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PET STUDIES ON THE MECHANISM OF MONOAMINERGIC DRUGS

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Cover illustration:

Horizontal PET images through the caudate putamen level after iv injection of [^{11}C]raclopride at baseline (left) and after administration of the antipsychotic drug quetiapine (right) in a healthy human subject

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ABSTRACT

Positron emission tomography (PET) is a molecular imaging technique that allows for examinations of neurochemistry directly in the living human brain. In the present thesis, PET was used to assess the pharmacological effects of two drugs representing the major classes of antipsychotic and antidepressant drugs. In addition, novel methodology for studies on antidepressant mechanism of action was developed further.

In study I, striatal D_2 dopamine receptor occupancy was examined in healthy subjects after administration of the antipsychotic drug quetiapine in two formulations, immediate-release (IR) and extended-release (XR), respectively. The D_2 occupancy at peak concentration was higher for the IR compared to the XR formulation (50±4% and 32±11% respectively). The result may explain observed pharmacodynamic differences between the two formulations, and also support the view that quetiapine may show antipsychotic effect at lower D_2 receptor occupancy than the first-generation of antipsychotic drugs.

In study II, changes in serotonin concentration were assessed in healthy subjects after administration of a single, clinically relevant dose of the antidepressant drug escitalopram. A competition-model with the 5-HT_{1B} receptor selective radioligand [^{11}C]AZ10419369 was used to indirectly measure changes in extracellular endogenous serotonin concentration after drug administration. Escitalopram was found to decrease serotonin concentrations in serotonergic projection areas. This observation directly in human subjects extends previous hypotheses on the mechanism of action of antidepressant drugs, derived primarily from experimental animals.

The aim of study III was primarily to evaluate the methodology of study II. The binding potential of the radioligand [^{11}C]AZ10419369 was determined in two consecutive PET measurements, performed on the same day in 8 healthy subjects. In serotonergic projection areas the mean difference in radioligand binding between PET 1 and PET 2 was minor, supporting the methodology and interpretation of the results of study II.

In study IV, the effect of age on 5-HT_{1B} receptor availability was examined. The 5-HT_{1B} receptor availability decreased significantly with age in cortical regions. On the contrary, the 5-HT_{1B} receptor availability in the caudate nucleus and the putamen remained stable over the investigated age range. This observation may indicate that the 5-HT_{1B} receptors in these regions are expressed on neurons with a different sensitivity to aging. The results highlight the importance of using age-matched controls in future clinical studies on the 5-HT_{1B} receptor.

LIST OF SCIENTIFIC PAPERS

I. **Nord M,** Nyberg S, Brogren J, Jucaite A, Halldin C, Farde L. Comparison of D2 dopamine receptor occupancy after oral administration of quetiapine fumarate immediate-release and extended-release formulations in healthy subjects. Int J Neuropsychopharmacol, 2011, 14, 1357-66.

II. **Nord M,** Finnema S. J, Halldin C, Farde L. Effect of a single dose of escitalopram on serotonin concentration in the non-human and human primate brain. Int J Neuropsychopharmacol, 2013, 16, 1577-86.

III. **Nord M,** Finnema S. J, Schain M, Halldin C, Farde L. Test-retest reliability of [11C]AZ10419369 binding to 5-HT1B receptors in human brain. Eur J Nucl Med Mol Imaging, 2014, 41, 301-7

IV. **Nord M,** Cselenyi Z, Forsberg A, Rosenqvist G, Tiger M, Lundberg J, Varrone A, Farde L. Distinct regional age effects on [11C]AZ10419369 binding to 5HT1B receptors in the human brain. Submitted.

NOT INCLUDED IN THE THESIS:

Nord M, Farde L. Antipsychotic Occupancy of Dopamine Receptors in Schizophrenia. CNS Neurosci Ther 2011, 17, 97-103.

CONTENTS

LIST OF ABBREVIATIONS

5-HIAA	5-Hydroxyindoleacetic acid
5-HT	Serotonin
AAL	Automated Anatomical Labeling
BBB	Blood Brain Barrier
BP	Binding Potential
cAMP	cyclic adenosine monophospate
CNS	Central Nervous System
COMT	Catechol-O-methyl transferase
CSF	cerebrospinal fluid
DAT	Dopamine Transporter
e-	electron
ECG	Electrocardiography
EPS	Extrapyramidal Symptoms
FWHM	Full Width Half Maximum
HRRT	High Resolution Reserach Tomograph
IR	immediate-release
iv	intravenously
MAO-A	Monoamine Oxidase A
MAO-B	Monoamine Oxidase B
MAOI	Monoamine Oxidase Inhibitors
max	maximal
MoA	Mechanism of Action
MRI	Magnetic resonance imaging
NET	Noradrenaline Transporter

NMDA	N-methyl-d-aspartate
Occ	Occupancy
p+	positron
PET	Positron Emission Tomography
PVE	Partial Volume Effect
PVEc	Partial Volume Effect correction
QTP	Quetiapine
ROI	Region of interest
SD	Standard deviation
SERT/ 5-HTT	Serotonin Transporter
SRTM	Simplified Reference Tissue Model
SSRI	Selective serotonin reuptake inhibitor
T	Tesla
t½	half-life
TCA	Tricyclic Antidepressant
VTA	Ventral Tegmental Area
XR	extended-release

1 INTRODUCTION

It is estimated that the human brain contains between 20 and 100 billion nerve cells (neurons). The neurons are organized in different groups and circuits, of which some remain to be identified. A neuron consists of a cell body (soma); dendrites which receives information from other neurons; and an axon which upon activation sends information to other neurons via a conjunction called the synapse. At activation a neuron-specific neurotransmitter such as dopamine or serotonin, is released from the terminal of the axon. The neurotransmitter passes the synaptic cleft and in many cases delivers a message to a receptor on the other side of the synapse, i.e. on the post-synaptic neuron (Figure 1).

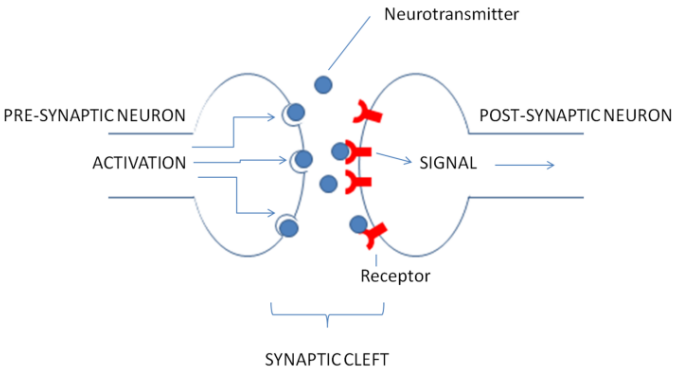

***Figure 1**: Scematic illustration of a synapse*

It has been estimated that each neuron on average has contact with 10.000 other neurons. Thus, in each individual brain, there are trillions of connections between nerve cells. Although the understanding of the central nervous system (CNS) has greatly improved during last decades, much of the organization and functional role of the neurotransmission systems is still not fully understood. For instance, genes encoding for over 700 G protein-coupled receptors have been identified in the human genome [1], but the function of many of these receptors remains to be clarified. Further, the regulation of the expression of these

receptors is only partly understood, as is their functional role in relation to human behavior.

The majority of pharmacological treatments for psychiatric disorders are targeting receptors, transporters or enzymes of the brain neurotransmission system. Initially such drugs were discovered through empirical observations, and their mechanism of action (MoA) was not defined until later. Nowadays, drugs are instead developed in a more rational fashion, to match current hypothesis of the pathophysiology of psychiatric disorders. However, a bottleneck for such developments is that the pathophysiology of major psychiatric disorders such as schizophrenia still remains largely unknown.

With the advent of positron emission tomography (PET) in the late 1970´s, it became possible to examine neuro-chemistry directly in the living human brain. PET has subsequently been applied to examine both the normal functioning of the CNS, the pathophysiology of psychiatric diseases as well as the molecular effects of drugs targeting the CNS [2]. In the present thesis, PET was used to assess the MoA of two drugs representing the major classes of antipsychotic and antidepressant drugs. To allow for studies on antidepressant MoA, the thesis includes examination of new methodology.

1.1 POSITRON EMISSION TOMOGRAPHY

1.1.1 General

PET is an imaging technique that allows for examinations of biochemistry and metabolism in vivo. The present thesis is focusing on the use of PET for examinations of biomarkers of neurotransmission in brain.

Crucial for the use of PET, is the development and availability of suitable positron-emitting radiotracers. While most PET radiotracers are receptor ligands that have been developed to bind to a specific target molecule in the brain, other tracers allow for measurements of blood flow or metabolic activity. Typically, radioligands intended for brain examinations are designed to fit the binding

pocket of a receptor, transporter or enzyme, with high selectivity. PET radioligands are commonly labeled with a short-lived radionuclide such as ^{11}C (half-life (t½) = 20.3 min) or ^{18}F (t½ = 110 min).

In a typical PET measurement, the radioligand is injected into a vein, and distributed to the brain via the blood stream. After passage over the blood brain barrier (BBB), the radioligand binds to the target molecule in brain. At decay of the radionuclide, a positron is emitted. The positron travels a short distance in the brain tissue (for ^{11}C typically about 1 mm) [3], until it collides with an electron. In the annihilation that follows, two gamma-particles (photons) are generated. The two photons move away from the site of annihilation in approximately opposite directions (i.e. 180 ± 0.25^{o}) [4], and are eventually detected by the PET system (Figure 2). When two photons arrive at two detectors within a short timeframe at an angle of 180^{o}, they are registered as a coincidence event. During a PET measurement, a large number of such coincidences are collected. The coincidences are subsequently used to reconstruct a 3D representation of the radioactivity distribution in the brain. The reconstruction process includes corrections for errors in the data collection procedure, such as scatter and random coincidences [3].

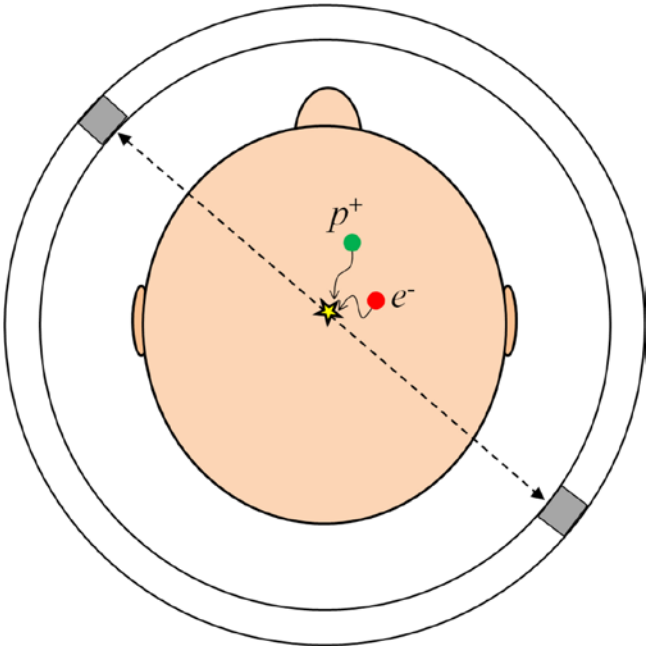

***Figure 2**. Schematic figure illustrating the collision between the positron (p^+) emitted at decay of the radionuclide, and an electron (e^-) within the brain tissue. As indicated by the broken line, the two photons generated move away from the site of annihilation and are eventually detected by the PET system (Figure made with help of Martin Schain)*

Spatial resolution can be described as the ability of a system to distinguish small structures as separate units. The smallest structures that can be separated define the resolution of the system. The PET system used in the present thesis, the High Resolution Research Tomography (HRRT; Siemens Molecular Imaging, USA), is dedicated for imaging of the CNS. The HRRT system has the highest resolution (approximately 1.5 mm) of all presently available PET systems for the human brain [5]. Thus, the HRRT system can be used to examine also small sub-regions of the brain.

1.1.2 Principles for applied PET studies

Radioligand binding to receptors, transporters or enzymes can be viewed as potential biomarkers of disease. Over the years, a large number of PET studies have been reported, addressing changes in such biomarkers in psychiatric disorders. Among the neurotransmission systems, the dopamine and serotonin systems have been the most extensively examined. While there is still no consensus regarding clinical use of biomarkers in psychiatric disorders, a more accepted disease biomarker is the beta-amyloid protein, which is expressed in high densities in patients with Alzheimer's disease [6].

PET can also be used in several steps of drug discovery and development [2]. Crucial for the effect of a neuropsychiatric drug is that it reaches the intended target in the brain. In the early stages of drug development, passage over the BBB in humans can be assured, by injection of a micro dose of the radiolabeled drug. This methodology can be very time- and cost-saving, as drugs shown not to pass the BBB can be discarded from further development at an early time.

PET can also be used to assure that the drug binds at the aimed target. This is usually established in two steps: First, a baseline PET measurement with a radioligand with confirmed binding to the target is performed. Then, a second PET measurement with the same radioligand is performed after administration of the drug. The difference in binding potential between PET 1 and PET 2 can be used to estimate the fraction of the target molecules that are occupied by the drug. This methodology can also be extended to cover the relationship between dose/plasmaconcentration of the drug and receptor occupancy in brain.

In patients, receptor occupancy in brain can be related to the clinical effect of the drug. This approach was early applied to define a therapeutic window for optimal dopamine D_2 receptor occupancy during treatment with antipsychotic drugs [7] .

1.2 BRAIN NEUROTRANSMISSION SYSTEMS

Most brain neurotransmission systems are defined by endogenous neurotransmitters. Approximately 100 neurotransmitters have been identified,

including peptides and hormones. The neurotransmitter-receptors can be divided in two major groups. G-protein coupled receptors are coupled to G proteins, which can stimulate or inhibit enzymes to produce a second messenger substance within the cell [8]. Other receptors are protein complexes forming ligand-gated ion-channels in nerve cell membranes, and regulate the opening and closing of the ion channel at activation. When an ion-channel receptor is activated, the effect is more or less immediate (< 1 millisecond), whereas the effect of activation of a second-messenger receptor is more slow (hundreds of milliseconds up to minutes) [9].

1.2.1 Monoamine neurotransmitters

Monoamine neurotransmitters are defined by their molecular properties, i.e. they are transmitters that contain an amine group connected to an aromatic ring. Dopamine, noradrenaline, histamine and serotonin are four examples of monoamine neurotransmitters that are of central importance for the human brain. These four monoamine transmitter systems share some common features. The cell bodies of the neurons are mainly located in the brain stem, from where they project axons to different parts of the brain, including the neocortex. The present thesis addresses aspects of two of the monoaminergic neurotransmitter systems, namely the dopamine and the serotonin system.

1.2.2 Dopamine

Dopamine was initially considered to be merely an inactive precursor of noradrenaline. During the 1950´s, experiments with reserpine were performed, to deplete catecholamine stores in animals. Arvid Carlsson and coworkers administered 3,4-dihydroxyphenylalanine (DOPA) to reserpine-treated rabbits, to replenish what was thought to be the noradrenaline stores. The DOPA administration reversed the reserpine-induced sedation and hypokinesia of the rabbits [10]. However, when brain tissues from these animals were analyzed, it was discovered that the tissue was still fully depleted of noradrenaline. Further experiments demonstrated that the anti-reserpine action of DOPA on animal

behavior was correlated to the restoration of dopamine levels in the brain [11, 12]. Subsequently, these results led to the suggestion that dopamine also is a neurotransmitter (For review, see [13]).

1.2.2.1 The dopamine system

The dopaminergic cell bodies are mainly located in the brain stem, within the substantia nigra and the ventral tegmental area (VTA). Three different dopaminergic pathways arise from these cell bodies [14] (for review see [15]):

1. The nigro-striatal pathway, with cell bodies in substantia nigra projecting to dorsal striatum [16]. This pathway has since long been known to be involved in motor function [17]. More recently involvement also in cognitive function have been described [18].
2. The mesolimbic pathway, with cell bodies in VTA projecting to nucleus accumbens and other parts of the limbic system. This pathway is thought to be involved in emotional processing, reward and motivation [17].
3. The mesocortical pathway (from VTA to frontal cortex), which might be involved in cognitive function and affective regulation [17].

A fourth pathway has its origin in the hypothalamus and regulates secretion of the hormone prolactin in the anterior pituitary gland. This pathway is called the tuberoinfundibular pathway [17].

A fifth dopaminergic pathway has recently been described in primates. This pathway arises from multiple sites within the upper brain stem and hypothalamus and projects to the thalamus [19]. The function of this thalamic pathway is not yet known, but it has been hypothesized to be involved in emotion, attention, cognition and motor control [20].

1.2.2.2 Dopamine synthesis and degradation

Dopamine does not pass the BBB, and is synthesized within the brain from the amino acid tyrosine. Initially, tyrosine is converted into L-DOPA by the enzyme

tyrosine hydroxylase. L-DOPA is further converted to dopamine by DOPA decarboxylase. The synthesized dopamine is stored into synaptic vesicles, where it remains until it is released into the synaptic cleft following neuronal firing [17].

After release, dopamine can travel across the synaptic cleft and reach receptors on the post-synaptic neuron. The activity of the released dopamine is mainly terminated by re-uptake into the releasing dopamine neuron by a presynaptic transporter (the DAT). Inside the dopamine neuron, dopamine can again be stored in synaptic vesicles for future use. Intra-neuronal dopamine that is not stored in synaptic vesicles is enzymatically degraded by monoamine oxidase A (MAO-A) or MAO-B. Extracellular dopamine can be degraded outside the neuron by the enzyme catechol-O- methyl transferase (COMT). Dopamine can also diffuse away from the synapse and be transported into noradrenergic neurons by the noradrenergic transporter (NET) [17].

1.2.2.3 Dopamine receptors

Five different dopamine receptor subtypes have been described and assigned numbers from D_1 to D_5. Depending on their coupling to second messenger systems, they have been further categorized into two families: The D_1-like family and the D_2-like family. The D_1 family comprises the D_1 and the D_5 receptor, and the D_2 family includes the D_2, D_3 and D_4 receptors. All dopamine receptors are G protein-coupled. The D_1-receptor family is coupled to G_s-type G proteins that activate adenylyl cyclase enzymes to synthesize cyclic adenosine monophosphate (cAMP), and the D_2-receptor family are coupled to G_i- and $G_o$type -proteins that inhibit adenylyl cyclase and cAMP formation [21].

Each of the five receptors has its own unique distribution in the brain. The D_1 subtype is the most abundant dopamine receptor in the human brain [21]. Despite this, the D_2 receptor, being a target for antipsychotic drugs, is probably the most extensively studied of the five dopamine receptor subtypes.

1.2.2.4 The D2 receptor

The highest density of the D_2 receptor is found in the striatum [22]. The D_2 receptor exists in a short and a long isoform – D_2S and D_2L [23]. The D_2L is located mainly postsynaptically and the D_2S is found mainly as a presynaptic auroreceptor on the dopamine neurons [24, 25]. The D_2 autoreceptor inhibits the release of dopamine and is situated both at the somatodendritic area of the dopamine neuron, and at the axon terminal. However, the largest amount of D_2 receptors is located at the post-synaptic side of the dopamine neuron.

1.2.2.5 Radioligands for the D_2 receptor

The first measurement of D_2 receptors in the living human brain was made in 1983 by Wagner and coworkers, using [^{11}C]N-methyl-spiperone and PET [26]. As methyl-spiperone binds also to 5-HT_2 receptors, there was a need for radioligands with a more selective binding to the D_2 receptor. [^{11}C]Raclopride, developed in the mid-1980´s [27] is selective for the D_2 and D_3 receptor, and is currently the most used radioligand for PET imaging of D_2 receptors. However, the affinity of [^{11}C]raclopride is not sufficiently high for measurements of extrastriatal D_2 receptors, which are expressed at low densities. For this purpose, [^{11}C]FLB457 [28] and [^{18}F]fallypride [29] have been developed.

1.2.3 Serotonin

The substance nowadays referred to as serotonin, was identified in the gastrointestinal system of animals in 1937, and denominated enteramine (for review, see [30]). About a decade later, a vasoconstrictive substance was isolated in serum, and named serotonin [31]. In 1952 it was uncovered that enteramine and serotonin indeed were the same substance [32]. Serotonin was eventually detected also in the CNS [33] and following studies could confirm its function as a neurotransmitter in the brain [34, 35].

1.2.3.1 The serotonin system in the CNS

The first anatomical description of serotonin pathways in the CNS was published in 1964 [36]. The serotonergic cell bodies are located in the brain stem, in small clusters referred to as the raphe nuclei. Their axons project to basically all parts of the brain as well as to the cerebellum and the spinal cord, making the serotonin system influential on several physiological functions. Among the raphe nuclei, the dorsal and median raphe nuclei are the primary nuclei for serotonergic innervation of the brain [37-39].

1.2.3.2 Serotonin synthesis and degradation

Serotonin, just as dopamine, does not pass the BBB, and is synthesized within the brain from the amino acid tryptophan, which is transported from the plasma into the brain. Inside the serotonin neuron, tryptophan is converted into 5-hydroxy-tryptophan via the enzyme tryptophan hydroxylase. Then, 5-hydroxytryptophan is converted to serotonin via another enzyme, the aromatic amino acid decarboxylase. Like dopamine, serotonin is stored in synaptic vesicles until it is released for neurotransmission [17].

The activity of a released serotonin molecule is terminated by re-uptake into the serotonergic neuron by the serotonin transporter (SERT or 5-HTT) or by enzymatic degradation by MAO-A. After re-uptake into the serotonin neuron, serotonin can again be stored in synaptic vesicles for future use [17].

1.2.3.3 Serotonin receptor subtypes

To date, 14 serotonin receptor subtypes have been identified in humans. All of these are G protein-coupled receptors, except for the 5-HT_3 receptor, which is an ion channel linked receptor [40-42].

Two of the receptor subtypes act as autoreceptors. The 5-HT_{1A} receptor is located at the somatodendritic area of the serotonin neuron in the raphe nuclei and regulates the firing rate of the serotonin neurons. The 5-HT_{1B} receptor is

located at axon terminals, and regulates the release of serotonin at the pre-synaptic level.

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

The nomenclature for the human 5-HT$_{1B}$ receptor has been confusing. Initially, the human 5-HT$_{1D}$ receptor was considered to be an equivalent of the 5-HT$_{1B}$ receptor in rodents. The human 5-HT$_{1D}$ receptor was later found to have two isoforms, 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$. It was eventually demonstrated that the receptor defined as human 5-HT$_{1D\beta}$, is the species equivalent of the rodent 5-HT$_{1B}$ receptor. Thus, the human 5-HT$_{1D\beta}$ receptor was re-named to 5-HT$_{1B}$, and the human 5-HT$_{1D\alpha}$ was called 5-HT$_{1D}$ without further subdivision (for review see[40].

The 5-HT$_{1B}$ receptor functions both as an autoreceptor as described above, and as a heteroreceptor, regulating the release of other neurotransmitters. Thus, it is expressed by both serotonin and non-serotonin cell populations. The highest densities of the 5-HT$_{1B}$ receptor are found in the globus pallidus and substantia nigra; lower densities are found in striatum, cortical regions, amygdala, hippocampus, hypothalamus and thalamus [43].

The 5-HT$_{1B}$ receptor has gained particular interest lately, as it has been implicated in both normal physiology and several psychiatric and somatic disorders. For example, the 5-HT$_{1B}$ receptor is considered to participate in the regulation of locomotion, feeding behavior, sleep, aggression and impulsivity (for review see [44] and [45]). A specific role of 5-HT$_{1B}$ in the pathophysiology of anxiety and depression (for review see [45]), as well as in Parkinson´s disease [46, 47] has also been suggested.

1.2.3.5 Radioligands for the 5-HT$_{1B}$ receptor

Currently, only two 5-HT$_{1B}$ receptor selective PET radioligands have been evaluated in human subjects: [^{11}C]AZ10419369 [48] (used in the present thesis) and [^{11}C]P943 [49]. However, in the development of [^{11}C]AZ10419369, a total

of eight radioligands were evaluated as potential PET radioligands for the 5-HT_{1B} receptor, in non-human primates [50]. As [^{11}C]AZ10419369 provided highest regional contrast, this radioligand was selected for further evaluation in human subjects [51].

The use of a competition-model with radioligands selective for the D_2 receptor is a well-recognized method for indirect measurements of endogenous extracellular dopamine levels [52, 53] (for review see [54]). A method for similar measurements of extracellular concentrations of endogenous serotonin in vivo has previously not been available. It is believed that autoreceptors – compared to heteroreceptors – may be particularly sensitive to their endogenous neurotransmitter. Therefore the two radioligands developed for the 5-HT_{1B} receptor have also been tested for their sensitivity to endogenous serotonin levels. Indeed, both [^{11}C]AZ10419369 [55, 56] and [^{11}C]P943 [57] have proven sensitive to changes in serotonin levels in non-human primates.

1.3 EXAMPLES OF PSYCHIATRIC DISORDERS RELATED TO MONOAMINERGIC NEUROTRANSMISSION AND THEIR TREATMENTS

1.3.1 Schizophrenia

Schizophrenia is a thoroughly disabling psychiatric disease, that affects approximately 0.5- 1 % of the population worldwide. Symptoms usually appear early in life, typically between age 16-30, and the disorder is life-long. The prevalence in men and women is equally high, but men tend to get the disease at an earlier age and have a worse outcome. The etiology of schizophrenia is unknown, but both genetic and environmental risk factors have been identified (for review see [58, 59]).

Schizophrenia symptoms are usually divided into positive, negative and cognitive symptoms. Positive symptoms are hallucinations, delusions and bizarre behavior. Negative symptoms include blunted affects, anhedonia and apathy. Although negative symptoms are less obvious than the positive ones,

negative symptoms are closely correlated to poor psychosocial functioning. Deficits in cognitive functions are common in schizophrenia, and may affect all cognitive domains. The cognitive deficits are also strongly correlated to functional outcome (for review see [58, 59]).

1.3.1.1 The dopamine hypothesis of schizophrenia

The dopamine hypothesis of schizophrenia was initially founded based on the discovery that antipsychotic agents block dopamine receptors [60, 61], and that dopamine-releasing drugs can induce psychotic symptoms [62]. Therefore, it was proposed that patients with schizophrenia have a hyperactive dopamine system. However, the hypothesis has later been revised, postulating that the dopamine system in schizophrenia patients is both hyper- and hypo-active, in different brain regions. Positive symptoms are assumed to be related to a hyperactivity of dopamine transmission in the mesolimbic pathway, and negative and cognitive symptoms are assumed to be related to an hypoactivity in the mesocortical system [63].

Direct evidence for an increased dopamine transmission in the striatum of schizophrenia patients, has been gained from brain imaging studies. When exposed to amphetamine (a dopamine releasing agent), patients with schizophrenia show a more pronounced reduction in radioligand binding to D_2 receptors than healthy controls. The interpretation is that amphetamine causes a larger increase of dopamine in patients with schizophrenia [64-66]. Some studies have also shown that the increase in dopamine is related to emergence or worsening of positive symptoms [65, 66].

1.3.2 Antipsychotics

The first antipsychotic drug – chlorpromazine - was discovered empirically in 1952 (for review see [67]) and its mechanism of action was initially entirely unknown. In the mid 1960´s, it was suggested that antipsychotic drugs act by blocking dopamine receptors [60, 61]. A decade later, a close correlation between D_2 receptor affinity in vitro and clinical antipsychotic effect could be

shown for a series of antipsychotic drugs [68, 69]. Further on, it was also demonstrated that antipsychotic drugs bind primarily to the dopamine D_2 receptor subtype [70]. The results were subsequently verified also in vivo, when high occupancy of D_2 receptors could be demonstrated in patients treated with antipsychotic drugs [71-74].

Traditionally, antipsychotics have been classified into two major groups: "typical" and "atypical" antipsychotics. The "typical" antipsychotics were all known to cause extrapyramidal side-effects (EPS) and raised serum prolactin levels. Clozapine, introduced in the early 1970´s, was the first "atypical" antipsychotic drug (for review see [75]). The term "atypical" initially referred to antipsychotic drugs not causing EPS or prolactin elevations, but the atypicality concept has later been widened. Currently there is no consensus on the definition of an "atypical" antipsychotic drug, but most pharmacologists and psychiatrists still seem to agree that a lower risk for EPS and prolactin elevations is a common property of "atypical" antipsychotics.

A number of new antipsychotics have been introduced on the market following clozapine. While all of these have been discussed as being "atypical", the term "second-generation" antipsychotic has also been used for the more recently introduced drugs. To explain the lower risk of EPS with these compounds, several hypotheses have been proposed. One hypothesis is based on the observation that all second-generation drugs have antagonistic effect on the 5-HT_{2A} receptor. Experimental studies have shown that the blocking of 5-HT_{2A} receptors enhance dopamine release in striatum, thereby counteracting the effect of the D_2 antagonism in this regions (for review see [76]).

In addition to EPS, antipsychotic drugs also have other severe side effects. Sedation, cardiac side effects (prolonged QT interval) and anticholinergic side effects are a few of these, and particularly second-generation drugs are associated with metabolic side effects. For clozapine, the risk of agranulocytosis limits its more widespread use. Another well-recognized problem in schizophrenia treatment is that cognitive and negative symptoms usually respond poorly to antipsychotic drugs (for review see [77]).

Although all antipsychotic drugs – both typical and atypical - bind to the D_2 receptor, most of them have affinity also to several other receptors. It has not yet

been conclusively demonstrated whether any of these receptors contribute to the antipsychotic effect.

1.3.2.1 D_2 dopamine receptor occupancy

All currently available antipsychotic drugs are antagonists or partial agonists at the D_2 receptor. Based on results from PET studies on patients with schizophrenia, a therapeutic window corresponding to approximately 65-80% striatal D_2 occupancy has been suggested. Even though the exact lower limit of this window has been difficult to define, occupancy above 80% is rather consistently related to extrapyramidal side effects [78-80] (Figure 3). Indirect support for an efficacy threshold around 65-70% D_2 occupancy, is provided by reports that clinical doses of more recently developed drugs also correspond to this occupancy level [81-86].

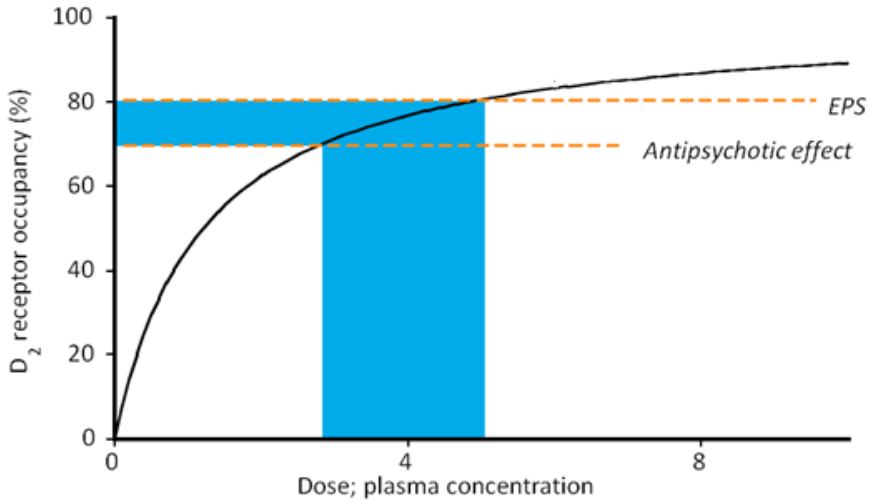

***Figure 3**: Relationship between dose/plasma concentration (arbitrary values) and striatal D_2 receptor occupancy with suggested thresholds for antipsychotic effect and extrapyramidal side effects (EPS). (Figure modified from Farde L, Trends Neurosci 1996 (6)).*

However, there are also drugs that do not fit this therapeutic window. First, D_2 occupancy during treatment with clinical doses of the antipsychotic drug aripiprazole, exceeds the therapeutic window [87-89]. However, as aripiprazole is a partial agonist at the D_2 receptor - in contrast to other antipsychotics that are antagonists – the high D_2 occupancy is thought to be functionally compensated for by a modest intrinsic activity [90].

Second, D_2 occupancy during clinical treatment with the atypical antipsychotic drug clozapine, is below the suggested therapeutic window [78, 91-93]. This is particularly intriguing, since clozapine despite lower D_2 occupancy has been shown to have superior efficacy in patients refractory to other antipsychotic drugs [94] (for review see [95]).

An unsolved question is whether high D_2 occupancy needs to be maintained during a certain time for mediation of antipsychotic effect. In initial studies of first-generation antipsychotics, high D_2 occupancy has been shown to be maintained throughout the dosing interval after oral administration [96-98]. Several second generation drugs have also been shown to maintain D_2 occupancy at rather stable levels throughout the dosing interval [99]. However, during maintenance treatment with injectable haloperidol in a depot formulation, it has been shown that relapse can be prevented despite moderate or low D_2 occupancy at the end of the four-week dosing interval [100]. Comparable results have been achieved in a study with risperidone in a depot formulation [101]. However, a fundamental question is whether the same time course of occupancy is required to prevent relapse as to treat acute psychosis.

1.3.2.2 Quetiapine

Quetiapine (QTP) is a second-generation antipsychotic drug, approved in 1997 [102]. The clinical effects are thought to be mediated both by QTP and its main active human metabolite nor-quetiapine [103]. Interestingly, like clozapine, QTP has been shown to induce D_2 receptor occupancy below the therapeutic window when administered in recommended doses [104-106]. It is possible that the low

D_2 occupancy can explain the low risk of EPS during quetiapine treatment [107]. However, quetiapine has a short elimination half-life and only transiently high plasma concentration [108]. It can thus not be excluded that maximal D_2 occupancy, estimated by PET during quetiapine treatment, has been underestimated [109, 110].

1.3.3 Depression

Depression is a common psychiatric disorder, with a life-time incidence of 10-20 %. Depression is almost twice as common in women compared to men (for review see [111]). The symptom severity can range from mild to very severe. As for schizophrenia, both genetic and environmental variables have been identified as risk factors for depression. Worth noting is that the genetic component may be smaller in depression compared to schizophrenia [111].

The diagnosis of depression (or more correctly "Major Depressive Disorder", MDD) [112] is based on the presence of several symptoms, with duration of at least two weeks. The main symptoms are depressed mood and anhedonia, but associated symptoms such as appetite or sleep disturbances; psychomotor changes; fatigue; diminished ability to concentrate; feelings of worthlessness or excessive guilt; and suicidal thoughts or attempts are also included in the diagnostic criteria.

1.3.3.1 The serotonin hypothesis of depression

The serotonin hypothesis of depression was introduced in the 1960s. The hypothesis was initially based on the finding that both tricyclic antidepressants (TCAs) and MAO inhibitors (MAOIs) increase the synaptic availability of serotonin. This observation lead to the assumption that reduced serotonin transmission had a causative effect in depression. The fact that clinically effective antidepressants affect the serotonin system is still one of the strongest arguments for the involvement of the serotonin system in depression.

Several other lines of evidence also support serotonergic involvement in depression.

For instance, already in the 1960´s, concentrations of serotonin and its metabolite 5-HIAA post-mortem were reported to be lower in depressed patients that had committed suicide, than in victims of sudden death due to e.g. coronary occlusion [113, 114].

As it has previously not been possible to measure serotonin directly in the living human brain, attempts have been made to indirectly assess serotonin levels in the brain by examinations of the cerebro-spinal fluid (CSF). Samples of CSF can be collected by lumbar puncture, and the concentration of serotonin and its metabolite 5-HIAA in the sample is assumed to reflect the extracellular serotonin concentration in the brain. Decreased levels of 5-HIAA in CSF has been reported in depressed patients compared to healthy controls [115-117]. A relation between low CSF 5-HIAA and suicide risk was first reported from our department in the 1970´s [118].

Tryptophan depletion is a method used to decrease brain levels of serotonin. It has been shown that patients in remission from depression experience a transient return of depressive symptoms after acute tryptophan depletion [119, 120]. However, consistent changes in mood after tryptophan depletion have not been shown in healthy subjects lacking risk factors for depression (for review see [121].

1.3.4 Antidepressants

The first antidepressant drugs were, just as the antipsychotic drugs, discovered empirically during the 1950´s. Most of currently available antidepressants are affecting the serotonin system, preferentially by blocking the serotonin transporter or enzymes that degrade serotonin.

The very first antidepressant drug, iproniazid, was initially used for treatment of tuberculosis. It was observed that patients with coexisting depression also improved their psychiatric symptoms during treatment with iproniazid [122]. Iproniazid was later shown to be an inhibitor of MAO (MAOI).

The second antidepressant drug, imipramine, was discovered to have antidepressive properties, when it was tested as an antipsychotic drug in patients with schizophrenia [122]. It was later discovered that imipramine, like other tricyclic antidepressants (TCAs), blocks the reuptake of both serotonin and noradrenalin, via inhibition of SERT/5-HTT and NET. TCAs also block muscarinic acetylcholine receptors, leading to unwanted atropine-like anticholinergic side effects, and are toxic in higher doses.

As both MAOIs and TCAs have severe side effects, the entry of selective serotonin reuptake inhibitors (SSRIs) on the market in the 1980's was well received. These drugs are less toxic than TCAs, and not associated with anticholinergic side effects. SSRIs will be further described below.

In addition to the three major groups of antidepressants (i.e. TCAs, MAOIs and SSRIs) there are other antidepressant drugs with different combinations of effects on the transporters and receptors of the serotonin and noradrenaline systems. For example, antidepressant drugs that block both SERT/5-HTT and NET, but do not inhibit acetylcholine receptors have been developed (i.e. serotonin-noradrenaline reuptake inhibitors, SNRIs). There are also antidepressant drugs that affect serotonergic receptors without blocking monoamine transporters. For instance, mirtazapine inhibits 5-HT_2- and $5HT_3$ receptors, while agomelatin is a melatonin receptor agonist and a 5-HT_{2C} receptor antagonist [17].

A general disadvantage with all of the above described antidepressants is that their therapeutic effect is not immediate. Usually about 2-4 weeks of treatment is required for an obvious antidepressive effect. Recently, a more immediate antidepressive effect has been reported after acute administration of low doses of the anesthetic drug ketamine. However, it has yet to be determined whether the antidepressive effect can be maintained for longer periods. Ketamine is a glutamate N-methyl-d-aspartate (NMDA) receptor antagonist. The exact mechanism behind the antidepressive effect of ketamine is unclear, but might be of importance for further understanding of the pathophysiology of depression and the development of more effective treatment options (for review see [123]).

1.3.4.1 SSRIs

The first SSRI, zimelidine [124] was introduced on the market in 1982. Zimelidine was soon withdrawn, due to the occasional occurrence of Guillain-Barré syndrome, a rare but serious side effect [125]. However, the development of zimelidine represented a milestone in rational drug development [126], and several other SSRIs were soon developed.

Though widely used since the 1980´s, the exact mechanism underlying the antidepressive effect of SSRIs remains unclear. PET studies have confirmed that SSRIs bind to the serotonin transporter in clinical treatment [127, 128]. High occupancy of the serotonin transporter is achieved already after a single dose of an SSRI [129]. Yet, the antidepressive effect is not ensued until after several weeks of treatment. This clinical observation indicates that inhibition of SERT is not the immediate cause of the antidepressive effect of SSRIs. Despite decades of research on animal models, the downstream effects of SSRIs in the human brain are not fully understood.

1.3.4.2 Escitalopram

Escitalopram is an antidepressant drug, belonging to the group of SSRIs. Escitalopram is the isolated S-enantiomer of the racemic compound citalopram, which contains equal amounts of the S- and R-enantiomer. It has been shown that it is mainly the S-enantiomer that accounts for the pharmacological effect on 5-HT reuptake inhibition. However, when equal doses of the S-enantiomers are administered, clinical efficacy seems to be superior for escitalopram compared to citalopram [130]. This has led to the hypothesis that the R-enantiomer may partly counteract the effect of the S-enantiomer on the serotonin transporter (for review see [131]).

It is commonly believed that the clinical effect of SSRIs is initiated by an increase of serotonin levels in the synapse. However, as suitable markers for endogenous serotonin levels have not been available, empirical testing of this assumption has not been possible in human subjects.

1.4 THE AGING BRAIN

Generally

Undoubtedly, the human body changes with age, and the brain is no exception. During childhood and adolescence the brain matures, which is associated with changes in grey and white matter volumes [132], as well as neuronal densities and synaptic morphology [133].

There are also age-related changes in the brain during adulthood. For example, grey matter volume decreases with age [134]. However, it has been observed that the actual number of neurons is generally not decreasing. Rather, there seem to be age-related changes in the connections between neurons in some brain regions (For review see [135]).

The monoamine systems

The first in vivo study reporting a decline in cerebral D_2 receptors with age was published in 1984 [136]. This finding has later been reproduced [137], and also several other mono-amine transporters and receptors have been shown to decrease by age. For example, declines have been reported for the D_1dopamine receptor, the dopamine transporter (DAT) (for review see [138]) and the noradrenalin transporter (NET) [139].

Age-related changes in the cerebral serotonin system have also been described, and proposed to be associated with behavioral changes observed particularly in the elderly population [140, 141]. Though only a few in vivo studies have been published on the effect of age on the serotonin system in humans, age related reductions in 5-HT_{2A} receptor binding [136, 142] and in serotonin transporter binding [143] have been described. The effect of age on 5-HT_{1A} receptor availability is less clear [144]. Previously, only one in vivo study on the age effect on the 5-HT_{1B} receptor has been published [145]. This study reported an age-related decline in 5-HT_{1B} binding, but as no conventional correction was made for possible age-related changes in brain volume, the results cannot be viewed as conclusive. As the 5-HT_{1B} receptor has been implicated in age-related diseases [47, 146], further understanding of the effect of normal aging on this receptor is of interest.

2 AIMS

The general aim of the present thesis was to extend the understanding of the mechanisms of action of psychiatric drugs in the human brain. Study I and study II included PET examinations of an antipsychotic drug and an antidepressant drug, respectively. The methodology used in study II was further evaluated in study III and study IV.

The specific aims were as follows:

Study I: To examine whether pharmacokinetic differences between two formulations of the antipsychotic drug quetiapine, would translate into differences in central D_2 dopamine receptor occupancy

Study II: To assess whether a single dose of the antidepressant drug escitalopram has effect on the endogenous serotonin concentration in brain

Study III: To evaluate the test-retest reliability of the radioligand [11C]AZ10419369

Study IV: To examine age-related changes in the availability of cerebral 5-HT_{1B} receptors, as assessed with PET and the radioligand [11C]AZ10419369

3 MATERIALS AND METHODS

All studies were approved by the local Ethics and Radiation Safety Committees. Studies I-III were also approved by the Medical Products Agency in Sweden, as were the original studies in study IV when applicable. All human subjects gave verbal and written informed consent before the study specific procedures were initiated.

The monkey experiments in study II, were approved by the Animal Research Ethical Committee of the Northern Stockholm region and were performed according to local (Drn 4820/06-600) and international guidelines [147].

3.1 STUDY SUBJECTS

All human subjects participating in study I-IV were healthy according to medical history and physical examination, including ECG, routine blood tests and MRI of the brain. Additionally, a urine drug screen was performed prior to inclusion and at the PET measurement day. Studies I-III included only male subjects, aged 20-30 years, while study IV included both male and female subjects across a larger age span (20-73 years).

Subjects in study I (n=11) were recruited from an AstraZeneca database of subjects that had expressed an interest to participate in clinical trials. Subjects in study II (n=10) and study III (n=8) were recruited by local advertisements. For study IV, data were pooled from subjects that had previously participated in PET examinations with the radioligand [^{11}C]AZ10419369 (n=51). In this study, data from subjects previously participating in studies II and III were also included.

In study II, three female cynomolgus monkeys (*Macaca fascicularis*) were examined. The monkeys were housed in the Astrid Fagreaus Laboratory of the Swedish Institute for Infectious Disease Control, Solna, Sweden, and were transported to the PET center on the day of the PET examinations. The monkeys weighed 3.3 – 5.1 kg and together participated in totally 14 PET measurements.

3.2 STUDY DRUGS

3.2.1 Quetiapine

Quetiapine (QTP) is a second-generation antipsychotic drug, which in addition has received approval for treatment of bipolar disorder, major depression and generalized anxiety disorder. The clinical effects are thought to be mediated both by QTP and its main active human metabolite nor-quetiapine [103]. While initially produced as an immediate release (IR) formula, more recently an extended release (XR) formula has been developed. Pharmacokinetic studies have shown that the exposure in terms of area under the curve (AUC) for the two formulations is comparable, when given in equivalent daily doses [148]. However, the XR formulation has a slightly lower peak plasma concentration (Cmax) than the IR formulation, and a longer half-life which allows for once-daily dosing. In study I, QTP was administered to healthy subjects to assess if the previously described differences in pharmacokinetics between the IR and the XR formulations also translate into different brain exposure and time-course of central D_2 receptor occupancy.

3.2.2 Escitalopram

Escitalopram is an antidepressive drug, belonging to the group of selective serotonin reuptake inhibitors (SSRIs). Escitalopram is considered to be among the most “selective” of the SSRIs [149]. Escitalopram was therefore the drug of choice in study II, where the aim was to assess the effect of a single dose SSRI on endogenous serotonin levels. The highest recommended dose for clinical treatment (20 mg) was chosen, as the dose was administered only once.

3.3 PET EXAMINATIONS

All PET examinations were performed using a High Resolution Research Tomograph (HRRT; Siemens Molecular Imaging, USA). The resolution of this system has been estimated to approximately 1.5 mm FWHM, when modeling of the point spread function is included in the image reconstruction [5].

Before initiation of the PET examinations, a plaster helmet was made individually for each human subject. The helmet was used during each PET measurement, to minimize head movements and to ensure maintenance of the same head positioning if the subject participated in more than one PET examination.

Monkeys were anesthetized before insertion into the PET system, and their heads were immobilized with a fixation device. Monkeys were supervised throughout the PET measurements and monitored for vital parameters.

For all human subjects, radioligands were injected intravenously (iv) as a bolus, followed by a rapid flush with 10 mL of physiological sodium chloride solution. Radioactivity in brain was measured in list mode fashion over 63 or 93 min, depending on the study specifications. The distribution of radioactivity from each PET measurement was reconstructed in 3D for a series of time frames consisting of eight 10-sec, five 20-sec, four 30-sec, four 1-min, four 3-min and seven 6-min frames for the 63 min measurement. For the 93 min measurement, the same initial sequences of frames were used, with the addition of five 6-min frames for the last 30 min.

In monkeys, the radioligand was administered iv according to a bolus-infusion protocol. With this protocol, the radioligand was injected as a bolus, at the same time as continuous infusion with a pump was initiated. Radioactivity in the brain was measured in a list mode fashion over 123 minutes and reconstructed in a series of 26 time frames (three 1-min, six 3-min and seventeen 6-min frames).

3.3.1 Radioligands

3.3.1.1 [^{11}C]Raclopride

[^{11}C]Raclopride is the most commonly used radioligand for imaging of D_2 dopamine receptors [72]. Being a selective antagonist with moderately high affinity to the D_2 receptor, [^{11}C]raclopride is useful for imaging of brain regions with a high density of D_2 receptors, such as the striatum [150]. [^{11}C]Raclopride was used in study I, and prepared by methylation of the corresponding desmethyl precursor using [^{11}C]methyl triflate, as previously described [151].

3.3.1.2 [^{11}C]AZ10419369

[^{11}C]AZ10419369 is a recently developed radioligand that binds selectively to 5-HT_{1B} receptors [48, 51]. In study III, the test-retest reliability of PET imaging with [^{11}C]AZ10419369 was studied. In study IV, [^{11}C]AZ10419369 was used for quantification of 5-HT_{1B} receptors in the brain.

[^{11}C]AZ10419369 has also proven useful to study regional changes in extracellular serotonin concentrations [55, 56], and it was used for this purpose in study II. In all three studies, [^{11}C]AZ10419369 was prepared by N-methylation of the corresponding desmethyl precursor using [^{11}C]methyl triflate as previously described [48].

3.3.2 Image processing

T1-weighted MR images were initially acquired using a 1.5 T Signa system and more recently a MR discovery 750 3T system (both systems from GE Medical Systems, Milwaukee, WI, USA). The 1.5 T Signa system was used for study I and part of study IV, and the 3 T system for studies II, III and most of study IV. The MR images were realigned so that the plane defined by the anterior-posterior commissure was placed horizontally.

In all studies, a region of interest (ROI) for the cerebellum was manually delineated on the realigned MR images for each subject, by means of Human

Brain Atlas software [152]. In studies I-III, all other ROIs were manually delineated in the same way. In study IV, a ROI template (the Automated Anatomical Labeling, AAL) [153] was used for definition of all ROIs except for the cerebellum.

MR images were co-registered to averaged PET images using statistical parametric software (SPM 5, Wellcome Department of Cognitive Neuroscience, UK). PET data were corrected for motion using frame-by-frame motion correction as previously described [154]. ROIs were then transferred to the series of PET images to generate time-activity curves.

3.3.2.1 Binding Potential and SRTM

The brain radioactivity measured with PET represents both the radioligand molecules that are specifically bound to the target receptor, and the molecules that are either un-bound or bound to other off-target proteins (= non-displaceable radioligand). Mathematical models are needed to translate the measured radioactivity into different parameters that describe radioligand kinetics in the brain.

In study I-IV, the binding potential (*BP*) was commonly used as such a parameter.

The *BP* of the radioligand is a term that is equivalent to the product of the density of the target receptor (B_{max}) and the binding affinity ($1/K_d$) [155]:

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

An important note is that the B_{max} and K_d cannot be separately determined in one PET measurement.

BP can be calculated by different models. The Simplified Reference Tissue Model (SRTM) was used to calculate *BP* in studies I-IV [156]. With this method, a brain region devoid of the target receptor is used as a reference region. The reference region is assumed to contain the same concentration of the non-

displaceable radioligand as the regions of interest. The binding potential derived with this method, the BP_{ND} [157], refers to the ratio between the concentration of the specifically bound radioligand and the concentration of the non-displaceable radioligand in tissue at equilibrium conditions.

The human cerebellum has been shown to contain negligible densities of D_2 and 5-HT_{1B} receptors [43, 158, 159]. The manually delineated cerebellum ROI was therefore for each subject used as the reference region in all studies.

3.3.2.2 Occupancy

In study I and study II, the aim was to compare radioligand binding before and after administration of the study drug. The drug-induced change in BP_{ND} was calculated according to the following equation:

$$\Delta = \frac{BP_{ND}^{basline} - BP_{ND}^{drug}}{BP_{ND}^{baseline}} \cdot 100$$

Where Δ is the drug-induced change in BP_{ND}, $BP_{ND}^{basline}$ is the BP_{ND} in the drug-free state, and BP_{ND}^{drug} is the BP_{ND} measured after drug administration. In study I, Δ represents D_2 receptor occupancy by quetiapine and in study II Δ represents 5-HT_{1B} receptor occupancy by endogenous serotonin.

3.3.2.3 $K_{i,app}$

If assuming a linear relationship between drug concentration in plasma and in brain, the relationship between receptor occupancy and the plasma concentration of a drug can be described as:

$$Occ = \frac{Occ_{\max} \cdot C_p}{K_{i,app} + C_p} \qquad (3)$$

Where Occ_{max} is the maximal occupancy induced by the drug, C_p is the plasma concentration and $K_{i,app}$ is an operationally defined affinity constant corresponding to the drug plasma concentration required to occupy 50% of the receptors.

In study I, $K_{i,app}$ was derived by fitting equation 3 to measured values of receptor occupancy and plasma concentrations, using a non-linear least squares minimization procedure. Occ_{max} was set to 100% , as previous studies have demonstrated that 100 % D_2 receptor occupancy is approached for [^{11}C]raclopride binding during treatment with high doses of antipsychotic drugs [160].

3.3.2.4 Partial Volume Effect correction (PVEc)

In study IV, cortical volume was expected to differ between subjects, as the grey matter volume has been shown to decrease with age [134]. The Partial Volume Effect (PVE) is larger in smaller volumes, i.e. a larger percentage of the radioactivity is spilling out from the ROI. Thus, a correction for PVE was required in this study. The PVE correction (PVEc) method described by Meltzer et al [161] was used in study IV. This PVEc method is correcting for spill-out of radioactivity from brain tissue in to the CSF. Consequently, regions at a greater distance from the CSF border will not be corrected for, and regions with a large area adjoining the CSF will be more heavily corrected.

3.4 STATISTICS

In study I, a two-tailed, two-sample t-test was used to compare mean plasma concentrations and mean D_2 receptor occupancy after administration of quetiapine XR and IR at peak and through concentrations.

In study II, a two-tailed paired t-test was used to evaluate regional BP_{ND} values obtained at baseline and after escitalopram administration. The minimum level of significance was designated as $p < 0.05$ both for study I and study II.

In study III, a t-test was not applicable for the main study purpose, i.e. to evaluate the test-retest-reliability of [^{11}C]AZ10419369. Instead, descriptive

statistics were used to compare the BP_{ND} at PET 1 and PET 2. This included mean, standard deviation, coefficient of variation, difference (%), variability (%) and intraclass correlation coefficient (ICC).

In study IV, a correlation analysis was performed for each ROI to determine the relationship between BP_{ND} and age. Multiple linear regression analysis was also performed with sex as covariate. The Bonferroni method was used to correct for multiple comparisons.

The ICC in study III was calculated using SPSS version 20. All other statistical analyses were performed using SAS statistical software JMP 8 or 10 (SAS Institute, Cary, NC, USA).

4 RESULTS AND COMMENTS

4.1 STUDY I: COMPARISION OF D_2 DOPAMINE RECEPTOR OCCUPANCY AFTER ORAL ADMINISTRATION OF QUETIAPINE FUMARATE IMMEDIATE-RELEASE AND EXTENDED-RELEASE FORMULATIONS IN HEALTHY SUBJECTS

Quetiapine (QTP) is an antipsychotic drug, produced in immediate-release (IR) and extended-release (XR) oral formulations. The primary aim of study I was to examine whether the pharmacokinetic differences described between the two formulations, translate into different time curves for central D_2 dopamine receptor occupancy. Eleven healthy subjects were administered QTP XR and IR in a one-sequence cross-over fashion. Subjects were examined with PET and the radioligand [^{11}C]raclopride at five occasions - at baseline, and after the last doses of quetiapine XR and IR at predicted times of peak and trough plasma concentrations (Figure 4).

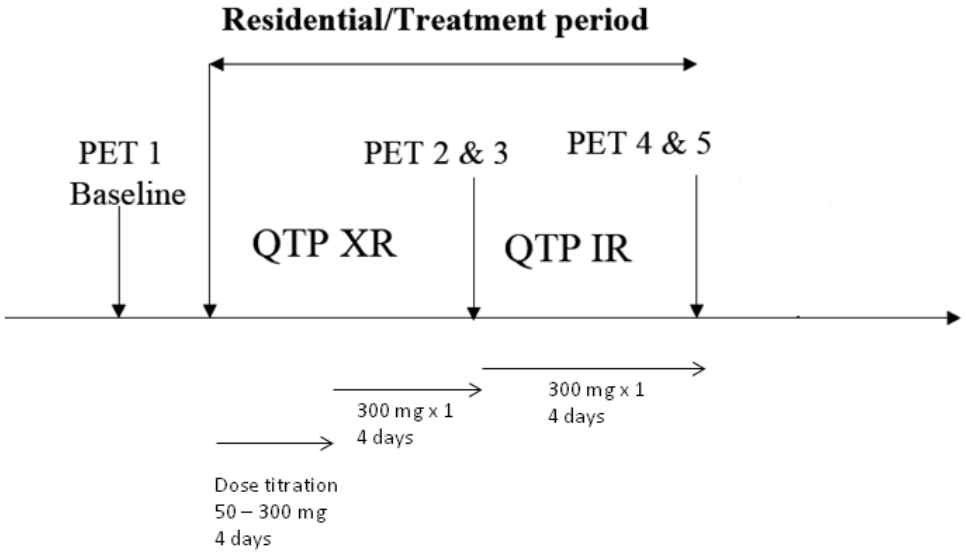

***Figure 4**: Schematic summary of the study design of study I*

The BP_{ND} was calculated using SRTM, and the striatal D_2 receptor occupancy at peak and trough concentrations of QTP were calculated for each subject. The peak D_2 receptor occupancy was significantly higher after administration of the IR formulation compared to the XR formulation (IR: 50±4%; XR: 32±11%, p =0.0003). The trough D_2 receptor occupancy was similarly low for both formulations (IR: 7±7%; XR: 8±6%, p=0.7) (Figure 5).

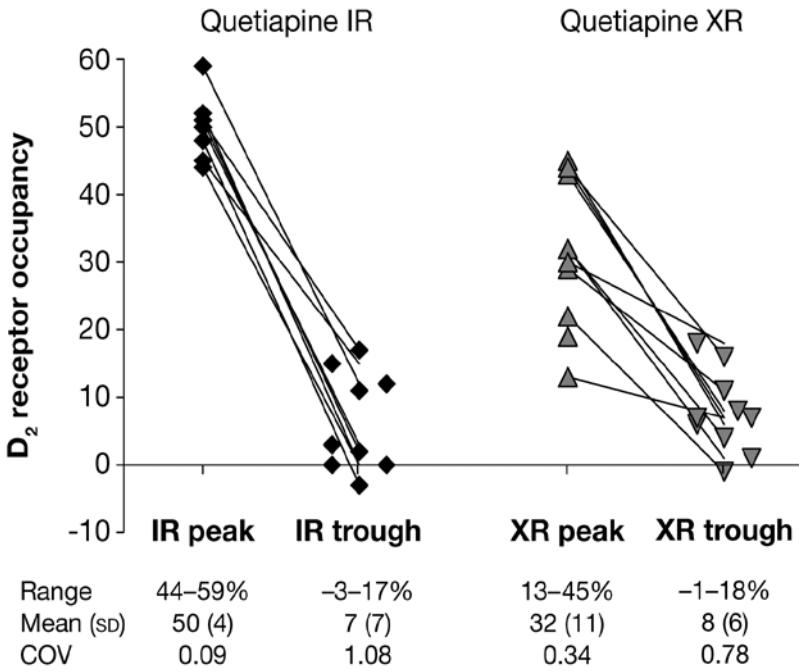

***Figure 5**: D_2 receptor occupancy after administration of quetiapine IR and XR, respectively*

The time curve for estimated D_2 receptor occupancy during a dosing interval was also calculated for each formulation, using equation 3 as described in the Materials and Methods section. After administration of the XR formulation, the peak D_2 receptor occupancy was less pronounced and diminished slower over time after peak, than after administration of the IR formulation (Figure 6).

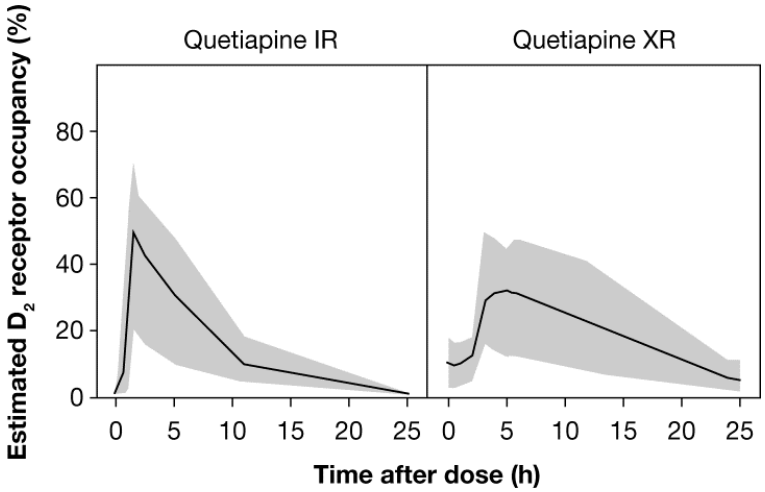

Figure 6: *Estimated D_2-dopamine receptor occupancy (%) versus time for quetiapine IR (left) and XR (right) formulations. The solid lines describe the occupancy-time curve for a typical individual. The grey areas represent the 95% prediction intervals.*

In clinical practice, QTP IR is usually administered twice-daily for the treatment of schizophrenia. As the primary aim of study I was to achieve a direct comparison of the XR and IR formulation, IR was administered only once-daily. The calculated D_2 receptor occupancy for the IR formulation can therefore not be directly translated to the more commonly used twice-daily dosing. The D_2 receptor occupancy associated with the standard 150 mg twice-daily treatment would in all probability be lower than the receptor occupancy in this study. However, larger fluctuation of the D_2 receptor occupancy during the dosing interval with the IR compared to the XR formulation would still be expected.

The lower peak receptor occupancy associated with quetiapine XR may explain previously observed pharmacodynamic differences between the formulations. Additionally, the results support the view that QTP may have antipsychotic effect at lower D_2 receptor occupancy than previously described for first-generation antipsychotic drugs.

4.2 STUDY II: EFFECT OF A SINGLE DOSE ESCITALOPRAM ON SEROTONIN CONCENTRATION IN THE NON-HUMAN AND HUMAN PRIMATE BRAIN

The primary aim of study II was to examine whether a single dose of the antidepressant drug escitalopram affects endogenous serotonin levels in human brain.

Initially, three monkeys were examined with PET and the 5-HT_{1B} receptor-selective radioligand [^{11}C]AZ10419369, before and after administration of a high single dose of escitalopram (2.0 mg/kg, iv) (fig 3). When a measurable effect of escitalopram on the BP_{ND} of [^{11}C]AZ10419369 in monkeys had been confirmed, the methodology was applied to human subjects using a lower, clinically relevant dose of escitalopram.

Nine male healthy subjects between 20 and 30 years of age were examined in the study. Each subject participated in two PET measurements with [^{11}C]AZ10419369: One in the morning during drug-free (baseline) conditions and one in the afternoon approximately 3 h after administration of a single oral dose of escitalopram (20 mg) (Figure 7).

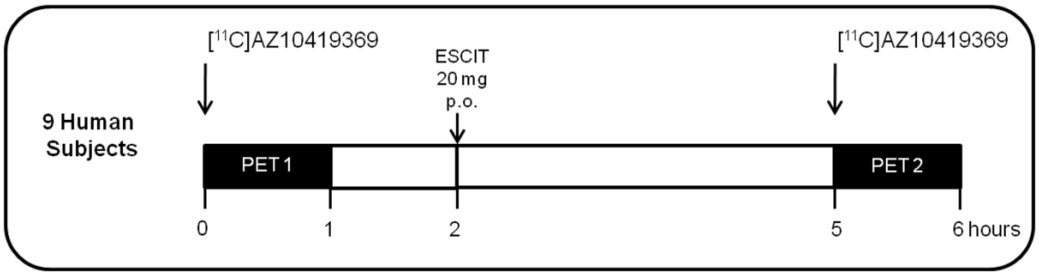

Figure 7: *Schematic summary of the study design of study II*

The BP_{ND} of [^{11}C]AZ10419369 for each PET measurement was defined in ROIs delineated for the raphe nuclei and for serotonergic projection areas. The difference in BP_{ND} between the pre- and post-dose PET measurements was used

as an index of the change in radioligand binding due to changes in extracellular serotonin levels.

In human subjects, the BP_{ND} of [^{11}C]AZ10419369 was increased in serotonergic projection areas after administration of escitalopram, indicating a decrease in the extracellular serotonin concentration. In the region defined for the raphe nuclei, there was on the contrary a trend to a decrease of the BP_{ND} (Figure 8).

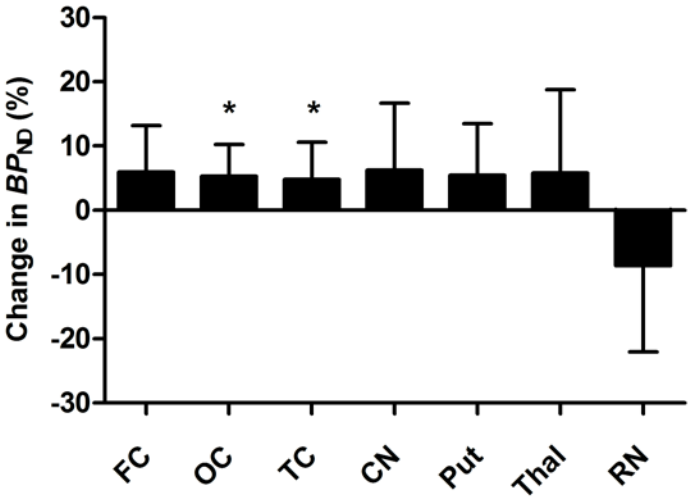

Figure 8: *Relative change in regional BP_{ND} of [^{11}C]AZ10419369 in human subjects after administration of escitalopram. FC = frontal cortex; OC = occipital cortex; TC = temporal cortex; CN = caudate nucleus; Put = putamen; Thal = thalamus; RN = raphe nuclei.*

This observation directly in human subjects extends previous hypotheses on the mechanism of action of antidepressant drugs, derived primarily from experimental animals. The results are consistent with the hypothesis that an increase of serotonin in the raphe nuclei induces stimulation of serotonergic

autoreceptors, which in turn decreases the firing and release of serotonin in projection areas. The observation of a decrease of serotonin in serotonergic projection areas after administration of a single dose of escitalopram, may contribute to the understanding of the time-lag between SERT/5HTT occupancy and the onset of the clinical effect of SSRIs.

4.3 STUDY III: TEST-RETEST RELIABILITY OF [^{11}C]AZ10419369 BINDING TO 5-HT$_{1B}$ RECEPTORS IN HUMAN BRAIN

To further evaluate the methodology used in study II, the test-retest reliability of [^{11}C]AZ10419369 binding was estimated in human subjects.

Eight male healthy subjects between 20 and 30 years were examined twice on the same day with PET and [^{11}C]AZ10419369, at baseline conditions.

The timing of the PET measurements were chosen to equal the times of PET 1 and 2 in study II, but for practical reasons the second PET measurement was performed 1 hour earlier than in study II (Figure 9). ROIs were delineated for the raphe nuclei and serotonergic projection areas (i.e. frontal cortex, occipital cortex, temporal cortex, putamen and thalamus).

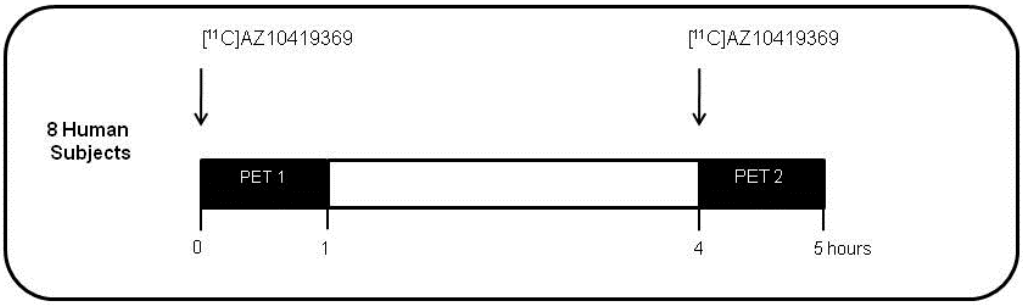

Figure 9: *Schematic summary of the study design of study III*

The BP_{ND} of [^{11}C]AZ10419369 was calculated using SRTM. The BP_{ND} in each ROI at PET 1 and PET 2 was compared using descriptive statistics such as mean, SD, difference (%) and variability (%). Overall, there was a good agreement between individual BP_{ND} values at PET 1 and PET 2 in serotonergic projection areas (Figure 10).

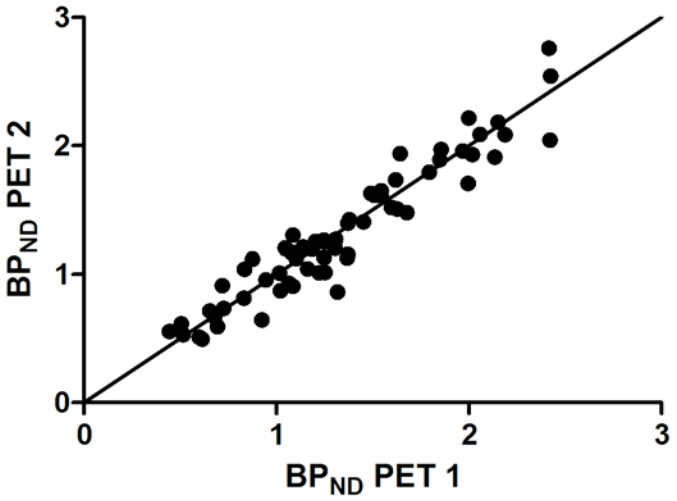

***Figure 10**: Relationship between individual BP_{ND} values at PET 1 and PET 2 in serotonergic projection areas. Each dot represents the value at PET 1 (x-axis) and PET 2 (y-axis) for one particular subject in one of the five regions. The solid line represents the line of identity for the BP_{ND} at PET 1 and PET 2*

The absolute mean differences in BP_{ND} between PET 1 and PET 2 was less than 3 % in all regions examined except for the raphe nuclei. The absolute variability was low (5-7%) in cortical areas but higher (20%) in the raphe nuclei.

The results support the interpretation of study II, i.e. that the increase in BP_{ND} in serotonergic projection areas observed in study II was indeed an effect of the escitalopram administration. The higher test-retest variability in the raphe nuclei

serves as a possible explanation for why it was difficult to obtain conclusive evidence for an effect of escitalopram in this region (Figure 11).

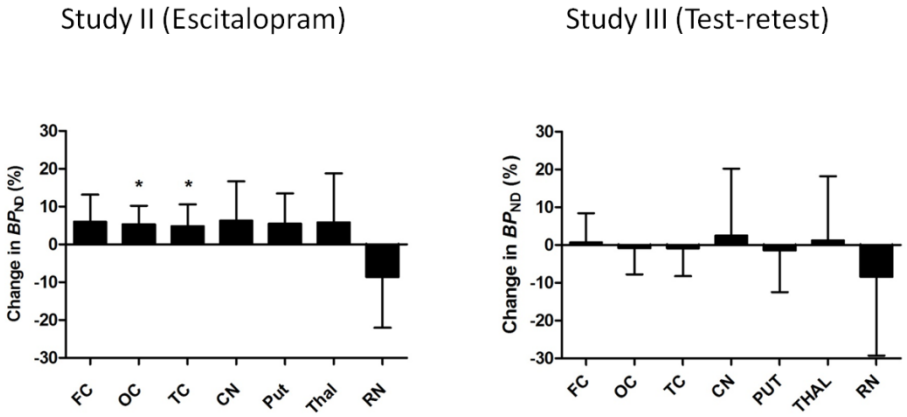

Figure 11: *Comparison of results from study II and study III; change in BP_{ND} between PET 1 and PET 2*

On a more general level, it can be concluded that the BP_{ND} of [^{11}C]AZ10419369 is highly reproducible in cortical areas and satisfactory in the subcortical serotonergic projection regions measured. As the variability was substantially higher in the raphe nuclei, larger sample sizes or larger treatment- or condition-related effects are required to assess a potential difference between subjects or between measurements in this region.

4.4 STUDY IV: DISTINCT REGIONAL AGE EFFECTS ON [^{11}C]AZ10419369 BINDING TO 5-HT$_{1B}$ RECEPTORS IN THE HUMAN BRAIN

In this study, the effect of age on 5-HT$_{1B}$ receptor availability, as assessed by [^{11}C]AZ10419369 binding, was examined in fifty-three healthy subjects, aged

between 20 and 73 years. The BP_{ND} in cortical and subcortical areas was calculated using the simplified reference tissue model (SRTM).

The grey matter volume has been shown to decrease with age [134]. Due to the large age-difference between subjects in this study, cortical volume was expected to differ between subjects. Thus, a correction for PVE was required.

The PVEc method described by Meltzer et al [161], was used in study IV. Other methods for PVEc could also have been considered. While the Meltzer method corrects for spill-out of radioactivity from brain tissue in to the CSF, the PVEc method described by Rousset et al [162] corrects also for spillover between brain regions. While this method probably provides a more thorough and "widespread" PVE correction, the lack of validation in our systems with the [^{11}C]AZ10419369 radioligand made it unsuitable for the present study.

A third PVEc method has been described by Muller-Gartner [163]. In this method, PVEc are made both for spillover to CSF and between grey and white matter. While this last step might seem as an improvement compared to the method described by Meltzer, the Muller-Gartner method assumes that the radioligand is evenly distributed within white respective grey matter regions. As radioligand pharmacokinetics in white matter can be markedly various within brain regions, this method might introduce errors in the quantification of data. Thus, the Meltzer method was considered the best option, although not free from disadvantages.

After correction for PVE, the correlation between age and regional BP_{ND} was examined. 5-HT_{1B} receptor availability decreased significantly with age in cortical regions, with an average decrease of 8 % per decade. In the ventral striatum, the age-related decrease was smaller (4% per decade). On contrary, the 5-HT_{1B} receptor availability in the dorsal striatal regions, the caudate nucleus and putamen, remained stable over age (Figure 12).

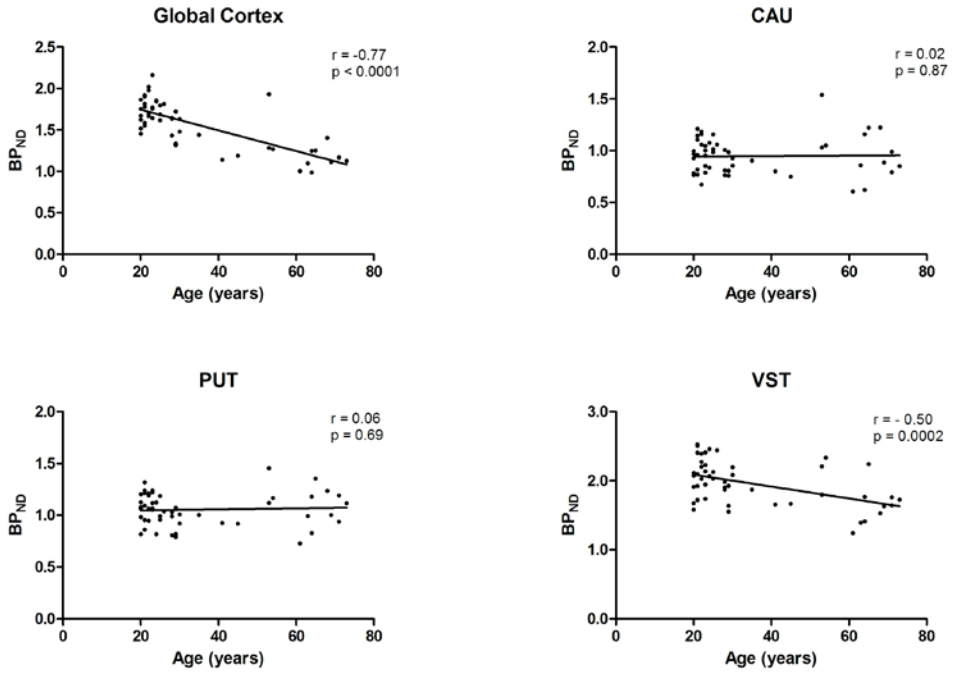

Figure 12: *Correlation between regional BP_{ND} and age. Each dot represents the BP_{ND} and age of one individual subject in the brain region specified in the figure. CAU = caudate nucleus, PUT = putamen, VST = ventral striatum.*

The findings support previous reports of decreases in biomarkers for cerebral monoaminergic neurotransmitter systems by age. The stable 5-HT_{1B} receptor availability in the caudate nucleus and the putamen is intriguing, and might indicate that 5-HT_{1B} receptors in these regions are expressed on neurons with a different sensitivity to aging.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

Study I: The lower peak D_2 receptor occupancy associated with quetiapine (QTP) XR compared to QTP IR may explain previously observed pharmacodynamic differences between the formulations. Further, the study provided additional support for the view that quetiapine, like clozapine, could have antipsychotic effect at lower D_2 receptor occupancy levels than the previously suggested therapeutic window.

While the lower D_2 receptor occupancy could explain the lower propensity for EPS with these drugs, the mechanism behind a possibly maintained antipsychotic effect despite moderate D_2 receptor occupancy remains to be elucidated. Hypothetically, this could be due to effects also on other receptor systems.

The different shapes of the estimated D_2 receptor occupancy time curves during a dosing interval with QTP XR compared to IR, also highlights the lack of knowledge regarding the relationships between duration of high D_2 occupancy and antipsychotic effect.

Both of the above mentioned issues need to be considered, for future development of antipsychotic drugs with improved efficacy and less side-effects. Further knowledge on the relationship between duration of D_2 receptor occupancy and antipsychotic effect, could also aid in optimizing the dosing of already available antipsychotic drugs.

Study II: Hypothetically, SSRIs initiate their clinical effect by increasing the serotonin concentration in the synapse. The results from study II indicate that extracellular serotonin levels in serotonergic projection areas decrease after a single, clinically relevant dose of an SSRI in human subjects. This observation may contribute to the understanding of the time-lag between SERT/5-HTT occupancy and clinical effect. However, further in vivo studies in humans are needed to evaluate the effect of SSRIs on extracellular serotonin concentrations after prolonged treatment, and whether there is a correlation between changes in serotonin concentrations and antidepressive effect. It would also be of interest to further examine the other downstream effects of SSRI treatment. Possibly this could aid in the development of antidepressant drugs with improved

effectiveness and with a shorter time-lag before the onset of the desired clinical effect.

Study III: A high reproducibility between repeated measurements of the BP_{ND} of the radioligand [^{11}C]AZ10419369, was demonstrated in serotonergic projection areas. This observation support the interpretation of the results of study II - i.e. that the change in BP_{ND} in serotonergic projection areas after escitalopram administration was indeed caused by escitalopram. However, the larger variability of the BP_{ND} in the area defined for the raphe nuclei, indicates the need for improved methodology for measurement of 5-HT_{1B} receptors in this region. As it is not possible to delineate the exact anatomical boundaries of the raphe nuclei on MR images, a more functionally based delineation directly on the PET images could be useful. This kind of delineation could be implemented if the distribution of [^{11}C]AZ10419369 within the raphe nuclei is sufficiently high compared to the surrounding tissue. However, this strategy has not yet been evaluated. Until then, larger sample sizes or larger effect sizes are required to assess a potential difference between subjects or between measurements in this region.

Study IV: The 5-HT_{1B} receptor availability, as assessed with PET and [^{11}C]AZ10419369, decreased significantly with age in cortical regions, but the 5-HT_{1B} receptor availability in the caudate nucleus and the putamen remained stable over the investigated age range. This observation might indicate that the 5-HT_{1B} receptors in the caudate nucleus and the putamen are expressed on neurons with a different sensitivity to aging. Further studies are needed to examine the fraction of pre- and post-synaptic expression of the $5HT_{1B}$ receptor in different brain regions.

The results from study IV also highlight the importance of using age-matched control subjects in future studies of the 5-HT_{1B} receptor. It remains to be clarified whether the age-related decline in 5-HT_{1B} receptor availability is related to changes in behavioral and cognitive functions in normal aging, or in relation to CNS disorders.

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7 REFERENCES

1. Bjarnadottir, T.K., et al., *Comprehensive repertoire and phylogenetic analysis of the G protein-coupled receptors in human and mouse.* Genomics, 2006. **88**(3): p. 263-73.

2. Lee, C.M. and L. Farde, *Using positron emission tomography to facilitate CNS drug development.* Trends Pharmacol Sci, 2006. **27**(6): p. 310-6.

3. Gulyas, B. and N. Sjoholm, *Principles of Positron Emission Tomography*, in *Functional Neuroimaging in Clinical Populations*, F.G. Hillary and J. DeLuca, Editors. 2007, The Guilford Press: New York.

4. Levin, C.S. and E.J. Hoffman, *Calculation of positron range and its effect on the fundamental limit of positron emission tomography system spatial resolution.* Phys Med Biol, 1999. **44**(3): p. 781-99.

5. Varrone, A., et al., *Advancement in PET quantification using 3D-OP-OSEM point spread function reconstruction with the HRRT.* Eur J Nucl Med Mol Imaging, 2009. **36**(10): p. 1639-50.

6. Sperling, R. and K. Johnson, *Biomarkers of Alzheimer disease: current and future applications to diagnostic criteria.* Continuum (Minneap Minn), 2013. **19**(2 Dementia): p. 325-38.

7. Farde, L., et al., *PET studies of dopamine receptors in relation to antipsychotic drug treatment.* Clin Neuropharmacol, 1992. **15 Suppl 1 Pt A**: p. 468A-469A.

8. Stahl, S.M., *Molecular neurobiology for practicing psychiatrists, part 2: how neurotransmitters activate second messenger systems.* J Clin Psychiatry, 1999. **60**(10): p. 647-8.

9. Greengard, P., *The neurobiology of slow synaptic transmission.* Science, 2001. **294**(5544): p. 1024-30.

10. Carlsson, A., M. Lindqvist, and T. Magnusson, *3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists.* Nature, 1957. **180**(4596): p. 1200.

11. Carlsson, A., *The occurrence, distribution and physiological role of catecholamines in the nervous system.* Pharmacol Rev, 1959. **11**(2, Part 2): p. 490-3.

12. Carlsson, A., et al., *On the presence of 3-hydroxytyramine in brain.* Science, 1958. **127**(3296): p. 471.

13. Benes, F.M., *Carlsson and the discovery of dopamine.* Trends Pharmacol Sci, 2001. **22**(1): p. 46-7.

14. Ungerstedt, U., *Stereotaxic mapping of the monoamine pathways in the rat brain.* Acta Physiol Scand Suppl, 1971. **367**: p. 1-48.

15. Vallone, D., R. Picetti, and E. Borrelli, *Structure and function of dopamine receptors.* Neurosci Biobehav Rev, 2000. **24**(1): p. 125-32.

16. Anden, N.E., et al., *DEMONSTRATION AND MAPPING OUT OF NIGRO-NEOSTRIATAL DOPAMINE NEURONS.* Life Sci, 1964. **3**: p. 523-30.

17. Stahl, S.M., *Stahl´s Essential Psychopharmacology*. 4th ed. 2013, New York: Cambridge University Press.

18. Backman, L., et al., *The correlative triad among aging, dopamine, and cognition: current status and future prospects.* Neurosci Biobehav Rev, 2006. **30**(6): p. 791-807.

19. Sanchez-Gonzalez, M.A., et al., *The primate thalamus is a key target for brain dopamine.* J Neurosci, 2005. **25**(26): p. 6076-83.

20. Garcia-Cabezas, M.A., et al., *Distribution of the dopamine innervation in the macaque and human thalamus.* Neuroimage, 2007. **34**(3): p. 965-84.

21. Missale, C., et al., *Dopamine receptors: from structure to function.* Physiol Rev, 1998. **78**(1): p. 189-225.

22. Hall, H., et al., *Distribution of D1- and D2-dopamine receptors, and dopamine and its metabolites in the human brain.* Neuropsychopharmacology, 1994. **11**(4): p. 245-56.

23. Dal Toso, R., et al., *The dopamine D2 receptor: two molecular forms generated by alternative splicing.* EMBO J, 1989. **8**(13): p. 4025-34.

24. Usiello, A., et al., *Distinct functions of the two isoforms of dopamine D2 receptors.* Nature, 2000. **408**(6809): p. 199-203.

25. Khan, Z.U., et al., *Prominence of the dopamine D2 short isoform in dopaminergic pathways.* Proc Natl Acad Sci U S A, 1998. **95**(13): p. 7731-6.

26. Wagner, H.N., Jr., et al., *Imaging dopamine receptors in the human brain by positron tomography.* Science, 1983. **221**(4617): p. 1264-6.

27. Farde, L., et al., *Substituted benzamides as ligands for visualization of dopamine receptor binding in the human brain by positron emission tomography.* Proc Natl Acad Sci U S A, 1985. **82**(11): p. 3863-7.

28. Halldin, C., et al., *Carbon-11-FLB 457: a radioligand for extrastriatal D2 dopamine receptors.* J Nucl Med, 1995. **36**(7): p. 1275-81.

29. Mukherjee, J., et al., *Brain imaging of 18F-fallypride in normal volunteers: blood analysis, distribution, test-retest studies, and preliminary assessment of sensitivity to aging effects on dopamine D-2/D-3 receptors.* Synapse, 2002. **46**(3): p. 170-88.

30. Gothert, M., *Serotonin discovery and stepwise disclosure of 5-HT receptor complexity over four decades. Part I. General background and discovery of serotonin as a basis for 5-HT receptor identification.* Pharmacol Rep, 2013. **65**(4): p. 771-86.

31. Rapport, M.M., A.A. Green, and I.H. Page, *SERUM VASOCONSTRICTOR (SEROTONIN) .4. ISOLATION AND CHARACTERIZATION.* Journal of Biological Chemistry, 1948. **176**(3): p. 1243-1251.

32. Erspamer, V. and B. Asero, *IDENTIFICATION OF ENTERAMINE, THE SPECIFIC HORMONE OF THE ENTEROCHROMAFFIN CELL SYSTEM, AS 5-HYDROXYTRYPTAMINE.* Nature, 1952. **169**(4306): p. 800-801.

33. Twarog, B.M., I.H. Page, and H. Bailey, *SEROTONIN CONTENT OF SOME MAMMALIAN TISSUES AND URINE AND A METHOD FOR ITS DETERMINATION.* American Journal of Physiology, 1953. **175**(1): p. 157-161.

34. Brodie, B.B. and P.A. Shore, *A CONCEPT FOR A ROLE OF SEROTONIN AND NOREPINEPHRINE AS CHEMICAL MEDIATORS IN THE BRAIN.* Annals of the New York Academy of Sciences, 1957. **66**(3): p. 631-642.

35. Shore, P.A., et al., *ROLE OF BRAIN SEROTONIN IN RESERPINE ACTION.* Annals of the New York Academy of Sciences, 1957. **66**(3): p. 609-617.

36. Dahlstroem, A. and K. Fuxe, *EVIDENCE FOR THE EXISTENCE OF MONOAMINE-CONTAINING NEURONS IN THE CENTRAL NERVOUS SYSTEM. I. DEMONSTRATION OF MONOAMINES IN THE CELL BODIES OF BRAIN STEM NEURONS.* Acta Physiol Scand Suppl, 1964: p. SUPPL 232:1-55.

37. Hornung, J.P., *The human raphe nuclei and the serotonergic system.* J Chem Neuroanat, 2003. **26**(4): p. 331-43.

38. Jacobs, B.L. and E.C. Azmitia, *Structure and function of the brain serotonin system.* Physiol Rev, 1992. **72**(1): p. 165-229.

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

40. Hannon, J. and D. Hoyer, *Molecular biology of 5-HT receptors.* Behav Brain Res, 2008. **195**(1): p. 198-213.

41. Barnes, N.M. and T. Sharp, *A review of central 5-HT receptors and their function.* Neuropharmacology, 1999. **38**(8): p. 1083-152.

42. Hoyer, D., J.P. Hannon, and G.R. Martin, *Molecular, pharmacological and functional diversity of 5-HT receptors.* Pharmacol Biochem Behav, 2002. **71**(4): p. 533-54.

43. Varnas, K., et al., *Autoradiographic mapping of 5-HT(1B) and 5-HT(1D) receptors in the post mortem human brain using [(3)H]GR 125743.* Brain Res, 2001. **915**(1): p. 47-57.

44. Sari, Y., *Serotonin1B receptors: from protein to physiological function and behavior.* Neurosci Biobehav Rev, 2004. **28**(6): p. 565-82.

45. Ruf, B.M. and Z. Bhagwagar, *The 5-HT1B receptor: a novel target for the pathophysiology of depression.* Curr Drug Targets, 2009. **10**(11): p. 1118-38.

46. Nicholson, S.L. and J.M. Brotchie, *5-hydroxytryptamine (5-HT, serotonin) and Parkinson's disease - opportunities for novel therapeutics to reduce the problems of levodopa therapy.* Eur J Neurol, 2002. **9 Suppl 3**: p. 1-6.

47. Varrone, A., et al., *Positron emission tomography imaging of 5-hydroxytryptamine receptors in Parkinson's disease.* Neurobiol Aging, 2013.

48. Pierson, M.E., et al., *[11C]AZ10419369: a selective 5-HT1B receptor radioligand suitable for positron emission tomography (PET). Characterization in the primate brain.* Neuroimage, 2008. **41**(3): p. 1075-85.

49. Gallezot, J.D., et al., *Kinetic modeling of the serotonin 5-HT(1B) receptor radioligand [(11)C]P943 in humans.* J Cereb Blood Flow Metab, 2010. **30**(1): p. 196-210.

50. Andersson, J.D., et al., *Development of a PET radioligand for the central 5-HT1B receptor: radiosynthesis and characterization in cynomolgus monkeys of eight radiolabeled compounds.* Nucl Med Biol, 2011. **38**(2): p. 261-72.

51. Varnas, K., et al., *Quantitative analysis of [11C]AZ10419369 binding to 5-HT1B receptors in human brain.* J Cereb Blood Flow Metab, 2011. **31**(1): p. 113-23.

52. Dewey, S.L., et al., *Amphetamine induced decreases in (18F)-N-methylspiroperidol binding in the baboon brain using positron emission tomography (PET).* Synapse, 1991. **7**(4): p. 324-7.

53. Laruelle, M., et al., *Imaging D2 receptor occupancy by endogenous dopamine in humans.* Neuropsychopharmacology, 1997. **17**(3): p. 162-74.

54. Laruelle, M., *Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review.* J Cereb Blood Flow Metab, 2000. **20**(3): p. 423-51.

55. Finnema, S.J., et al., *Fenfluramine-induced serotonin release decreases [(11)C]AZ10419369 binding to 5-HT(1B)-receptors in the primate brain.* Synapse, 2010. **64**(7): p. 573-577.

56. Finnema, S.J., et al., *Confirmation of fenfluramine effect on 5-HT(1B) receptor binding of [(11)C]AZ10419369 using an equilibrium approach.* J Cereb Blood Flow Metab, 2012. **32**(4): p. 685-95.

57. Cosgrove, K.P., et al., *Assessing the sensitivity of [(1)(1)C]p943, a novel 5-HT1B radioligand, to endogenous serotonin release.* Synapse, 2011. **65**(10): p. 1113-7.

58. Mueser, K.T. and S.R. McGurk, *Schizophrenia.* Lancet, 2004. **363**(9426): p. 2063-72.

59. van Os, J. and S. Kapur, *Schizophrenia.* Lancet, 2009. **374**(9690): p. 635-45.

60. Carlsson, A. and M. Lindqvist, *Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain.* Acta Pharmacol Toxicol (Copenh), 1963. **20**: p. 140-4.

61. van Rossum, J.M., *The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs.* Arch Int Pharmacodyn Ther, 1966. **160**(2): p. 492-4.

62. Angrist, B.M. and S. Gershon, *The phenomenology of experimentally induced amphetamine psychosis--preliminary observations.* Biol Psychiatry, 1970. **2**(2): p. 95-107.

63. Davis, K.L., et al., *Dopamine in schizophrenia: a review and reconceptualization.* Am J Psychiatry, 1991. **148**(11): p. 1474-86.

64. Breier, A., et al., *Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method.* Proc Natl Acad Sci U S A, 1997. **94**(6): p. 2569-74.

65. Laruelle, M., et al., *Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects.* Proc Natl Acad Sci U S A, 1996. **93**(17): p. 9235-40.

66. Abi-Dargham, A., et al., *Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort.* Am J Psychiatry, 1998. **155**(6): p. 761-7.

67. Lopez-Munoz, F., et al., *History of the discovery and clinical introduction of chlorpromazine.* Ann Clin Psychiatry, 2005. **17**(3): p. 113-35.

68. Creese, I., D.R. Burt, and S.H. Snyder, *Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs.* Science, 1976. **192**(4238): p. 481-3.

69. Seeman, P., et al., *Antipsychotic drug doses and neuroleptic/dopamine receptors.* Nature, 1976. **261**(5562): p. 717-9.

70. Peroutka, S.J. and S.H. Snyder, *Relationship of neuroleptic drug effects at brain dopamine, serotonin, alpha-adrenergic, and histamine receptors to clinical potency.* Am J Psychiatry, 1980. **137**(12): p. 1518-22.

71. Cambon, H., et al., *In vivo assay for neuroleptic receptor binding in the striatum. Positron tomography in humans.* Br J Psychiatry, 1987. **151**: p. 824-30.

72. Farde, L., et al., *Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET.* Science, 1986. **231**(4735): p. 258-61.

73. Smith, M., et al., *Serial [18F]N-methylspiroperidol PET studies to measure changes in antipsychotic drug D-2 receptor occupancy in schizophrenic patients.* Biol Psychiatry, 1988. **23**(7): p. 653-63.

74. Maziere, B., et al., *In vivo quantitative imaging of dopamine receptors in human brain using positron emission tomography and [76Br]bromospiperone.* Eur J Pharmacol, 1985. **114**(3): p. 267-72.

75. Wenthur, C.J. and C.W. Lindsley, *Classics in chemical neuroscience: clozapine.* ACS Chem Neurosci, 2013. **4**(7): p. 1018-25.

76. Horacek, J., et al., *Mechanism of action of atypical antipsychotic drugs and the neurobiology of schizophrenia.* CNS Drugs, 2006. **20**(5): p. 389-409.

77. Tandon, R., *Antipsychotics in the treatment of schizophrenia: an overview.* J Clin Psychiatry, 2011. **72 Suppl 1**: p. 4-8.

78. Farde, L., et al., *Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects.* Arch Gen Psychiatry, 1992. **49**(7): p. 538-44.

79. Kapur, S., et al., *Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia.* Am J Psychiatry, 2000. **157**(4): p. 514-20.

80. Nordstrom, A.L., et al., *Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients.* Biol Psychiatry, 1993. **33**(4): p. 227-35.

81. Nyberg, S., et al., *Suggested minimal effective dose of risperidone based on PET-measured D2 and 5-HT2A receptor occupancy in schizophrenic patients.* Am J Psychiatry, 1999. **156**(6): p. 869-75.

82. Nordstrom, A.L., et al., *Positron emission tomography finding of a high striatal D2 receptor occupancy in olanzapine-treated patients.* Arch Gen Psychiatry, 1998. **55**(3): p. 283-4.

83. Kapur, S., et al., *5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: a PET investigation.* Am J Psychiatry, 1998. **155**(7): p. 921-8.

84. Mamo, D., et al., *A PET study of dopamine D2 and serotonin 5-HT2 receptor occupancy in patients with schizophrenia treated with therapeutic doses of ziprasidone.* Am J Psychiatry, 2004. **161**(5): p. 818-25.

85. Corripio, I., et al., *Striatal dopaminergic D2 receptor occupancy and clinical efficacy in psychosis exacerbation: a 123I-IBZM study with ziprasidone and haloperidol.* Prog Neuropsychopharmacol Biol Psychiatry, 2005. **29**(1): p. 91-6.

86. Nyberg, S. and L. Farde, *Non-equipotent doses partly explain differences among antipsychotics - implications of PET studies.* Psychopharmacology (Berl), 2000. **148**(1): p. 22-3.

87. Kegeles, L.S., et al., *Dose-occupancy study of striatal and extrastriatal dopamine D2 receptors by aripiprazole in schizophrenia with PET and [18F]fallypride.* Neuropsychopharmacology, 2008. **33**(13): p. 3111-25.

88. Mamo, D., et al., *Differential effects of aripiprazole on D(2), 5-HT(2), and 5-HT(1A) receptor occupancy in patients with schizophrenia: a triple tracer PET study.* Am J Psychiatry, 2007. **164**(9): p. 1411-7.

89. Grunder, G., et al., *Brain and plasma pharmacokinetics of aripiprazole in patients with schizophrenia: an [18F]fallypride PET study.* Am J Psychiatry, 2008. **165**(8): p. 988-95.

90. Grunder, G., A. Carlsson, and D.F. Wong, *Mechanism of new antipsychotic medications: occupancy is not just antagonism.* Arch Gen Psychiatry, 2003. **60**(10): p. 974-7.

91. Nordstrom, A.L., et al., *D1, D2, and 5-HT2 receptor occupancy in relation to clozapine serum concentration: a PET study of schizophrenic patients.* Am J Psychiatry, 1995. **152**(10): p. 1444-9.

92. Pilowsky, L.S., et al., *Clozapine, single photon emission tomography, and the D2 dopamine receptor blockade hypothesis of schizophrenia.* Lancet, 1992. **340**(8813): p. 199-202.

93. Farde, L., et al., *D1- and D2-dopamine receptor occupancy during treatment with conventional and atypical neuroleptics.* Psychopharmacology (Berl), 1989. **99 Suppl**: p. S28-31.

94. Kane, J., et al., *Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine.* Arch Gen Psychiatry, 1988. **45**(9): p. 789-96.

95. Remington, G. and S. Kapur, *Atypical antipsychotics: are some more atypical than others?* Psychopharmacology (Berl), 2000. **148**(1): p. 3-15.

96. Farde, L., et al., *Central D2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs.* Arch Gen Psychiatry, 1988. **45**(1): p. 71-6.

97. Nyberg, S., et al., *Positron emission tomography studies on D2 dopamine receptor occupancy and plasma antipsychotic drug levels in man.* Int Clin Psychopharmacol, 1995. **10 Suppl 3**: p. 81-5.

98. Baron, J.C., et al., *Striatal dopamine receptor occupancy during and following withdrawal from neuroleptic treatment: correlative evaluation by positron emission tomography and plasma prolactin levels.* Psychopharmacology (Berl), 1989. **99**(4): p. 463-72.

99. Catafau, A.M., et al., *Pharmacokinetics and time-course of D(2) receptor occupancy induced by atypical antipsychotics in stabilized schizophrenic patients.* J Psychopharmacol, 2008. **22**(8): p. 882-94.

100. Nyberg, S., et al., *D2 dopamine receptor occupancy during low-dose treatment with haloperidol decanoate.* Am J Psychiatry, 1995. **152**(2): p. 173-8.

101. Uchida, H., et al., *Monthly administration of long-acting injectable risperidone and striatal dopamine D2 receptor occupancy for the management of schizophrenia.* J Clin Psychiatry, 2008. **69**(8): p. 1281-6.

102. Lieberman, J.A., et al., *Drugs of the psychopharmacological revolution in clinical psychiatry.* Psychiatr Serv, 2000. **51**(10): p. 1254-8.

103. Jensen, N.H., et al., *N-desalkylquetiapine, a potent norepinephrine reuptake inhibitor and partial 5-HT1A agonist, as a putative mediator of quetiapine's antidepressant activity.* Neuropsychopharmacology, 2008. **33**(10): p. 2303-12.

104. Gefvert, O., et al., *Time course of central nervous dopamine-D2 and 5-HT2 receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel) in patients with schizophrenia.* Psychopharmacology (Berl), 1998. **135**(2): p. 119-26.

105. Gefvert, O., et al., *D(2) and 5HT(2A) receptor occupancy of different doses of quetiapine in schizophrenia: a PET study.* Eur Neuropsychopharmacol, 2001. **11**(2): p. 105-10.

106. Mamo, D.C., et al., *Quetiapine extended-release versus immediate-release formulation: a positron emission tomography study.* J Clin Psychiatry, 2008. **69**(1): p. 81-6.

107. Baldwin, C.M. and L.J. Scott, *Quetiapine extended release: in schizophrenia.* CNS Drugs, 2009. **23**(3): p. 261-9.

108. DeVane, C.L. and C.B. Nemeroff, *Clinical pharmacokinetics of quetiapine: an atypical antipsychotic.* Clin Pharmacokinet, 2001. **40**(7): p. 509-22.

109. Tauscher-Wisniewski, S., et al., *Quetiapine: an effective antipsychotic in first-episode schizophrenia despite only transiently high dopamine-2 receptor blockade.* J Clin Psychiatry, 2002. **63**(11): p. 992-7.

110. Kapur, S., et al., *A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D2 receptor occupancy.* Arch Gen Psychiatry, 2000. **57**(6): p. 553-9.

111. Belmaker, R.H. and G. Agam, *Major depressive disorder.* N Engl J Med, 2008. **358**(1): p. 55-68.

112. American Psychiatric Association, *Diagnostic and statistical manual of mental disorders : DSM-5.* 2013.

113. Shaw, D.M., F.E. Camps, and E.G. Eccleston, *5-Hydroxytryptamine in the hind-brain of depressive suicides.* Br J Psychiatry, 1967. **113**(505): p. 1407-11.

114. Bourne, H.R., et al., *Noradrenaline, 5-hydroxytryptamine, and 5-hydroxyindoleacetic acid in hindbrains of suicidal patients.* Lancet, 1968. **2**(7572): p. 805-8.

115. Agren, H., *Symptom patterns in unipolar and bipolar depression correlating with monoamine metabolites in the cerebrospinal fluid: I. General patterns.* Psychiatry Res, 1980. **3**(2): p. 211-23.

116. Ashcroft, G.W., et al., *5-hydroxyindole compounds in the cerebrospinal fluid of patients with psychiatric or neurological diseases.* Lancet, 1966. **2**(7472): p. 1049-52.

117. Asberg, M., et al., *"Serotonin depression"--a biochemical subgroup within the affective disorders?* Science, 1976. **191**(4226): p. 478-80.

118. Asberg, M., L. Traskman, and P. Thoren, *5-HIAA in the cerebrospinal fluid. A biochemical suicide predictor?* Arch Gen Psychiatry, 1976. **33**(10): p. 1193-7.

119. Delgado, P.L., et al., *Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan.* Arch Gen Psychiatry, 1990. **47**(5): p. 411-8.

120. Smith, K.A., C.G. Fairburn, and P.J. Cowen, *Relapse of depression after rapid depletion of tryptophan.* Lancet, 1997. **349**(9056): p. 915-9.

121. Toker, L., et al., *The biology of tryptophan depletion and mood disorders.* Isr J Psychiatry Relat Sci, 2010. **47**(1): p. 46-55.

122. Lopez-Munoz, F. and C. Alamo, *Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today.* Curr Pharm Des, 2009. **15**(14): p. 1563-86.

123. Salvadore, G. and J.B. Singh, *Ketamine as a fast acting antidepressant: current knowledge and open questions.* CNS Neurosci Ther, 2013. **19**(6): p. 428-36.

124. Ogren, S.O., et al., *The pharmacology of zimelidine: a 5-HT selective reuptake inhibitor.* Acta Psychiatr Scand Suppl, 1981. **290**: p. 127-51.

125. Fagius, J., et al., *Guillain-Barre syndrome following zimeldine treatment.* J Neurol Neurosurg Psychiatry, 1985. **48**(1): p. 65-9.

126. Carlsson, A., *A paradigm shift in brain research.* Science, 2001. **294**(5544): p. 1021-4.

127. Meyer, J.H., et al., *Occupancy of serotonin transporters by paroxetine and citalopram during treatment of depression: a [(11)C]DASB PET imaging study.* Am J Psychiatry, 2001. **158**(11): p. 1843-9.

128. Meyer, J.H., et al., *Serotonin transporter occupancy of five selective serotonin reuptake inhibitors at different doses: an [11C]DASB positron emission tomography study.* Am J Psychiatry, 2004. **161**(5): p. 826-35.

129. Lundberg, J., et al., *PET measurement of serotonin transporter occupancy: a comparison of escitalopram and citalopram.* Int J Neuropsychopharmacol, 2007. **10**(6): p. 777-85.

130. Montgomery, S., T. Hansen, and S. Kasper, *Efficacy of escitalopram compared to citalopram: a meta-analysis.* Int J Neuropsychopharmacol, 2011. **14**(2): p. 261-8.

131. Sanchez, C., *The pharmacology of citalopram enantiomers: the antagonism by R-citalopram on the effect of S-citalopram.* Basic Clin Pharmacol Toxicol, 2006. **99**(2): p. 91-5.

132. Giedd, J.N., et al., *Brain development during childhood and adolescence: a longitudinal MRI study.* Nat Neurosci, 1999. **2**(10): p. 861-3.

133. Huttenlocher, P.R., *Synaptic density in human frontal cortex - developmental changes and effects of aging.* Brain Res, 1979. **163**(2): p. 195-205.

134. Resnick, S.M., et al., *Longitudinal magnetic resonance imaging studies of older adults: a shrinking brain.* J Neurosci, 2003. **23**(8): p. 3295-301.

135. Burke, S.N. and C.A. Barnes, *Neural plasticity in the ageing brain.* Nat Rev Neurosci, 2006. **7**(1): p. 30-40.

136. Wong, D.F., et al., *Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain.* Science, 1984. **226**(4681): p. 1393-6.

137. Nordstrom, A.L., et al., *PET analysis of central [11C]raclopride binding in healthy young adults and schizophrenic patients - reliability and age effects.* Human psychopharmacology, 1992. **7**: p. 157-165.

138. Kaasinen, V. and J.O. Rinne, *Functional imaging studies of dopamine system and cognition in normal aging and Parkinson's disease.* Neurosci Biobehav Rev, 2002. **26**(7): p. 785-93.

139. Ding, Y.S., et al., *PET imaging of the effects of age and cocaine on the norepinephrine transporter in the human brain using (S,S)-[(11)C]O-methylreboxetine and HRRT.* Synapse, 2010. **64**(1): p. 30-8.

140. Meltzer, C.C., et al., *Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging.* Neuropsychopharmacology, 1998. **18**(6): p. 407-30.

141. Fidalgo, S., D.K. Ivanov, and S.H. Wood, *Serotonin: from top to bottom.* Biogerontology, 2013. **14**(1): p. 21-45.

142. Uchida, H., et al., *Effects of aging on 5-HT(2A) R binding: a HRRT PET study with and without partial volume corrections.* Int J Geriatr Psychiatry, 2011. **26**(12): p. 1300-8.

143. Yamamoto, M., et al., *Age-related decline of serotonin transporters in living human brain of healthy males.* Life Sci, 2002. **71**(7): p. 751-7.

144. Costes, N., et al., *A 18F-MPPF PET normative database of 5-HT1A receptor binding in men and women over aging.* J Nucl Med, 2005. **46**(12): p. 1980-9.

145. Matuskey, D., et al., *Age effects on serotonin receptor 1B as assessed by PET.* J Nucl Med, 2012. **53**(9): p. 1411-4.

146. Garcia-Alloza, M., et al., *Differential involvement of 5-HT(1B/1D) and 5-HT6 receptors in cognitive and non-cognitive symptoms in Alzheimer's disease.* Neuropsychopharmacology, 2004. **29**(2): p. 410-6.

147. Garber, J.C., et al., *Guide for the Care and Use of Laboratory Animals*. 2011: The National Academies Press,Washington D.C., USA.

148. Figueroa, C., et al., *Pharmacokinetic profiles of extended release quetiapine fumarate compared with quetiapine immediate release.* Prog Neuropsychopharmacol Biol Psychiatry, 2009. **33**(2): p. 199-204.

149. Carrasco, J.L. and C. Sandner, *Clinical effects of pharmacological variations in selective serotonin reuptake inhibitors: an overview.* Int J Clin Pract, 2005. **59**(12): p. 1428-34.

150. Hall, H., et al., *Raclopride, a new selective ligand for the dopamine-D2 receptors.* Prog Neuropsychopharmacol Biol Psychiatry, 1988. **12**(5): p. 559-68.

151. Langer, O., et al., *Precursor synthesis and radiolabelling of the dopamine D2 receptor ligand [11C]raclopride from [11C]methyl triflate.* Journal of Labelled Compounds and Radiopharmaceuticals, 1999(42): p. 1183-1193.

152. Roland, P.E., et al., *Human Brain Atlas:For high-resolution functional and anatomical mapping.* Human Brain Mapping, 1994. **1**: p. 173-184.

153. Tzourio-Mazoyer, N., et al., *Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain.* Neuroimage, 2002. **15**(1): p. 273-89.

154. Schain, M., et al., *Quantification of serotonin transporter availability with [11C]MADAM--a comparison between the ECAT HRRT and HR systems.* Neuroimage, 2012. **60**(1): p. 800-7.

155. Mintun, M.A., et al., *A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography.* Ann Neurol, 1984. **15**(3): p. 217-27.

156. Lammertsma, A.A. and S.P. Hume, *Simplified reference tissue model for PET receptor studies.* Neuroimage, 1996. **4**(3 Pt 1): p. 153-8.

157. Innis, R.B., et al., *Consensus nomenclature for in vivo imaging of reversibly binding radioligands.* J Cereb Blood Flow Metab, 2007. **27**(9): p. 1533-9.

158. Hall, H., et al., *Autoradiographic localization of extrastriatal D2-dopamine receptors in the human brain using [125I]epidepride.* Synapse, 1996. **23**(2): p. 115-23.

159. Varnas, K., C. Halldin, and H. Hall, *Autoradiographic distribution of serotonin transporters and receptor subtypes in human brain.* Hum Brain Mapp, 2004. **22**(3): p. 246-60.

160. Nyberg, S., et al., *D(2)- and 5-HT(2) receptor occupancy in high-dose neuroleptic-treated patients.* Int J Neuropsychopharmacol, 1998. **1**(2): p. 95-101.

161. Meltzer, C.C., et al., *Correction of PET data for partial volume effects in human cerebral cortex by MR imaging.* J Comput Assist Tomogr, 1990. **14**(4): p. 561-70.

162. Rousset, O.G., Y. Ma, and A.C. Evans, *Correction for partial volume effects in PET: principle and validation.* J Nucl Med, 1998. **39**(5): p. 904-11.

163. Muller-Gartner, H.W., et al., *Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects.* J Cereb Blood Flow Metab, 1992. **12**(4): p. 571-83.