PhD Thesis

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Imaging Serotonin 2A Receptors in Schizophrenia
Patients Before and After First Antipsychotic Treatment
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Hans Rasmussen, Copenhagen, May 2009
Summary

Post-mortem investigations and the receptor affinity profile of atypical antipsychotics have implicated the serotonin 2A receptors (5-HT$_{2A}$R) in the pathophysiology of schizophrenia. Most post-mortem studies point towards lower frontal cortical 5-HT$_{2A}$R binding in schizophrenia patients as compared to healthy controls. However, \textit{in vivo} studies of 5-HT$_{2A}$R binding report conflicting results, presumably because sample sizes have been small or because schizophrenia patients who were not antipsychotic-naïve were included. Furthermore, the relationships between 5-HT$_{2A}$R binding, psychopathology, and central neurocognitive deficits in schizophrenia are unclear. Finally, there are no \textit{in vivo} studies of 5-HT$_{2A}$R in first episode antipsychotic-naïve schizophrenia patients before and after sustained treatment with an atypical antipsychotic compound rendering the relationship between 5-HT$_{2A}$R occupancy and treatment effect unknown.

In Paper 1 we assessed \textit{in vivo} brain 5-HT$_{2A}$R binding potentials in antipsychotic-naïve first episode schizophrenia patients and matched healthy controls, and examined possible associations with psychopathology, memory, attention and executive functions. The participants were 30 patients and 30 matched healthy control subjects.

The patients were subsequently treated with the atypical antipsychotic compound quetiapine for 6 months in flexible doses according to their clinical need.

In Paper 2 we measured 5-HT$_{2A}$R occupancy in the same patients after 6 months of quetiapine treatment and explored the relationship with quetiapine and its active metabolite nor-quetiapine plasma levels, dose and the treatment effect. Fifteen patients completed the follow-up PET scan.

The main outcome measure was \textit{in vivo} 5-HT$_{2A}$R binding as measured using positron emission tomography (PET) and the 5-HT$_{2A}$R-specific radioligand, [18F]altanserin, in a bolus infusion steady state model. Psychopathology was assessed using the Positive and Negative Syndrome Rating Scale (PANSS) and both patients and controls underwent a neuropsychological test battery. After the treatment period 5-HT$_{2A}$R occupancy was determined from an occupancy plot of the regional distribution volumes in the unblocked and the partially blocked condition. Treatment effect was defined as the difference between PANSS scores at baseline and PANSS scores at the follow-up scan.
At baseline schizophrenia patients had significantly lower 5-HT$_{2A}$R binding in frontal cortex than control subjects. A significant negative correlation was observed between frontal cortical 5-HT$_{2A}$R binding and positive psychotic symptoms in the male patients. No correlations were found between cognitive functions and 5-HT$_{2A}$R binding.

At follow up we found a one site binding hyperbolic relationship between 5-HT$_{2A}$R occupancy, quetiapine dose and plasma concentration. Furthermore, the data revealed a modest effect on positive symptoms up until a 5-HT$_{2A}$R occupancy level of approximately 60%, after which a considerable increase in efficacy was found. The mean dose of quetiapine was 383 mg in the present study, corresponding to a 5-HT$_{2A}$R occupancy of 64%. This occupancy level is in the middle range of 60-70% where we found quetiapine to exert the highest reduction in the positive symptoms. The mean dose is in the lower part of the recommended dose-range of quetiapine (300-800 mg). As such this study provides support for using low doses of quetiapine in first episode schizophrenia patients.

Our results suggest that frontal cortical 5-HT$_{2A}$R are involved in the pathophysiology of schizophrenia. Furthermore, the study supports that the 5-HT$_{2A}$R has an important therapeutic role in the treatment of positive symptoms with quetiapine and suggests that measurements of quetiapine plasma concentrations provide guidance in terms of dosing and 5-HT$_{2A}$R blockade.
Dansk titel (Danish title)
Visualisering af serotonin 2A receptorer hos skizofrene patienter før og efter første antipsykotiske behandling

Dansk Resumé (Danish summary)
Post mortem undersøgelser og receptor affiniteten af atypiske antipsykotika har indikeret at serotonin 2A receptoren (5-HT$_{2A}$R) er relateret til patofysiologien ved skizofreni. Post mortem undersøgelser har generelt vist en lavere frontal cortical 5-HT$_{2A}$R binding hos skizofrene patienter sammenholdt med kontrolpersoner. *In vivo* undersøgelser af 5-HT$_{2A}$R har vist modstridende resultater, formodentlig fordi patientgrupperne har været for små eller fordi studierne inkluderede kroniske skizofrene patienter, som ikke var antipsykotika-naive. Herudover er der en uklar sammenhæng mellem 5-HT$_{2A}$R, psykopatologi og centrale neurokognitive deficits ved skizofreni. Ligeledes eksisterer der ingen *in vivo* undersøgelser af 5-HT$_{2A}$R i debuterende antipsykotika-naive skizofrene patienter før og efter længerevarende behandling med et atypisk antipsykotikum og forholdet mellem 5-HT$_{2A}$R blokade og behandlingseffekt er derfor ukendt.

I Artikel 1 ønskede vi, at måle *in vivo* 5-HT$_{2A}$R binding hos debuterende antipsykotika-naive skizofrene patienter samt hos matchede raske kontrol personer. Endvidere ønskede vi, at undersøge mulige sammenhænge med psykopatologi, hukommelse, opmærksomhed og eksekutive funktioner. Deltagerne var 30 patienter og 30 matchede raske kontrol personer.

Efterfølgende blev patienterne behandlet i 6 måneder med det atypiske antipsykotikum quetiapin individuelt doseret efter klinisk effekt.

I Artikel 2 ønskede vi, at måle 5-HT$_{2A}$R blokaden efter 6 måneders quetiapin behandling i de samme patienter samt at undersøge forholdet mellem quetiapin og nor-quetiapin plasmaniveauer, dosis og behandlingseffekt. Femten patienter gennemførte opfølgningsundersøgelserne.

Vores outcome parameter var *in vivo* 5-HT$_{2A}$R binding målt ved hjælp af positron emissions tomografi (PET) med den 5-HT$_{2A}$R-specifikke radioligand, [¹⁸F]altanserin, i en bolus infusions steady state model. Psykopatologien blev vurderet ved hjælp af the positive and negative syndrome...
scale (PANSS) og både patienter og kontrol personer gennemgik et neuropsykologisk test batteri. 5-HT\textsubscript{2A}R blokade efter behandlingen blev bestemt ud fra et okkupansplot af de regionale distributionsvolumina i den ublokerede og i den delvist blokerede tilstand. Behandlingseffekt blev defineret som forskellen mellem PANSS scores ved baseline og PANSS scores ved follow-up.

Før behandling havde de skizofrene patienter en signifikant lavere 5-HT\textsubscript{2A}R binding i frontal cortex sammenlignet med raske kontrolpersoner. Yderligere fandt vi en signifikant negativ korrelation mellem frontal cortical 5-HT\textsubscript{2A}R binding og positive psykotiske symptomer hos de mandlige patienter. Der var ingen signifikante korrelationer mellem kognitive funktioner og 5-HT\textsubscript{2A}R binding.

Efter behandlingsperioden fandt vi, at behandlingseffekten på positive psykotiske symptomer var beskeden op til en 5-HT\textsubscript{2A}R blokering på 60 %. Ved en 5-HT\textsubscript{2A}R blokering mellem 60-70 % fandtes en udtalt reduktion i de positive psykotiske symptomer. Den gennemsnitlige quetiapin dosis var 383 mg svarende til en 5-HT\textsubscript{2A}R blokering på 64 %. Dette blokeringsniveau ligger i midten af det område hvor quetiapin viste sig at være mest effektiv (mellem 60 og 70 % blokering). Den korresponderende dosis er i den lave ende af det anbefalede dosisområde for quetiapin (300-800 mg), hvorfor denne undersøgelse støtter anvendelse af lave doser quetiapin til debuterende skizofrene patienter.

Overordnet tyder resultaterne på, at frontale 5-HT\textsubscript{2A}R er involveret i patofysiologien ved skizofreni, samt at 5-HT\textsubscript{2A}R har en vigtig terapeutisk rolle i behandlingen af positive psykotiske symptomer med quetiapin samt at plasma koncentrationsmålinger kan vejlede omkring dosering og 5-HT\textsubscript{2A}R blokade.
List of publications

The thesis is based on the following publications, which are presented in the appendices:


List of abbreviations

- **5-HT**: 5-hydroxytryptamine, serotonin
- **5-HT\textsubscript{2A}R**: The serotonin 2A receptor
- **\textsuperscript{18}F-altanserin**: \textsuperscript{18}F-labeled 3-(2-[4-(4-fluorobenzoyl)-1-piperidinyl]-ethyl)-2,3-dihydro-2-thioxo-4-quinazolinone
- **BP\textsubscript{T}**: Binding potential
- **Bq**: Becquerel
- **CANTAB**: Cambridge neuropsychological test automated battery
- **Da**: Dalton
- **D\textsubscript{2}**: The dopamine 2 receptor
- **DMSO**: Dimethyl sulfoxide
- **DSM-IV**: Diagnostic and Statistical Manual of Mental Disorders
- **EPS**: Extrapyramidal symptoms
- **ESRS**: Extrapyramidal Symptom Rating Scale
- **FGA**: First generation antipsychotic drug
- **HPLC**: High-performance liquid chromatography
- **IED**: Intra-extradimensional set shifting
- **ICD-10**: International Statistical Classification of Diseases
- **K\textsubscript{off}**: Dissociation rate constant
- **MPRAGE**: Magnetization-prepared rapid-gradient echo
- **NMR**: Nuclear magnetic resonance spectroscopy
- **MRI**: Magnetic resonance imaging
- **NET**: Norepinephrine transporter
- **PANSS**: Positive and Negative Syndrome Scale
- **PET**: Positron emission tomography
- **RF**: Radio frequency
- **RVP**: Rapid visual information processing
- **SCAN-2.1**: Schedules for Clinical Assessment in Neuropsychiatry
- **SGA**: Second generation antipsychotic drug
- **SSRI**: Selective serotonin reuptake inhibitor
- **SOC**: Stockings of cambridge
- **SWM**: Spatial working memory
- **THF**: Tetrahydrofuran
- **VOI**: Volume of interest
- **V\textsubscript{T}**: Distribution volume
Background

Schizophrenia

Schizophrenia is a severe and heterogeneous brain disease with a prevalence of approximately 1% in the general population (Andreasen, 2000). According to the World Health Organization schizophrenia is among the seven most disabling diseases in the age group between 20 and 45 thereby surpassing diabetes, cardiovascular disease, and HIV-AIDS (Okasha and Okasha, 2009). The symptoms typically start in young adulthood and are commonly classified in: positive symptoms (hallucinations, delusions and thought disorder), negative symptoms (affective flattening, poverty of speech, anhedonia) and cognitive deficits (attention, memory and executive functions) (Schultz and Andreasen, 1999; Weickert et al., 2000).

Schizophrenia is characterized by disturbances in brain biology and function, but has an extraordinarily complex etiology affected by both genetic liability and environmental influence (Osvan et al., 2008). However, most candidate genes for schizophrenia are related to neural plasticity, synaptogenesis, or transmitter function within brain circuits that are involved in information processing (Harrison and Weinberger, 2005). In accordance with this, multiple neurotransmitters have been implicated in the disturbances in early information processing and higher cognitive functions that are believed to constitute core features in schizophrenia (Glenthoj et al., 2009). Disturbances in information processing are primarily genetically determined and considered to be important markers for the disease and to predispose for development of the positive and negative schizophrenia symptoms (Carlsson, 2006; Geyer et al., 2001) and these disturbances are therefore central for most neurobiological hypotheses of schizophrenia (Glenthoj et al., 2009).
Transmitter systems in schizophrenia

Several brain transmitter systems are involved in the pathophysiological processes in schizophrenia (see table 1).

<table>
<thead>
<tr>
<th>Transmitter</th>
<th>Receptor</th>
<th>Mechanism</th>
<th>Examples of mechanisms for treatment of schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>D1, D3 “D1-like”</td>
<td>GPCR</td>
<td>D2 antagonism (typical and atypical antipsychotics), D2 enhancement (PFC).</td>
</tr>
<tr>
<td></td>
<td>D2, D3, D4 “D2-like”</td>
<td>GPCR</td>
<td>5-HT2A modulation by most atypical antipsychotics, combined 5-HT1A/D2 modulation.</td>
</tr>
<tr>
<td>Serotonin</td>
<td>5-HT1, 2, 4, 5, 6, 7</td>
<td>GPCR</td>
<td>Several atypical antipsychotics interact with the NE system (α2 antagonism).</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>α1, α2</td>
<td>GPCR</td>
<td>D-serine, glycine, mGluR2-3 agonist (LY2140023), Ampakines, mGluR5 agonists.</td>
</tr>
<tr>
<td></td>
<td>β1, β2</td>
<td>GPCR</td>
<td>GABAα2 receptor subunit agonist (MK-0777).</td>
</tr>
<tr>
<td>Glutamate</td>
<td>NMDA</td>
<td>Ionotropic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMPA</td>
<td>Ionotropic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kainate</td>
<td>Ionotropic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mGluR1-8</td>
<td>GPCR</td>
<td></td>
</tr>
<tr>
<td>GABA</td>
<td>GABA_A</td>
<td>Ionotropic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GABA_B</td>
<td>GPCR</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Transmitter systems implicated in the pathophysiology of schizophrenia, adapted from (Glenthoj et al., 2009)

In addition, other neurobiological hypotheses of schizophrenia also involve the cholinergic system, cannabinoid receptors, histamine, nitric oxide, peptide neurotransmitters (e.g. neurokinin, neotensin, cholecystokinin) and potential disturbances in a vast number of tropic factors and intracellular processes (Glenthoj et al., 2009).

Given the heterogenic character of the disease, different transmitter systems within different brain loops are likely to be involved in different patients. Furthermore, the disturbances observed in the patients might be secondary adaptive changes to primary dysfunctions. Nevertheless, an abundant literature has demonstrated transmitter disturbances in patients with schizophrenia and pharmacological treatment is the cornerstone for all other interventions in this disease (Glenthoj et al., 2009).
The two transmitter systems that have been most extensively investigated in schizophrenia are the dopamine system and the serotonin system, especially dopamine D$_2$ receptors and the serotonin 5-HT$_{2A}$ receptors (5-HT$_{2A}$R) (Meltzer et al., 2003; Glenthoj and Hemmingsen, 1997).

**Dopamine D$_2$ receptors**

Dopamine D$_2$ receptors have been in focus in schizophrenia research ever since the relation between D$_2$ receptor affinity and antipsychotic effect was established for first generation antipsychotics (Farde et al., 1988; Seeman et al., 1976a). Dopamine receptors are divided into a D$_1$ like family (D$_1$ and D$_5$ receptors) and a D$_2$ like family (D$_2$, D$_3$, and D$_4$ receptors). They are G-protein-coupled receptors hence they do not directly gate ion-channels in contrast to fast responding receptors. Instead, stimulation of the receptor induces a cascade of intracellular events whereby dopamine modulates the response of a neuron to other transmitter systems or induces long-term changes in synaptic plasticity (Sunahara et al., 1991; Van Tol et al., 1991; Tiberi et al., 1991; Sokoloff et al., 1990).

All marketed antipsychotic drugs affect the D$_2$ receptors. The D$_2$ receptors are found in high density in the basal ganglia and in low concentrations in extrastriatal areas such as the thalamus, the temporolimbic region, and the frontal cortex. In addition to the postsynaptic D$_2$ receptors, there are also presynaptic D$_2$ autoreceptors. In this way, D$_2$ receptors regulate both dopamine release and dopamine neuronal activity (Glenthoj et al., 2009).

The classical dopamine hypothesis of schizophrenia suggests that schizophrenia is the result of increased dopamine activity (Carlsson and Lindqvist, 1963; Carlsson, 1974). This hypothesis has been supported by numerous studies demonstrating a relationship between affinity for striatal dopamine D$_2$ receptors and antipsychotic effect (Farde et al., 1988; Seeman et al., 1976b) and also by the psychotogenic effect of dopamine enhancing compounds (Lieberman et al., 1990).

Preclinical and clinical findings point to a sensitized subcortical dopaminergic presynaptic activity in schizophrenia and an association between phasic increases in striatal dopamine release, positive psychotic symptoms and frontal dysfunction (Abi-Dargham et al., 2000; Breier et al., 1997; Glenthoj et al., 1993; Glenthoj et al., 1999; Grace, 1991; Laruelle et al., 1996; Abi-Dargham et al., 1998; Laruelle, 2000). Preclinical studies have further demonstrated a reverse relationship between frontal and striatal dopamine activity (Weinberger et al., 1988). In a previous study from our group,
we have also identified an association between prefrontal D2 activity and positive schizophrenia symptoms in antipsychotic naïve first episode schizophrenia patients (Glenthoj et al., 2006).

**Serotonin 2A receptors**

In the 1950s it was discovered that the monoamine neurotransmitter, serotonin (5-hydroxytryptamine, 5-HT), clinically had similar effects as lysergic acid diethylamide (LSD), a drug known to cause psychotic like symptoms (Gaddum and Hameed, 1954). This observation led to a hyper serotonin hypothesis of schizophrenia, which was mainly focused on the 5-HT2A receptor subtype.

5-HT is synthesized from the essential amino acid tryptophan and released by nerve cells in the raphe nuclei throughout the brain. 5-HT2AR belong to a family of serotonin receptors constituted of 15 different receptors encoded by distinct genes which are divided into seven major classes: 5-HT1, 5-HT2, 5-HT3, 5-HT4, 5-HT5, 5-HT6, and 5-HT7. Most classes can be divided in subtypes, e.g. the 5-HT2 into 5-HT2A, 5-HT2B, and 5-HT2C. Except for the 5-HT3 subtype all of the 5-HT receptors, are members of the G protein-coupled superfamily (Gray and Roth, 2001). The 5-HT2AR has a wide distribution in the brain with a high density in cortical areas, lower density in the midbrain and thalamic areas and negligible expression in the cerebellum (Adams et al., 2004).

Numerous studies have, either directly or via interactions with the dopaminergic system, implicated the 5-HT2AR in the pathophysiology of schizophrenia (Glenthoj and Hemmingsen, 1997; Glenthoj and Hemmingsen, 1999; Meltzer et al., 2003; Meltzer et al., 1989).

Post-mortem studies of brain tissue from schizophrenia patients suggest cortical serotonergic dysfunction. Eleven (Arora and Meltzer, 1991; Bennett, Jr. et al., 1979; Burnet et al., 1996; Dean and Hayes, 1996; Dean et al., 1998; Dean et al., 1999; Gurevich and Joyce, 1997; Laruelle et al., 1993; Matsumoto et al., 2005; Mita et al., 1986; Pralong et al., 2000) out of fifteen (Dean et al., 1996; Joyce et al., 1993; Reynolds et al., 1983; Whitaker et al., 1981) post-mortem studies report decreased 5-HT2A/C receptor expression in cortical areas, in particular in the frontal cortex. However, these studies might have been confounded by chronicity and previous treatment with antipsychotic drugs, which likely decrease 5-HT2AR expression (Dean, 2003). Furthermore, the techniques used to analyze post mortem tissue differ between studies (Dean et al., 2008).
With the introduction of selective 5-HT\textsubscript{2A}R PET ligands, it is now possible to examine 5-HT\textsubscript{2A}R density in the living human brain and study how antipsychotic medication blocks these receptors. In antipsychotic-naïve schizophrenia patients only a few PET studies of the 5-HT\textsubscript{2A}R have been carried out in a very limited number of patients (n<15) and the results are conflicting. Three PET studies (n<10) found no difference in 5-HT\textsubscript{2A}R binding between schizophrenia patients and healthy controls (Lewis et al., 1999; Okubo et al., 2000; Trichard et al., 1998). One study (n=6) found decreased binding in the left lateral frontal cortex in schizophrenia (Ngan et al., 2000). In a previous preliminary publication on a subgroup of the present cohort (n=15) we found increased 5-HT\textsubscript{2A}R binding in the caudate nucleus (Erritzoe et al., 2008).

**Serotonin 2A receptors and antipsychotic compounds**

Twenty years ago it was shown that the atypical antipsychotic drug clozapine (Kane et al., 1988) is superior to typical antipsychotic drugs such as haloperidol and chlorpromazine in treating positive and negative symptoms of treatment-resistant schizophrenia while producing minimal extrapyramidal symptoms (EPS). Since then atypical antipsychotic drugs such as risperidone, olanzapine, quetiapine and ziprasidone have been developed and modeled on clozapine and they are now referred to as second-generation antipsychotic drugs (SGAs) (Lohr and Braff, 2003).

The past two decades of neuropharmacological studies have focused on the mechanism of action by which SGAs produce their therapeutic efficacy. SGAs have a complex pharmacology, for example, clozapine has high affinity for a number of serotonin (5-HT\textsubscript{2A}, 5-HT\textsubscript{2C}, 5-HT\textsubscript{6}, 5-HT\textsubscript{7}), dopamine (D\textsubscript{4}), muscarinic (M\textsubscript{1}, M\textsubscript{2}, M\textsubscript{3}, M\textsubscript{4}, M\textsubscript{5}), adrenergic (\(\alpha_1\)- and \(\alpha_2\)-subtypes) and other biogenic amine receptors (Roth et al., 2004b). However, according to the serotonin-dopamine hypothesis as proposed by (Meltzer et al., 1989) a common feature shared by SGAs is a relatively potent blockade of 5-HT\textsubscript{2A}R coupled with a weaker antagonism of dopamine D\textsubscript{2} receptors. Neuroimaging studies show that for FGAs such as haloperidol, a 70 % D\textsubscript{2} receptor occupancy is the optimal level for antipsychotic response, and that occupancies above 80 % are associated with EPS (Farde and Nordstrom, 1992; Pilowsky et al., 1997). However, during optimal dosing of SGAs, the occupancy of 5-HT\textsubscript{2A}R in the cortex by far exceeds D\textsubscript{2} occupancy in the striatum (Farde et al., 1995; Kapur et al., 1998). Cortical 5-HT\textsubscript{2A}R occupancies of clozapine, risperidone, olanzapine and ziprasidone at therapeutic doses are reported as very high (over 90 %) (Kapur and Remington, 1996; Kapur et al., 1999; Nyberg et al., 1999; Mamo et al., 2004) while quetiapine and aripiprazole show slightly lower occupancy (60–70 %) of 5-HT\textsubscript{2A}R (Gefvert et al., 1998; Mamo et al., 2004; Mamo et al., 2007).
In opposition to the serotonin-dopamine hypothesis it has been suggested by Kapur and Seeman (Kapur and Seeman, 2001) that the difference between FGAs and SGAs might be fully explained by the pharmacokinetics of their interaction with the D2 receptor by transiently high D2 occupancy and a fast dissociation rate (k_{off}). This theory implies that the atypical antipsychotic effect can be produced by appropriate modulation of the D2 receptor alone, while the blockade of the 5-HT_{2A}R and other receptor systems may neither be necessary nor sufficient. It therefore predicts that low doses of FGAs such as haloperidol could achieve most, if not all, of the benefits of e.g. clozapine with regard to antipsychotic action and EPS (Kuroki et al., 2008).

Conversely, it is argued by Meltzer and colleagues that 5-HT_{2A}R antagonism might confer atypicality on drugs with relatively weak D2 receptor antagonism due to a differential modulating effect of 5-HT_{2A}R on dopaminergic activity in different brain regions. Furthermore, it is argued that only low-affinity drugs such as quetiapine and clozapine have fast dissociation rates and that SGAs like iloperidone, blonanserin, olanzapine, risperidone and ziprasidone, have slow k_{off} properties. Also sertindole, an SGA with essentially no EPS in its clinical range has a slower k_{off} than haloperidol. Similarly, olanzapine has a k_{off} that is only marginally faster than chlorpromazine and much slower than quetiapine and clozapine. Thus Meltzer and collegues argue that the k_{off} hypothesis as a general model is not supported (Meltzer et al., 2003). Similarly, it has been suggested that 5-HT_{2} receptor blockade inhibits phasic increases in dopamine synthesis and release in the striatum, for references please see (Glenthoj and Hemmingsen, 1999). In this way, 5-HT_{2} receptor antagonism can potentiate D2 receptor antagonism and facilitate a reduction in positive symptoms by closure of the striato-thalamic filter (Glenthoj and Hemmingsen, 1999).

**Quetiapine**

Quetiapine is an SGA characterized by a high affinity for the 5-HT_{2A}R and modest affinity and a fast k_{off} for the D2 receptors. Clozapine has a similar profile (Kapur and Seeman, 2000), however according to clinical guidelines, clozapine is not recommended as a drug of first choice in first-episode schizophrenia (Kerwin, 2007). Quetiapine produces two metabolites 7-hydroxy-quetiapine and nor-quetiapine which are pharmacologically active on the 5-HT_{2A}R (Mauri et al., 2007). Nor-quetiapine also has norepinephrine transporter (NET) antagonist and partial 5-HT_{1A} agonist properties, which have been suggested to explain why quetiapine can be used for treatment of
bipolar depression and relieves depressive symptoms in schizophrenia (Jensen et al., 2008; Goldstein et al., 2007; Nyberg et al., 2007).

Few studies have investigated the relationship between plasma quetiapine concentrations and clinical outcome in schizophrenia. Within a treatment period of 6 weeks or less, no clear association between quetiapine plasma concentration and clinical response was found, and no optimal therapeutic range for quetiapine could be identified (Small et al., 1997; Fabre, Jr. et al., 1995). A recent review suggests that measurements of plasma quetiapine concentrations provide poor guidance in terms of dosing (Mauri et al., 2007).

Only a few PET studies of quetiapine have been carried out. One study found a curvilinear hyperbolic relation between 5-HT2AR occupancy and plasma quetiapine concentration (Kapur et al., 2000). In 12 chronic and previously medicated schizophrenia patients a quetiapine dose between 300 and 600 mg/day resulted in 5-HT2AR occupancy between 57% and 78% in the frontal cortex. In another study in a similar patient group (n=5) frontal cortical 5-HT2AR occupancies were determined as 74 and 57% at doses of 750 and 450 mg/day, respectively (Gefvert et al., 2001). These results, however, might have been confounded by disease progress and effects of previous medication.

Serotonin 2A receptors and cognition in schizophrenia

Cognitive deficits represent a core feature in schizophrenia and have substantial impact on the course of the illness, compliance, and on psychosocial functioning (Heinrichs and Zakzanis, 1998; McGurk et al., 2007). Studies have suggested that 5-HT2AR antagonism improves cognition in general (Roth et al., 2004a) presumably through an increase in prefrontal dopamine turnover and a consequent improvement of the cognitive functions that are mediated by the prefrontal cortex (Friedman et al., 1999).

It is however unclear how 5-HT2A activity is associated with the most commonly found clinical cognitive deficits in schizophrenia (Weickert et al., 2000; Gur et al., 2001), i.e. deficits in attention, executive functions and spatial working memory. It has been proposed that working memory could be one of the central cognitive markers or endophenotypes of schizophrenia (Conklin et al., 2005; Jindal and Keshavan, 2008; Meneses, 2002).
Recent research has shown that the affinity of antipsychotic drugs to the 5-HT$_{2A}$R is associated with cognition in a subtle way. Spatial working memory has been suggested to improve by stimulation rather than blockade of 5-HT$_{2A}$R in both pre-clinical and clinical studies (Tyson et al., 2004; Tyson et al., 2006; Williams et al., 2002). Furthermore, blockade of 5-HT$_{2A}$R by the 5-HT$_{2A}$R antagonist ketanserin in healthy control subjects impaired memory more than combined escitalopram ketanserin treatment (Wingen et al., 2007). SGAs with a high affinity for the 5-HT$_{2A}$R may therefore have a less beneficial effect on spatial working memory than low affinity drugs (Tyson et al., 2004). These studies support a linkage between impaired working memory and 5-HT$_{2A}$R function in the human brain.

**Positron Emission Tomography**

With positron emission tomography (PET) receptor binding in the living human brain can be measured. Short-lived positron emitting isotopes such as $[^{11}\text{C}]$ (half-life 20 minutes) and $[^{18}\text{F}]$ are made in an on site cyclotron, and integrated into a ligand. After purification the radio-labeled ligand, also called a “tracer”, is injected intravenously and hereafter it will distribute throughout the body. An annihilation process occurs when a positron emitted by the tracer encounters an electron, resulting in the radiation of two gamma photons in opposite directions – each with energy of 511 kilo electron Volts. The detector ring around the anatomical site of interest registers these photons (see figure 1).

![Detector ring](https://example.com/detector-ring.png)

**Figure 1: Physical principle of PET**
The tracers \(^{18}\text{F}\)altanserin, \(^{11}\text{C}\)MDL100907 and \(^{18}\text{F}\)setoperone have been used for PET-imaging of 5-HT\(_2\)AR in previous studies. However, due to the moderate affinity of \(^{18}\text{F}\)setoperone for the dopamine D\(_2\) receptor this tracer is limited to detection of 5-HT\(_2\)AR in cortical regions only where the D\(_2\) receptor is only sparsely distributed. \(^{18}\text{F}\)altanserin has a high selectivity and affinity for the 5-HT\(_2\)AR (see figure 2) (Kristiansen et al., 2005) which makes it more suitable for imaging the 5-HT\(_2\)AR. Since the cerebellum contains a negligible level of 5-HT\(_2\)AR and as such can be assumed to account for non specific binding only (Pazos et al., 1987) it can be used as a reference region.

Aims and hypotheses

The present knowledge point towards two major challenges in schizophrenia research of importance for a further understanding of this complex disorder and the development of more specific treatment strategies:
The majority of patients studied in clinical trials have been ill for several years, have had multiple admissions, and have tried several different antipsychotic medications. To genuinely understand the underlying basis of the illness, it is critical to examine the patients before their brains have been affected by repeated relapses, related social deprivation and antipsychotic medication, i.e. to study antipsychotic-naïve first-episode schizophrenia patients and prodromal patients, even if these patients are very hard to recruit and examine before they are medicated. *The present PhD thesis focuses on first-episode schizophrenia patients who had never received any antipsychotic medication before baseline examinations.*

Most studies engaged in schizophrenia research are cross-sectional or shorter lasting interventional or naturalistic studies. While such studies are relatively straightforward to conduct, they do not provide a longitudinal perspective of the response to treatment. *The present study involved a longer lasting intervention of 6 months of treatment with one specific antipsychotic compound (quetiapine) which is characterized by loose binding to the brain D2 receptors and relatively high affinity to the 5-HT2A R.*

Aim 1. To examine 5-HT2A R binding with [18F]altanserin-PET in first-episode antipsychotic-naïve schizophrenia patients and matched healthy control subjects. A decrease in frontal cortical 5-HT2A R was expected in the patients. Furthermore, we expected to replicate our preliminary finding of an increased 5-HT2A R binding in the caudate nucleus. Also, we wanted to explore possible associations between 5-HT2A R, psychopathology and central cognitive deficits, specifically spatial working memory, attention and executive functions. An association between spatial working memory and 5-HT2A R binding was expected.

Aim 2. To relate 5-HT2A R occupancy after sustained quetiapine treatment for 6 months to plasma levels of quetiapine and nor-quetiapine, dose and clinical effect. A relationship between 5-HT2A R occupancy and treatment effect on positive symptoms was expected. Moreover, a relationship between levels of nor-quetiapine and treatment effect on depression scores was expected.
Materials and methods

Study design

The patients underwent a comprehensive test battery in the antipsychotic naïve state and after 6 months of treatment with quetiapine (see figure 3). Data regarding MRI/fMRI scanning, psychophysiology and neuropsychology are under submission as part of three collaborative Ph.D. studies. The present thesis relates to PET, psychopathology and aspects of the neuropsychological test battery.

Figure 3: Flowchart of the study
Participants

Baseline

Thirty-three (26 male and 7 female) patients underwent a PET scan after voluntary first-time referral to a psychiatric unit of one of the affiliated university hospitals in the Capital Region of Copenhagen (Bispebjerg Hospital, Rigshospitalet, Psychiatric University Center Glostrup or Psychiatric University Center Gentofte).

Thirty of the 33 patients fulfilled the diagnostic criteria for schizophrenia according to both ICD-10 and DSM-IV. Three patients proved to have a diagnosis of schizotypal personality disorder at a later stage of the study, and were therefore excluded. All patients (mean age: 26.4 years, SD=5.5) included were antipsychotic naïve. The diagnosis of schizophrenia was verified by means of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) interview (Wing et al., 1990).

Thirty healthy control subjects (mean age: 26.4 years, SD=5.7) matched for age, gender and parental socioeconomic status were recruited from the community by advertisement. None of the healthy control subjects had present or prior psychiatric disorder or any history of psychotropic medication as determined by SCAN interviews.

None of the participants had a history of significant head injury or non-psychiatric disorder. All participants had a normal neurological and physical examination, and showed no clinical pathological findings in a structural magnetic resonance imaging (MRI) scan of the brain as evaluated by a neuroradiologist.

Six patients were prior (n=4) or present (n=2) users of antidepressant medication (in all cases selective serotonin reuptake inhibitors (SSRIs), see table 2).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Antidepressant</th>
<th>Mean daily dose</th>
<th>Treatment period</th>
<th>Discontinuation before PET scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>Citalopram</td>
<td>N/A*</td>
<td>14 days</td>
<td>2 years</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>Citalopram</td>
<td>20 mg</td>
<td>1 day</td>
<td>13 days</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>Citalopram</td>
<td>40 mg</td>
<td>60 days</td>
<td>Current</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>Citalopram</td>
<td>10 mg</td>
<td>12 days</td>
<td>5 days</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>Sertraline</td>
<td>40 mg</td>
<td>28 days</td>
<td>14 days</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>Fluoxetine</td>
<td>40 mg</td>
<td>6 years</td>
<td>Current</td>
</tr>
</tbody>
</table>

Table 2: Previous (4) or present (2) treatment with antidepressive medication, *N/A: Not available
Benzodiazepines were allowed, albeit not on the day of the examinations. Eight patients fulfilled lifetime criteria for substance abuse. All abuse diagnoses were clearly secondary to the diagnosis of schizophrenia. Substance dependence was an exclusion criterion. DSM-IV diagnoses of substance abuse were: alcohol abuse, in sustained full remission (n=2); cannabis abuse, in a controlled environment, (n=1); other abuse, sustained full remission (n=1); other abuse, moderate, (n=1); other abuse, in a controlled environment (n=2); and other abuse, early partial remission (n=1). In four of the patients the diagnosis ‘other abuse’ covered mixed cannabis and alcohol abuse, and in one patient the diagnosis covered a history of amphetamine and cocaine use. Three patients had no history of abuse for the past year, and four patients had no abuse for the past month. All subjects had a negative urine screening for substance intake prior to the PET scan.

Eighteen of the patients and 6 of the control subjects were smokers. None of the participants smoked 2 hours before the PET investigations. Smoking status was not a matching criterion since we in a recent study on 136 healthy subjects study had found no effect of smoking on 5-HT2A R binding (Erritzoe et al., 2009).

**Follow-up**

In the period between baseline and follow-up 15 patients dropped out, due to diverse reasons, i.e. intolerable side effects, lack of efficacy, non-compliance or unwillingness to be re-scanned, resulting in 15 patients (5 females, mean age: 28.9 years, SD=5.4) completing the follow-up study. During the treatment period (mean=6.8 months, SD=0.9), patients were treated with quetiapine in flexible doses according to their clinical condition (average dose: 383 ±145 mg per day or 5.2±2.2 mg/kg bodyweight per day).

While at baseline patients received no treatment, at follow-up they received their normal daily quetiapine dose 165 minutes prior to the PET scan. This time period was based on a pilot PET study on one healthy control subject: the participant was administered one dose of 100 mg of quetiapine, and the time interval between quetiapine administration and maximum $[^{18}\text{F}]$altanserin displacement was determined as 165 minutes (see figure 4).
Figure 4: Pilot study, showing the displacement of $[^{18}F]$altanserin by quetiapine in a healthy control subject

As for the baseline examinations concomitant treatment with benzodiazepines was allowed on an “if needed basis”, except on the days of testing. Nine patients were smokers. Smoking does not affect quetiapine metabolism (DeVane and Nemeroff, 2001). Of the 15 patients participating in the follow-up 4 patients had a previous history of substance abuse according to DSM-IV: alcohol abuse, in sustained full remission (n=2); cannabis abuse, sustained full remission (n=1), other abuse, sustained full remission and other abuse, early partial remission (n=1). The diagnosis ‘other abuse’ covered mixed cannabis and alcohol abuse. During the treatment period none of the 15 patients had any substance abuse as determined by regular clinical contacts. Also at follow-up all subjects had a negative urine screening for substance intake prior to the PET scans.

At follow-up two patients were treated with the selective serotonin reuptake inhibitors (SSRIs) fluoxetine (n=1) and citalopram (n=1) in stable doses (40 mg/day for both compounds), throughout the investigation period. Thirteen patients had no lifetime history of antidepressant exposure.
Experimental Procedures

Psychopathological ratings

Symptom severity was assessed by trained raters using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Interviews were recorded on DVD for validation purposes. A subsample of 10 randomly selected PANSS ratings showed an intra-class correlation coefficient of 0.85 between the raters in a two-way fixed effect model (McGraw and Wong, 1996).

The depression cluster (PANSS-D) of the PANSS scale (items: somatic concern (G1), anxiety (G2), guilt feelings (G3) and depression (G6)) (Marder et al., 1997; Emsley et al., 1999) was used to examine the relationship between nor-quetiapine plasma concentration and the level of depressive symptoms. The PANSS-D has been found to strongly correlate with other scales specifically designed to measure depressive symptoms (El et al., 2002).

Neurocognitive testing

The cognitive examinations were performed as part of a collaborative PhD study (Rune Andersen) involving a larger sample of patients and controls. At baseline memory, executive functions and attention were assessed with the following subtests from the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Sahakian and Owen, 1992): Spatial Working Memory (SWM), Stockings of Cambridge (SOC), Intra-Extradimensional Set Shifting (IED) and Rapid Visual Information Processing (RVP).

Magnetic resonance imaging

High-resolution 3D T1-weighted, sagittal, magnetization-prepared rapid-gradient echo (MPRAGE) scans of the whole head (TI/TE/TR=800/3.93/1540 ms, flip angle 9°; matrix: 256x256; 192 slices) using an eight-channel head array coil were acquired in all subjects on a 3 tesla TRIO scanner (Siemens, Erlangen, Germany) at the MR department of the Copenhagen University Hospital, Hvidovre, Denmark. The structural MRI scans were done as part of a collaborative PhD study (Bjorn H. Ebdrup).
**PET: Radiosynthesis and administration**

The radiosynthesis of \([^{18}F]altanserin\) has been described previously (Lemaire et al., 1991). Quality control was performed using thin-layer chromatography and high-performance liquid chromatography (HPLC). The absence of residual solvents (methanol, THF, and DMSO) in the final formulation was confirmed by \(^1\)H NMR. For each PET study, 0.3–3.5 GBq of \([^{18}F]altanserin\) was produced with a radiochemical yield exceeding 95 %. Catheters were inserted in both cubital veins for tracer infusion and blood sampling, respectively. \([^{18}F]altanserin\) was administered as a bolus injection followed by continuous infusion to obtain steady state of the tracer in blood and tissue. The bolus infusion ratio was 1.75 h, as previously described (Pinborg et al., 2003). Subjects received a maximum dose of 3.7 MBq/kg body weight \([^{18}F]altanserin\).

**PET imaging**

PET scans were acquired in tracer steady-state conditions with an 18-ring GE-Advance scanner (GE, Milwaukee, WI, USA), operating in 3D-acquisition mode, producing 35 image slices with an interslice distance of 4.25 mm. The total axial field of view was 15.2 cm with an approximate in-plane resolution down to 5 mm. During steady state, the fraction of unmetabolized tracer in venous plasma was determined at five time points using HPLC analysis. Reconstruction, attenuation, and scatter correction procedures were conducted as previously described (Pinborg et al., 2003).

The subjects were placed in the scanner 90 min after the bolus injection of \([^{18}F]altanserin\). The subjects were aligned in the scanner using a laser system so that the detectors were parallel to the orbitomeatal line and positioned to include the cerebellum in the field of view using a short 2-min transmission scan. An individual head holder was made to ensure relative immobility. All subjects were scanned in a resting state. A 10-min transmission scan was obtained for correction of tissue attenuation using retractable \(^{68}\)Ge/\(^{68}\)Ga pin sources. The transmission scans were corrected for tracer activity by a 5-min emission scan performed in 2D mode. Dynamic 3D emission scans (five frames of 8 min) were started 120 min after tracer administration.

Data were reconstructed into a sequence of 128 ×128 ×35 voxel matrices, each voxel measuring 2.0 ×2.0 ×4.25 mm, with software provided by the manufacturer. A 3D reprojection algorithm with a transaxial Hann filter (6 mm) and an axial ramp filter (8.5 mm) was applied. Corrections for dead-time, attenuation, and scatter were performed.
**Blood samples**

Five venous blood samples were drawn at mid-frame 4, 12, 20, 28, and 36 min after starting the dynamic scanning sequence. The samples were immediately centrifuged, and 0.5 ml of plasma was counted in a well-counter for determination of radioactivity. Three of the five blood samples drawn at 4, 20, and 36 min were also analyzed for percentage of parent compound ([18F]altanserin) using reverse-phase HPLC following a previously described method (Adams et al., 2004).

For quetiapine and nor-quetiapine plasma concentration measurements, five 7 mL venous blood samples were drawn during the scanning and analyzed according to a previously described method (Hasselstrom and Linnet, 2003).

In addition, the free fraction of [18F]altanserin in plasma, $f_P$, was estimated using equilibrium dialysis, following a modified procedure (Videbaek et al., 1993). The dialysis was performed using Teflon-coated dialysis chambers (Harvard Bioscience, Amika, Holliston, MA, USA) with a cellulose membrane that retains proteins >10 000 Da. A small amount of [18F]altanserin (approximately 1 MBq) was added to 10-ml plasma samples drawn from the subjects. A 500-$\mu$l portion of plasma was then dialyzed at 37°C for 3 h against an equal volume of buffer, since pilot studies had shown that a 3-h equilibration time yielded stable values. The buffer consisted of 135 mM NaCl, 3.0 mM KCl, 1.2 nM CaCl$_2$, 1.0 mM MgCl$_2$, and 2.0 mM phosphate (pH 7.4). After the dialysis, 400 $\mu$l of plasma and buffer were counted in a well counter, and $f_P$ of [18F]altanserin was calculated as the ratio of DPM$_{\text{buffer}}$/DPM$_{\text{plasma}}$.

**MR/PET co-registration**

PET images and 3D T1 weighted MRI scans were co-registered using a Matlab (Mathworks Inc., Natick, MA, USA)-based program (Willendrup P et al., 2008), where PET images and MRIs are fitted through manual translation and rotation of the PET image with subsequent visual inspection in three planes (Adams et al., 2004).

**Volumes of interest and partial volume correction**

Volumes of interest (VOIs) were automatically delineated on each individual's transaxial MRI slices in a strictly user-independent manner (Svarer et al., 2005). This approach allowed automatic co-registration of a template set of 10 MRIs to a new subject's MRI. The identified transformation
parameters were used to define VOIs in the new subject MRI space, and through the co-registration these VOIs were transferred onto the PET images.

For study 1, a frontal cortex region was defined for each subject and served as the primary VOI. The frontal cortex VOI consisted of a volume-weighted average of left and right cortical regions and included: orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, and anterior cingulate cortex (Svarer et al., 2005).

Other regions included were: amygdala, caudate nucleus, entorhinal cortex, hippocampus, hypothalamus, insula, occipital cortex, parietal cortex, posterior cingulate cortex, putamen, sensorimotor cortex, superior temporal cortex, medial inferior temporal cortex and thalamus. The cerebellum was used for estimation of non-specific binding.

To enable partial volume correction of the PET data, MRIs corrected for RF inhomogeneities using the N3 software (Sled et al., 1998) were segmented into gray matter, white matter, and cerebrospinal fluid tissue classes using Statistical Parametric Mapping (SPM2) (Wellcome Department of Cognitive Neurology, London, UK). Partial volume correction was performed according to the Müller Gartner method (Muller-Gartner et al., 1992; Quarantelli et al., 2004). The white matter value was extracted from the uncorrected PET image as the mean voxel value from a brain region containing predominantly white matter (centrum semiovale).

**Outcome measures**

The outcome measure was the binding potential of specific tracer binding (BP\textsubscript{p}). The cerebellum was used as a reference region, since it represents non-specific binding only. In steady state, BP\textsubscript{p} is defined as

\[
BP_p = \frac{C_{\text{VOI}} - C_{\text{Reference}}}{C_{\text{Plasma}}} = f_p \frac{B_{\text{max}}}{K_d} \quad (\text{ml/ml})
\]

where \(C_{\text{VOI}}\) and \(C_{\text{Reference}}\) are the steady-state mean count density in the VOI and in the reference region, respectively \(C_{\text{Plasma}}\) is the steady-state activity of non-metabolized tracer in plasma; \(f_p\) is the free fraction of radiotracer; \(B_{\text{max}}\) is the density of receptor sites available for tracer binding; and \(K_d\) is the affinity constant of the radiotracer to the receptor.
The distribution volume ($V_T$) of a radioligand is defined as the ratio of the radioligand concentration in tissue target region ($C_T$, kBq·cm$^{-3}$) to that in plasma ($C_P$, kBq·mL$^{-1}$) at equilibrium (Innis et al., 2007). $C_P$ represents the concentration of parent radioligand in plasma.

$$V_T = \frac{C_T}{C_P} \quad (2)$$

A global measure of 5-HT$_{2A}$R occupancy ($O$) was calculated from the distribution volume in the unblocked ($V_T$) and in the partially blocked condition ($V_{T,b}$).

$$0 = 1 - \frac{V_{T,b} - V_{ND}}{V_T - V_{ND}} \quad (3)$$

where $V_{ND}$ is the distribution volume of the nondisplicable tracer, i.e., the free and nonspecifically bound tracer. Rearrangement of equation 2 leads to:

$$V_{T,b} = (1-O) V_T + O V_{ND} \quad (4)$$

By inserting corresponding values for each measured brain region in the unblocked and partially blocked condition, an occupancy plot (figure 5) can be made for each individual, and hence, an estimate of the global occupancy can be determined in each individual using linear regression analysis (Pinborg et al., 2007).
A one site binding hyperbola was used to evaluate the relationship between 5-HT$_{2A}$R occupancy and the corresponding plasma quetiapine concentration and dose using the following equation:

$$O = \frac{E_{\text{max}} \cdot X}{EC_{50} + X}$$  \hspace{1cm} (5)$$

where $E_{\text{max}}$ is the maximum receptor occupancy (100 %), $X=$quetiapine plasma concentration (ng/mL) or dose (mg) and $EC_{50}$ is the estimated quetiapine plasma concentration (ng/mL) or dose (mg) associated with 50 % maximal receptor occupancy.

Michaelis-Menten kinetics was applied to fit the relation between quetiapine and nor-quetiapine plasma concentration, from which the maximal velocity ($V_{\text{max}}$) and the constant ($K_m$) for the conversion of quetiapine into nor-quetiapine could be determined. Since the metabolism may be far
from saturation, a linear fit was also tested, and the goodness of fit used to assess if the metabolism was rate limited.

**Statistics**

All analyses were performed using SPSS® software. Between-group (patients, controls) comparisons of all reported outcome measures were performed using parametric analysis after verifying that the data were normally distributed according to the Kolmogorov–Smirnov test. Potential outliers were detected with Grubb’s outlier test (Grubb F, 1969), and subsequently excluded from analysis. The planned comparison in frontal 5-HT$_{2A}$R binding between patients and controls was performed with an independent samples Student’s t-test (one-tailed, because of our directional hypothesis). In addition, an ANOVA was performed with between factor group (patient or control) and within factor region (frontal or other (a variable consisting of the combination of all other regions)), to test whether a potential effect of group was more a global effect across all regions than a regional effect principally affecting frontal cortex. Furthermore, to test for additional regional group differences in binding an ANOVA was performed with between factors group (patient or control) and within factor region (the different regions, as specified in table 3). Independent samples Student’s t-tests (two-tailed) were only performed when these ANOVAs indicated statistical significant results.

Independent sample Student’s t-tests were further used to test for differences between patients and controls with regards to neurocognitive and psychopathological measures (two tailed). Correlation analyses were performed using the Pearson product-moment correlation coefficient. The potential effect on the results of antidepressive medication, benzodiazepines and substance abuse was examined by including these parameters in a multiple analysis of covariance (MANCOVA) as covariates.

Linear regression analysis was used to calculate global 5-HT$_{2A}$R occupancies. Differences in PANSS scores between baseline and follow-up were examined with paired samples t-tests. Regression analysis was used to examine the extent to which global 5-HT$_{2A}$R occupancy was associated with treatment effects. The latter were calculated as the difference in PANSS scores between baseline and follow-up. P=0.05 (two-sided) was employed as the level of significance for all tests. Curvefitting was performed using GraphPad Prism®.
Results and discussion

Study 1: Baseline data

5-HT2A binding
The planned comparison of frontal cortical binding revealed reduced 5-HT2A binding in patients compared to controls (t=2.54, df=58, p<0.01). The ANOVA on region and group revealed significant main effects of group [F(1,58) = 5.58, p<0.01] and region [F(17,42) = 82.19, p<0.001], and a significant region x group interaction effect [F(17,986) = 5.77, p<0.001].

Further analysis of these results indicated that 5-HT2A binding in the patients was significantly reduced not only in the frontal cortex but also in a number of other cortical - but not subcortical - regions (see table 3 and figure 6). Therefore, to test whether the frontal cortical region showed an even lower 5-HT2A binding than the other cortical regions a post-hoc ANOVA was performed with within factor region (frontal cortex or other regions, see table 3) and between factor group. This ANOVA revealed main effects of region [F(1,58) = 1109, p<0.001] and group [F(1,58) = 6.00, p<0.05] as well as a first order interaction between region and group [F(1,58) = 7.78, p<0.01], indicating a more pronounced reduction in 5-HT2A binding in the frontal cortical region than in the other cortical regions (see figure 7).
<table>
<thead>
<tr>
<th>Region</th>
<th>Patients</th>
<th>SEM</th>
<th>Controls</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>2.91</td>
<td>0.12</td>
<td>3.37</td>
<td>0.14</td>
<td>0.007</td>
</tr>
<tr>
<td>Orbitofrontal cortex</td>
<td>2.89</td>
<td>0.13</td>
<td>3.42</td>
<td>0.15</td>
<td>0.004</td>
</tr>
<tr>
<td>Medial inferior frontal cortex</td>
<td>3.07</td>
<td>0.12</td>
<td>3.50</td>
<td>0.13</td>
<td>0.065</td>
</tr>
<tr>
<td>Superior frontal cortex</td>
<td>3.34</td>
<td>0.14</td>
<td>3.85</td>
<td>0.15</td>
<td>0.008</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>2.34</td>
<td>0.09</td>
<td>2.68</td>
<td>0.13</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>Other regions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.68</td>
<td>0.04</td>
<td>0.77</td>
<td>0.05</td>
<td>NS. (0.15)</td>
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<tr>
<td>Caudate nucleus</td>
<td>0.60</td>
<td>0.04</td>
<td>0.65</td>
<td>0.04</td>
<td>NS. (0.34)</td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>1.11</td>
<td>0.05</td>
<td>1.21</td>
<td>0.06</td>
<td>NS. (0.20)</td>
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<tr>
<td>Hippocampus</td>
<td>0.74</td>
<td>0.04</td>
<td>0.81</td>
<td>0.05</td>
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<td>Hypothalamus</td>
<td>0.34</td>
<td>0.04</td>
<td>0.38</td>
<td>0.04</td>
<td>NS. (0.50)</td>
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<tr>
<td>Insula</td>
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<td>0.08</td>
<td>2.10</td>
<td>0.09</td>
<td>0.038</td>
</tr>
<tr>
<td>Medial inferior temporal cortex</td>
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<td>0.13</td>
<td>0.014</td>
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<tr>
<td>Occipital cortex</td>
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<td>0.11</td>
<td>2.97</td>
<td>0.12</td>
<td>0.012</td>
</tr>
<tr>
<td>Parietal cortex</td>
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<td>0.13</td>
<td>3.70</td>
<td>0.14</td>
<td>0.012</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>2.57</td>
<td>0.11</td>
<td>2.93</td>
<td>0.12</td>
<td>0.032</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.41</td>
<td>0.05</td>
<td>0.48</td>
<td>0.03</td>
<td>NS. (0.08)</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>2.72</td>
<td>0.11</td>
<td>3.13</td>
<td>0.11</td>
<td>0.012</td>
</tr>
<tr>
<td>Superior temporal cortex</td>
<td>2.68</td>
<td>0.11</td>
<td>3.03</td>
<td>0.12</td>
<td>0.004</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.48</td>
<td>0.03</td>
<td>0.52</td>
<td>0.03</td>
<td>NS. (0.39)</td>
</tr>
</tbody>
</table>

Table 3: Mean binding potentials of the specific [18F]altanserin binding (BP$_{v}$) in frontal cortex and sub-regions of interest in patients (n=30) and controls (n=30), respectively.
Figure 6: Parametric [$^{18}$F]altanserin PET image of averaged binding potential values in the 30 schizophrenia patients (top) and the 30 healthy control subjects (bottom) of study 1.
Figure 7: Mean frontal cortical and total 5-HT$_{2A}$ receptor binding (±/ 1 SEM) in 30 antipsychotic-naïve first-episode schizophrenia patients and 30 matched healthy controls

The data are in agreement with the vast number of post-mortem studies suggesting decreased cortical 5-HT$_{2A}$R binding in schizophrenia patients. Our results are based on the hitherto largest sample studied with PET, whereas earlier PET studies of 5-HT$_{2A}$R have reported on n<10 antipsychotic naïve patients (Ngan et al., 2000;Trichard et al., 1998;Okubo et al., 2000;Lewis et al., 1999). The majority of these studies, including our own previous preliminary study based on the first 15 patients included (Erritzoe et al., 2008), were unable to identify differences in cortical 5-HT$_{2A}$R binding between schizophrenic patients and healthy control subjects. In that preliminary study we found increased 5-HT$_{2A}$R binding in the caudate nucleus. This nucleus is a region with a relatively low 5-HT$_{2A}$R density; hence, the post-hoc analyses were more prone to type II errors (Haugbol et al., 2007). The present study does not confirm our preliminary finding of increased
binding in the caudate nucleus, but it does support the study by Ngan and colleagues (Ngan et al., 2000), who reported a lowered 5-HT$_{2A}$R binding in frontal cortex of six neuroleptic-naïve schizophrenic subjects. Similarly, Hurlemann and colleagues (Hurlemann et al., 2005;Hurlemann et al., 2008) reported a decreased cortical 5-HT$_{2A}$R binding in subjects at high risk of developing schizophrenia.

Decreased frontal 5-HT$_{2A}$R binding may reflect either a primary pathophysiological disturbance in schizophrenia or a compensatory down-regulation of receptors in response to altered endogenous serotonin levels. Alternatively, the finding could indicate a down-regulation compensating for hyperactive second messenger systems or primary changes in other systems interacting with 5-HT$_{2A}$R, such as the dopaminergic system.

5-HT$_{2A}$R binding and neurocognition

The cognitive data represent a sub-sample of a larger dataset published elsewhere (Andersen et al., submitted). Patients had significantly lower neurocognitive scores than healthy control subjects in the following tests: SWM strategy, SWM total errors and SWM between errors, IED total errors, and IED total number of trials on all stages attempted. There were no significant differences in SOC or RVP (see table 4). Neither in the frontal cortex, nor in the other VOIs did the 5-HT$_{2A}$R binding correlate significantly with any of the neurocognitive measures.
Table 4: Neurocognitive performance of memory, executive functions and attention of schizophrenia patients compared with healthy controls.

As expected, patients showed significantly lower performance in spatial working memory and aspects of executive functions than healthy control subjects. This is in agreement with previous studies which have shown that spatial working memory and executive functions are central impaired neurocognitive domains in schizophrenia (Weickert et al., 2000; Gur et al., 2001).

We detected no correlations between the cognitive parameters and 5-HT$_{2A}$R binding in any of the VOIs. Hence, our data do not support previous findings relating 5-HT$_{2A}$R to cognition in general (Roth et al., 2004a), and spatial working memory in particular (Tyson et al., 2004; Tyson et al., 2006). However, the interaction between serotonin and cognition is complex. Indeed, the interactions between serotonin, dopamine, norepinephrine and the cholinergic system have been suggested to mediate cognitive behavior (Tyson et al., 2006). Moreover, studies differ in design with regard to subjects (rodents, healthy controls or patients), type of serotonin manipulation.
(global, specific depletion or stimulation), serotonin receptor subtype (currently 15 serotonin receptor subtypes have been identified), and the cognitive tests being used (Tyson et al., 2004).

**5-HT$_{2A}$R binding, psychopathology and gender effects**

In the patients, the PANSS scores were: positive 20.0 (SEM=0.93), negative 22.0 (SEM=1.20), general 38.5 (SEM=1.30), and total 80 (SEM=2.60). A significantly negative correlation ($r=-0.571$, $p<0.01$) was found between 5-HT$_{2A}$R binding in the frontal cortex and positive symptoms in the larger group of male patients. An explorative *post hoc* analysis showed significant negative correlations between frontal 5-HT$_{2A}$R binding and the following sub-items of the positive PANSS scale: P1 delusions ($r=-0.47$, $p=0.027$) and P6 suspiciousness ($r=-0.53$, $p=0.011$) (see figure 8). No significant differences were found between the other VOIs and psychopathology. There was no gender effect on symptom severity or 5-HT$_{2A}$R binding.

![Figure 8: Negative correlation in male schizophrenia patients between mean frontal cortical 5-HT$_{2A}$ receptor binding, positive PANSS symptoms ($r=0.571$, $p=0.007$) and subitems P1 delusions ($r=-0.47$, $p=0.027$) and P6 suspiciousness ($r=-0.53$, $p=0.011$).](image)
The correlation between 5-HT₂AR binding and positive psychotic symptoms was only present in the male patients. Various aspects of schizophrenia, including age of onset, pathophysiology, symptomatology, course of illness, and treatment response have previously been shown to be related to gender. These gender differences might indicate a potential role of gonadal hormones and for an interaction of these hormones with neurotransmitters (for a review, see (Halbreich and Kahn, 2003)). Indeed, we have previously reported gender differences in antipsychotic-naïve schizophrenia patients with regards to the dopamine system, namely a correlation between D₂ receptor binding in the frontal cortex and positive psychotic symptoms in male patients only (Glenthoj et al., 2006).

Data has shown a more rapid remission from acute psychotic symptoms and an improved general health in female schizophrenia patients on combined antipsychotic and estrogen treatment as compared to treatment with antipsychotic medication alone (Kulkarni et al., 1996). A number of studies suggest that the serotonin system is influenced by sex hormones. Different hormonal levels in females may influence the 5-HT₂AR (Moses et al., 2000), and psychotic symptoms have been shown to improve during the high estrogen phase of the menstrual cycle (Riecher-Rossler et al., 1994). Similarly, a preclinical study suggests that estrogen modulates the serotonin system by increasing the expression of the 5-HT₂AR genes and the serotonin transporter in the dorsal raphe nucleus and the forebrain of rats (Sumner and Fink, 1998).

A prefrontal increase of the 5-HT₂AR has been demonstrated after estrogen administration in female subjects (Kugaya et al., 2003). It has also been argued that sex differences in response to citalopram are caused by the modulatory role of estrogen on the serotonergic system (Young EA et al., 2008). Conversely, testosterone has been shown to modulate serotonin receptor expression (Zhang et al., 1999). Finally, gender differences in antipsychotic drug response have been reported (Usall et al., 2007). Estrogen may therefore have a protective effect on schizophrenic symptomatology (Kulkarni et al., 2001; Kulkarni et al., 2008). Thus, our results lead to the suggestion that future studies should collect detailed data on the menstrual cycle of the patients.
Methodological considerations

Four patients were on prior (n=4) or current (n=2) SSRI treatment, which targets the serotonergic system. However, we have previously found that [18F]altanserin binding to 5-HT2AR is insensitive to an acute citalopram challenge increasing extracellular 5-HT (Pinborg et al., 2004). Furthermore, the effect of chronic SSRI treatment on 5-HT2AR density is unclear since SSRI’s have different effects on 5-HT2AR. Fluoxetine has been reported to have either no effect on 5-HT2AR number or actually increase receptor number. Similarly, paroxetine has been shown to increase or have no effect on 5-HT2AR density. In contrast, chronic citalopram treatment has been shown to down-regulate 5-HT2AR (Gray and Roth, 2001). For these reasons, we initially chose to include patients on previous or current antidepressants but controlled for the potential effect in a post hoc analysis where these patients were removed from the analyses. This did not change the results. Similarly, one patient had a history of amphetamine and cocaine abuse. These substances are known to affect serotonergic innervation in the brain, however the patient did not differ in 5-HT2AR binding and exclusion of the patient from the analyses did not alter the results.

Study 2: Follow-up data

5-HT2AR occupancy, dose, plasma concentration and treatment effect

The equation of the one site binding hyperbola that was used to fit 5-HT2AR occupancy and quetiapine plasma concentration revealed an EC50 value of 201.7 ng/mL, with a 95 % confidence interval of 147.2 to 256.3 ng/mL, r²=0.68 (see figure 10A).

The mean dose of quetiapine was 383 mg (range 100-600 mg) in the present study, corresponding to a 5-HT2AR occupancy of 64 % and a plasma concentration of 352 ng/mL (range 74-735 ng/mL) (see also figure 9).
Figure 9: [18F]altanserin PET images of two axial brain slices illustrating 5-HT$_{2A}$ receptor binding in one of the male schizophrenia patients before (top) and after approximately 6 months of treatment with 300 mg/day quetiapine (bottom). 5-HT$_{2A}$ receptor occupancy=57 % (165 min post quetiapine administration).

Similarly, the equation of the one site binding hyperbola that was used to fit 5-HT$_{2A}$ R occupancy and quetiapine dose resulted in a EC$_{50}$ value of 231 mg with a 95 % confidence interval of 170 to 293 mg, r$^2$=0.67 (see figure 10B).

The average PANSS score of positive symptoms was significantly reduced from 19.5 (SD=5.4) to 15.7 (SD=6.6), p=0.004 after quetiapine treatment. There were non-significant reductions in PANSS negative (from 20.3 (SD=6.1) to 18.4 (SD=6.5), p=0.37), general (from 38.0 (SD=8.7) to 33.0 (SD=11.1), p=0.11) and total scores (from 77.8 (SD=17.1) to 67.0 (SD=22.8), p=0.07).
A significant nonlinear (logarithmic) relationship was found between 5-HT$_{2A}$R occupancy and treatment effect on positive symptoms. ($r^2=0.75$, $P<0.001$) (figure 10C). Quetiapine was most effective on positive symptomatology between 60 and 70 % 5-HT$_{2A}$R occupancy. No significant relationship was found between 5-HT$_{2A}$R occupancy and treatment effect on negative symptoms, general, total symptoms scores or the PANSS-D cluster.

Michaelis-Menten kinetics applied to quetiapine and nor-quetiapine plasma concentration revealed a $V_{\text{max}}$ of 384.7 and a $K_m$ value of 396.6, with a 95 % confidence interval of 0.0 to 954.4, $r^2=0.59$ (see figure 10D). Plasma concentrations of quetiapine and nor-quetiapine did not correlate significantly with treatment effect on the PANSS-D cluster. Assuming a linear relationship within the dose range did not improve the goodness of fit ($r^2=0.53$). The relation between 5-HT$_{2A}$R occupancy and the combined quetiapine plus nor-quetiapine plasma concentration adjusted for their different affinities to the 5-HT$_{2A}$R ($K_i$ quetiapine$=38$, $K_i$ nor-quetiapine$=2.93$ (Goldstein et al., 2007)) was also plotted. Using an affinity weighted combined plasma concentration did not improve the goodness of fit ($r^2=0.68$ vs. 0.66). This implies that additional measurements of nor-quetiapine plasma levels are clinically irrelevant. Finally, we did not find a significant relationship between treatment effect on PANSS-D and quetiapine or nor-quetiapine plasma concentration. As such, the data do not support recent reports on efficacy of quetiapine or nor-quetiapine on depressive symptoms in schizophrenia (Jensen et al., 2008;Goldstein et al., 2007;Nyberg et al., 2007).

The data revealed a modest effect on positive symptoms up until a 5-HT$_{2A}$R occupancy level of approximately 60 %, after which a considerable increase in efficacy was found. The mean dose of quetiapine was 383 mg in the present study, corresponding to a 5-HT$_{2A}$R occupancy of 64 %. This occupancy level is in the middle range (between 60 and 70 %) where we found quetiapine to exert the highest reduction in the positive symptoms.
Figure 10: A The relationship between 5-HT$_{2A}$ receptor occupancy and quetiapine plasma concentration (ng/mL). The curve has been fit to the following equation occupancy=100(plasma concentration/(plasma concentration + 202 ng/mL)), where 201.7 ng/mL is the level of 50 % occupancy and the 95 % confidence interval for this constant is 147 to 256 ng/mL, $r^2=0.68$. B The relationship between 5-HT$_{2A}$ receptor occupancy and quetiapine dose (mg). The curve has been fit to the following equation occupancy=100(dose/(dose + 231 mg)), where the 231 mg is the level of 50 % occupancy and the 95 % confidence interval for this constant is 170 to 293 mg, $r^2=0.67$. C The nonlinear (logarithmic) relationship between 5-HT$_{2A}$ receptor occupancy and treatment effect on positive PANSS scores ($r^2=0.75, \ P<0.001$). D Quetiapine plasma concentration (ng/mL) and nor-quetiapine plasma concentration (ng/mL) fitted to Michaelis Menten kinetics, $V_{\text{max}}$=385, $K_m$=397, with a 95 % confidence interval of 0.0 to 954, $r^2=0.59$.

The study points to a therapeutic role of the 5-HT$_{2A}$R in the treatment of positive psychotic symptoms in schizophrenia either directly or indirectly via interactions with the dopaminergic system or other receptor systems (see below). Our imaging findings further indicate that quetiapine plasma concentration is a valid measure of the drug at one of its primary targets in the brain i.e. the 5-HT$_{2A}$R. Furthermore, contrary to a recent review (Mauri et al., 2007) the data suggest that
measurements of plasma quetiapine concentrations can provide guidance in terms of dosing and 5-HT$_{2A}$R occupancy.

Plasma concentration measurements would be of particular relevance in cases where pharmacokinetics are likely to be altered, e.g. in children (Gerlach et al., 2007), the elderly (Grimm et al., 2006; Sotaniemi et al., 1997), in patients with renal or hepatic impairment (Gunasekara and Spencer, 1998) and in patients concomitantly treated with compounds that affect the enzyme CYP3A4 (Grimm et al., 2006; DeVane and Nemeroff, 2001) by which quetiapine is predominately metabolized. Furthermore, in cases of non-response and adverse effects plasma monitoring seems appropriate.

**Other receptor systems**

In a $[^{11}\text{C}]$raclopride and $[^{11}\text{C}]$N-methylspiperone PET study by Gefvert et al. (Gefvert et al., 1998) it was found that two hours after the last dose of 450 mg of quetiapine the D$_2$ receptor occupancy was 44 % (range 21-68) in the putamen and caudate nucleus while 5-HT$_2$ receptor occupancy in the frontal cortex was 72 % (range 58-82). The patients were previously long term medicated with different antipsychotics before their shift to a quetiapine treatment period of 29 days. It can be argued that the reported D$_2$ receptor occupancy of 44 % might be overestimated since previous long term treatment with antipsychotics has been shown to increase the number of D$_2$ receptors of around 30 % as a result of receptor induction (Silvestri et al., 2000).

In the present study, quetiapine was administered 165 minutes before the PET scan. Considering the results of Gefvert et al. it can be reasoned that the D$_2$ receptor occupancy at 165 minutes is relatively lower than 5-HT$_{2A}$R occupancy. In our sample the mean 5-HT$_{2A}$R occupancy was 64 %, suggesting that the D$_2$ receptor occupancy is well below the traditionally proposed therapeutic window of 65-70 % for a D$_2$ mediated effect (Farde et al., 1995; Kapur et al., 1996; Nordstrom et al., 1993).

We found that 5-HT$_{2A}$R occupancy was associated with treatment effect on positive symptoms. Based on our data, however, it is not possible to directly determine the role of the D$_2$ receptor on the present findings, since we did not assess this receptor. For that reason, we cannot make any definite conclusions with regard to a direct or an indirect causal association between 5-HT$_{2A}$R and
psychopathology. It is possible that 5-HT2 receptor blockade inhibits phasic increases in dopamine synthesis and release in the striatum thereby potentiating D2 receptor antagonism and facilitating a reduction in positive symptoms by closure of the striato-thalamic filter (Glenthoj and Hemmingsen, 1999). Likewise, Meltzer et al. (Meltzer et al., 2003) have suggested that atypical antipsychotic action might be modulated by a differential effect of 5-HT2AR on dopaminergic activity in different brain regions, see also (Schmidt et al., 1993; Svensson et al., 1995). For example, clozapine has been shown to exert a preferential effect on dopamine release in the prefrontal cortex (Youngren et al., 1999; Kuroki et al., 2008).

Furthermore, as mentioned earlier a fast dissociation theory stating that atypical antipsychotic action is mediated by a transient D2 occupancy and a fast dissociation rate (koff), has been put forward by Kapur and Seeman (Kapur and Seeman, 2001). In fact Kapur et al. (Kapur et al., 2000) have argued that transiently high D2 occupancy may be sufficient for the antipsychotic effect of quetiapine, thereby questioning the assumption that continuously high D2 occupancy is required for response.

Finally, quetiapine also has affinity for other receptor systems. It has been found that some SGAs have a relatively high affinity for the 5-HT1A receptors. Clozapine, ziprasidone and quetiapine are partial 5-HT1A receptor agonists (Newman-Tancredi et al., 1998; Kuroki et al., 2008) and it has been suggested that 5-HT1A receptor agonism in conjunction with 5-HT2AR antagonism, might be related to clinical benefits of SGAs (Araneda and Andrade, 1991; Wadenberg and Ahlenius, 1991; Millan, 2000; Kuroki et al., 2008).

**Dosing**

To achieve a full therapeutic response, it has been suggested that some patients might require higher than licensed dosages, i.e., a quetiapine dose above 800 mg/day (Citrome et al., 2005; Pierre et al., 2005; Khazaal et al., 2007). As for first-episode schizophrenia patients, our data suggest that low doses of quetiapine (around 400 mg) are sufficient. This supports the conclusion of a recent meta-analysis (Sparshatt et al., 2008) on dose and clinical response of quetiapine, supporting that in first-episode patients a dose of 300-400 mg/day is optimal.

The use of high dose quetiapine is common in clinical practice, although support for this practice builds on case reports (Sparshatt et al., 2008). For example, 7 patients refractory to treatment with
quetiapine in doses up to 800 mg/day, who were subsequently treated with doses of 1200 to 2400 mg/day, showed modest to moderate clinical improvements in terms of positive psychotic symptoms, behavioral disturbances, violent behavior, and sociability. Adverse effects were e.g. sedation and orthostasis (Pierre et al., 2005) probably due to quetiapine’s antinoradrenergic and antihistaminergic action (Nemeroff et al., 2002). The adverse effects responded to dose reduction.

Currently, it cannot be ruled out that some patients have an additional therapeutic effect by very high doses of quetiapine, but this needs to be confirmed in rigorously controlled trials. From the present results, however, it seems unlikely that this additional effect is directly related to 5-HT2A R blockade. Rather it can be reasoned that patients benefitting from very high quetiapine doses, are patients who require a higher D2 blockade than can be obtained with low quetiapine doses. This high D2 blockade might then be brought about by very high quetiapine doses. As such, the present study suggests that patients who respond to quetiapine, already respond at lower doses (around 400 mg) and that non-responders, might require a stronger D2 blockade. Therefore, these non-responders to low quetiapine doses may obtain a better treatment effect and fewer adverse effects by switching to another atypical compound with a more potent D2 blockade. For example, amisulpride has high affinity for the D2/3 receptors and negligible affinity to other receptor systems while showing atypical clinical characteristics (Natesan et al., 2008).

**Previous studies of quetiapine**

Only a few PET studies have reported on 5-HT2A R occupancy after quetiapine treatment (Gefvert et al., 1998; Kapur et al., 2000; Gefvert et al., 2001).

In a [11C]N-methylspiperone study of 5 schizophrenia patients frontal 5-HT2A R occupancies were determined as 74 % and 57% at quetiapine doses of 750 and 450 mg/day respectively, with PET scanning performed 2 hours post administration (Gefvert et al., 2001). 750 mg is beyond the dose range of the present study; however we found that a quetiapine dose of 450 mg resulted in a global 5-HT2A R occupancy of 67 % with PET scanning performed 165 minutes post administration.

In a [18F]setoperone PET study by Kapur et al. (Kapur et al., 2000) it was shown in 12 patients that 300 to 600 mg/day of quetiapine occupies 57% to 78% of frontal 5-HT2A R. In our study 300 and 600 mg of quetiapine gave rise to comparable global occupancies of respectively 56 % and 70%.
However, the studies are not readily comparable because of a number of methodological issues. For example in the present study only first-episode antipsychotic-naïve patients were included at baseline, whereas previous studies (Gefvert et al., 1998; Kapur et al., 2000; Gefvert et al., 2001) included patients who were chronically ill and previously medicated with both typical and atypical compounds before their shift to quetiapine. Previous treatment with antipsychotics that antagonize the 5-HT$_2$AR can induce a paradoxical down-regulation of the receptor, both in vivo and in vitro (Dean, 2003; Gray and Roth, 2001). In contrast, previous antipsychotic treatment can increase the number of D$_2$ receptors as a result of receptor induction (Silvestri et al., 2000).

Furthermore, the present study had a longitudinal design where the patients were their own controls, while the study by Kapur et al (Kapur et al., 2000) used a cross-sectional design where baseline measures of 5-HT$_2$AR used in the calculation of occupancy, were derived from a different cohort of both patients and healthy controls. The latter approach is based on the assumption that in vivo 5-HT$_2$AR densities are similar in patients and healthy subjects. However, the interindividual variability in 5-HT$_2$AR densities is large and, as shown in study 1 schizophrenia patients have a decreased cortical 5-HT$_2$AR binding as compared to healthy controls.

**Methodological considerations**

Importantly, the tracers $[^{18}$F$]$setoperone and $[^{11}$C$]$N-methylspiperone used in previous studies (Gefvert et al., 1998; Kapur et al., 2000; Gefvert et al., 2001) are limited by their relatively poor selectivity for the 5-HT$_2$AR. In conjunction with the poor selectivity of the tracers, a lower ratio of 5-HT$_2$AR to D$_2$ receptors in subcortical areas compared to cortical areas makes these ligands inadequate to measure subcortical binding.

$[^{18}$F$]$altanserin has a 200 to 500-fold 5-HT$_2$/D$_2$ selectivity measured as 1/(5-HT$_2$/D$_2$ $K_i$)=1/(0.13–0.3/62 nM)=1/(0.002–0.005) (Kristiansen et al., 2005; Tan et al., 1999) making it between 8 and 50 times more selective for the 5-HT$_2$AR than $[^{18}$F$]$setoperone ((1/(1/10–25 nM)=1/(0.1–0.04)=10–25-fold 5-HT$_2$/D$_2$ selectivity (Lewis et al., 1999). In addition, the affinity of $[^{18}$F$]$altanserin for the 5-HT$_2$AR is at least 20-fold higher than for other 5-HT subtypes (Tan et al., 1999). We have previously demonstrated that $[^{18}$F$]$altanserin PET with a bolus infusion design is a highly reproducible method for reliable quantification of 5-HT$_2$AR (Haugbol et al., 2007).
Poor radiotracer selectivity might complicate the interpretation of its binding measurements. \[^{18}\text{F}]\text{altanserin}\) has its second highest affinity for the serotonin 2C receptor subtype (5-HT\(_{2C}\)R) after the 5-HT\(_{2A}\)R (Tan et al., 1999). It could be considered that group differences between cortical \[^{18}\text{F}]\text{altanserin}\) binding might reflect changes in 5-HT\(_{2C}\)R rather than 5-HT\(_{2A}\)R. However, this is unlikely since \[^{18}\text{F}]\text{altanserin}\) has a 5-HT\(_{2A}\)R vs. 5-HT\(_{2C}\)R selectivity ratio of 20 (Tan et al., 1999) and the expression of cortical 5-HT\(_{2A}\)R is higher than 5-HT\(_{2C}\)R (Nichols and Nichols, 2008). In addition brain homogenate binding studies have shown that blockade with the 5-HT\(_{2B/2C}\) selective compound SB 206553 does not alter \[^{18}\text{F}]\text{altanserin}\) binding (Kristiansen et al., 2005).

One of the general requirements of a radiotracer is that it should preferably not yield any radiolabeled metabolites crossing the blood brain barrier (Lammertsma and Hume, 1996). However, after systemic injection, \[^{18}\text{F}]\text{altanserin}\) gives yield to radiolabeled metabolites of which primarily radiolabeled altanserinol crosses the blood-brain barrier (Price et al., 2001) and with a bolus-infusion protocol, the lipophilic metabolites accumulate and increase the signal from non-specific binding over time (Pinborg et al., 2003; Adams et al., 2004). This can however be compensated for in the steady-state approach where the contribution from radiolabeled lipophilic metabolites can be subtracted directly from the reference region void of receptors i.e. the cerebellum. According to equation 1 in the methods section the calculation of the outcome measure BP\(_p\) includes the non-specific binding and C\(_p\). As such an overestimation of non-specific binding and/or C\(_p\) would underestimate BP\(_p\). In contrast, a high plasma free fraction of radiotracer, f\(_p\), would lead to a high BP\(_p\).

Currently another tracer \[^{11}\text{C}]\text{MDL 100907}\) is available for imaging 5-HT\(_{2A}\)R. This tracer is highly comparable with \[^{18}\text{F}]\text{altanserin}\) (Kristiansen et al., 2005). Both \textit{in vitro} and \textit{in vivo} experiments reveal that both tracers have high affinity, selectivity and a satisfactory ratio of specific to non-specific binding for 5-HT\(_{2A}\)R (Herth et al., 2009; Kristiansen et al., 2005). However, the selectivity of \[^{11}\text{C}]\text{MDL 100907}\) for the 5-HT\(_{2A}\)R as compared to \[^{18}\text{F}]\text{altanserin}\) is slightly higher and metabolites of \[^{11}\text{C}]\text{MDL 100907}\) do not enter the brain to any significant extent. Notwithstanding, a major advantage of \[^{18}\text{F}]\text{altanserin}\) over \[^{11}\text{C}]\text{MDL 100907}\) is the possibility to perform steady state scannings lasting several hours based on the 110 min half-life of \[^{18}\text{F}]\text{fluorine}\) (Herth et al., 2009). However, for both radioligands, the binding seen in PET studies is not directly influenced by changes in endogenous 5-HT levels. This is further discussed in the section on research perspectives below.
The present study is limited by the fact that we at follow-up only obtained a binding measure in a medicated state. Therefore we cannot make direct inferences regarding a potential paradoxical down-regulation of 5-HT$_{2A}$R caused by the quetiapine treatment (Dean, 2003; Gray and Roth, 2001) which might lead to an overestimation of occupancy. Ideally, two scans could have been performed at follow up, one in the medicated state and one at a quetiapine plasma level of zero after discontinuation providing a more dynamic measure of occupancy. However, for obvious ethical and logistical reasons this was not performed.
Conclusions

Based on in vivo PET-measurements in the, until now, largest cohort of antipsychotic naive first episode schizophrenia patients we have identified a decreased 5-HT_{2A}R binding in the frontal cortex and a relationship between this binding and positive psychotic symptoms in male patients. Since no correlations were found between binding and cognition, this study does not support the involvement of 5-HT_{2A}R in cognitive deficits in this early stage of the disease. Furthermore, we have shown that 5-HT_{2A}R blockade has an important therapeutic role in the treatment of positive psychotic symptoms either directly or via interactions with the dopaminergic system or other transmittersystems. From a clinical perspective the study shows that a low dose of quetiapine (around 400 mg) is recommendable for first episode schizophrenia patients and that measurements of plasma quetiapine concentrations provide guidance in terms of dosing and the level of 5-HT_{2A}R blockade.
Research perspectives

It would be of importance to expand the current study design to include a D₂ tracer in order to directly evaluate the potential influence of D₂ receptors on the present findings. Another interesting development of the present study would be to use the same longitudinal design however focusing on other important serotonergic markers and to examine the molecular genetics of 5-HT₂A R.

The serotonin transporter (SERT) is essential for the regulation of serotonergic neurotransmission since it controls serotonin availability at postsynaptic receptors by high affinity reuptake of released serotonin (Blakely et al., 1994). Increasing evidence suggests an association between variation in SERT levels and 5-HT₂A R function (Jennings et al., 2008). To our knowledge only one study of SERT in schizophrenia has been carried out, however in a small sample of chronic and previously medicated schizophrenia patients (Frankle et al., 2005).

Methodological developments to improve the study of in vivo dynamics of human serotonergic neurotransmission would include the development of new high-affinity tracers for the measurement of endogenous serotonin release and for the imaging of new targets like the serotonin 4 and 2C receptors.

Evidence from pharmacological and molecular genetic studies suggests a role for the 5-HT₄R in the consequences of antipsychotic treatment in particular dyskinesia and weight gain (Reynolds et al., 2005). 5-HT₄R is also considered as an important pharmacological target in the treatment of disorders related to dopaminergic neuronal dysfunction in schizophrenia and in other neuropsychiatric disorders such as depression, Parkinson's disease and drug addiction. It has also been shown that the 5-HT₄R controls activated dopaminergic neurons by modulating the neuronal firing (Berg et al., 2008).

In autoradiographical studies with serotonin manipulation in rodents, the 5-HT₄ receptor has been positively correlated to serotonin levels (Licht et al., submitted). As such, measurements of the 5-HT₄ receptor might further improve the indirect characterization of synaptic serotonin levels.
An additional analysis to include in the present study would be the molecular genetics of 5-HT$_{2A}$R. Estimations from twin studies show that schizophrenia is highly heritable (Cardno et al., 1999), and we have recently shown that the cortical 5-HT$_{2A}$R binding pattern in the human brain is strongly genetically determined (Pinborg et al., 2008). Also, cortical 5-HT$_{2A}$ mRNA expression is lower in schizophrenia patients (Hernandez and Sokolov, 2000) than in healthy subjects which is consistent with evidence from genetic studies linking polymorphisms in the 5-HT$_{2A}$R with symptom severity (Quednow et al., 2008), clinical response to the SGA clozapine in schizophrenia patients (Arranz et al., 1998b; Arranz et al., 1998a), and overall risk of developing schizophrenia (Abdolmaleky et al., 2004; Golimbet et al., 2007). This could indicate that genetically determined alterations in 5-HT$_{2A}$R function during brain maturation predisposes to the development of schizophrenia; a hypothesis which is in line with the observations made in the studies by Hurleman and colleagues (Hurleman et al., 2005; Hurleman et al., 2008) who reported decreased 5-HT$_{2A}$R binding already during the prodromal states of schizophrenia. Furthermore, 5-HT$_{2A}$R stimulation affects the firing rate of serotonergic cells bodies in the raphe nuclei through GABAergic interneurons (Liu et al., 2000). As such, an altered 5-HT$_{2A}$ receptor function may have an overall effect on global serotonin availability and, thereby, affect serotonin-regulated neuroplasticity (Azmitia, 2007) during the development of the brain. 5-HT$_{2A}$ receptor alterations also affect other neurotransmitter-systems, like the dopamine system (Alex and Pehek, 2007; Glenthoj and Hemmingsen, 1999), which is related to working memory performance (Williams and Goldman-Rakic, 1995) and positive psychotic symptoms (Glenthoj et al., 2006). Through these mechanisms, the 5-HT$_{2A}$ receptor function may set the cerebral scene for schizophrenia, long before symptoms evolve.
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Appendices
Decreased Frontal 5-HT_{2A} Receptor Binding in Antipsychotic-Naïve First-episode Schizophrenia Patients

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Abstract

Context: Post-mortem investigations and the receptor affinity profile of atypical antipsychotics have implicated the participation of serotonin 2A (5-HT$_{2A}$) receptors in the pathophysiology of schizophrenia. Most post-mortem studies point towards lower cortical 5-HT$_{2A}$ binding in schizophrenic patients. However, in vivo studies of 5-HT$_{2A}$ binding report conflicting results, presumably because sample sizes have been small or because schizophrenic patients who were not antipsychotic-naïve were included. Furthermore, the relationships between 5-HT$_{2A}$ binding, psychopathology, and central neurocognitive deficits in schizophrenia are unclear.

Objectives: To assess in vivo brain 5-HT$_{2A}$ binding potentials in a large sample of antipsychotic-naïve schizophrenic patients and matched healthy controls, and to examine possible associations with psychopathology, memory, attention and executive functions.

Design: Case-control study

Setting: University Hospital, Denmark

Participants: A sample of 30 first-episode antipsychotic-naïve schizophrenic patients, 23 males and 7 females, and 30 matched healthy control subjects.

Main Outcome Measures: 5-HT$_{2A}$ binding was measured using positron emission tomography and the 5-HT$_{2A}$-specific radioligand, [F$^{18}$]altanserin, in a bolus infusion approach. The binding potential of specific tracer binding was used as the outcome parameter. Psychopathology was assessed using the positive and negative symptom rating scale and both patients and controls underwent a neuropsychological test battery.
**Results:** Schizophrenic patients had significantly lower $5\text{-HT}_{2A}$ binding in frontal cortex than control subjects. A significant negative correlation was observed between frontal cortical $5\text{-HT}_{2A}$ binding and positive psychotic symptoms in the male patients. No correlations were found between cognitive functions and $5\text{-HT}_{2A}$ binding.

**Conclusion:** This study of $5\text{-HT}_{2A}$ receptor binding in first-episode antipsychotic-naïve schizophrenic patients shows a decreased binding in the frontal cortex and a negative correlation with positive symptoms in male patients. The results suggest that frontal cortical $5\text{-HT}_{2A}$ receptors are involved in the pathophysiology of schizophrenia.

**Introduction**

A growing body of evidence points towards impairment of the serotonin 2A ($5\text{-HT}_{2A}$) receptor function in schizophrenia. The initial serotonin-hypothesis of schizophrenia was sparked by the observation that lysergic acid diethylamide (LSD), a drug with structural similarities to serotonin and a high affinity to $5\text{-HT}_{2A}$ receptors, has hallucinogenic properties similar to schizophrenic symptoms. This hypothesis is backed by eleven\textsuperscript{1-11} out of fifteen\textsuperscript{12-15} post-mortem studies reporting decreased $5\text{-HT}_{2A/C}$ binding in cortical areas, especially in frontal cortex. However, these reports were primarily based on chronic, medicated patients and the techniques used to analyze post mortem tissue differed between studies\textsuperscript{16}

Indirect support for the involvement of the $5\text{-HT}_{2A}$ receptor in schizophrenia arises from the association between the receptor affinity profile and the clinical characteristics of new, atypical antipsychotic drugs. Atypical antipsychotic drugs have a complex
pharmacology. For example, clozapine has high affinity for a number of serotonin (5-HT$_{2A}$, 5-HT$_{2C}$, 5-HT$_{6}$, 5-HT$_{7}$), dopamine (D$_{4}$), muscarinic (M, M$_{2}$, M$_{3}$, M$_{4}$, M$_{5}$), adrenergic ($\alpha_{1}$- and $\alpha_{2}$-subtypes) and other biogenic amine receptors $^{17}$. However, compared with typical antipsychotic drugs, which primarily bind to the dopamine2 (D$_{2}$) receptors, most atypical antipsychotics have higher affinity to cortical 5-HT$_{2A}$ receptors than to striatal D$_{2}$ receptors$^{18,19}$. This may account for the reduced extrapyramidal side effects (EPS) of atypical antipsychotics and their effect on negative symptoms$^{20}$.

It is unclear how 5-HT$_{2A}$ activity is associated with the most commonly found clinical cognitive deficits in schizophrenia$^{21,22}$, e.g. attention, executive functions and spatial working memory. It has been proposed that working memory could be one of the central cognitive markers or endophenotypes of schizophrenia$^{23-25}$. In general, the literature suggests that 5-HT$_{2A}$ receptor antagonism improves cognition in schizophrenia$^{26}$. Recent research has shown that the affinity of antipsychotic drugs to the 5-HT$_{2A}$ receptor is associated with cognition in a subtle way. Spatial working memory has been suggested to improve by stimulation rather than blockade of 5-HT$_{2A}$ receptors in both pre-clinical and clinical studies$^{27-29}$. Conversely, blockade of 5-HT$_{2A}$ receptors by the 5-HT$_{2A}$ antagonist ketanserin in healthy control subjects impaired memory more than combined escitalopram ketanserin treatment$^{30}$. Atypical antipsychotic drugs with a high antagonistic action on 5-HT$_{2A}$ may therefore benefit spatial working memory tasks less than low affinity drugs$^{27}$. These studies support a linkage between impaired working memory and decreased 5-HT$_{2A}$ availability or function in the human brain.
The introduction of selective 5-HT$_{2A}$-receptor radioligands for positron emission tomography (PET) made it possible to examine the 5-HT$_{2A}$-receptor density in the living human brain. However, only few PET studies on first-episode antipsychotic-naïve schizophrenic patients have so far been performed, and the results are inconsistent. Three studies found no difference in 5-HT$_{2A}$ binding between schizophrenic patients and healthy control subjects$^{31-33}$, and one study found a decreased binding potential in the left lateral frontal cortex in six patients$^{34}$. These studies are limited by small sample sizes and by their use of the radioligands [18F]setoperone and [$^{11}$C]N-methylspiperone, which have a relatively low 5-HT$_{2A}$-receptor selectivity$^{35}$.

The radioligand [18F]altanserin is highly selective for the 5-HT$_{2A}$ receptor and allows measurements of 5-HT$_{2A}$ receptor availability in both cortical and subcortical regions$^{31,36,37}$. In a previous, preliminary study, we reported the use of this radioligand in 15 antipsychotic-naïve schizophrenic patients$^{35}$. We were then unable to confirm our hypothesis of decreased frontal 5HT$_{2A}$ binding. However, in a post hoc analysis we found an increased 5-HT$_{2A}$ binding in the caudate nucleus. This result was considered a preliminary finding due to the modest receptor density of 5-HT$_{2A}$ in subcortical brain regions. Larger sample sizes were deemed to be required to exclude type II errors$^{38}$.

The aim of the present PET study is therefore to use [18F]altanserin-PET to investigate cortical and subcortical 5-HT$_{2A}$ binding in an extended group of first-episode antipsychotic-naïve schizophrenic patients and matched healthy control subjects. Fifteen of the patients were identical to the patients included in our previous preliminary study.$^{35}$
A decrease in 5-HT$_{2A}$ binding in frontal cortex in these patients compared with matched healthy control subjects was expected a priori. We also expected to confirm our preliminary finding of 5-HT$_{2A}$ receptor up-regulation in the caudate nucleus. As an additional and new approach, we explored possible associations between 5-HT$_{2A}$ binding, psychopathology and central cognitive deficits, specifically spatial working memory, attention and executive functions.

**Materials and methods**

The study was approved by the Ethics Committee of Copenhagen and Frederiksberg (KF)11-061/03). The subjects participated after receiving a full explanation of the study and providing written informed consent according to the declaration of Helsinki II.

**Participants**

Thirty-three (26 male and 7 female) patients were recruited after voluntary first-time referral to a psychiatric unit of one of the affiliated university hospitals in the Capital Region of Copenhagen (Bispebjerg Hospital, Rigshospitalet, Psychiatric University Center Glostrup or Psychiatric University Center Gentofte).

Thirty of the 33 patients fulfilled the diagnostic criteria for schizophrenia according to both ICD-10 and DSM-IV. Three patients proved to have a diagnosis of schizotypal personality disorder at a later stage of the study, and were therefore excluded. All patients (mean age: 26.4 years, SD=5.5) included were antipsychotic naïve. The diagnoses of schizophrenia were verified by means of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) interview$^{39}$. 
Thirty healthy control subjects (mean age: 26.4 years, SD=5.7) matched for age, gender and parental socioeconomic status were recruited from the community by advertisement. None of the healthy control subjects had present or prior psychiatric disorder or any history of psychotropic medication as determined by SCAN interviews.

Six patients were prior (n=4) or present (n=2) users of antidepressant medication (in all cases selective serotonin reuptake inhibitors (SSRIs)). Benzodiazepines were allowed, albeit not on the day of the PET scan. Eight patients fulfilled lifetime criteria for substance abuse. All abuse diagnoses were clearly secondary to the diagnosis of schizophrenia. Substance dependence was an exclusion criterion. DSM-IV diagnoses of substance abuse were: alcohol abuse, in sustained full remission (n=2); cannabis abuse, in a controlled environment, (n=1); other abuse, sustained full remission (n=1); other abuse, moderate, (n=1); other abuse, in a controlled environment (n=2); and other abuse, early partial remission (n=1). In four of the patients the diagnosis ‘other abuse’ covered mixed cannabis and alcohol abuse, and in one patient the diagnosis covered a history of amphetamine and cocaine use. Three patients had no history of abuse for the past year, and four patients had no abuse for the past month. All subjects had a negative urine screening for substance intake prior to the PET scan.

60 % of the patients and 20 % of the control subjects were smokers. None of the participants smoked 2 hours before the PET investigations. Smoking status was not a matching criterion since we in a recent study on 136 healthy subjects study had found no effect of smoking on 5-HT_{2A} binding.
No subjects had a history of significant head injury or non-psychiatric disorder. All subjects had a normal neurological interview and examination, and a structural magnetic resonance imaging (MRI) scan of the brain without clinical pathological findings as evaluated by a neuroradiologist.

**Psychopathological ratings**

Symptom severity was assessed by trained raters using the Positive and Negative Syndrome Scale (PANSS). All interviews were recorded on DVD for validation purposes. A sub-sample of 10 randomly selected PANSS ratings showed an intra-class correlation coefficient of 0.85 between the raters in a two-way fixed effect model.

**Neurocognitive testing**

Memory, executive functions and attention were assessed with the following subtests from the Cambridge Neuropsychological Test Automated Battery (CANTAB): Spatial Working Memory (SWM), Stockings of Cambridge (SOC), Intra-Extradimensional Set Shifting (IED) and Rapid Visual Information Processing (RVP).

**Magnetic resonance imaging**

High-resolution 3D T1-weighted, sagittal, magnetization-prepared rapid-gradient echo (MPRAGE) scans of the whole head (TI/TE/TR=800/3.93/1540 ms, flip angle 9°; matrix: 256 x 256; 192 slices) using an eight-channel head array coil were acquired in all subjects on a 3 tesla TRIO scanner (Siemens, Erlangen, Germany) at the MR department of the Copenhagen University Hospital, Hvidovre, Denmark.
**PET: Radiosynthesis and administration**

The radiosynthesis of \([^{18}F]altanserin\) has been described previously\(^44\). Quality control was performed using thin-layer chromatography and high-performance liquid chromatography (HPLC). The absence of residual solvents (methanol, THF, and DMSO) in the final formulation was confirmed by \(^1\)H NMR. For each PET study, 0.3–3.5 GBq of \([^{18}F]altanserin\) was produced with a radiochemical yield exceeding 95%. Catheters were inserted in both cubital veins for tracer infusion and blood sampling, respectively. \([^{18}F]altanserin\) was administrated as a bolus injection followed by continuous infusion to obtain steady state of the tracer in blood and tissue. The bolus infusion ratio was 1.75 h, as previously described\(^45\). Subjects received a maximum dose of 3.7 MBq/kg body weight \([^{18}F]altanserin\).

**PET imaging**

PET scans were acquired in tracer steady-state conditions with an 18-ring GE-Advance scanner (GE, Milwaukee, WI, USA), operating in 3D-acquisition mode, producing 35 image slices with an interslice distance of 4.25 mm. The total axial field of view was 15.2 cm with an approximate in-plane resolution down to 5 mm. During steady state, the fraction of unmetabolized tracer in venous plasma was determined at five time points using HPLC analysis. Reconstruction, attenuation, and scatter correction procedures were conducted as previously described\(^45\).

The subjects were placed in the scanner 90 min after the bolus injection of \([^{18}F]altanserin\). The subjects were aligned in the scanner using a laser system so that the detectors were parallel to the orbitomeatal line and positioned to include the cerebellum.
in the field of view using a short 2-min transmission scan. An individual head holder was made to ensure relative immobility. All subjects were scanned in a resting state. A 10-min transmission scan was obtained for correction of tissue attenuation using retractable $^{68}$Ge/$^{68}$Ga pin sources. The transmission scans were corrected for tracer activity by a 5-min emission scan performed in 2D mode. Dynamic 3D emission scans (five frames of 8 min) were started 120 min after tracer administration.

Data were reconstructed into a sequence of 128 x 128 x 35 voxel matrices, each voxel measuring 2.0 x 2.0 x 4.25 mm, with software provided by the manufacturer. A 3D reprojection algorithm with a transaxial Hann filter (6 mm) and an axial ramp filter (8.5 mm) was applied. Corrections for dead-time, attenuation, and scatter were performed.

**Blood samples**

Five venous blood samples were drawn at mid-scan times 4, 12, 20, 28, and 36 min after starting the dynamic scanning sequence. The samples were immediately centrifuged, and 0.5 ml of plasma was counted in a well-counter for determination of radioactivity. Three of the five blood samples drawn at 4, 20, and 36 min were also analyzed for percentage of parent compound ($[^{18}$F$]$altanserin) using reverse-phase HPLC following a previously described method\(^{46}\).

In addition, the free fraction of $[^{18}$F$]$altanserin in plasma, $f_P$, was estimated using equilibrium dialysis, following a modified procedure\(^{47}\). The dialysis was performed using Teflon-coated dialysis chambers (Harvard Bioscience, Amika, Holliston, MA, USA) with a cellulose membrane that retains proteins >10 000 Da. A small amount of $[^{18}$F$]$altanserin
(approximately 1 MBq) was added to 10-ml plasma samples drawn from the subjects. A 500-µl portion of plasma was then dialyzed at 37°C for 3 h against an equal volume of buffer, since pilot studies had shown that a 3-h equilibration time yielded stable values. The buffer consisted of 135 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl₂, 1.0 mM MgCl₂, and 2.0 mM phosphate (pH 7.4). After the dialysis, 400 µl of plasma and buffer were counted in a well counter, and \( f_P \) of [¹⁸F]altanserin was calculated as the ratio of DPM_{buffer}/DPM_{plasma}.

**MR/PET co-registration**

PET images and 3D T1 weighted MRI scans were co-registered using a Matlab (Mathworks Inc., Natick, MA, USA)-based program, where PET images and MRIs are brought to fit through manual translation and rotation of the PET image with subsequent visual inspection in three planes.

**Volumes of interest and partial volume correction**

Volumes of interest (VOIs) were automatically delineated on each individual's transaxial MRI slices in a strictly user-independent manner. This approach allowed automatic co-registration of a template set of 10 MRIs to a new subject's MRI. The identified transformation parameters were used to define VOIs in the new subject MRI space, and through the co-registration these VOIs were transferred onto the PET images.

A frontal cortex region was defined for each subject and served as the primary VOI. The frontal cortex VOI consisted of a volume-weighted average of left and right cortical
regions and included: orbitofrontal cortex, medial inferior frontal cortex, superior frontal cortex, and anterior cingulate cortex\textsuperscript{49}.

Other regions included were: amygdala, caudate nucleus, entorhinal cortex, hippocampus, hypothalamus, insula, occipital cortex, parietal cortex, posterior cingulate cortex, putamen, sensorimotor cortex, superior temporal cortex and thalamus. The cerebellum was used for estimation of non-specific binding.

To enable partial volume correction of the PET data, MRIs corrected for RF inhomogeneities using the N3 software\textsuperscript{50} were segmented into gray matter, white matter, and cerebrospinal fluid tissue classes using Statistical Parametric Mapping (SPM2) (Wellcome Department of Cognitive Neurology, London, UK). Partial volume correction was performed according to the Müller Gartner method\textsuperscript{51,52}. The white matter value was extracted from the uncorrected PET image as the mean voxel value from a brain region containing predominantly white matter (centrum semiovale).

**Quantification of the 5-HT\textsubscript{2A} receptor binding**

The outcome measure was the binding potential of specific tracer binding ($B_{PP}$). The cerebellum was used as a reference region, since it represents nonspecific binding only. In steady state, $B_{PP}$ is defined as

\[
B_{PP} = \frac{C_{VOI} - C_{Reference}}{C_{Plasma}} = f_p \cdot \frac{B_{max}}{K_d} \text{ (ml/ml)}
\]

where $C_{VOI}$ and $C_{Reference}$ are the steady-state mean count density in the VOI and in the reference region, respectively $C_{Plasma}$ is the steady-state activity of non-metabolized tracer in plasma; $f_p$ is the free fraction of radiotracer; $B_{max}$ is the density of receptor sites.
available for tracer binding; and $K_d$ is the affinity constant of the radiotracer to the receptor.

**Statistics**

All analyses were performed using SPSS® software. Between-group (patients, controls) comparisons of all reported outcome measures were performed using parametric analysis after verifying that the data were normally distributed according to the Kolmogorov–Smirnov test. Potential outliers were detected with Grubb’s outlier test\(^5\), and subsequently excluded from analysis. The planned comparison in frontal 5-HT\(_{2A}\) binding between patients and controls was performed with an independent samples Student’s t-test (one-tailed, because of our directional hypothesis). In addition, an ANOVA was performed with between factor group (patient or control) and within factor region (frontal or other), to test whether a potential effect of group was more a global effect across all regions than a regional effect principally affecting frontal cortex. Furthermore, to test for additional regional group differences in binding an ANOVA was performed with between factors group (patient or control) and within factor region (the different regions, as specified in Table 1). Independent samples Student’s t-tests (two-tailed) were only performed when these ANOVAs indicated statistical significant results.

Independent sample Student’s t-tests were further used to test for differences between patients and controls with regards to neurocognitive and psychopathological measures (two tailed). Correlation analyses were performed using the Pearson product-moment correlation coefficient. The potential effect on the results of antidepressive medication, benzodiazepines and substance abuse was examined by including these parameters in a multiple analysis of covariance (MANCOVA) as covariates.
Results

5-HT2A binding

The planned comparison of frontal cortical binding revealed reduced 5-HT2A binding in patients compared to controls (t=2.54, df=58, p<0.01). The ANOVA on region and group revealed significant main effects of group [F(1,58) = 5.58, p<0.01] and region [F(17,42) = 82.19, p<0.001], and a significant region x group interaction effect [F(17,986) = 5.77, p<0.001]. Further analysis of these results indicated that 5-HT2A binding in patients was significantly reduced not only in the frontal cortex (see above) but also in a number of other cortical - but not subcortical - regions (see Table 1). Therefore, to test whether the frontal cortical region showed an even lower 5-HT2A receptor binding than the other cortical regions a post-hoc ANOVA was performed with within factor region (frontal cortex or other regions, see Table 1) and between factor group. This ANOVA revealed main effects of region [F(1,58) = 1109, p<0.001] and group [F(1,58) = 6.00, p<0.05] as well as a first order interaction between region and group [F(1,58) = 7.78, p<0.01], indicating a more pronounced reduction in 5-HT2A receptor binding in the frontal cortical region than in the other cortical regions (see Figure 1).

In the control group, the Grubb’s test indicated one significant outlier with an increased binding in the frontal cortex. After exclusion of this outlier, the differences in 5-HT2A binding remained significant. None of the results changed when the subjects on prior (n=4) or current antidepressant treatment (n=2) or cocaine and amphetamine abuse (n=1) were excluded from the analyses. Data related to antidepressive medication is described
in detail in Table 2. Furthermore, use of benzodiazepines did not covary significantly. The two groups did not differ significantly with regard to body mass index (BMI), injected radioactive dose, plasma free fraction, and specific radioactivity of $^{[18]F}$ altanserin (see Table 3). The patients had a significantly lower non-specific binding than the healthy control subjects.

5-HT$_{2A}$ binding and neurocognition

The cognitive data represent a sub-sample of a larger dataset published elsewhere (Andersen et al., submitted). Patients had significantly lower neurocognitive scores than healthy control subjects in the following tests: SWM strategy, SWM total errors and SWM between errors, IED total errors, and IED total number of trials on all stages attempted. There were no significant differences in SOC or RVP (see Table 4). In the frontal cortex, no significant correlations were detected between 5-HT$_{2A}$ binding and the neurocognitive measures. No significant correlations were found between the other VOIs and neurocognitive performance.

5-HT$_{2A}$ binding and psychopathology

In the patients, the PANSS scores were: positive 20.0 (SEM=0.93), negative 22.0 (SEM=1.20), general 38.5 (SEM=1.30), and total 80 (SEM=2.60). A significantly negative correlation ($r=-0.571$, $p<0.01$) was found between 5-HT$_{2A}$ binding in the frontal cortex and positive symptoms in the larger group of male patients. An explorative post hoc analysis showed significant negative correlations between frontal 5-HT$_{2A}$ binding and the following sub-items of the positive PANSS scale: P1 delusions ($r=-0.47$, $p=0.027$) and P6
suspiciousness ($r=-0.53$, $p=0.011$) (see Figure 2). No significant differences were found between the other VOIs and psychopathology. There was no gender effect on symptom severity or 5-HT$_{2A}$ binding.

**Discussion**

In this study of 5-HT$_{2A}$ binding in antipsychotic-naïve first-episode schizophrenic patients, we confirmed our hypothesis of a lower frontal cortical 5-HT$_{2A}$ binding in patients than in matched healthy control subjects. 5-HT$_{2A}$ binding was also reduced in a number of other cortical regions, but the reduction in the frontal cortical region was more pronounced. Furthermore, the reduction in 5-HT$_{2A}$ receptor binding in the frontal cortical region was more pronounced than in the other regions. This is in agreement with the vast number of post-mortem studies suggesting decreased cortical 5-HT$_{2A}$ receptor binding in schizophrenic patients. Moreover, the data revealed a significant, negative correlation between frontal cortical 5-HT$_{2A}$ binding and positive psychotic symptoms in male patients. We were not, however, able to confirm correlations between cognitive functions and 5-HT$_{2A}$ binding, even though the patients performed significantly worse in spatial working memory and aspects of executive function than did the healthy controls.

Our results are based on the hitherto largest sample studied with PET, whereas earlier PET studies have reported results based on 6-15 patients$^{31-35}$. The majority of these studies, including our own$^{54}$, were unable to identify differences in cortical 5-HT$_{2A}$ binding between schizophrenic patients and healthy control subjects. In our previous study we found increased 5-HT$_{2A}$ receptor binding in the caudate nucleus. This nucleus is a region with a relatively low 5-HT$_{2A}$ receptor density; hence, the post-hoc analyses were
more prone to type II errors. The present study does not confirm our preliminary finding of increased binding in the caudate nucleus, but it does support the study by Ngan and colleagues, who reported a lowered 5-HT$_{2A}$ binding in frontal cortex of six neuroleptic-naïve schizophrenic subjects. Similarly, Hurlemann and colleagues reported a decreased cortical 5-HT$_{2A}$ binding in subjects at high risk of developing schizophrenia.

Decreased frontal 5-HT$_{2A}$ binding and the relation with positive psychotic symptoms may reflect either a primary pathophysiological disturbance in schizophrenia or a compensatory down-regulation of receptors in response to altered endogenous serotonin levels. Alternatively, the finding could indicate a down-regulation compensating for hyperactive second messenger systems or hyperactivity in other systems on which the 5-HT$_{2A}$ receptors have a modifying effect. Finally, the finding could imply that frontal 5-HT$_{2A}$ receptors are important targets for antipsychotics.

The correlation between 5-HT$_{2A}$ binding and symptoms was only present in the male subjects. Various aspects of schizophrenia, including age of onset, pathophysiology, symptomathology, course of illness, and treatment response have previously been shown to be related to gender. These gender differences supply evidence for a potential role of gonadal hormones and for an interaction of these hormones with neurotransmitters (for a review, see ). Indeed, we have previously reported gender differences in drug-naïve schizophrenic patients with regards to the dopamine system, namely a correlation between D$_2$ receptor binding in the frontal cortex and positive psychotic symptoms in male patients only.
As expected, patients showed significantly poorer performance in spatial working memory and aspects of executive functions than healthy control subjects. This is in agreement with previous studies which have shown that spatial working memory and executive functions are central impaired neurocognitive domains in schizophrenia\textsuperscript{21,22}. However, we detected no correlations between the cognitive parameters and 5-HT$_{2A}$ binding in any of the VOIs. Hence, our data do not support previous findings relating 5-HT$_{2A}$ receptor to cognition in general\textsuperscript{26}, and spatial working memory in particular\textsuperscript{27,28}. The interaction between serotonin and cognition is complex. Indeed, the interactions between serotonin, dopamine, norepinephrine and the cholinergic system have been suggested to mediate cognitive behavior\textsuperscript{28}. Moreover, studies differ in design with regard to subjects (rodents, healthy controls or patients), type of serotonin manipulation (global, specific depletion or stimulation), serotonin receptor subtype (currently 15 serotonin receptor subtypes have been identified), and the cognitive tests being used\textsuperscript{27}.

There are some methodological issues in the study that need to be addressed. Four patients were on prior (n=4) or current (n=2) SSRI treatment, which is known to affect serotonergic innervation. However, we have previously found that [\textsuperscript{18}F]altanserin binding to 5-HT$_{2A}$ receptors is insensitive to a citalopram challenge increasing extracellular 5-HT\textsuperscript{59}. Furthermore, the effect of chronic SSRI treatment on 5-HT$_{2A}$ density is unclear since SSRI’s have different effects on 5-HT$_{2A}$ receptors. Fluoxetine has been reported to have either no effect on 5-HT$_{2A}$ receptor number or actually increase receptor number. Similarly, paroxetine has been shown to increase or have no effect on 5-
HT$_{2A}$ receptor density. In contrast, chronic citalopram treatment has been shown to
down-regulate 5-HT$_{2A}$ receptors$^{60}$.

For the above reasons, we initially chose to include patients on previous or current
antidepressants but controlled for the potential effect in a post hoc analysis where these
patients were removed from the analyses. This did not change the results.

Similarly, one patient had a history of amphetamine and cocaine abuse. These substances
are known to affect serotonergic innervation in the brain, however the patient did not
differ in 5-HT$_{2A}$ receptor binding and exclusion of the patient from the analyses did not
alter the results.

Finally, there was a significantly lower binding in the cerebellum in patients as compared
to control subjects. We have no explanation for the non-specific binding being lower in
the patients; the fraction of $[^{18}F]$altanserin metabolites in venous blood was the same in
both groups. However, a lower non-specific cerebellar binding in the patients is not likely
to bias the results since a relative underestimation of non-specific binding in the patients
would lead to an overestimation of the composite measure BP$_T$ (see equation 1).
Conclusion

This study of 5-HT$_{2A}$ receptor binding in first-episode antipsychotic-naïve schizophrenic patients shows a decreased binding in the frontal cortex and a negative correlation with positive symptoms in male patients. The results suggest that frontal cortical 5-HT$_{2A}$ receptors are involved in the pathophysiology of schizophrenia. Since no correlations were found between binding and cognition, this study does not support the involvement of 5-HT$_{2A}$ receptors in cognitive deficits in this early stage of the disease.
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Figure 1: Mean frontal cortical and total 5-HT$_{2A}$ receptor binding (+/- 1 SEM) in 30 antipsychotic-naïve first-episode schizophrenic patients and 30 matched healthy controls.
Figure 2: Negative correlation in male schizophrenic patients between mean frontal cortical 5-HT$_{2A}$ receptor binding, positive PANSS symptoms ($r=0.571$, $p=0.007$) and subitems P1 delusions ($r=-0.47$, $p=0.027$) and P6 suspiciousness ($r=-0.53$, $p=0.011$).
<table>
<thead>
<tr>
<th>Region</th>
<th>Patients</th>
<th>SEM</th>
<th>Controls</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>2.91</td>
<td>0.12</td>
<td>3.37</td>
<td>0.14</td>
<td>0.007</td>
</tr>
<tr>
<td>Orbitofrontal cortex</td>
<td>2.89</td>
<td>0.13</td>
<td>3.42</td>
<td>0.15</td>
<td>0.004</td>
</tr>
<tr>
<td>Medial inferior frontal cortex</td>
<td>3.07</td>
<td>0.12</td>
<td>3.50</td>
<td>0.13</td>
<td>0.065</td>
</tr>
<tr>
<td>Superior frontal cortex</td>
<td>3.34</td>
<td>0.14</td>
<td>3.85</td>
<td>0.15</td>
<td>0.008</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>2.34</td>
<td>0.09</td>
<td>2.68</td>
<td>0.13</td>
<td>0.019</td>
</tr>
<tr>
<td>Other regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.68</td>
<td>0.04</td>
<td>0.77</td>
<td>0.05</td>
<td>NS. (0.15)</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.60</td>
<td>0.04</td>
<td>0.65</td>
<td>0.04</td>
<td>NS. (0.34)</td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>1.11</td>
<td>0.05</td>
<td>1.21</td>
<td>0.06</td>
<td>NS. (0.20)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.74</td>
<td>0.04</td>
<td>0.81</td>
<td>0.05</td>
<td>NS. (0.30)</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.34</td>
<td>0.04</td>
<td>0.38</td>
<td>0.04</td>
<td>NS. (0.50)</td>
</tr>
<tr>
<td>Insula</td>
<td>1.82</td>
<td>0.08</td>
<td>2.10</td>
<td>0.09</td>
<td>0.038</td>
</tr>
<tr>
<td>Medial inferior temporal cortex</td>
<td>2.66</td>
<td>0.11</td>
<td>3.08</td>
<td>0.13</td>
<td>0.014</td>
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<tr>
<td>Occipital cortex</td>
<td>2.56</td>
<td>0.11</td>
<td>2.97</td>
<td>0.12</td>
<td>0.012</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>3.26</td>
<td>0.13</td>
<td>3.70</td>
<td>0.14</td>
<td>0.012</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>2.57</td>
<td>0.11</td>
<td>2.93</td>
<td>0.12</td>
<td>0.032</td>
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<tr>
<td>Putamen</td>
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<td>0.05</td>
<td>0.48</td>
<td>0.03</td>
<td>NS. (0.08)</td>
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<td>Sensorimotor cortex</td>
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<td>0.11</td>
<td>3.13</td>
<td>0.11</td>
<td>0.012</td>
</tr>
<tr>
<td>Superior temporal cortex</td>
<td>2.68</td>
<td>0.11</td>
<td>3.03</td>
<td>0.12</td>
<td>0.004</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.48</td>
<td>0.03</td>
<td>0.52</td>
<td>0.03</td>
<td>NS. (0.39)</td>
</tr>
</tbody>
</table>

Table 1: Mean binding potentials of the specific [18F]altanserin binding (BPn) in frontal cortex and sub-regions of interest in patients (n=30) and controls (n=30), respectively.
Table 2: Antidepressive medication

*N/A: Not available

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender</th>
<th>Antidepressant</th>
<th>Mean daily dose</th>
<th>Treatment period</th>
<th>Discontinuation before PET scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>Citalopram</td>
<td>N/A*</td>
<td>14 days</td>
<td>2 years</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>Citalopram</td>
<td>20 mg</td>
<td>1 day</td>
<td>13 days</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>Citalopram</td>
<td>40 mg</td>
<td>60 days</td>
<td>Current</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>Citalopram</td>
<td>10 mg</td>
<td>12 days</td>
<td>5 days</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>Sertraline</td>
<td>40 mg</td>
<td>28 days</td>
<td>14 days</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>Fluoxetine</td>
<td>40 mg</td>
<td>6 years</td>
<td>Current</td>
</tr>
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</table>

Table 3: PET-related data.
<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Patient (mean)</th>
<th>SEM</th>
<th>Control (mean)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
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<tr>
<td><strong>MEMORY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SWM strategy</td>
<td>30.57</td>
<td>1.13</td>
<td>26.48</td>
<td>1.0</td>
<td>0.009</td>
</tr>
<tr>
<td>SWM total errors</td>
<td>19.07</td>
<td>3.15</td>
<td>10.10</td>
<td>1.83</td>
<td>0.016</td>
</tr>
<tr>
<td>SWM between errors</td>
<td>18.71</td>
<td>3.11</td>
<td>9.73</td>
<td>1.80</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>EXECUTIVE FUNCTIONS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOC problems solved in minimum moves</td>
<td>9.32</td>
<td>.32</td>
<td>9.20</td>
<td>.38</td>
<td>NS. (0.80)</td>
</tr>
<tr>
<td>SOC mean number of moves</td>
<td>4.15</td>
<td>.09</td>
<td>4.13</td>
<td>.088</td>
<td>NS. (0.91)</td>
</tr>
<tr>
<td>IED total errors</td>
<td>14.52</td>
<td>2.16</td>
<td>9.86</td>
<td>.50</td>
<td>0.045</td>
</tr>
<tr>
<td>IED completed stage errors</td>
<td>12.14</td>
<td>1.35</td>
<td>9.86</td>
<td>.49</td>
<td>NS. (0.12)</td>
</tr>
<tr>
<td>IED EDS errors</td>
<td>6.04</td>
<td>1.35</td>
<td>2.07</td>
<td>.23</td>
<td>0.007</td>
</tr>
<tr>
<td>IED total number of trials on all stages attempted</td>
<td>77.44</td>
<td>3.63</td>
<td>68.55</td>
<td>1.19</td>
<td>0.026</td>
</tr>
<tr>
<td><strong>ATTENTION</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVP A’</td>
<td>.985</td>
<td>.0026</td>
<td>.989</td>
<td>.0017</td>
<td>NS. (0.15)</td>
</tr>
<tr>
<td>RVP total hits 3-5-7</td>
<td>69.89</td>
<td>.76</td>
<td>71.16</td>
<td>.46</td>
<td>NS. (0.15)</td>
</tr>
<tr>
<td>RVP total misses 3-5-7</td>
<td>4.07</td>
<td>.73</td>
<td>2.83</td>
<td>.46</td>
<td>NS. (0.16)</td>
</tr>
</tbody>
</table>

Table 4: Neurocognitive performance of memory, executive functions and attention of schizophrenic patients compared with healthy controls.
Archival Report

Serotonin 2A receptor blockade and clinical effect in first-episode schizophrenia patients treated with quetiapine

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Abstract

Objective: There is converging evidence that the serotonin 2A receptor (5-HT2AR) is an important therapeutic target in schizophrenia. Until now no longitudinal studies of 5-HT2AR in first-episode antipsychotic-naïve schizophrenia patients have reported on the relationship between 5-HT2AR occupancy and treatment effect sustained treatment with an atypical antipsychotic compound. The objective was to measure 5-HT2AR occupancy in these patients after 6 months of quetiapine treatment and to explore the relationship with plasma levels of quetiapine and its active metabolite nor-quetiapine, dose, and treatment effect.

Method: In fifteen antipsychotic-naïve schizophrenia patients the 5-HT2AR binding before and after 6 months of quetiapine treatment was measured with [18F]altanserin positron emission tomography (PET). Treatment effect was defined as the difference between the PANSS scores at baseline and follow-up.

Results: The data revealed a modest effect on positive symptoms up until a 5-HT2AR occupancy level of approximately 60 %, after which a considerable increase in efficacy was found. The mean dose of quetiapine was 383 mg in the present study, corresponding to a 5-HT2AR occupancy of 64 %. This occupancy level was in the middle range of 60-70 % where we found quetiapine to exert the highest reduction in positive symptoms.

Conclusions: This study suggests a therapeutic role of 5-HT2AR for treatment of positive symptoms and suggests that measurements of plasma drug concentrations provide guidance in terms of dosing and 5-HT2AR occupancy. The data further indicate that in first-episode schizophrenia patients, low-dose quetiapine treatment is recommendable.
Background

Increasing evidence points towards a role of the serotonin 2A receptor (5-HT$_{2A}$R) in schizophrenia. Eleven out of fifteen published post-mortem studies report that 5-HT$_{2A}$R binding is decreased in cortical, and especially frontal cortical brain regions (see(1) for references). These findings are supported in vivo by our recent PET study in 30 first-episode antipsychotic-naïve schizophrenia patients, reporting a decreased 5-HT$_2$R binding in the frontal cortex(2).

Indirect support for the involvement of the 5-HT$_{2A}$R in schizophrenia comes from the association between the receptor affinity profile and the clinical characteristics of second generation antipsychotic drugs (SGAs). In contrast to first generation antipsychotic drugs (FGAs), that primarily are dopamine 2 receptor (D$_2$R) antagonists, most SGAs have higher affinity to 5-HT$_{2A}$R than to D$_2$R(3). It has been suggested that 5-HT$_2$R blockade inhibits phasic increases in dopamine synthesis and release in the striatum(4). In this way, 5-HT$_2$R antagonism can potentiate D$_2$R antagonism and facilitate a reduction in positive psychotic symptoms by closure of the striato-thalamic filter(4). This mechanism may also account for the reduced extrapyramidal side effects (EPS) induced by SGAs, and contribute to their effect on positive and negative psychotic symptoms(5).

In vivo studies of the 5-HT$_{2A}$R in first-episode antipsychotic-naïve schizophrenia patients treated with the same SGA over a long period are absent. In this study, quetiapine was chosen because of its high affinity for the 5-HT$_{2A}$R and modest affinity and a fast
dissociation rate ($k_{off}$) for the $D_2R$. Clozapine has a rather similar profile\(^6\), however according to clinical guidelines, clozapine is not recommended as a first choice in first-episode schizophrenia\(^7\). Quetiapine produces two metabolites 7-hydroxy-quetiapine and nor-quetiapine which are pharmacologically active on the 5-HT\(_{2A}\)R\(^8\). Nor-quetiapine also has norepinephrine transporter (NET) antagonist and partial 5-HT\(_{1A}\) agonist properties\(^9\)-\(^11\), which may be the reason why quetiapine is used for treatment of bipolar depression and might relieve depressive symptoms in schizophrenia.

Few studies have investigated the relationship between plasma quetiapine concentrations and clinical outcome. Within a treatment period of 6 weeks or less, no clear association between quetiapine plasma concentration and clinical response was found, and no optimal therapeutic range for quetiapine was identified\(^12\),\(^13\). A recent review suggests that measurements of plasma quetiapine concentrations provide poor guidance in terms of dosing\(^8\). One study using \(^{[18}F\)setoperone PET found a curvilinear hyperbolic relation between 5-HT\(_{2A}\)R occupancy and plasma quetiapine concentration\(^14\). In 12 chronic and previously medicated schizophrenic patients a quetiapine dose between 300 and 600 mg/day resulted in a 5-HT\(_{2A}\)R occupancy between 57% and 78%. These results might have been confounded by disease progress and effects of previous medication.

In the present study we aimed in antipsychotic-naïve first-episode schizophrenia patients to examine the relation between 5-HT\(_{2A}\)R occupancy and clinical effect after 6 months of sustained treatment with quetiapine. Cerebral 5-HT\(_{2A}\)R occupancy as measured with \(^{[18}F\)altanserin-PET, was related to plasma levels of quetiapine and its metabolite nor-
quetiapine, dose, and clinical effect. We expected to find a relationship between 5-HT$_{2A}$R occupancy and treatment effect on positive symptoms. Moreover, we hypothesized to find a relationship between levels of nor-quetiapine and treatment effect on depression scores as assessed with the PANSS-D cluster.

**Methods and Materials**

The study was approved by the Ethics Committee of Copenhagen and Frederiksberg ((KF)11-061/03, (KF)12 291906 and (KF) 11-323091). After complete description of the study to the subjects, written informed consent was obtained.

**Participants**

Thirty antipsychotic-naïve patients (7 female) diagnosed with schizophrenia according to both ICD-10 and DSM-IV were recruited after voluntary first-time referral to a psychiatric unit of one of the affiliated university hospitals in the Capital Region of Copenhagen. The patients were identical to those included in a previously published PET study on cerebral 5-HT$_{2A}$R binding in the antipsychotic-naïve state(2) The schizophrenia diagnoses were verified by means of the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1) interview(15). None of the patients had a history of significant head injury or non-psychiatric disorder. All of the patients had normal neurological and physical examinations, and structural magnetic resonance imaging (MRI) brain scans were without abnormalities.
In the period between baseline and follow-up 15 patients dropped out because of intolerable side effects, lack of efficacy, non-compliance or unwillingness to be re-scanned, resulting in 15 patients (5 females, mean age: 28.9 years, SD=5.4) completing the study.

Of the 15 patients participating in the follow-up 4 patients were diagnosed as having a history of substance abuse according to DSM-IV: alcohol abuse, in sustained full remission (n=2); cannabis abuse, sustained full remission (n=1), other abuse, sustained full remission and other abuse, early partial remission (n=1). The diagnosis ‘other abuse’ covered mixed cannabis and alcohol abuse. During the treatment period none of the 15 patients had any substance abuse as determined by regular clinical contacts. All subjects had a negative urine screening for substance intake prior to the PET scans. At follow-up 2 patients were treated with the selective serotonin reuptake inhibitors (SSRIs) fluoxetine (n=1) and citalopram (n=1) in stable doses (40 mg/day for both compounds), throughout the investigation period. Thirteen patients had no lifetime history of antidepressant exposure.

**Experimental design**

All 15 subjects were tested twice: once at inclusion and once after a period of as close to 6 months as possible (mean=6.8 months, SD=0.9). During that period, patients were treated with quetiapine in flexible doses according to their clinical condition (average dose: 383 ±145 mg per day or 5.2±2.2 mg/kg bodyweight per day). Concomitant treatment with benzodiazepines was allowed on an “if needed basis”, except on the test
days. Nine patients were smokers. Smoking was not allowed up until 2 hours before the PET radioligand administration.

While at baseline patients received no antipsychotic treatment, at follow-up they received their normal daily quetiapine dose 165 minutes prior to the PET scan. This time period was based on a pilot PET study on one healthy control subject: the participant was given one dose of 100 mg of quetiapine, and the time interval between quetiapine administration and maximum [$^{18}$F]altanserin displacement was determined as 165 minutes (see figure 1).

**Psychopathological ratings**

Symptom severity was assessed at the time of both PET scans by trained raters using the Positive and Negative Syndrome Scale (PANSS)(16). An intra-class correlation coefficient of 0.85 was achieved(2). The depression cluster (PANSS-D) of the PANSS scale (items: somatic concern (G1), anxiety (G2), guilt feelings (G3) and depression (G6))(17,18) was used to examine the relationship between nor-quetiapine plasma concentration and the level of depressive symptoms. The PANSS-D has been found to strongly correlate with other scales specifically designed to measure depressive symptoms(19).

**Imaging**

The 5-HT$_2$A binding was imaged with [$^{18}$F]altanserin according to a method described previously(20). In short: after bolus-infusion of the tracer, emission scans (five frames of 8 min each) were acquired in tracer steady state conditions with an 18-ring GE-Advance
tomograph (GE, Milwaukee, Wisconsin) operating in three-dimensional acquisition mode. The total axial field of view was 15.2 cm with an approximate in-plane resolution of 6 mm. After 2 hours, when steady state had been obtained, the fraction of unmetabolized tracer in venous plasma was determined at three time points with high-performance liquid chromatography analysis. Reconstruction, including attenuation correction and scatter correction, is described in detail previously(20). Subjects received a maximum dose of 3.7 MBq/kg bodyweight [18F]altanserin. High-resolution 3D T1-weighted, sagittal, magnetization-prepared rapid-gradient echo (MPRAGE) scans of the whole head were acquired in all subjects on a 3 tesla TRIO scanner (Siemens, Erlangen, Germany)

**MR/PET co-registration**

The five PET image frames for each subject were aligned using the AIR program(21) and subsequently a mean PET image was calculated. The mean PET images and 3D T1-weighted MRI scans were co-registered using a Matlab (Mathworks Inc., Natick, MA, USA)-based program(22), where PET images and MRIs were fitted through manual translation and rotation of the PET image with subsequent visual inspection in three planes(23).

**Volumes of interest**

Volumes of interest (VOIs) covering the whole brain included (left and right): orbitofrontal, medial inferior frontal, superior frontal, anterior cingulate, posterior cingulate, entorhinal, occipital, parietal, sensorimotor, medial inferior temporal and
superior temporal cortex, amygdala, caudate nucleus, hippocampus, hypothalamus, insula, putamen, and thalamus. The cerebellum was used for determination of non-specific binding.

The VOIs were automatically delineated on each individual's transaxial MRI slices in a strictly user-independent manner(24). This approach allowed automatic co-registration of a template set of 10 MRIs to a new subject's MRI. The identified transformation parameters were used to define VOIs in the new subject MRI space, and through the co-registration these VOIs were transferred onto the PET images. The cerebellum was used for determination of non-specific binding.

**Blood samples**

For quetiapine and nor-quetiapine plasma concentration measurements, five 7 mL venous blood samples were drawn during the scanning and analyzed according to a previously described method(25).

**Quantification of 5-HT$_{2A}$ occupancy**

The distribution volume ($V_T$) of a radioligand is defined as the ratio of the radioligand concentration in tissue target region ($C_T$, kBq·cm$^{-3}$) to that in plasma ($C_P$, kBq·mL$^{-1}$) at equilibrium(26). $C_P$ represents the concentration of parent radioligand in plasma.

$$V_T = \frac{C_T}{C_P} \quad (1)$$

A global measure of 5-HT$_{2A}$R occupancy ($O$) was calculated from the distribution volumes in the unblocked ($V_T$) condition and in the partially blocked condition ($V_{T,b}$).
where \( V_{\text{ND}} \) is the distribution volume of the nondisplaceable tracer, i.e., the free and non-specifically bound tracer. Rearrangement of equation 2 leads to:

\[
V_{T,b} = (1-O) V_T + O \ V_{\text{ND}} \quad (3)
\]

By inserting corresponding values for each measured brain region in the unblocked and partially blocked condition, an occupancy plot (figure 2) can be made for each individual, and hence, an estimate of the global occupancy can be determined in each individual using linear regression analysis(27).

A one site binding hyperbola model (14) was used to evaluate the relationship between 5-HT\(_{2A}\)R occupancy and the corresponding plasma quetiapine concentration and dose using the following equation:

\[
O = \frac{E_{\text{max}} \cdot X}{\text{EC}_{50} + X} \quad (4)
\]

where \( E_{\text{max}} \) is the maximum receptor occupancy (100%), \( X \) = quetiapine plasma concentration (ng/mL) or dose (mg) and \( \text{EC}_{50} \) is the estimated quetiapine plasma concentration (ng/mL) or dose (mg) associated with 50 % maximal receptor occupancy.
Michaelis-Menten kinetics was applied to fit the relation between quetiapine and nor-quetiapine plasma concentration, from which the maximal velocity ($V_{\text{max}}$) and the constant ($K_m$) for the conversion of quetiapine into nor-quetiapine could be determined. Since the metabolism may be far from saturation, a linear fit was also tested, and the goodness of fit used to assess if the metabolism was rate limited.

**Statistics**

Linear regression analysis was used to calculate global 5-HT$_2$A R occupancies. Differences in PANSS scores between baseline and follow-up were examined with paired samples t-tests. Regression analysis was used to examine the extent to which global 5-HT$_2$A R occupancy was associated with treatment effects. The latter were calculated as the difference in PANSS scores between baseline and follow-up. All analyses were performed with SPSS®. $P=0.05$ (two-sided) was employed as the level of significance for all tests. Curvefitting was performed using GraphPad Prism®.

**Results**

**5-HT$_2$A R occupancy**

The equation of the one site binding hyperbola that was used to fit 5-HT$_2$A R occupancy and quetiapine plasma concentration revealed an $EC_{50}$ value of 201.7 ng/mL, with a 95 % confidence interval of 147.2 to 256.3 ng/mL, $r^2=0.68$ (see figure 4A).

The mean dose of quetiapine was 383 mg (range 100-600 mg) corresponding to a 5-HT$_2$A R occupancy of 64 % and a plasma concentration of 352 ng/mL (range 74-735 ng/mL) (see also figure 3). Similarly, the equation that was used to fit 5-HT$_2$A R
occupancy and quetiapine dose resulted in a EC\textsubscript{50} value of 231 mg with a 95 % confidence interval of 170 to 293 mg, $r^2=0.67$ (see figure 4B).

**5-HT\textsubscript{2A}R occupancy and psychopathology**

The mean PANSS score of positive symptoms was significantly reduced from 19.5 (SD=5.4) to 15.7 (SD=6.6), $t=3.5$, df=13, $p<0.01$ after quetiapine treatment. There were non-significant reductions in PANSS negative (from 20.3 (SD=6.1) to 18.4 (SD=6.5), $p=0.37$), general (from 38.0 (SD=8.7) to 33.0 (SD=11.1), $p=0.11$) and total scores (from 77.8 (SD=17.1) to 67.0 (SD=22.8), $p=0.07$).

A significant nonlinear (logarithmic) relationship was found between 5-HT\textsubscript{2A}R occupancy and treatment effect on positive symptoms. ($r^2=0.75$, $p<0.001$) (figure 4C). There was a modest effect on positive symptoms up until a 5-HT\textsubscript{2A}R occupancy level of approximately 60 %, after which a considerable increase in efficacy was found (between 60 and 70 %). No significant relationship was found between 5-HT\textsubscript{2A}R occupancy and treatment effect on the other PANSS subscales.

**Nor-quetiapine**

Michaelis-Menten kinetics applied to quetiapine and nor-quetiapine plasma concentration revealed a $V_{\text{max}}$ of 384.7 and a $K_m$ value of 396.6, with a 95 % confidence interval of 0.0 to 954.4, $r^2=0.59$ (see figure 4D). Assuming a linear relationship within the dose range did not improve the goodness of fit ($r^2=0.53$). The relation between 5-HT\textsubscript{2A}R occupancy
and the combined quetiapine plus nor-quetiapine plasma concentration adjusted for their different affinities to the 5-HT$_{2A}$R ($K_i$$_{quetiapine}$=38, $K_i$$_{nor-quetiapine}$=2.93(10)) was also plotted. Using an affinity weighted combined plasma concentration did not improve the goodness of fit ($r^2$ =0.68 vs. 0.66). Plasma concentrations of quetiapine and nor-quetiapine did not correlate significantly with treatment effect on the PANSS-D.

**Discussion**

In this study we found a one site binding hyperbolic relationship between 5-HT$_{2A}$R occupancy, quetiapine dose and plasma concentration. The data revealed a modest effect on positive psychotic symptoms up until approximately 60 % 5-HT$_{2A}$R occupancy after which a considerable increase in efficacy was found. The mean dose of quetiapine of 383 mg corresponded to a plasma concentration of 352 ng/mL and a 5-HT$_{2A}$R occupancy of 64 %. This occupancy level is in the middle range between 60 and 70 % where we found quetiapine to exert the highest reduction in the positive symptoms. The mean dose is in the lower limit of the recommended dose-range of quetiapine (300-800 mg). However, it has been suggested that in some patients, higher than recommended dosages are required for full therapeutic effect (28-30). The present data support the conclusion of a recent meta-analysis(31) on dose and clinical response of quetiapine, suggesting an optimal dose of 300-400 mg/day, and is not in accordance with ‘a high-dose theory’ of quetiapine(28,29), at least not in first-episode patients.

Furthermore, contrary to a recent review(8) the data suggest that measurements of plasma quetiapine concentrations can provide guidance in terms of dosing and also 5-HT$_{2A}$R receptor occupancy. Plasma concentration measurements would be of particular
relevance in cases where pharmacokinetics are likely to be altered, e.g. in children(32), the elderly(33,34), in patients with renal or hepatic impairment(35) and in patients concomitantly treated with compounds that affect the enzyme CYP3A4(33,36) by which quetiapine is predominately metabolized. Furthermore, in cases of non-response and adverse effects plasma monitoring seems appropriate.

Using an affinity weighted combined plasma concentration did not improve the goodness of fit, implying that additional measurements of nor-quetiapine plasma levels are clinically irrelevant. Finally, we did not find a significant relationship between treatment effect on PANSS-D and quetiapine or nor-quetiapine plasma concentration. As such, our data do not support recent reports on efficacy of quetiapine or nor-quetiapine on depressive symptoms in schizophrenia(9-11).

The involvement of the D2R in the present findings should be considered. In a $[^{11}\text{C}]$raclopride and $[^{11}\text{C}]$N-methylspiperone PET study by Gefvert et al.(37) it was found that two hours after the last quetiapine dose of 450 mg the D2R occupancy was 44 % (range 21-68) in the putamen and caudate nucleus while 5-HT$_2$ receptor occupancy in the frontal cortex was 72 % (range 58-82). In the present study, quetiapine was administered 165 minutes before the PET scan. Considering the results of Gefvert et al. it can be reasoned that the D$_2$ occupancy at 165 minutes is relatively lower than 5-HT$_2$AR occupancy. It can be argued that the reported D$_2$ receptor occupancy reported by Gefvert et al.(37) of 44 % might be overestimated since previous long term treatment with antipsychotics has been shown to increase the number of D$_2$ receptors of around 30 % as a result of receptor induction(38). In our sample the mean quetiapine dose gave rise to a
5-HT$_2A$R occupancy of 64 $\%$, suggesting that the D$_2$ occupancy is well below the traditionally proposed therapeutic window of 65-70 $\%$ for a D$_2$ mediated effect(3,39,40). In the present study we found that 5-HT$_2A$R occupancy was associated with treatment effect on positive symptoms. Based on our data, however, it is not possible to directly determine the role of the D$_2$R on the present findings, since we did not assess this receptor. For that reason, we cannot make any definite conclusions with regard to a direct or an indirect causal association between 5-HT$_2A$R and psychopathology; i.e. whether the association is a direct result of blockade of 5-HT$_2A$R, or is caused by e.g. a decreased subcortical and increased prefrontal dopamine release induced by 5-HT$_2A$R blockade(4) or related to the affinity of quetiapine for other receptor systems. Importantly, it has also been suggested by Kapur et al.(14) that transiently high D$_2$ occupancy may be sufficient for the antipsychotic effect of quetiapine, thereby questioning the assumption that continuously high D$_2$ occupancy is required for response.

Only a few PET studies have reported on 5-HT$_2A$R occupancy after quetiapine treatment(14,37,41). In a [${}^{[11]}$C]N-methylspiperone study of 5 chronic and previously medicated schizophrenia patients 5-HT$_2A$R receptor occupancies were determined as 74 and 57$\%$ at quetiapine doses of 750 and 450 mg/day respectively, with PET scanning performed 2 hours post administration(41). 750 mg is beyond the dose range of the present study; however we found that a quetiapine dose of 450 mg resulted in a 5-HT$_2A$R occupancy of 67 $\%$ with PET scanning performed 165 minutes post administration.
In a $[^{18}F]$setoperone PET study by Kapur et al.(14) it was shown in 12 patients that 300 to 600 mg/day of quetiapine occupies 57% to 78% of 5-HT$_2\text{A}$R. In our study 300 and 600 mg of quetiapine gave rise to comparable occupancies of respectively 56 % and 70%.

However, the studies are not readily comparable because of a number of methodological issues. For example in the present study only first-episode antipsychotic-naïve patients were included at baseline, whereas previous studies(14,37,41) included patients who were chronically ill and previously medicated with both typical and atypical compounds before their shift to quetiapine. Previous treatment with antipsychotics that antagonize the 5-HT$_2\text{A}$R has been shown to induce a paradoxical down-regulation of the receptor, both \textit{in vivo} and \textit{in vitro}(42,43). Furthermore the tracers $[^{18}F]$setoperone and $[^{11}C]$N-methylspiperone used in previous studies(14,37,41) are limited by their relatively poor selectivity for the 5-HT$_2\text{A}$R. In comparison $[^{18}F]$altanserin has a 200 to 500-fold 5-HT$_2\text{A}$/D$_2$ selectivity(44,45) making it between 8 and 50 times more selective for the 5-HT$_2\text{A}$R than $[^{18}F]$setoperone which has a 10–25-fold 5-HT$_2\text{A}$/D$_2$ selectivity(46). In addition, the affinity of $[^{18}F]$altanserin for the 5-HT$_2\text{A}$R is at least 20-fold higher than for other 5-HT subtypes(45). We have previously demonstrated that $[^{18}F]$altanserin PET with a bolus infusion design is a highly reproducible method for reliable quantification of 5-HT$_2\text{A}$R(47)

There are some limitations in the present study that need to be addressed. At follow-up we only obtained a binding measure in a medicated state. Therefore we cannot make direct inferences regarding a potential paradoxical down-regulation of 5-
HT_2A_R caused by the quetiapine treatment(42,43) which might lead to an overestimation of occupancy. Ideally, two scans should have been performed at follow up, one in the medicated state and one at a quetiapine plasma level of zero after discontinuation. However, for obvious ethical and logistical reasons this was not performed.

Two out of the 15 patients had SSRIs during the treatment period. However the effect of chronic SSRI treatment on 5-HT_2A_R density is unclear, since different SSRIs show different effects on 5-HT_2A_R. Fluoxetine has been reported to have either no effect or to increase 5-HT_2A_R density or to increase receptor number(43). In contrast, chronic citalopram treatment down-regulates 5-HT_2A_R(43). However, we have previously found that [^{18}F]altanserin binding to 5-HT_2A_R is insensitive to an acute citalopram challenge increasing extracellular 5-HT(48). For these reasons, we performed a post hoc analysis, where we excluded the two patients on SSRI treatment from the analyses. This did not change the curve fits significantly.

In conclusion, the present study supports a therapeutic role of the 5-HT_2A_R for treatment of positive symptoms in schizophrenia either directly or indirectly via interactions with the dopaminergic system or other receptor systems. Furthermore, the study suggests that measurements of plasma quetiapine concentrations can provide guidance in terms of both dosing and 5-HT_2A_R blockade and that low doses of quetiapine in first-episode schizophrenia patients is recommendable.
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Figure 1: Pilot study, showing the displacement of $[^{18}\text{F}]$altanserin by quetiapine in a healthy control subject
Figure 2: Occupancy plot in one of the patients showing paired (left and right) distribution volumes of the VOIs in the unblocked ($V_T$) and partially blocked situation ($V_{T,b}$). Regression line: $Y=0.6034x + 0.7499$, $r^2=0.9677$, 5-HT$_{2A}$ receptor occupancy=40%
Figure 3: $^{[18\text{F}]}$altanserin PET images of two axial brain slices illustrating 5-HT$_{2\text{A}}$ receptor binding in one of the male schizophrenic patients before (top) and after approximately 6 months of treatment with 300 mg/day quetiapine (bottom). 5-HT$_{2\text{A}}$ receptor occupancy=57% (165 min post quetiapine administration)
Figure 4: A The relationship between 5-HT$_{2A}$ receptor occupancy and quetiapine plasma concentration (ng/mL). The curve has been fit to the following equation occupancy = 100(plasma concentration/(plasma concentration + 202 ng/mL)), where 201.7 ng/mL is the level of 50% occupancy and the 95% confidence interval for this constant is 147 to 256 ng/mL, $r^2$=0.68. B The relationship between 5-HT$_{2A}$ receptor occupancy and quetiapine dose (mg). The curve has been fit to the following equation occupancy = 100(dose/(dose + 231 mg)), where the 231 mg is the level of 50% occupancy and the 95% confidence interval for this constant is 170 to 293 mg, $r^2$=0.67. C The nonlinear (logarithmic) relationship between 5-HT$_{2A}$ receptor occupancy and treatment effect on positive PANSS scores ($r^2$=0.75, P<0.001). D Quetiapine plasma concentration (ng/mL) and nor-quetiapine plasma concentration (ng/mL) fitted to Michaelis Menten kinetics, $V_{\text{max}}$=385, $K_m$=397, with a 95% confidence interval of 0.0 to 954, $r^2$=0.59.