through tissue, skull and air before the coincidences are detected by the surrounding PET system. The divergence from the 180° route, the non-collinearity effect, depends on the isotope used, with its momentum of β^+ and β^- at annihilation[160]. The β^+ range and the non-collinearity effect set the physical limitations to image resolution. The intensity of the γ-rays are attenuated by the passage through tissue and skull. To compensate for this a transmission scan normally precedes the acquisition of emission data, to enable individual attenuation correction.
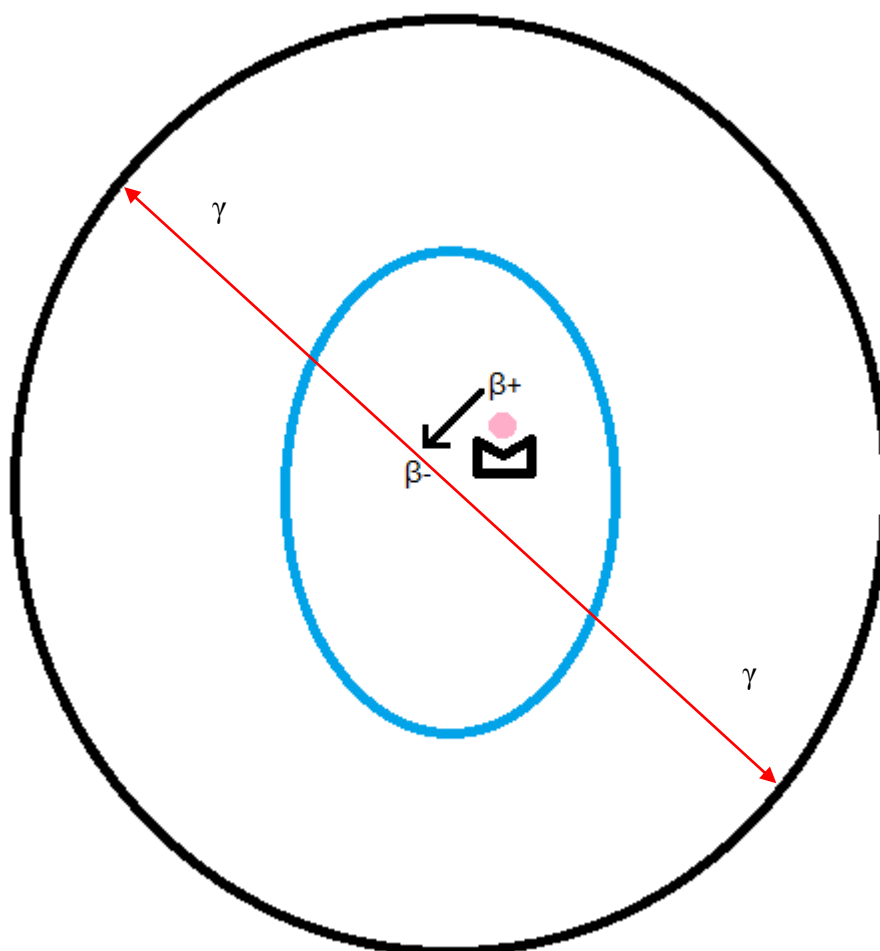
Figure 3. Schematic picture of the principle of PET. The radioligand (pink circle) passes through the BBB and enters the brain (blue circle) where it binds to the target protein. A positron, β+, is emitted from the radioligand, encountering its antiparticle the electron, β-, within a few millimetres distance. The annihilation of this particle pair results in two gamma (γ) rays travelling at approximately 180 degrees from each other, detected by the PET system (black circle).

The spatial resolution is the ability of the imaging system to separate objects that are located in close proximity to one another. It is expressed in terms of the full width at half maximum (FWHM) of the image representing an object. With the Gaussian representation of a perfect point source, FWHM is defined as the distance where the intensity in the image is half of the maximal value. In practice the image resolution is mainly determined by the PET system used: see chapter 3.3.

1.5   PET RADIOLIGANDS FOR THE SEROTONIN SYSTEM

The molecular diversity of the serotonin system has created a vast array of druggable targets for radioligand development. In the recent review by Zimmer and Le Bars, 13 PET radioligands for 5-HT receptors tested in humans are counted, binding to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_4, or 5-HT_6 receptors[7]. PET radioligands for imaging of the 5-HT7 receptors are under development[161]. The most successful radioligands for the 5-HTT are [^{11}C]DASB(3-amino-4-(2-diethylamino-methyl-phenylsulfanyl)-benzonitrile)

and [11C]MADAM(N,N-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine)[6]. In paper I [11C]MADAM was applied. In paper II-IV the 5-HT$_{1B}$ receptor selective radioligand [11C]AZ10419369 was used.

1.5.1 Imaging the serotonin transporter

The success story of antidepressant and anxiolytic drugs that inhibit 5-HT reuptake spurred the development of radioligands for imaging of serotonin transporters in the living human brain[6]. The first attempts to make tracers specific for 5-HTT were carbon-11 or fluorine-18 modifications of SSRIs. Despite displaying high affinity and selectivity for 5-HTT in vitro, the radiolabeled SSRIs have had questionable binding specificity in vivo, as well as slow binding kinetics[162].

The first 5-HTT selective PET radioligand was the isoquinoline derivative [11C](+)McN5652 (*trans*-1,2,3,5,6,10-β-hexahydro-6-[4-(methylthio)phenyl]pyrrolo-[2,1-α]isoquinoline)[163]. However, this compound was not optimal for 5-HTT quantification, given its high non-specific binding and slow brain uptake[135].

Subsequently, a number of radioligands have been derived from the diaryl sulfide series. In a comparison of the properties of four of these and the reference 5-HTT agent [11C](+)McN5652, [11C]DASB was singled out for its faster kinetic uptake, enabling shorter scanning times[164]. The position of [11C]DASB as the leading 5-HTT PET radioligand has thereafter been challenged by a relative newcomer in the diaryl sulfide family, [11C]MADAM[165].

1.5.1.1 [11C]MADAM

Our research group has developed the high affinity 5-HTT radioligand [11C]MADAM, a close analogue to [11C]DASB[166]. [11C]MADAM binds selectively and reversibly to 5-HTT[165].

The rank order of binding potentials in different brain regions in humans has been shown to be in accordance with post mortem 5-HTT binding[129, 130, 133, 167]. [11C]MADAM binding in the cerebellum was sufficiently low to allow for simplified quantitative methods, with cerebellum as reference region[133].

Good to excellent reproducibility has been demonstrated in test-retest measurements with [11C]MADAM[168]. 5-HTT occupancy has been shown with the SSRI citalopram and its S-enantiomer escitalopram[169].

1.5.2 Imaging the 5-HT$_{1B}$ receptor

The development of 5-HT$_{1B}$ receptor radioligands has been delayed by the misinterpretation that this receptor existed only in rodents. Relatively recently two 5-HT$_{1B}$ receptor antagonists have emerged for imaging of 5-HT$_{1B}$ receptors: [11C]P943 (R-1-[4-(2-methoxy-isopropyl)-3-[2-(4-methyl-piperazin-1-yl)benzyl]-pyrrolidin-2-one) and [11C]AZ10419369 (5-methyl-8-(4-methyl-piperazin-1-yl)-4-oxo-4H-chromene-2-carboxylic acid(4-morhpholin-4-yl-phenyl)-amide)(figure 4). Both [11C]AZ10419369 and [11C]P943 have high affinity for 5-HT$_{1B}$ receptors (K_D=0.4 and

$K_D=1.2$ nM respectively)[6]. Sensitivity to pharmacologically enhanced levels of endogenous serotonin has been demonstrated in non-human primates for the above 5-HT_{1B} receptor antagonists[170-173], although no significant displacement of [^{11}C]AZ10419369 binding occurred after a single dose of SSRI in humans[148].
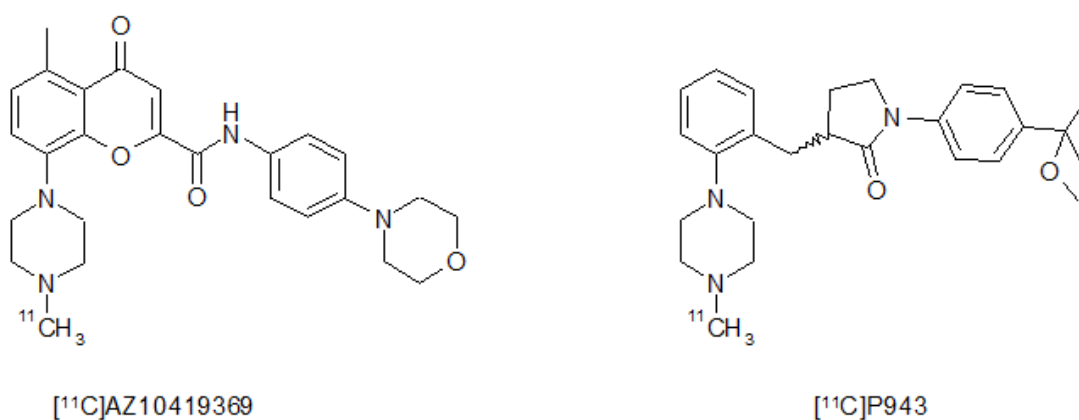

[^{11}C]AZ10419369   [^{11}C]P943

Figure 4. Structural formulas for 5-HT$_{1B}$ receptor radioligands

1.5.2.1 [^{11}C]AZ10419369

The development of the 5-HT$_{1B}$ receptor selective radioligand [^{11}C]AZ10419369 was initially a joint venture, involving Astra Zeneca Pharmaceuticals and the PET group at Karolinska Institutet[174]. In vivo studies have demonstrated rapid brain uptake of [^{11}C]AZ10419369 [147] and no significant amounts of metabolites entering the CNS after injection[146]. Reduced [^{11}C]AZ10419369 binding after pretreatment with a 5-HT$_{1B}$ receptor selective antagonist has confirmed specific 5-HT$_{1B}$ receptor binding[147].

The [^{11}C]AZ10419369 radioactivity distribution has been in agreement with know 5-HT$_{1B}$ receptor densities[145-147]. The radioactivity was distinctly the lowest in cerebellum[146], a region practically devoid of 5-HT$_{1B}$ receptors[145]. Binding potentials calculated with reference tissue models, with cerebellum as reference region, correlated with those obtained with kinetic compartment analysis[146].

The test-retest properties of [^{11}C]AZ10419369 binding are high in cortical regions and satisfactory in subcortical regions[175]. 5-HT$_{1B}$ receptor occupancy has been demonstrated with PET and [^{11}C]AZ10419369[176].

1.6 PET STUDIES OF MAJOR DEPRESSION

In the early days of PET, neuroreceptor imaging of major depression was hampered by a lack of suitable radioligands[177]. The earliest PET literature on depression consists mainly of functional studies. Early on, the limbic system and in particular the anterior cingulate cortex was implicated in the postulated neurocircuitry of depression[178].

Functional PET studies have turned the subgenual prefrontal cortex (SGPFC), a subdivision of the anterior cingulate cortex, ventral to the genu of corpus callosum (Brodmann area 25), into a prime region of interest in depressive disorder[179]. In a

pioneering PET study Drevets et al. found 7.7% lower blood flow in the subgenual prefrontal cortex in depressives relative to controls[180]. This could partially be explained by lower grey matter volume in the subgenual prefrontal cortex in the depressed subjects, as measured with MRI. The lower blood flow and metabolism in SGPFC is a replicated finding in major depressive disorder (MDD)[181-183]. The reduced SGPFC volume in MDD has been corroborated in a meta-analysis of volumetric studies[184]. It has been suggested that, when correcting for the consequent partial volume effects, metabolism in the SGPFC might actually be increased in depressed subjects[179]. The main argument put forward is the decreased blood flow in the SGPFC seen during improvement associated with deep brain stimulation of the SGPFC[185].

During the last two decades radioligand development has boomed. At present, there are several monoaminergic targets available for neuroreceptor imaging[186]. The bulk of molecular imaging studies of MDD are dedicated to targets in the serotonin system[186].

The possibly most replicated finding in receptor imaging of depression is lower 5-HT_{1A} receptor binding in the raphe nuclei and limbic and cortical regions in subjects with MDD compared with controls[187-189]. This is in agreement with the reduced 5-HT_{1A} receptor mRNA concentrations found post mortem in the hippocampus in suicide victims with a history of depression[190]. The reduced 5-HT_{1A} receptor levels did not change with antidepressant treatment[187] and persisted after recovery from depression[188], implying that the lower 5-HT_{1A} receptor binding might reflect a vulnerability trait for depression, rather than the depressive state in itself. However, elevated 5-HT_{1A} receptor binding has also been reported in depression[191]. Shreshta et al. hypothesized that the reduced 5-HT_{1A} receptor BP_{ND} in different serotonergic projection areas might have been an artefact, caused by differing 5-HT_{1A} receptor binding in the grey matter of the reference region cerebellum. The authors instead advocated the use of cerebellar white matter for reference[192]. Still, lower 5-HT_{1A} receptor BP_{ND} in depression has also been demonstrated with cerebellar white matter as reference region[193].

Imaging of the serotonin transporter in MDD has provided partially inconsistent findings, with higher, lower or no difference in 5-HTT binding when comparing depressed subjects with controls[186, 194]. Two intriguing findings imply that depression might primarily correspond to increased 5-HTT binding. Firstly, Cannon et al. demonstrated a correlation between increased 5-HTT binding in the thalamus and depression severity[195]. Secondly, [^{11}C]DASB binding in serotonergic projection areas was strongly associated with the severity of negativistic dysfunctional attitudes[196]. However, either decreases or no change in serotonin transporter binding has been found in the cerebral cortex in depressed subjects post mortem[197].

The data on 5-HT_{2A} receptors in depression do not show any clear general trend[186]. Medications might have confounded some of the results, since reduced 5-HT_{2A} receptor binding has been observed after SSRI treatment, at least in young subjects[198]. Increased 5-HT_{2A} receptor binding has been reported in a subgroup of depressed subjects with extremely dysfunctional attitudes[199]. Bhagwagar and co-workers

replicated a correlation between dysfunctional attitudes score and 5-HT$_{2A}$ binding potential, albeit in patients recovered from depression[200].

As yet, there is only one case-control PET study of 5-HT$_{1B}$ receptor binding in MDD, reporting lower [^{11}C]P943 binding in the ventral striatum in depressed subjects compared with controls[158]. The intracellular protein p11 is important for 5-HT$_{1B}$ receptor function, and has been implicated in depressive behaviour in p11 knockout mice[201]. In this elegant study by Svenningsson et al., lower p11 mRNA and protein in the ACC in depressed subjects was also reported[201].

The clinical heterogeneity inherent within the depressive syndrome[30] poses a challenge for the research on the pathophysiology of major depression. One way to increase the phenotypic homogeneity in MDD is to study patients with recurrent major depressive disorder, a condition that is likely more hereditary than are solitary depressive episodes[10]. In a recent position paper, Savitz and Drevets summarized the current state of knowledge on neuroimaging for depression, concluding that although there are promising results, there are currently no brain imaging biomarkers that can be used for diagnosing depression or predicting antidepressant treatment response[202].

1.7 PET STUDIES OF ANTIDEPRESSANTS

PET studies of the effect of antidepressants have mainly served two purposes: in vivo confirmation of in vitro and ex vivo derived hypotheses of mechanisms of action and clinical dose finding. The pioneering PET studies of D$_2$ dopamine receptor occupancy in relation to antipsychotic effect and extrapyramidal side effects have constituted a model for clinical occupancy studies[203-205]. Based on the results it was hypothesized that a therapeutic window of 70-80 % D$_2$ dopamine receptor occupancy would be optimal for antipsychotic treatment with classical neuroleptics[5]. With the advent of selective serotonin reuptake inhibitors, the serotonin transporter became a rational target for occupancy studies with antidepressants[194]. In the 5-HTT occupancy studies so far, attempts have been made to relate occupancy to clinical effect but not to side effects, possibly since the serotonergic side effects are not as distinctly dose-responsive as the extrapyramidal symptoms that might occur with neuroleptic treatment.

A [^{11}C]DASB PET study quickly confirmed 5-HTT occupancy in the living human brain with SSRI treatment[206]. Suhara et al. reported 5-HTT occupancy with a tricyclic antidepressant as well as with an SSRI, with surprisingly high 5-HTT occupancies at low doses[62]. Meyer and co-workers have conducted the largest PET study of 5-HTT occupancy so far, with five different SSRIs and 82 subjects[61]. No association between 5-HTT occupancy and depression symptom ratings was found. Despite this lack of correlation between 5-HTT occupancy and clinical effect a threshold of 80 % 5-HTT occupancy was suggested for therapeutic effect, based on the striatal 5-HTT occupancies seen with SSRI treatment in the clinical dosing range. This postulated minimum of 5-HTT occupancy has been applied in a dose-finding study for the SNRI duloxetine[207].

Most PET studies on SSRI have indeed demonstrated 5-HTT occupancies of 80 % or more[194]. Already 2003, Suhara et al. noted that further dose escalation beyond this level would have a minimal effect on 5-HTT blockade[62]. With high SSRI doses 85 % striatal 5-HTT occupancy has been reported[208]. Lower 5-HTT occupancies, around 70 %, have been demonstrated after single doses of SSRIs[169, 209].

With the development of (S,S)-[^{18}F]Me-NER-D2, the norepinephrine transporter (NET) can be visualized in the human brain in vivo[210], enabling studies of the other main antidepressant pathway proposed – inhibition of noradrenaline reuptake[211] . NET occupancy after nortriptyline administration was first demonstrated in healthy volunteers[212] and subsequently in depressed patients responding to nortriptyline treatment[213]. Based on data from this clinical PET study, the recommended NET occupancy threshold for antidepressant treatment with nortriptyline was 50%[213].

One of the most intriguing new findings in the PET studies of compounds with antidepressive properties is a recent [^{11}C]AZ10419369 and [^{11}C]DASB PET study of ketamine effect in non-human primates, with potential bearing also on imaging studies of the serotonin system in MDD. Yamanaka and co-workers reported a significant increase in 5-HT$_{1B}$ receptor binding in the nucleus accumbens and ventral pallidum, as well as a significantly reduced 5-HTT binding in these brain regions upon ketamine administration[214].

2 AIMS

The overall objective of this thesis was to contribute to the PET studies of the serotonin system in major depression and its treatment. The specific aims were:

1. To confirm 5-HTT occupancy as a shared mechanism of action in the living brain for first and second generation antidepressants, TCAs and SSRIs, in patients who previously had responded to treatment.
2. To examine the effect of cognitive behavioural therapy for depression on 5-HT_{1B} receptor binding.
3. To compare 5-HT_{1B} receptor binding in unmedicated patients with an ongoing moderate depressive episode within recurrent major depressive disorder with age- and sex matched controls.
4. To assess the sensitivity of [^{11}C]AZ10419369 binding to basal serotonin levels, by correlating BP_{ND} with CSF concentrations of serotonin and its metabolite 5-HIAA in healthy volunteers.

3 MATERIALS AND METHODS

3.1 ETHICS

We adhered strictly to the Declaration of Helsinki in all studies, which emphasizes that no medical research on humans should be conducted without informed consent. All studies were approved by the relevant ethical review boards and by the Radiation Safety Committee at the Karolinska University Hospital. No experimental procedures were performed before the subjects had given informed consent.

The patients in paper II got treatment with internet-based CBT through participation in the study. These patients were informed that they could get treatment even without being enrolled in the study. The excluded patients that were eligible for internet-CBT were offered this treatment. Otherwise the subjects did not receive any personal gains from participating in the studies, apart from a small economical compensation for their efforts.

Basically, the risk from the exposure to a low dose of radiation for the subjects was to be weighed against the increase in knowledge of the serotonin system in relation to depression and its treatment gained through the studies. In paper IV, there was also a risk for post lumbar puncture headache. According to our judgement, approved by ethical review boards, the increased knowledge about the pathophysiology of depression and the mechanisms of actions of its treatment obtained through these studies outweighed the above risks.

3.2 SUBJECTS

The studies in this thesis used only human participants. The examinations were approved by the relevant ethical review boards and by the Radiation Safety Committee at the Karolinska University Hospital. The subjects were physically essentially healthy, according to medical history, physical examination, lab tests and magnetic resonance imaging (MRI) of the brain.

3.2.1 Patients

In paper I, responders to pharmacological treatment of depression in monotherapy, with any of seven different antidepressants at different doses, were recruited from psychiatric outpatient clinics or primary health care centres. Twenty patients were examined: 6 males and 14 females, aged 22-59 years.

In papers II-III ten patients with an on-going and untreated major depressive episode of moderate severity within recurrent major depressive disorder were included. Patients with bipolar disorder, on-going psychopharmacological treatment (wash out-period>1 month), organic brain disorder, drug or alcohol abuse, and suicidal patients were excluded. Six males and four females, aged 24-68 years, were examined.

3.2.2 Controls

The controls were deemed healthy according to the above medical assessment. In addition, the controls were mentally healthy, according to medical history and a structured clinical interview. The controls for paper II-III were matched to the patients by age (±3 years) and sex. Seventeen controls were examined, 8 males and 9 females, aged 20-69 years.

3.3 POSITRON EMISSION TOMOGRAPHY AND MAGNETIC RESONANCE IMAGING

Two different PET systems were used in this thesis: ECAT EXACT HR 47 (Siemens, Berlin and Munich, Germany), which was run in the three-dimensional mode[215] (paper I) or ECAT HRRT (High Resolution Research Tomograph, Siemens Molecular Imaging) (papers II-IV). The inplane and axial resolution of the ECAT EXACT HR 47 was ~3.8 and 4.0 mm, respectively, FWHM. The spatial resolution of the HRRT system was approximately 1.5 mm in the centre and 2.4 mm at 10 cm off-centre FWHM with the current protocol[216].

Brain radioactivity was measured in a list mode fashion during 93 (papers I-III) or 63 (paper IV) minutes. Scatter correction was performed as described in the literature[215, 216]. Emission data were attenuation-corrected to compensate for the loss of signal through tissue propagation, as estimated by the preceding transmission scan. The resulting images were reconstructed in a series of time frames either with filtered back projection with a 2 mm FWHM Hann filter (ECAT EXACT HR 47) or with the three-dimensional ordinary Poisson-ordered subset expectation maximization algorithm (3D-OP-OSEM) (HRRT). 3D-OP-OSEM has been optimized to 10 iterations and 16 subsets for the HRRT.

Movement correction algorithms were applied in papers II-IV. For most subjects, each minute block of frames was realigned to a summation of the first four time frames[217]. For some, subjects the head movements during PET exceeded the limits of this protocol. Hence the PET images were reconstructed manually using frame-specific attenuation-data.

MRI was performed before PET with either a 1.5 Tesla system or a 3 Tesla system. The MRI protocol included a T2-weighted sequence to rule out pathology and a T1-weighted 3-dimensional sequence for optimal visualization of anatomy and for coregistration with PET images. The T1 sequence was a 3D FSPGR BRAVO protocol in the axial plane with the following settings: repetition time 8.132 ms, echo time 3.18 ms, flip angle 12°, field of view 256 mm, matrix 256 × 256×176, slice thickness 1.0 mm, number of excitations = 1.

The MR images were adjusted to position the anterior-posterior commissural (AC-PC) line in the horizontal plane and the interhemispheric plane orthogonal to the AC-PC plane. The MR images were then resampled and cropped to generate a 256X256X141 matrix with 1 mm^2 pixels. This matrix was coregistered to the PET images with rigid body transformation using SPM2 or SPM5[218].

In paper III depressed patients were compared with controls. Lower volumes in the subgenual cingulate cortex in patients with mood disorders is a replicated finding[184]. The partial volume effect increases with smaller volumes, when a larger percentage of the radioactivity spills out of the ROI. Therefore the PET files in paper III were corrected for partial volume effects with the method developed by Meltzer et al.[219].

3.4 EXAMINATION PROCEDURE

Before each PET examination a plaster helmet was made for each subject according to an established procedure[220], to fit into a head fixation system, for reproducible position alignment and minimization of head movements during PET. [^{11}C]MADAM (paper I) or [^{11}C]AZ10419369 (papers II-IV) was synthesized as previously described[147, 166]. The subject was placed recumbent with his or her head in the PET system. A sterile physiological phosphate buffer (pH=7.4) solution containing the radioligand was prepared for injection with either 300 MBq (paper I) or 400 MBq (papers II-IV) and diluted with saline to a volume of 10 ml. The radioligand solution was then injected as a bolus into an antecubital vein, which was flushed with 10 ml saline immediately thereafter. Brain radioactivity was measured as described in 3.3.

3.5 REGIONS OF INTEREST

In papers I-III regions of interest (ROIs) were drawn manually on MR images using the Human Brain Atlas software[221]. In paper I ROIs for putamen and cerebellum were delineated on five consecutive horizontal sections. In papers II-III ROIs were defined for orbitofrontal cortex, anterior cingulate cortex, subgenual prefrontal cortex, amygdala, hippocampus, ventral striatum, dorsal brain stem and cerebellum. The ROIs in papers II-III were delineated as previously described[146], except for the ROI for the dorsal brain stem, which was drawn on 5 consecutive horizontal sections of the dorsal half of the brain stem, ranging from the lower limit of the inferior colliculi to the isthmus.

In paper IV ROIs for the occipital cortex and the caudate nucleus were defined with the Automated Anatomical Labeling (AAL) template[222].

3.6 QUANTIFICATION OF RADIOLIGAND BINDING

Within each defined region the accumulated radioactivity was measured in each time-frame. The radioactivity represented three fractions of radioligand: free, non-specifically bound and specifically bound. With kinetic analyses of regional time-activity curves the measured radioactivity can be transformed into an estimation of radioligand binding. Since the studies reported in papers I-III were clinical studies and since reference tissue modelling has been found suitable for the applied radioligands[133, 146], invasive measurements of arterial input were avoided in this thesis.

The binding capacity of the radioligand depends on the density of the target protein, B_{max}, and the affinity of the radioligand for the target protein, $1/K_D$. These two factors cannot be distinguished from each other by in vivo measurements with tracer

methodology in human subjects, as in PET measurements. Instead the binding potential, BP, has been introduced as a concept to estimate target protein densities[223]. BP is the product of target protein density, B_{max}, and the affinity of the radioligand, $1/K_D$:

$$BP = \frac{B_{max}}{K_D} \qquad (1)$$

The BP model assumes that the radioligand is homogenously distributed over the examined compartments.

When a reference tissue model is used, the resulting binding potential is referred to as non-displaceable binding potential (BP_{ND})[224]. The binding potential is then calculated by comparing the radioactivity in the ROI with that of the reference region, which ideally should be devoid of the target protein, and therefore of specific binding to displace. The cerebellum was used as reference region in this thesis, since it has negligible densities of 5-HTT and 5-HT$_{1B}$ receptors[129-132, 145]. Reference tissue modelling carry an inherent risk, since the results in the ROIs are calculated relative to data in the reference region. A difference in BP_{ND} between examined groups could thus depend on both differing radioactivity in the ROI, but also on differences in the reference region. Global differences in BP_{ND} should thus be treated with caution in studies of pathophysiology.

3.6.1 The simplified reference tissue model

The simplified reference tissue model (SRTM) was used to calculate BP_{ND} in papers I, III and IV[225]. The radioactivity in each ROI was compared with the radioactivity in the cerebellum, which served as reference region, providing an approximation of the radioactivity for non-specifically bound and free radioligand. The concentration of non-displaceable radioligand was assumed to be the same in the ROIs as in the cerebellum.

3.6.2 The linear graphics analysis

In studies II-III [^{11}C]AZ10419369 BP_{ND} was also calculated with the non-invasive Logan plot[226]. The linear graphic analysis for reversible ligand binding to target proteins was developed by Logan et al.[227] and initially required an arterial input function (invasive Logan plot).

The slope of the plot when the linear phase has been reached corresponds to the plasma volume plus the total volume of distribution of the radioligand. The volume of distribution is defined as the amount of plasma needed to correspond to the amount of radioligand in 1 cm^3 tissue in the brain[224].

Similarly, the slope of the linear part of the non-invasive Logan plot can provide the distribution volume ratio (DVR), from which BP_{ND} can be derived as follows:

$$DVR = V_T/V_{ND} = BP_{ND} + 1 \qquad (2)$$

V_T is the volume of distribution in the tissue region of interest and V_{ND} the volume of distribution in the reference region, here the cerebellum.

3.6.3 Wavelet-aided parametric imaging

In papers II and III 7 or 8 brain ROIs were analyzed. The dorsal brain stem ROI was delineated to encompass the median raphe nucleus, hypothesized to be of importance in major depression and its treatment[111, 228]. The 5-HT_{1B} receptor mRNA expression in the median raphe is relatively low[229]. Therefore the signal-to-noise ratio was expected to be lower in this region compared to the other examined ROIs. To compensate for this a Wavelet-Aided Parametric Imaging approach (WAPI) was used to filter out noise. The wavelet transform separated signal components with lower frequency from noise with higher frequency[230]. The number of iterations (depth of decomposition) and filter kernel have previously been optimized to 3 and 16, respectively[231]. 3-D stationary WAPI (S-WAPI) is translation-invariant and has been validated for in vivo-measurements in the human brain[232]. S-WAPI was used in papers II-III. Since WAPI is computationally demanding, the relatively economical linear graphics analysis described in 3.6.2 was applied in the calculation of BP_{ND} values.

3.6.4 Occupancy and $K_{i\ dose}$

PET is an excellent method for determining drug receptor occupancy in vivo[233], and this technique has been used to determine 5-HTT occupancy in a dose-finding study for the antidepressant duloxetine[207]. Occupancy is here defined as the percentage of target proteins that are occupied by drug binding. With two PET examinations, one with drug treatment and one without, occupancy can be calculated with the given formula:

$$occupancy = \left(1 - \frac{BP_{treatment}}{BP_{reference}}\right) \times 100 \qquad (3)$$

Equation (3) was used to determine 5-HTT occupancy in paper I. $BP_{treatment}$ was the BP_{ND} in the putamen in patients with antidepressant treatment at steady state. This was compared with the average BP_{ND} in the putamen of an untreated reference group, *BPreference*.

The relationship between occupancy and dose can be described by a curvilinear function given by the following equation (4):

$$B = \frac{B_{max} \times F}{K_{i\ app} + F} \qquad (4)$$

where B is the concentration of ligand bound to 5-HTT, B_{max} the concentration of available 5-HTT, F the concentration of unbound ligand and $K_{i\ app}$ the apparent inhibition constant.

In paper I the inhibition constant was referred to as $K_{i\,dose}$, since the calculations were based on dose, for estimation of the 5-HTT affinity of the drug, based on the occupancy achieved with the prescribed dose. If a linear relationship between antidepressant dose and drug concentration in the brain is assumed, F may be substituted with *dose*. Accordingly, equation (4) may be rewritten as follows:

$$occupancy = \frac{occ_{max} \times dose}{K_{i\,dose} + dose} \qquad (5)$$

where occ_{max} is the maximal occupancy (here assumed to be 100%) and dose constitutes the daily dose in mg of the drug.

4 RESULTS AND COMMENTS

4.1 STUDY I

The aim of this study was to compare serotonin transporter occupancy in responders to seven different antidepressants to see to which extent serotonin reuptake inhibition is a shared mechanism of action. Patients with different doses of four SSRIs, citalopram, fluoxetine, sertraline, and venlafaxine, where compared with patients treated with two first-generation antidepressants, the TCAs amitriptyline and clomipramine. Mirtazapine was included as a 5-HTT "dummy." Twenty patients on monotherapy were recruited.

For all antidepressants but mirtazapine [^{11}C]MADAM BP_{ND} in the putamen was lower in the patients compared to the reference group, figure 5. Mirtazapine did not occupy the serotonin transporter. The mean 5-HTT occupancy for the SSRIs and the TCAs was 67%, range 28-86 %, figure 6. The occupancy was lower for the TCAs than for the SSRIs, albeit not significantly so (mean 61 % versus 70 %, median 69 % versus 73 %, Independent Samples Kruskal Wallis Test: sig = 0.165). The doses of amitiptyline were on the lower end of the therapeutic range.
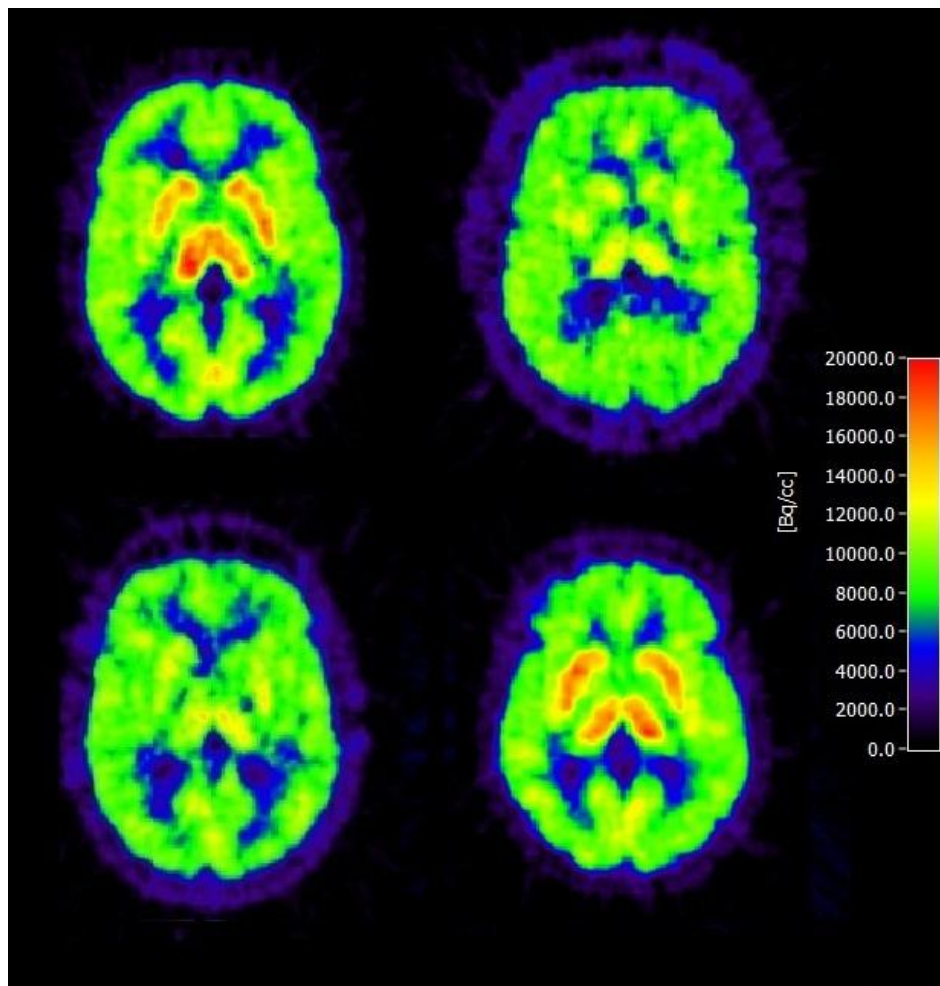
Figure 5. Examples of summated PET images (frame 6-20) of an untreated reference subject (top left), of subjects treated with an SSRI (citalopram, top right), a TCA (clomipramine, bottom left) and mirtazapine (bottom right).
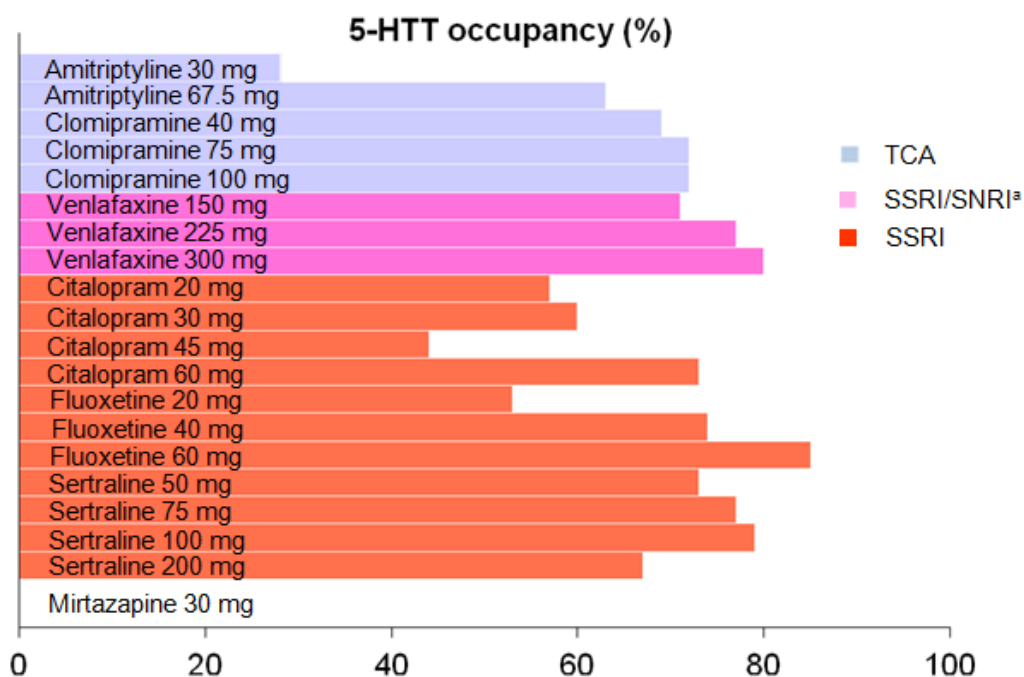
Figure 6. Histogram illustrating the relationship between drug, daily dose, and 5-HTT occupancy.

[a] Venlafaxine is sometimes also referred to as a serotonin norepinephrine reuptake inhibitor (SNRI), due to its affinity *in vitro* for both the norepinephrine transporter (NET) and the 5-HTT.

The drug affinities correlated with the corresponding recommended clinical dose (Spearmans rho = 0.93, $p < 0.05$; table 1[234]), which provides further support for 5-HTT occupancy as a mode of action in the treatment of depression.

The average 5-HTT occupancy was significantly lower than the 80 % suggested as important for antidepressant effect[61]. Since we recruited responders to antidepressant treatment no baseline data were available. The 5-HTT BP_{ND} in the treated patients were instead compared with those of a male reference group. 14 of the patients were female. Gender differences in [11C]MADAM binding has previously been reported, with lower BP_{ND} in females than in males[235]. Furthermore, the mean age of the patients, 40 years, was 13 years higher than that of the reference group. Age related decline in serotonin transporter binding has been found in healthy male subjects[236]. The mismatch of age and gender between the patients and the reference group could thus possibly have resulted in an overestimation of 5-HTT occupancy.

Table 1. The calculated *in vivo* affinity for 5-HTT and recommended dose for each drug. The $K_{i\ dose}$ is the median dose associated with 50% occupancy. $2K_{i\ dose}$ is associated with 67% occupancy.

Substance	$K_{i\ dose}$ (mg)	$2\ K_{i\ dose}$ (mg)	Recommended daily dose (mg)
Fluoxetine	14	28	20
Citalopram	21	42	20
Sertraline	22	44	50
Clomipramine	28	56	75
Amitriptyline	59	118	75
Venlafaxine	66	132	75

4.2 STUDY II

The purpose of this study was to examine the effect of cognitive behavioural therapy (CBT) on 5-HT$_{1B}$ receptor binding. Ten patients with an untreated depressive episode within recurrent major depressive disorder were included and examined with PET and [^{11}C]AZ10419369 before initiation of treatment. CBT was delivered through an internet platform, with weekly mail contact with a trained psychologist. A second PET measurement with the same radioligand was performed after treatment.

All patients responded to treatment with a mean reduction of 18.6 points on the MADRS score (p<0.001) and average Clinical Global Impression-Severity scores going down from 3.7 to 1.5 (p<0.001). The assessed MADRS score correlated with selfratings before and after CBT (ρ=0.65, p<0.05 and ρ=0.71, p<0.05, respectively).

The [^{11}C]AZ10419369 BP_{ND} was reduced by 33 % (p=0.001) in the Dorsal Brain Stem (DBS) after CBT(table 2, figure 7). There was a trend towards reduced binding post-treatment also in the hippocampus (p=0.09). There were no other significant differences in [^{11}C]AZ10419369 binding in the brain with treatment.

Similar results were obtained from the voxel-based comparisons of [^{11}C]AZ10419369 BP_{ND} at PET1 and PET2. There was only one cluster in the brain with a significant change in BP_{ND} (p=0.01 corrected for multiple comparisons), a cluster of 190 voxels (1520 mm^3) with significant decrease in BP_{ND}, whose distribution and size resembles that of the ROI for the DBS (1309 mm^3). This cluster resided primarily in the dorsal brain stem, although parts of it spilled over into surrounding structures. There was a significant correlation between BP_{ND} change (%) in the voxel cluster and the DBS ROI (Spearman´s ρ =0.74; p<0.05).
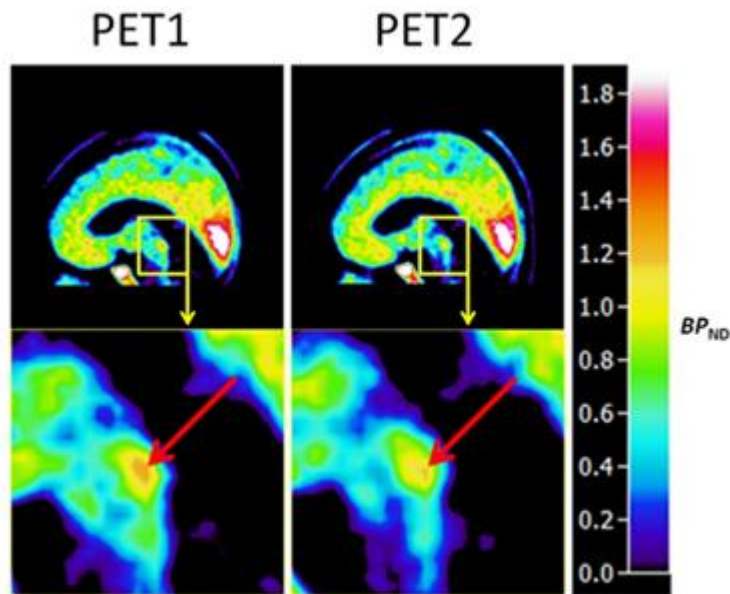
Figure 7. Midline sagittal view of the hemisphere (top) and the brainstem (bottom) of average files (average of summation images of eight subjects), from PET1 (left) and PET2 (right). The red arrows indicate the median raphe nuclei.

There was a strong negative correlation between age and [^{11}C]AZ10419369 BP_{ND} in DBS, both at PET1 and PET2 (ρ=-0.90; p<0.001 and ρ=-0.89; p<0.001). No significant correlation was found between [^{11}C]AZ10419369 BP_{ND} in the DBS and MADRS or CGI at PET1 or PET2, when corrected for age (partial correlation, ρ=-0.41, p=0.27 or ρ=-0.25, p=0.51). No significant correlation between Δ[^{11}C]AZ10419369 BP_{ND} and ΔMADRS was found when introducing MADRS as a regressor in the voxel based analysis (p > 0.05; cluster level, Gaussian random-field theory corrected).

The DBS ROI was drawn to include the median raphe nuclei, which cannot be distinguished on the MR image. The overinclusive DBS inevitably contains a portion of white matter, which attenuates the signal from the ROI, leading to an underestimation of B_{max}. The actual difference in binding in the raphe nuclei between first and second PET might thus be more pronounced than reported here.

[^{11}C]AZ10419369 binds selectively to the 5-HT$_{1B}$ receptor and has also been shown to be sensitive to pharmacologically induced serotonin release. The reduced binding in the DBS could either reflect lower 5-HT$_{1B}$ receptor densities or increased serotonin levels. Since 5-HT$_{1B}$ receptors inhibit serotonin release, the reduced [^{11}C]AZ10419369 BP_{ND} would result in a stimulation of the serotonin system in both cases. Given the lack of correlation between [^{11}C]AZ10419369 binding and serotonin in the CSF (reported in study IV) we find reduced 5-HT$_{1B}$ receptor levels the more likely explanation.

Table 2. [^{11}C]AZ10419369 BP_{ND} for different ROIs

ROI	PET1	PET2	Change(%)	SD	p	t
OFC	0.99	0.93	-2	0.17	0.313	1.069
VST	1.79	1.71	-2	0.31	0.421	0.842
Hippocampus	0.26	0.21	-13	0.08	0.09	1.922
DBS	0.56	0.38	-33	0.13	0.001*ᵃ	4.565
ACC	0.80	0.81	0	0.17	0.911	-0.115
Amygdala	0.81	0.73	-10	0.27	0.359	0.968
SGPFC	0.71	0.74	4	0.13	0.46	-0.766

OFC = Orbitofrontal Cortex
VST = Ventral Striatum
DBS = Dorsal Brain Stem
ACC= Anterior Cingulate Cortex
SGPFC = Subgenual Prefrontal Cortex
Degrees of freedom for all ROIs: 9
a= p=0.007 with Bonferroni correction for multiple comparisons

4.3 STUDY III

The aim of this study was to compare 5-HT$_{1B}$ receptor levels in the brain in ten patients with an ongoing and untreated depressive episode within recurrent major depressive disorder with age and sex-matched controls. PET and the 5-HT$_{1B}$ receptor selective radioligand [^{11}C]AZ10419369 was used. The PET data from the depressed patients were acquired from baseline PET in study II.

The [^{11}C]AZ10419369 binding was 25% lower in the anterior cingulate cortex in depressed patients compared with controls (p=0.003) (table 3). A significant difference was also found in the subgenual prefrontal cortex, corresponding to Brodmann area 25, with 20 % lower BP_{ND} for the patients than for the controls (p=0.044). The largest difference was found in the hippocampus ROI, where the average BP_{ND} was 45 % lower in the depressed subjects compared with the controls (p=0.029). For other regions in the brain, including the ventral striatum and pallidum, there were no significant differences in BP_{ND} between patients and controls (table 3). The ROI volumes in the patients did not differ significantly from those of the controls.

The lower [^{11}C]AZ10419369 binding potentials in the depressed patients were found in two different parts of the cingulate cortex, considered important in the neurocircuitry of depression[178, 237]. The subgenual prefrontal cortex, Brodmann area 25, closely corresponds to the target region for deep brain stimulation for treatment-resistant depression[185]. The [^{11}C]AZ10419369 BP_{ND} in the anterior cingulate cortex and subgenual prefrontal cortex did not change with psychotherapy, despite treatment response. With no treatment effect on radioligand binding in ACC or SGPFC, the reported decreases in depression are most likely trait-dependent, reflecting either vulnerabilities for depression or a consequence of the disorder. Based on the lack of

correlation between [11C]AZ10419369 binding and CSF serotonin described in study IV the lower radioligand binding in ACC and SGPFC in depression likely reflects lower levels of 5-HT$_{1B}$-receptors in these brain regions. We could not replicate the previously reported difference in 5-HT$_{1B}$ receptor binding in the ventral striatum or pallidum between depressed subjects and controls [158].

Table 3 [11C]AZ10419369 binding results

ROI	Controls $BP_{ND}\pm SD$	Patients $BP_{ND}\pm SD$	p
Orbitofrontal cortex	1.49±0.15	1.27±0.37	0,07
ACC	1.38±0.22	1.10±0.22	0,003**
SGPFC	1.22±0.17	1.02±0.21	0,044*
Ventral striatum	1.98±0.34	1.70±0.45	0,242
Pallidum	3.33±0.56	3.04±0.66	0,422
Amygdala	1.06±0.21	0.96±0.38	0,486
Hippocampus	0.48±0.12	0.33±0.13	0,029*
Dorsal brain stem	0.55±0.28	0.70±0.28	0,087

OC= Occipital Cortex, ACC=Anterior Cingulate Cortex, SGPFC=Subgenual Prefrontal Cortex. SD=Standard Deviation.

4.4 STUDY IV

The purpose of this study was to assess the serotonin sensitivity level for the radioligand [11C]AZ10419369 by parallel measurements of serotonin and its metabolite 5-HIAA in the cerebrospinal fluid (CSF). Twelve subjects underwent PET and within a month lumbar puncture between L3/L4, where 5 ml of cerebrospinal fluid was collected. The mean serotonin concentration in the CSF (±s.d.) was 0,58 (±0.12) ng/ml. In one subject 5-HIAA levels in the CSF were below the limit of detection. The average 5-HIAA concentration (±s.d.) for the rest of the group was 1.49 (±1.22) ng/ml. When the two outliers with high 5-HIAA (>3 ng/ml) were removed from the analysis, an obvious correlation between CSF concentrations of 5-HIAA and serotonin was found (figure 8, p=0.938, p<0.001).
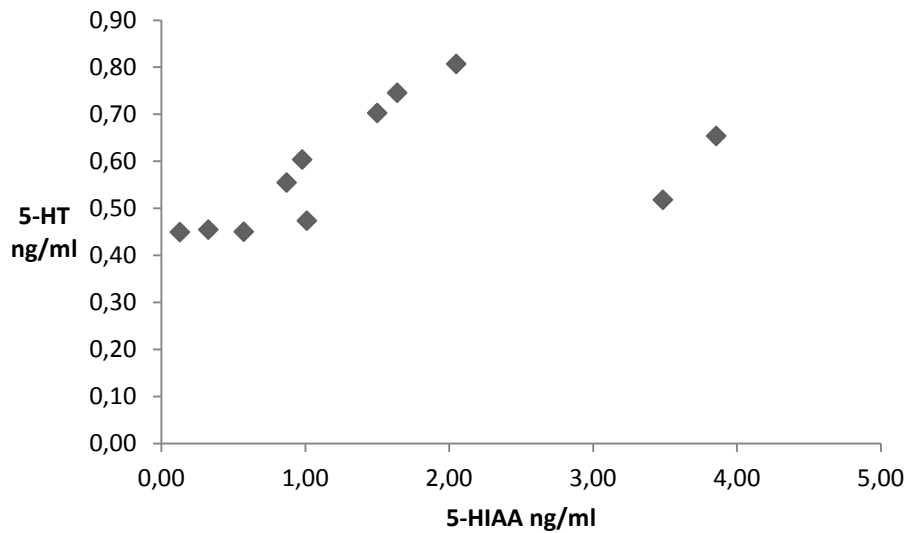
Figure 8. Scatterplot depicting the relationship between serotonin and 5-HIAA in the CSF.

There was no correlation between [11C]AZ10419369 BP_{ND} in the whole brain and CSF concentrations of serotonin (p=0.471) and its metabolite 5-HIAA (figures 9A and 10A, p=0.675). Neither [11C]AZ10419369 binding in the occipital cortex nor in the caudate nucleus correlated with CSF serotonin or 5-HIAA (figures 9B-C and 10 B-C). In previous post mortem studies correlations between concentrations of 5-HIAA in the CSF and in several brain regions have been demonstrated[119]. In the same report the serotonin concentration in the caudate nucleus correlated with that in the CSF[119]. With the lack of correlation between [11C]AZ10419369 BP_{ND} in the whole brain, and in particular the caudate nucleus, and CSF serotonin and 5-HIAA the hypothesis of sensitivity to physiological serotonin levels could not be corroborated.
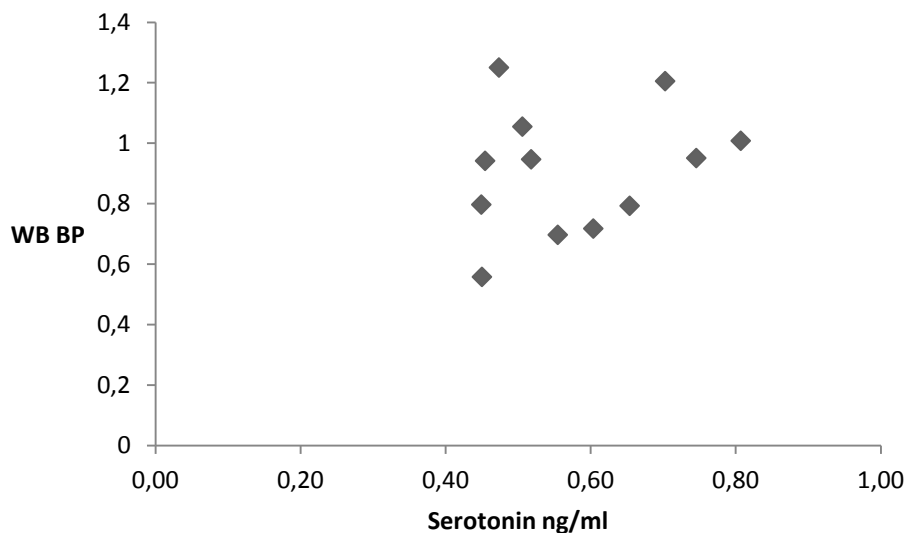
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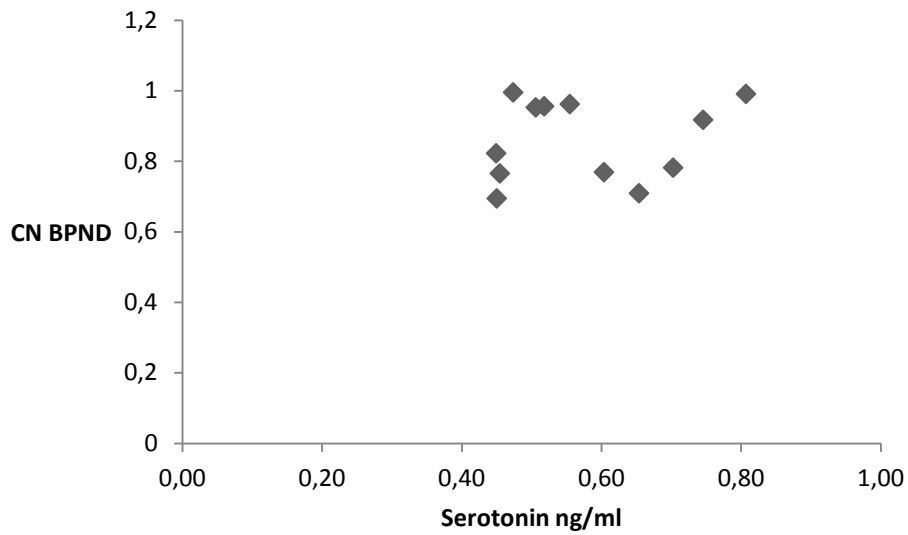
Figure 9B
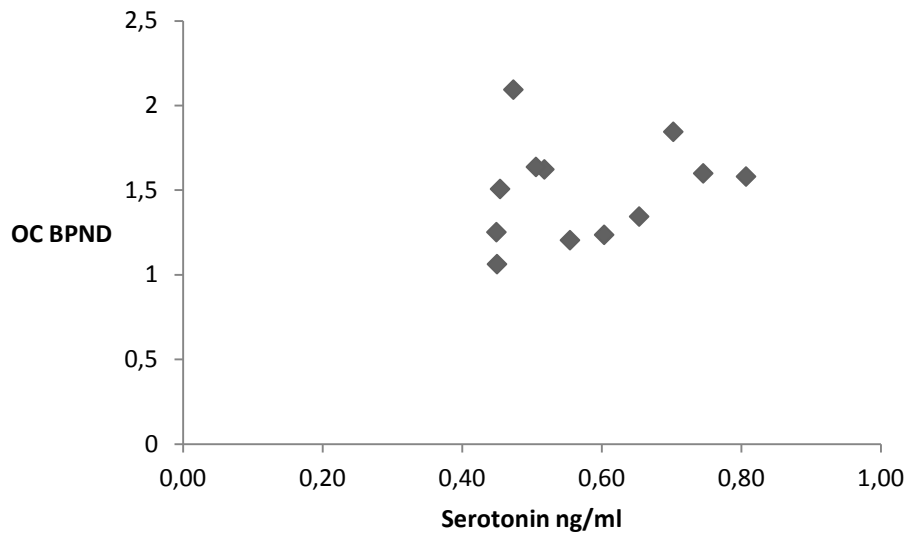
Figure 9C

Figures 9A-C. Scatterplots demonstrating the relationship between [^{11}C]AZ10419369 BP$_{ND}$ in the whole brain (A), in the caudate nucleus (B), and in the occipital cortex (C) and serotonin in the CSF. WB=Whole Brain, CN=Caudate nucleus, OC=Occipital Cortex.
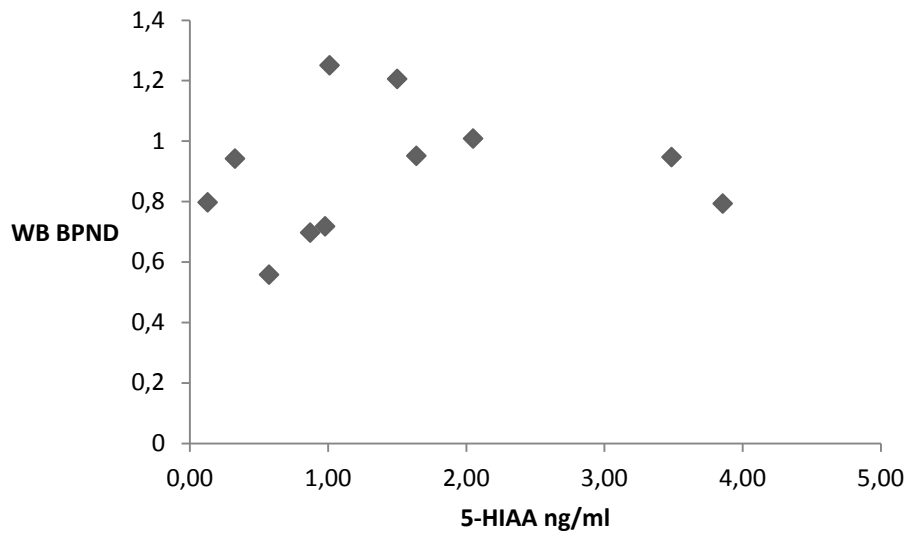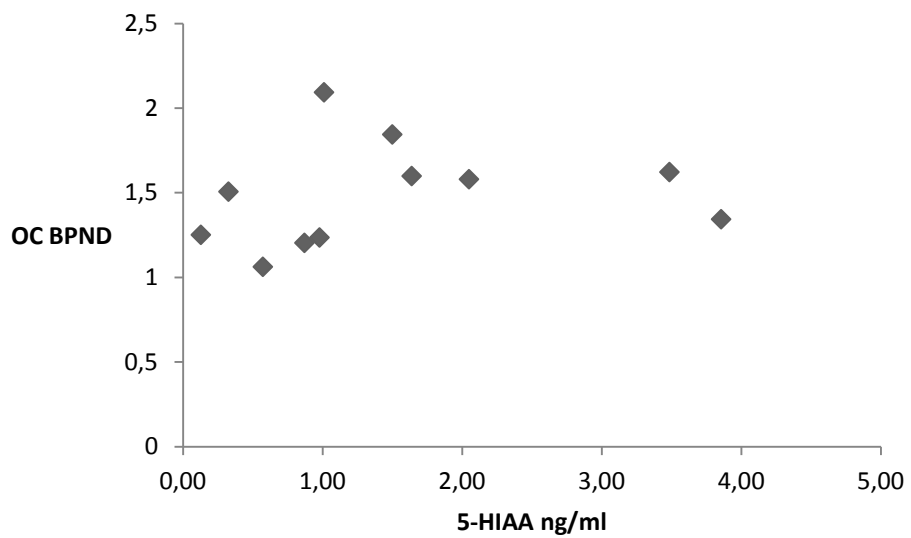
Figure 10A
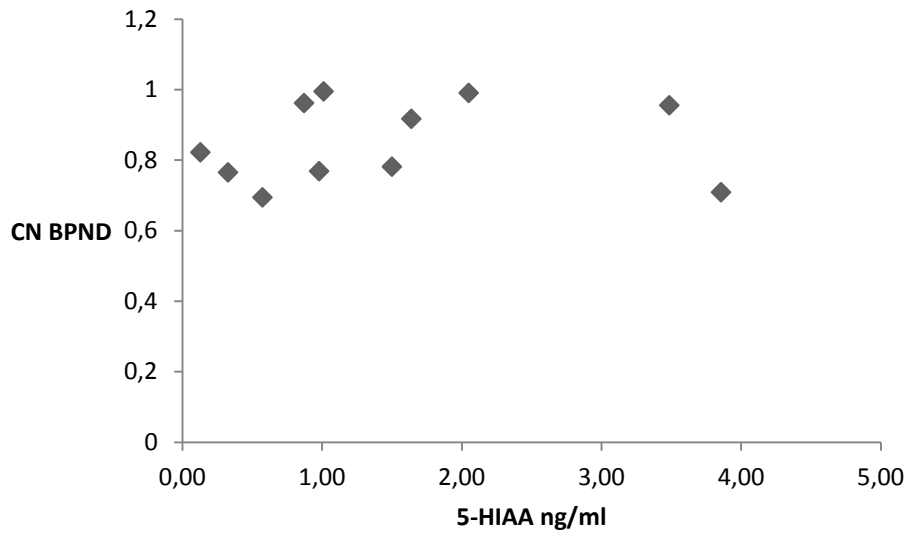
Figure 10B

Figure 10C

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Figures 10A-C. Scatterplots demonstrating the relationship between 5-HIAA in the CSF and [^{11}C]AZ10419369 BP$_{ND}$ in the whole brain (A), in the caudate nucleus (B), and in the occipital cortex (C). WB=Whole Brain, CN=Caudate Nucleus, OC=Occipital Cortex.

5   SUMMARY OF FINDINGS

5.1   ON SEROTONIN TRANSPORTER OCCUPANCY WITH ANTIDEPRESSANTS

The study corroborates 5-HTT as a shared target in the living human brain for both first and second generation antidepressants, TCAs and SSRIs. There was no significant difference in 5-HTT occupancy between the 5 patients treated with TCAs and the 14 patients on SSRI treatment. The underlying pharmacodynamics behind the superior antidepressant efficacy of TCAs compared with SSRIs[74] are thus likely to be sought elsewhere.

The 5-HTT affinities of the examined antidepressants correlated with recommended clinical dose, further corroborating 5-HTT occupancy in the mechanism of action of TCAs and SSRIs. Mirtazapine did not occupy the serotonin transporter, demonstrating alternative modes of action.

The 5-HTT occupancy with treatment was significantly lower (61-74 %, 95 % CI, figure 1, table 2) than the postulated 80 % for antidepressant effect with SSRIs[61]. In our material, remission could be maintained with SSRI treatment on average occupying 70 % of the serotonin transporters.

5.2   ON THE EFFECT OF COGNITIVE BEHAVIOUR THERAPY ON [11C]AZ10419369 BINDING

All patients in the study responded to cognitive behavioural therapy for depression. The [11C]AZ10419369 binding in the dorsal brain stem was reduced by 33 % after the given treatment. There were no other significant changes in radioligand binding in the brain between first and second PET.

Although increased serotonin levels cannot be ruled out, the reduced [11C]AZ10419369 binding in the dorsal brain stem is more likely due to decreased 5-HT$_{1B}$ receptor densities. Reduced 5-HT$_{1B}$ receptor activity would in theory result in increased serotonin release, through decreased inhibition. The dorsal brain stem contains the raphe nuclei, from which the serotonergic neurons project. Increased activity in the raphe neurons after CBT could have a global impact on the serotonin system, corroborating the serotonin hypothesis of depression.

5.3   ON [11C]AZ10419369 BINDING IN PATIENTS WITH MAJOR DEPRESSIVE DISORDER COMPARED WITH CONTROLS

The patients had lower [11C]AZ10419369 binding in the anterior cingulate cortex and the subgenual prefrontal cortex, likely corresponding to lower 5-HT$_{1B}$ receptor densities. These parts of the cingulate cortex are replicated hot spots in the neurocircuitry of depression. The absence of change in BP_{ND} in these regions with effective treatment against the depressive state implies that the lower [11C]AZ10419369

binding rather corresponds to a trait in major depressive disorder. Based on the results of study IV the lower [^{11}C]AZ10419369 BP_{ND}s likely reflect lower 5-HT$_{1B}$ receptor densities. There were no significant differences in the other regions of interest in the brain. The earlier reported lower 5-HT$_{1B}$ receptor levels in the ventral striatum and striatum in depressed subjects[158] was not replicated.

5.4 ON [^{11}C]AZ10419369 BINDING IN RELATION TO 5-HIAA AND SEROTONIN IN CEREBROSPINAL FLUID

We found no correlations between [^{11}C]AZ10419369 BP_{ND} binding in the whole brain or the brain regions of interest, and concentrations of serotonin and 5-HIAA in the CSF. The hypothesis was that [^{11}C]AZ10419369 is sensitive to physiological serotonin levels. With the lack of correlation between serotonin measurements in the CSF and radioligand binding, this hypothesis could not be corroborated. For low to medium concentrations of 5-HIAA, there was a distinct correlation with serotonin concentrations in the CSF, providing support for 5-HIAA as a serotonergic marker, at least at low to medium 5-HIAA levels.

6 FINAL REMARKS AND FUTURE PERSPECTIVES

As research goes, we have ended up with more questions than we started with. In study I we could confirm 5-HTT occupancy with TCAs as well as with SSRIs, with no significant difference in the degree of occupancy. Still, with the time of onset of antidepressant effect, a black box remains between inhibition of serotonin reuptake and treatment effect with the currently available drugs for depression. Further studies of the contents in this black box of antidepressant effect are of vital importance for the development of new antidepressants.

The relationship between 5-HTT occupancy and its effects is less well studied than that of D_2 dopamine receptor occupancy, antipsychotic effect and extrapyramidal side effects. The main obstacle with SSRI treatment is the risk for sexual side effects, such as loss of libido. Dose-finding studies correlating 5-HTT occupancy with antidepressant effect and sexual side effects with different doses of SSRI would be of value for optimizing the clinical use of SSRIs.

Earlier studies have shown superior efficacy with TCAs compared with SSRIs, at least in a subgroup of patients. The similar 5-HTT occupancies in these two groups of antidepressants indicate that the potential advantage in antidepressant effect of TCAs is mediated via additional mechanisms of action. The anticholinergic side effects of TCAs, together with the putative antidepressant effects of the anticholinergic drug scopolamine and the use of vagus nerve stimulation in the treatment of depression all provide a rationale for further exploration of the effects of modulating the acetylcholine system in depression. Finally, the lack of 5-HTT occupancy with mirtazapine illustrates that there are other mechanisms of action than inhibition of serotonin reuptake in the pharmacological treatment of depression.

In study II we found a significant reduction of $[^{11}C]$AZ10419369 binding in the dorsal brain stem after internet-CBT for depression. This reduction did not correlate with alleviated symptoms as rated with MADRS. One reason for this lack of correlation was the homogeneity of the patient population, in which all patients responded to the given treatment. Further studies with bigger samples, including non-responders, are needed to more adequately address the question of correlation between reduced $[^{11}C]$AZ10419369 binding in the DBS and improvement from depression. Future studies of the brain stem in depression and its treatment would benefit from improved methods to visualize this challenging brain region, especially when it comes to delineating the raphe nuclei.

The lower $[^{11}C]$AZ10419369 binding in the anterior cingulate cortex and associated regions in depressed patients compared with controls reported in paper III is likely constituted of a difference in 5-HT$_{1B}$ receptor density. With the current methodology we cannot tell if this difference is due to fewer 5-HT$_{1B}$ receptor containing neurons, fewer synapses expressing 5-HT$_{1B}$ receptors or down-regulation of 5-HT$_{1B}$ receptors. To further explore this intriguing finding, studies with methods such as autoradiography would be needed.

In contrast with the previous PET study of 5-HT$_{1B}$ receptors in MDD there was no significant difference in 5-HT$_{1B}$ receptor binding in the ventral striatum and the pallidum between depressed subjects and controls. Head to head comparisons between the 5-HT$_{1B}$ receptor radioligands [^{11}C]P943 and [^{11}C]AZ10419369 could possibly elucidate the conflicting findings in the two case-control studies of 5-HT$_{1B}$ receptor binding in vivo performed in depression.

There was a trend towards higher [^{11}C]AZ10419369 BP in the DBS in the depressed patients than in the controls in study III, albeit not significant. High BP in the DBS would be in line with the BP reduction in the DBS after treatment with CBT. The patient sample in this study was small, and the risk of type II errors evident. Studies with larger samples or pooled analyses, such as with a meta-analysis, would increase the power and hence reduce this risk.

Studies II and III implicate the 5-HT$_{1B}$ receptor as a possible target in the treatment of depression.

Study IV addressed the sensitivity of [^{11}C]AZ10419369 binding to baseline serotonin concentrations. No significant correlations were found between CSF concentrations of serotonin and 5-HIAA and [^{11}C]AZ10419369 BP_{ND} in the whole brain, the caudate nucleus or in the occipital cortex. The serotonin concentration threshold for displacement of [^{11}C]AZ10419369 is likely to be somewhere in between baseline serotonin levels in the brain and those brought about with pharmacological serotonin enhancement.

The development of agonist tracers targeting 5-HT$_{1B}$ receptors would in theory yield radioligands more sensitive to endogenous serotonin, since they, like serotonin itself, only bind to receptors in the high affinity state.

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