PhD Thesis

Frontal Dopamine $D_{2/3}$ Receptors and Brain Structure in Antipsychotic-Naïve Schizophrenia Patients Before and After Their First Antipsychotic Treatment

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Content

Preface

The present PhD study was carried out at Center for Neuropsychiatric Schizophrenia Research, CNSR & Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research, CINS Mental Health Centre Glostrup, University of Copenhagen.

Examinations were carried out at:

- CNSR, Copenhagen University Hospital, Psychiatric Center Bispebjerg (now Mental Health Centre Copenhagen)
- Danish Research Centre for Magnetic resonance (DRCMR), MR-department, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark
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List of Publications

This thesis is based on the following two manuscripts:

Paper One

Frontal $D_{2/3}$ receptor availability in schizophrenia patients before and after their first antipsychotic treatment: Relation to cognitive functions and psychopathology. Henrik Nørbak-Emig, Bjørn H. Ebdrup, Birgitte Fagerlund, Claus Svarer, Hans Rasmussen, Lars Friberg, Peter N. Allerup, Egill Rostrup, Lars H. Pinborg, Birte Y Glenthøj. Int J Neuropsychopharmacol. 2016 Jan 27. pii: pyw006. doi: 10.1093/ijnp/pyw006

Paper Two

Extrastriatal dopamine $D_{2/3}$ receptors and cortical grey matter volumes in antipsychotic-naïve schizophrenia patients before and after initial antipsychotic treatment. Henrik Nørbak-Emig, Lars Pinborg, Jayachandra M. Raghava, Claus Svarer, William Baaré, Peter Allerup, Lars Friberg, Egill Rostrup, Birte Glenthøj, Bjørn H. Ebdrup. In review

Summary

For decades, the dopamine system has been in focus in neurobiological schizophrenia research since blockade of striatal dopamine D_2 receptors has shown antipsychotic effect. Also, there is strong evidence that schizophrenia is associated with structural brain volume changes though the pathophysiological and pathogenetic underpinnings are still unresolved. In recent years, antipsychotics have been related to grey matter changes with long-term studies finding brain volume loss to be associated with exposure to antipsychotic medication. Currently, all commercialized antipsychotics block dopamine D_2 receptors.

The overall objective of this thesis was to investigate effects of dopamine D_2 receptor blockade in a group of initially antipsychotic-naïve patients with first-episode schizophrenia. More specifically, the objectives were to examine the effects of dopamine D_2 receptor blockade on cortical grey matter volume as well as cognitive measures of attention, processing speed and executive functions in patients who had never previously been treated with antipsychotic compounds. In addition, we wished to examine whether baseline frontal D_2 binding potential (BP_{ND}) values can be used to predict outcome with regard to positive symptoms and whether grey matter volume reductions at follow-up would be associated with D_2 receptor blockade and negative symptoms.

In the PhD thesis, structural magnetic resonance (MRI) and single photon emission computed tomography (SPECT) scans were used to examine effects of the first antipsychotic treatment in patients with first-episode schizophrenia. The participants were also examined with a selected number of tests from CANTAB (Cambridge Neuropsychological Test Automated Battery) and patients had their psychopathology assessed with PANSS (Positive and Negative Syndrome Scale).

In *Study 1* we used MRI and SPECT scans with the dopamine $D_{2/3}$ receptor ligand [^{123}I]epidepride to assess brain structure and dopamine $D_{2/3}$ receptor BP_{ND} in extrastriatal regions. Cognitive functions were assessed with selected tests from CANTAB and psychopathology with PANSS. The subjects were 25 antipsychotic-naïve first-episode schizophrenia patients and matched healthy controls. After three months of treatment with either risperidone or zuclopenthixol, twenty-two patients were re-examined. The aim was to relate extrastriatal dopamine $D_{2/3}$ receptor blockade to cognitive functions. Moreover, we wished to explore if frontal BP_{ND} at baseline was associated with the reduction in positive psychotic symptoms after the patients' first treatment with an antipsychotic compound.

We found that blockade of extrastriatal dopamine $D_{2/3}$ receptors was correlated with decreased attentional focus and planning time. Moreover, baseline frontal dopamine $D_{2/3}$ BP_{ND} and positive symptom reduction correlated positively. Our data supported a negative influence of $D_{2/3}$ receptor blockade on specific cognitive functions in schizophrenia. This is highly clinically relevant given the well-established association between severity of cognitive disturbances and a poor functional outcome in schizophrenia. Additionally, the findings supported associations between frontal $D_{2/3}$ receptor BP_{ND} at baseline and the effect of antipsychotic treatment on positive symptoms.

In S*tudy 2* we also used MRI and SPECT scans with the dopamine $D_{2/3}$ receptor ligand [^{123}I]epidepride. Here, 22 patients and 20 healthy controls from the same cohort were examined at

baseline. Further, patients had their psychopathology assessed using PANSS. After three months of treatment with either risperidone or zuclopenthixol, 20 patients were re-examined. The aim was to investigate associations between extrastriatal dopamine $D_{2/3}$ receptor $BP_{ND,}$ blockade and grey matter volume changes before and after three months of antipsychotic treatment. There was no significant overall change in brain volumes over time, and neither extrastriatal $D_{2/3}$ receptor BP_{ND} at baseline nor blockade at follow-up was related to regional cortical volume changes. However, after excluding three patients with cannabis abuse higher $D_{2/3}$ receptor blockade was significantly associated with a relative grey matter volume increase in right frontal cortex. Moreover, higher risperidone dose was associated with a relative increase in frontal grey matter volume after three months.

Our results do not support an association between extrastriatal $D_{2/3}$ receptor blockade and extrastriatal grey matter loss, at least not in the early phases of schizophrenia. If anything, they suggest a relation between $D_{2/3}$ receptor blockade and a subtle frontal volume increase.

Dansk titel (Danish Title)

Frontale dopamin $D_{2/3}$ receptorer og hjernestruktur hos antipsykotika-naïve patienter med skizofreni før og efter deres første behandling med antipsykotika

Dansk Resumé (Danish Summary)

I årtier har dopaminsystemet været i fokus i neurobiologisk skizofreniforskning, da blokade af striatale dopamin D_2 receptorer har antipsykotisk virkning. Der er også stærk evidens for, at skizofreni er forbundet med strukturelle ændringer i hjernevolumen, selvom de patofysiologiske og patogenetiske årsager hertil er ukendte. I de senere år har antipsykotika været relateret til grå substans ændringer, idet forløbsundersøgelser har fundet, at tab af hjernevolumen var associeret med udsættelse for antipsykotisk medicin. For nuværende blokerer alle markedsførte antipsykotika dopamin D_2 receptorer.

Det overordnede formål med denne afhandling var at undersøge effekten af dopamin D_2 receptor blokade i en gruppe af initielt antipsykotisk-naive patienter med debuterende skizofreni. Mere specifikt var formålet at undersøge effekten af blokade af dopamin D_2 receptorer på grå substans volumen i kortex såvel som kognitive mål for opmærksomhed, forarbejdningshastighed og eksekutive funktioner i patienter, der ikke tidligere havde været behandlet med antipsykotika. Derudover ønskede vi at undersøge, om højere frontale D_2 bindingspotentiale (BP_{ND}) værdier ved baseline kunne forudsige behandlingseffekt med hensyn til positive symptomer, og om reduktion i grå substans volumen ved follow-up ville være forbundet med D_2 receptor blokade og negative symptomer.

I Ph.d. afhandlingen blev strukturelle magnetiske resonans (MRI) og single photon emission computed tomography (SPECT) scanninger anvendt til at undersøge virkningerne af den første antipsykotiske behandling hos patienter med første episode skizofreni. Deltagerne blev også undersøgt med en række udvalgte prøver fra CANTAB (Cambridge Neuropsychological Test Automated Battery) og patienter fik vurderet deres psykopatologi ved hjælp af PANSS (Positive and Negative Syndrome Scale).

I *studium 1* anvendte vi MRI og SPECT scanninger med dopamin $D_{2/3}$ receptor liganden [123I] epidepride til at undersøge hjernens struktur og dopamin $D_{2/3}$ BP_{ND} i ekstrastriatale regioner. Kognitive funktioner blev undersøgt med udvalgte tests fra CANTAB og psykopatologi ved hjælp af PANSS. Deltagerne var 25 antipsykotiske-naive første-episode patienter med skizofreni og matchede kontrolpersoner. Efter tre måneders behandling med enten risperidon eller zuclopenthixol blev 22 patienter genundersøgt. Formålet var at relatere ekstrastriatal dopamin $D_{2/3}$-receptor blokade til kognitive funktioner. Desuden ønskede vi at undersøge, om BP_{ND} ved baseline var associeret med reduktionen i positive psykotiske symptomer efter patienternes første behandling med et antipsykotisk præparat.

Vi fandt, at blokade af ekstrastriatale dopamin $D_{2/3}$-receptorer var korreleret med nedsat opmærksomhed og planlægning. Desuden var baseline frontal dopamin $D_{2/3}$ BP_{ND} positivt

korreleret med reduktionen i positive symptomer. Vores data støttede, at $D_{2/3}$-receptor blokade havde en negativ påvirkning på specifikke kognitive funktioner hos patienter med skizofreni. Dette er klinisk yderst relevant i betragtning af den veletablerede sammenhæng mellem sværhedsgraden af kognitive forstyrrelser og dårligt funktionsniveau hos patienter med skizofreni. Derudover støttede resultaterne en sammenhæng mellem frontal $D_{2/3}$ receptor BP_{ND} ved baseline og effekten af antipsykotisk behandling på positive symptomer.

I *studium 2* anvendte vi også MRI og SPECT scanninger med dopamin $D_{2/3}$ receptor liganden [123I] epidepride. Her blev 22 patienter og 20 raske kontroller fra samme kohorte undersøgt ved baseline. Endvidere fik patienterne vurderet deres psykopatologi med PANSS. Efter tre måneders behandling med enten risperidon eller Zuclopenthixo blev 20 patienter genundersøgt. Formålet var at undersøge sammenhængen mellem extrastriatal dopamin $D_{2/3}$ receptor BP_{ND}, blokade og volumenændringer i grå substans før og efter tre måneders antipsykotisk behandling. Der var overordnet set ingen signifikant ændring i hjernevolumen over tid og hverken extrastriatal $D_{2/3}$ receptor BP_{ND} ved baseline, eller blokade ved follow-up, var relateret til regionale kortikale volumenændringer. Efter eksklusion af tre patienter med cannabis misbrug var højere $D_{2/3}$-receptor blokade imidlertid signifikant associeret med en relativ stigning i grå substans volumen i højre frontal kortex. Derudover var højere risperidon dosis forbundet med en relativ stigning i grå substans volumen frontalt efter tre måneder.

Vores resultater støtter ikke en sammenhæng mellem ekstrastriatal $D_{2/3}$-receptor blokade og tab af ekstrastriatal grå substans, i det mindste ikke i de tidlige faser af skizofreni. Hvis noget, foreslår de en relation mellem $D_{2/3}$-receptor blokade og en diskret volumen stigning frontalt.

List of Abbreviations

ANOVA	Analysis of variance
BP_{ND}	Binding potential (ND reference to Non-Displaceable tracer binding)
CANTAB	Cambridge Neuropsychological Test Automated Battery
CSTC	Cortico thalamic striatal cortical (loop)
CSF	Cerebro spinal fluid
DUP	Duration of untreated psychosis
EPS	Extrapyramidal side effects
FGA	First generation antipsychotic compound
FOV	Field of view
FSL	FMRIB (Functional Magnetic resonance imaging of the brain) software library
FWHM	Full width at half maximum
GM	Grey matter
HPLC	High performance liquid chromatography
ICD-10	International Classification of Diseases 10^{th} revision
ICV	Intracranial volume
MB_q	Megabecquerel
MPRAGE	Magnetization prepared rapid gradient echo
MRI	Magnetic resonance imaging
MSN	Medium spiny neuron
NMDA	N-Methyl-D-Aspartate
OM	Orbitomeatal
PANSS	Positive and Negative Syndrome Scale
PET	Positron emission tomography
PFC	Prefrontal cortex
P-SES	Parental socioeconomic status

rmANOVA	Repeated measures analysis of variance
ROI	Region of interest
SCAN	Schedules for Clinical Assessment in Neuropsychiatry
SGA	Second generation antipsychotic compound
SN	Substantia Nigra
SPECT	Single photon emission computed tomography
SPSS	Statistical package for the social sciences
T	Tesla
TE	Echo time
TR	Repetition time
TBV	Total brain volume
WM	White matter
V_{ND}	Distribution volume of non-displaceable ligand
V_T	Total volume of distribution of ligand
VTA	Ventral tegmental area

Background

Schizophrenia

Schizophrenia is a severe mental brain disease with a lifetime prevalence of approximately 0.7% peaking at the age of 22 (McGrath et al. 2008; Pedersen et al. 2014; van der Werf et al. 2014). The severity is underscored by the World Health Organisation (WHO) report from 2010 on chronic diseases in Europe listing neuropsychiatric diseases (including schizophrenia) second with regard to disease burden (Busse et al. 2010). Moreover, patients with schizophrenia have markedly premature mortality (Crump et al. 2013) which might partly be explained by the risk of suicide (MacDonald and Schulz 2009).

The clinical symptoms of schizophrenia can be divided into different domains and the diagnosis relies on the clinical presentation of some of these domains. Typically, symptoms appear gradually during adolescence increasing in strength to the point of psychosis. The symptom domains are normally classified into **positive symptoms** (hallucinations, delusions and thought disorder) and **negative symptoms** (affective blunting, anhedonia, poverty of speech, social withdrawal) with negative symptoms being qualities that people have in general but which are reduced or lacking in patients with schizophrenia. Cognitive deficits (deficits in attention, memory and executive functions) are also present in a majority of patients with schizophrenia and have been suggested to be a core deficit of the disorder (Schultz and Andreasen 1999).

Various hypotheses for schizophrenia regarding neurodevelopment, neurodegeneration and transmitter disturbances exist though the aetiology remains largely unknown (Insel 2010). Nevertheless, it is generally accepted that both genetic and environmental factors contribute to development of schizophrenia and disturbances in both brain structure and function are present (van OJ et al. 2008; van OJ et al. 2010). Robust evidence has shown that the dopamine system is a key neurotransmitter system implicated in the pathophysiology of schizophrenia and psychosis.

The dopamine system

Since the discovery in 1957 by Arvid Carlsson that 3-hydroxytyramine (dopamine) was more than a precursor for other catecholamines as it had neurotransmitter properties (Carlsson et al. 1957), dopamine has been implicated in attention, (Clark and Noudoost 2014), working memory (Goldman-Rakic 1995), reward processing (Schultz 1998) and decision making (St Onge et al. 2011). Besides attributing to the above-mentioned functions, dopamine is also implicated in diseases such as Parkinson's disease, attention deficit hyperactivity disorder (ADHD), drug abuse and schizophrenia.

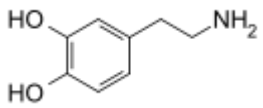

Figure 1. Dopamine

Anatomy of the Dopamine System

In the brain, dopamine is primarily synthesized in neurons located in the midbrain in the ventral tegmental area (VTA) and substantia nigra (SN). Neurons in VTA and SN are topographically arranged projecting to various brain regions through several major pathways. The **nigrostriatal pathway** projects from the SN to the putamen and part of the caudate nucleus (dorsal striatum) affecting motor control functions. The **mesolimbic pathway** projects from VTA to the nucleus accumbens (ventral striatum), caudate nucleus (associative striatum), amygdala and hippocampus and is, among others, related to reward processing (Schultz 1998). The **mesocortical pathway** projects from VTA and SN to the prefrontal cortex (PFC) and has been implicated in information processing and cognitive functions (Puig et al. 2014). The mesolimbic and mesocortical pathways overlap and have been referred to as the **mesocorticolimbic pathway.** Finally, the **tubero-infundibular pathway** projects from the hypothalamus to the pituitary gland regulating prolactin secretion (Figure 2).

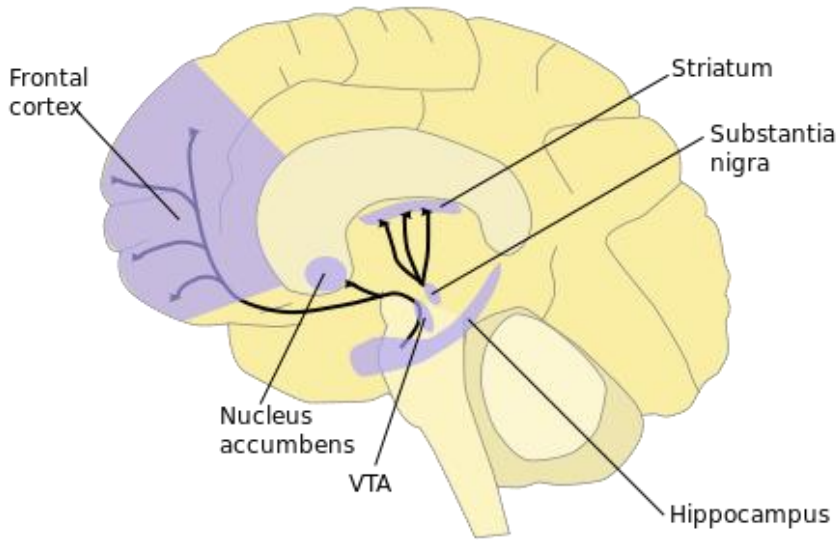

Figure 2. Schematic illustration of dopaminergic projections from the midbrain. From Wikipedia; https://en.wikipedia.org/wiki/Dopamine#/media/File:Dopamine_pathways.svg

The connections between the midbrain and striatum (nigrostriatal and mesolimbic pathways) and back are arranged in an inverse dorsal-ventral topographic order (Haber 2014). Connections from striatum to cortex run in different parallel cortico-striato-thalamico-cortical (CSTC) loops (Alexander et al. 1990) demonstrating that cortex and the basal ganglia are heavily interconnected anatomically as well as functionally (Handbook of basal ganglia function 2010). Preclinical studies show that prefrontal neurons are topographically organized with the striatum in a manner where distinct prefrontal areas are being linked to selected areas in striatum (Gerfen 1989; Levey et al.

1993; Haber and McFarland 1999) i.e. the dorsolateral prefrontal cortex is connected with the caudate nucleus in associative striatum. Apart from CSTC loops to the frontal cortex, other loops are present. The CSTC loops can be divided into three functionally different sub-types: associative, limbic and sensorimotor loops. The organization of thalamus is described later in the section on thalamus on p. 20.

The associative loops send ascending connections from the midbrain (SN) to the caudate nucleus and on to dorsolateral prefrontal cortex and other cortical areas related to associative learning. The limbic loops project from the VTA and SN to the ventral part of striatum (nucleus accumbens). From there connections pass through the mesocorticolimbic pathway to medial prefrontal cortex, orbital prefrontal cortex, anterior cingulate cortex, amygdala, hippocampus and the olfactory bulb. The last functional loop is the sensorimotor loop projecting from SN to dorsal part of the caudate nucleus and putamen to primary motor cortex, premotor cortex and supplementary motor cortex (Martinez et al. 2003). For further details, please see fig. 4 - and the sections on "physiology of the dopamine system" as well as on "cortico-striatal-thalamic-cortical loops".

Dopamine Firing

Dopaminergic neurons in the midbrain exert neuronal activity that has been characterized into four states with two active firing states; tonic firing and burst firing (Grace and Bunney 1984a; Grace and Bunney 1984b). Firing is regulated by inhibitory input from the ventral part of hippocampus and excitatory input from the peduncolupontine tegmentum (Floresco et al. 2001; Floresco et al. 2003; Grace et al. 2007). Dopamine is released from vesicles into the synaptic cleft, where it activates postsynaptic dopamine receptors and is then cleared by reuptake from presynaptic auto-receptors. The tonic firing state is regarded as an intrinsic pacemaker maintaining a level of baseline activity. In contrast, burst firing is related to changes in dopamine concentration in target areas such as prefrontal cortex (Grace et al. 2007; Marinelli and McCutcheon 2014). Burst firing is believed to be the functionally relevant signal sent to postsynaptic sites modulating reward and goal directed behaviour (Schultz 1998; Trantham-Davidson et al. 2004; St Onge et al. 2012). Other transmitter systems, e.g. glutamate, GABA, serotonin and acetylcholine, modulate dopamine firing (Carlsson 2006; Cachope and Cheer 2014).

Dopamine Receptors

The effect of dopamine is mediated through dopamine receptors, which are G protein-coupled receptors. Dopamine receptors can be divided into two subfamilies. The first subgroup is D_1 receptor-like receptors consisting of D_1 and D_5 receptors both activating adenylate cyclase, which stimulate production of cyclic adenosine monophosphate (cAMP). The second subgroup is D_2 receptor-like receptors consisting of D_2, D_3 and D_4 receptors inhibiting adenylate cyclase. D_2 receptors can be in two different states (D_2^{High} and D_2^{Low}) where they activate more easily in the high affinity state. Separating dopamine D_2^{High} receptors from D_2^{Low} receptors in vivo in humans are

at present difficult, if not impossible, since current ligands bind to both states (Seeman 2006; Seeman 2011).

The expression of the receptors throughout the brain is differentiated according to their receptor type. D_1 and D_5 receptors are present in multiple brain regions such as the prefrontal cortex, hippocampus, striatum, and thalamus and cingulate cortex. D_2, D_3 and D_4 receptors are abundant in the striatum but are also expressed in frontal and temporal cortex (Beaulieu and Gainetdinov 2011). In cortex, dopamine receptors follow a rostral-caudal gradient from frontal cortex to occipital cortex with the highest density in frontal cortex and the lowest in the occipital lobe. Moreover, dopamine D_2 receptors are mainly located in cortical layer V, whereas D_1 receptors are found in layer II and III (Lidow et al. 1991).

Physiology of the Dopamine System

The functional aspects of the dopamine system have been extensively studied. Based on anatomical and functional findings three major areas of interest are the prefrontal cortex, the basal ganglia and Thalamus.

In general, the dopamine system works as a neuromodulator affecting other transmitter systems (glutamate and GABA), which then mediate information processing (Durstewitz et al. 2000; Trantham-Davidson et al. 2004; Surmeier et al. 2007; Surmeier et al. 2011).

Prefrontal Cortex

In prefrontal cortex the dopaminergic neuromodulation is mediated through neurons expressing dopamine D_1 or D_2 receptors. An advanced computational model, the *two-state dynamic model of dopamine function in PFC* (Seamans and Yang 2004), has proposed that D_1 or D_2 receptors have somewhat opposite actions. Predominant prefrontal D_2 activity will cause a net reduction in prefrontal inhibition allowing multiple inputs to be represented in the network at the same time (open gate), but it will also result in transient network instability. The authors call this network state 1. The effect of D_2 receptors is primarily mediated via inhibition of $GABA_A$ and NMDA receptors resulting in increased glutamategic activation.

In contrast, predominant D_1 receptor modulation will result in a net increase in the inhibition of intruding stimuli and initiation of goal-related representations in working memory (closure of the gate) resulting in a stabilization of the network. This is called network state 2. In state 2, already presented information is retained in information buffers facilitating the encoding of specific stimuli at the expense of other internally or externally derived representations (Seamans and Yang 2004). In this way state 1 makes the organism able to respond to significant stimuli whereas state 2 intensifies already present representations and avoid further internal or external stimuli to be represented in the network. Differential dopamine receptor activation is established by changes in dopamine concentrations where high concentrations preferably activate D_2 receptors and low

concentrations activate D_1 receptors. In this model, information is a general term describing different sensations and perceptions (Seamans and Yang 2004).

Another feature of the dopamine system in the prefrontal cortex is highlighted by the seminal work of Patricia Goldman-Rakic and her group (Arnsten 2013). Their work linked dopamine and working memory in dorsolateral prefrontal cortex. Initially, Fuster showed that pyramidal neurons in dorsolateral prefrontal cortex were able to maintain persistent firing across the delay period and termed these cells "memory cells" (Fuster 1985). These cells were later identified as pyramidal neuron cells, located in cortical layer two and three and had dopamine D_1 receptors which responded to dopamine stimulation following an inverted U-curve (Williams and Goldman-Rakic 1995; Goldman-Rakic 1995) (Figure 3). In short, low as well as high concentrations of dopamine resulted in reduced firing and more noise in the system thus showing a small window where D_1 receptors worked in optimal conditions.

Recently, others have shown that both dopamine D_1 and D_2 receptors may be involved in other cognitive functions such as flexibility and decision making in prefrontal cortex (St Onge et al. 2011; St Onge et al. 2012; Floresco 2013; Fagerlund et al. 2013). This underscores that the balance between D_1 and D_2 receptor activation is required for optimal prefrontal functioning.

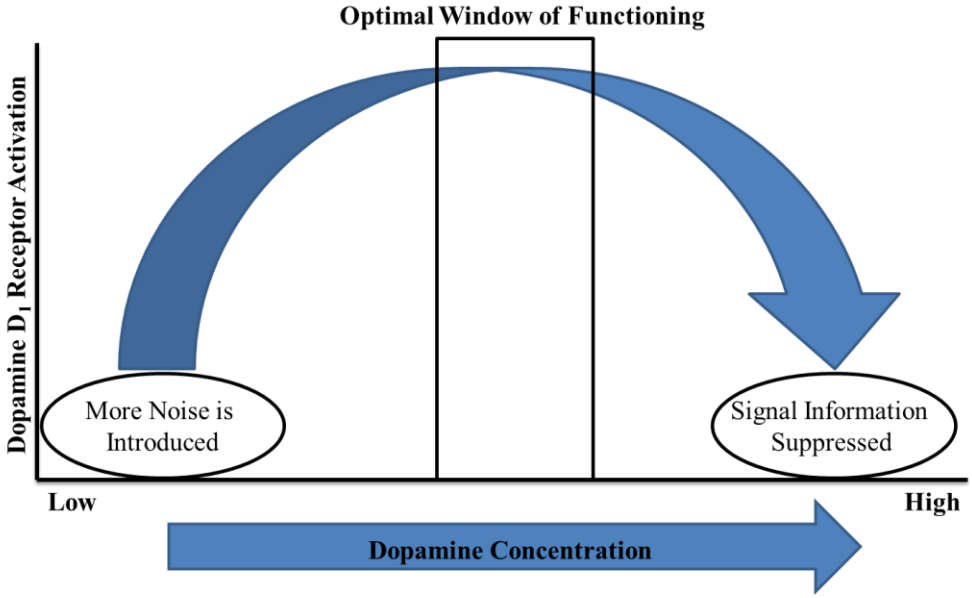

Figure 3. The relation between D_1 receptor activation and dopamine concentration in dorsolateral prefrontal cortex. At low dopamine concentrations, D_1 receptors will be less active and noise will be introduced in the system since neurons for non-preferable inputs will keep firing. At high dopamine concentrations D_1 receptors will also be less active but here all neurons will have reduced firing, effectively suppressing information. Hence the inverted U-curve describes a window where optimal conditions between information and noise exist. Adapted from (Arnsten 2013).

The Basal Ganglia

The other extensively examined area of interest related to the dopamine system is the basal ganglia that comprises the caudate nucleus, nucleus accumbens, putamen and globus pallidus (Steiner and Tseng 2010). Neurons in the basal ganglia expressing dopamine receptors are referred to as medium spiny neurons (MSN). MSNs are functionally divided into two distinct pathways; the direct and indirect pathway. The D_1 and D_2 receptors have somewhat opposite directed actions. Medium spiny neurons expressing D_1 receptors will through the direct pathway mediate increased information flow to the cortex by increasing dopamine firing in VTA. Medium spiny neurons expressing D_2 receptors will through the indirect pathway decrease information flow to cortex through a decrease in VTA firing (Keeler et al. 2014; Surmeier et al. 2007; Gerfen et al. 1990). The differentiated response of D_1 and D_2 receptors by dopamine is very complex. Overall it is suggested that tonic dopamine release predominantly activates D_2 receptors increasing inhibition and phasic dopamine activates D_1 receptors (Grace et al. 2007; Surmeier et al. 2011). This model may be an oversimplification since phasic dopamine has recently been shown to activate D_2 receptors on MSNs (Marcott et al. 2014). Moreover, it has recently been proposed that MSN in the ventral striatum can influence MSNs in the dorsal striatum through the midbrain dopamine cells. This is done through feed forward loops between the striatum and the midbrain in an ascending spiral starting in the ventral striatum (nucleus accumbens) and ending in dorsal striatum (putamen) (Figure 3)(Haber 2014). This is functionally important when assessing the role of the CTSC loops combining the frontal cortex and the basal ganglia.

Thalamus

Thalamus is located between the midbrain and telencephalon and comprises numerous nuclei. Functionally, thalamus can be seen as an information hub where multiple stimuli are relayed on to cortex (Herrero et al. 2002). Thalamus also has a gating function where incoming sensory input are filtered before being passed on to cortex (Carlsson 1988; Sherman and Guillery 2002). The anatomical organisation is complex and locating the different thalamic nuclei in vivo is not without considerable problems (Byne et al. 2009). Here emphasis is put on the functional organisation of the thalamus with regard to the CTSC loops and the dopamine system but for a more detailed description of the thalamic nuclei please see (Herrero et al. 2002).

Studies of dopamine function in the thalamus in healthy subjects are sparse and usually include thalamus as a part of examining extrastriatal areas. One study by Rieck et al. specifically examined dopamine D2 receptors in sub-regions of the thalamus. The authors examined both post-mortem brains of healthy individuals using epidepride and in vivo examination of other healthy individuals using fallypride (Rieck et al. 2004). They showed that the highest dopamine D_2 receptor density (binding potential) was located to the anterior midline regions and with lower but still moderate density in the mediodorsal regions.

Different parts of the thalamus projects to various parts of the forebrain. The thalamic anterior nuclear complex has been linked to the limbic CTSC loop and it is believed to be a key area connecting with the cingulate gyrus (van Veen and Carter 2002) and Papez circuit (Jankowski et al. 2013). The mediodorsal thalamus is connected with prefrontal cortex through strong cortico-cortical

projections receiving input from cortical layer V and VI (Mitchell 2015) and is thus associated with the associative CTSC loop (Mitchell and Chakraborty 2013). For the sensorimotor CTSC loop it has been suggested to involve the centromedian nucleus (Sadikot et al. 1992). The pulvinar, another thalamic complex, has been assumed to be an in between complex, where the anterior part has a similar function as the mediodorsal complex whereas the posterior part resembles the centromedian nucleus (Byne et al. 2009).

Cortico-Thalamic-Striatal-Cortical Loops

As mentioned previously, there are three functionally different cortico-striato-thalamo-cortical loops (Figure 4). First, the limbic loop has been demonstrated to be related to brain functions such as reward, motivation and affect regulation. This loop has also been suggested to be involved in the pathophysiology of schizophrenia though this was based on preclinical findings (see below). The associative loop is thought to be involved in working memory and executive functioning and clinical studies suggest this loop is associated with the pathophysiology in schizophrenia (see below). Finally, the sensorimotor loop is associated with planning of movements and executing motor actions. Integrating the interaction between the midbrain and the basal ganglia into the function of these CSTC loops is complex. However, it has been proposed that there is a cortical information flow from frontal cortex toward parietal cortex mediated through the CSTC loops (Haber and Knutson 2010; Keeler et al. 2014).

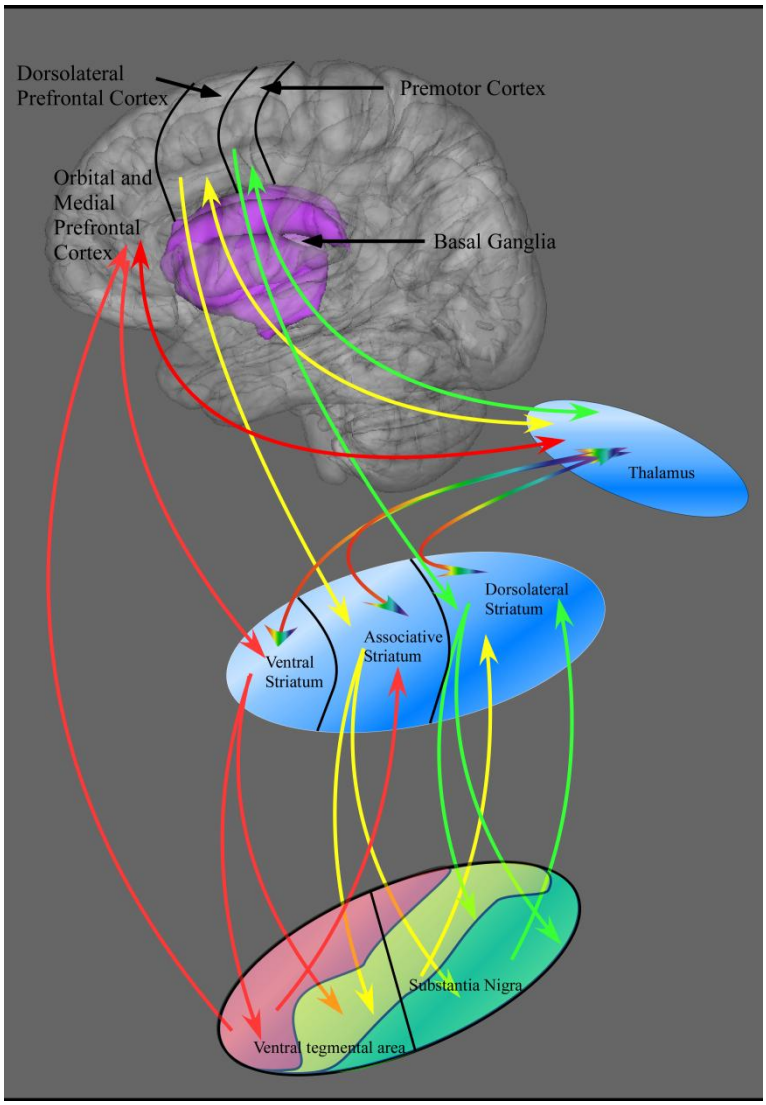

Figure 4. Schematic illustration of the cortico-thalamic-striatal-cortical loops. In the midbrain, dopamine containing neurons in substantia nigra and the ventral tegmental area project to the basal ganglia. Red arrows indicate projections to the ventral striatum, yellow the associative striatum and green to the dorsolateral striatum. Though overly simplified, red arrows are to represent the limbic loop, yellow the associative loop and green the sensorimotor loop.
The ascending and descending loops between the midbrain and striatum create a "spiral" of information. This spiral makes information from the dopaminergic neurons in the ventral tegmental area via loops ending in substantia nigra through a ventromedial to a dorsolateral gradient in the striatum. From the striatum, information flows to Thalamus, then cortex and back. Multi-coloured arrows between striatum and thalamus are to illustrate that information from red, yellow and green loops are ascending and descending to all parts of the striatum. Adapted from (Haber 2014).
Note other structures (hippocampus, amygdala, lateral habenula, rostromedial tegmental nucleus, subthalamic nucleus) are also involved in these circuits but are left out here.

In summary, one can see the basal ganglia working as a stimulus-response interface sending sensory information on to cortex via thalamus and in this way being involved in reward based (Redgrave et

al. 1999; Keeler et al. 2014) and associative learning (Puig et al. 2014). Moreover, it has been proposed that there is an inverse relationship between frontal and striatal dopamine interactions (Wilkinson 1997), though the nature of these interactions are much more complex (Keeler et al. 2014; Puig et al. 2014; Grace et al. 2007; Surmeier et al. 2011). However, our current understanding of the dopamine system establishes a framework on which dopamine hypotheses on schizophrenia can been built.

Dopamine Hypotheses on Schizophrenia

Since Arvid Carlsson's discovery in 1957, together with the discovery of antipsychotics in the 1950's which was later shown to be dopamine antagonists (Seeman et al. 1976), the role of dopamine in schizophrenia has led to several versions of the dopamine hypothesis on schizophrenia. The first hypothesis drew on studies showing that low doses of dopamine agonists could trigger psychotic symptoms in patients with schizophrenia (Janowsky et al. 1973; Angrist and Gershon 1970) and higher doses could trigger psychotic symptoms in healthy individuals (Randrup and Munkvad 1967). Hence Carlsson stated that a hyper-dopaminergic state was present in patients with schizophrenia (Carlsson 1978). This was later revised and expanded pointing to an involvement of the glutamategic system. Carlsson proposed that cortical glutamatergic dysfunction would disinhibit GABAergic control of thalamus and coupled with striatal dopaminergic hyperactivity this resulted in "overload" of the thalamic filter and eventually psychosis (Carlsson 1988).

In 1991 the second version of the dopamine hypothesis was presented by Davis et al (Davis et al. 1991). Based on the, at that time accessible literature, Davis et al. suggested that dopamine disturbances were differentiated into a subcortical "hyperdopaminergia" mediated through the mesolimbic pathway and prefrontal "hypodopaminergia" via the mesocortical pathway. It was proposed that reduced levels of dopamine in prefrontal cortex would result in increased dopamine in the striatum (Davis et al. 1991). Grace (Grace 1991) proposed that the decreased cortical dopamine levels would cause a prolonged decrease in subcortical tonic dopamine levels in ventral striatum. This down-regulation in baseline dopamine tone would result in an increased responsivity of the dopamine system. Thus, behaviourally relevant stimuli that activate dopamine neuron firing would produce abnormally large phasic dopamine responses in the compensated ventral striatum (Grace 1991). To explain the pathophysiological connection between psychosis and the dopamine alterations, the salience hypothesis was proposed (Kapur 2003). According to this hypothesis, striatal "hyperdopaminergia" would lead to aberrant assignment of salience, i.e. irrelevant information would be assigned an important cue and thus, psychosis would be a product of aberrant salience of internal representations (Kapur 2003). The implication of the ventral striatum (nucleus accumbens) as the point of subcortical hyperdopaminergia was initially based on preclinical studies implicating the limbic loop in the pathophysiology of schizophrenia (Crow et al. 1977; Flagstad et al. 2004). Later, clinical PET imaging studies have shown that patients with schizophrenia in average have an increased dopamine synthesis capacity located to the functional associative striatum (precommissural dorsal caudate) (Elkashef et al. 2000; Meyer-Lindenberg et al. 2002; Howes et al. 2011; Demjaha et al. 2012). This increased synthesis capacity in associative striatum

has been linked to psychotic symptoms (Howes et al. 2012) and increased synthesis capacity has been described as the final common pathway to psychosis (Howes and Kapur 2009). According to this hypothesis, changes in multiple transmitter systems and genetic as well as environmental exposures eventually lead to striatal dopaminergic hyperfunction and psychosis. Hence, the finding of increased synthesis capacity in associative striatum implicates the associative loop and not the limbic loop as suggested by preclinical studies.

Besides, being a filter for internal and external stimuli, thalamus has also been implicated in the pathophysiology of schizophrenia. Buchsbaum et al. have shown decreased metabolic rate in thalamus compared to healthy controls (Buchsbaum et al. 1996). The same group also showed lower glucose metabolism in the left thalamus and this was associated with more positive symptoms whereas relative lower glucose metabolism in mediodorsal nucleus was associated with negative symptoms and worse performance on verbal memory task (Hazlett et al. 2004). A recent review of thalamic neuroimaging findings also concludes that especially the mediodorsal nucleus is affected in schizophrenia (Pergola et al. 2015). Thus, there is evidence of pathology in thalamus in schizophrenia (Byne et al. 2009).

Dopamine D_2 Receptors in Schizophrenia

Numerous studies have investigated whether D_2 receptor density is altered in schizophrenia patients compared to healthy control subjects. Most of these studies were carried out before the dopamine synthesis capacity studies. Since the antipsychotic effect is linked to blockade of dopamine D_2 receptors it was hypothesized that patients would have higher receptor density compared to healthy controls.

Early findings suggested that there might be differences between patients and healthy controls with patients having elevated dopamine $D_{2/3}$ binding potential (BP_{ND}) suggesting higher receptor density (Wong et al. 1986). Later, others found no differences (Farde et al. 1990; Pilowsky et al. 1994). Inconsistent results were also seen for extrastriatal regions with some studies reporting decreased BP_{ND} in patients compared to healthy controls in frontal cortex (Suhara et al. 2002) opposed to others finding no changes (Glenthoj et al. 2006; Talvik et al. 2003). Lower BP_{ND} in patients has also been found in temporal cortex (Tuppurainen et al. 2003) and thalamus (Talvik et al. 2003; Yasuno et al. 2004; Buchsbaum et al. 2006; Kessler et al. 2009). In a more recent study examining both striatal and extrastriatal dopamine $D_{2/3}$ receptors BP_{ND} using a dual ligand paradigm, patients had increased BP_{ND} in thalamus and caudate nucleus and lower BP_{ND} in uncus (temporal lobe) (Kegeles et al. 2010c).

Three meta-analyses overall conclude (Laruelle 1998; Zakzanis and Hansen 1998; Kestler et al. 2001) that there might be an elevation of striatal $D_{2/3}$ receptor density. This increase is in the vicinity of 10-20 % compared to healthy controls though the results from studies are heterogeneous (Howes et al. 2009). When assessing antipsychotic-naïve patients, no elevation has been found. For extrastriatal regions, there are still few studies. In a recent meta-analysis (Kambeitz et al. 2014), the

authors found no evidence of altered $D_{2/3}$ receptor BP_{ND} in temporal cortex, too few studies of frontal cortex to conduct a meta-analysis and a numerical though non-significant decrease in patients in the thalamus.

Moreover, it has been proposed that patients with schizophrenia are supersensitive to dopamine due to an elevated level of D_2^{High} receptors. Clinical studies using a dopamine depletion method where participants are scanned using dopamine D_2 receptor ligands before and after oral administration of α-MPT (Abi-Dargham et al. 2000; Kegeles et al. 2010a) have showed an increased level of BP_{ND} in patients compared to controls after dopamine depletion in striatal regions. In the study by Kegeles et al. increase in BP_{ND} was located to the associative striatum (precommissural dorsal caudate) (Kegeles et al. 2010a). Other authors have suggested these findings indicate a larger availability of D_2^{High} receptors in patients (Seeman 2013) and that a small increase in receptor density might have large functional implications. As such it is proposed that a small increase in density might be enough to cause psychotic symptoms (Seeman 2013; Seeman and Seeman 2014).

Prefrontal "hypodopaminergia"

The prefrontal "hypodopaminergia" suggested by initial findings (Knable and Weinberger 1997; Weinberger et al. 1988; Weinberger and Berman 1988) is less validated since direct measurement of frontal dopamine levels in vivo in humans is complicated. Even so, a recent study indicated a deficit in the capacity for dopamine release in the dorsolateral prefrontal cortex in patients with schizophrenia (Slifstein et al. 2015). The study used a novel method to measure dopamine release and even if the results support the hypothesis of "hypodopaminergia", replication in independent samples should be considered before drawing conclusions. The prefrontal cortex has mostly been linked with cognitive deficits and "hypofunction" though it has also been implicated in psychosis. Psychosis can be seen as formation of inappropriate associations between stimuli, thoughts and percepts (Murray et al. 2010) suggesting that the prefrontal cortex may be involved in psychosis. One model already mentioned is the computational *two-state dynamic model of dopamine function in the prefrontal cortex* (Seamans and Yang 2004). To recapitulate, D_2 receptors activity results in an open gate whereas D_1 receptor activity leads to a closed gate. These assumptions can model positive and negative symptoms respectively. In patients with schizophrenia, increased prefrontal D_2 receptor (more open gate) activity will result in random, tangential or intrusive thoughts leading to the development of positive symptoms. Our group has previously demonstrated a correlation between frontal dopamine D_2 receptor BP_{ND} and positive symptoms in antipsychotic-naïve schizophrenia patients (Glenthoj et al. 2006). For increased D_1 receptor activity (more closed gate) a narrowing of stimuli selected for action will lead to negative symptoms. However, positive symptoms may also arise from low D_1 receptor (inverse closed gate) activity due to premature termination of information in working-memory buffers prior to the completion of thoughts or actions (Figure 5).

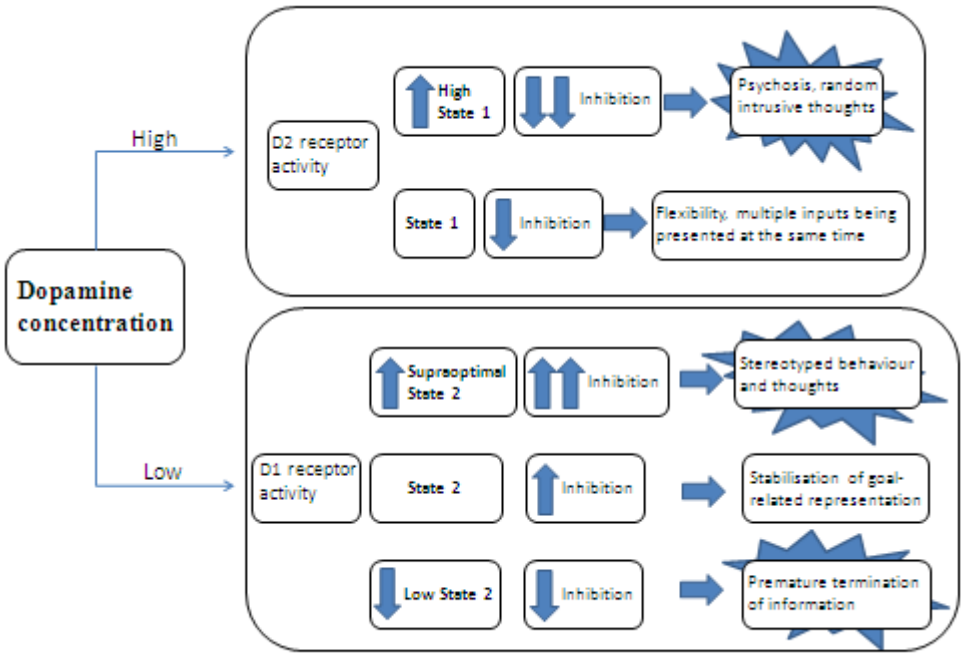

Figure 5. Simplified illustration of the two-state dynamic model of dopamine function in prefrontal cortex. Dopamine concentrations in the frontal cortex will differentiate activation between dopamine receptor subtypes. High concentrations will preferentially activate D_2 receptors whereas low concentrations will activate D_1 receptors and switching between the two states is crucial for normal information processing. A high state 1 one can result in psychotic symptoms such as delusions. A low state two is proposed to cause premature termination of information which may also lead to disrupted information processing. A supraoptimal state 2 will result in increased inhibition leading to negative symptoms. Antipsychotic medication causing D_2 receptor blockade might force a supraoptimal state 2 resulting in secondary negative symptoms or stabilize existing psychotic symptoms (Seamans and Yang 2004).

It is now widely accepted that several other transmitter systems, like the serotonergic- (Rasmussen et al. 2011; Ebdrup et al. 2011a), glutamatergic- (Kahn and Sommer 2014; Howes and Kapur 2014), the GABAergic- (Schmidt and Mirnics 2014), and the noradrenergic (Arnsten 2004) systems are also involved in the pathophysiology of schizophrenia. Moreover, dysfunction in one part of the prefrontal-striatal-thalamic network can alter functions in other areas in the network as well (Meyer-Lindenberg et al. 2002; Kellendonk et al. 2006; Fusar-Poli et al. 2010; Fusar-Poli et al. 2011b).

Antipsychotic Medication

In the 1950ies chlorpromazine (an antihistamine) was discovered to have antipsychotic effect. Later this effect was linked to blockade of dopamine D_2 receptors (Seeman et al. 1976) and today antipsychotic medication continues to be the first choice for treating psychosis (Leucht et al. 2013). The therapeutic action of antipsychotics has been linked to striatum. Initially it was thought that it was blockade of dopamine D_2 receptors in the limbic regions. This, was based on preclinical

findings (Crow et al. 1977) and because dopamine D_2 receptors are abundant in these regions (Beaulieu and Gainetdinov 2011). With clinical findings showing increased presynaptic dopamine synthesis capacity in associative striatum (caudate nucleus) it seems plausible that blockade of postsynaptic dopamine D_2 receptors in associative striatum is responsible for the antipsychotic effect. More than 50 studies have examined striatal dopamine D_2 receptors and a meta-analysis from 2009 concluded that blockade of striatal dopamine D_2 receptors was associated with improvement in positive symptoms (Howes et al. 2009).

Therapeutic Window

Occupancy studies have suggested that there is a therapeutic window. This window corresponds to a striatal dopamine D_2 receptor blockade in the range from 65% - 72% and results in the highest treatment response and the lowest incidence of side effects (Kapur et al. 2000; Nord and Farde 2011). However, an important notion is the large individual variation in occupancy between patients receiving identical antipsychotic doses complicating treatment regimens (Howes et al. 2009).

The "classic" side effects of the first generation of antipsychotics (FGA) are extrapyramidal side effects (EPS) (parkinsonism, dystonia, dyskinesia and akathesia) and hormonal disturbances (hyperprolactinemia) (Chouinard and Margolese 2005; Novick et al. 2010). These side effects have also been related to blockade of dopamine D_2 receptors with increasing likelihood above 72% occupancy (Farde et al. 1992; Nordstrom et al. 1993; Kapur et al. 1995; Kapur et al. 2000). The mechanism of action is proposed to be blockade of D_2 receptors in the nigro-striatal pathway (Stahl 2013).

With the introduction of clozapine came the second generation of antipsychotics (SGA). The SGAs were characterized by having a lower incidence of EPS and clozapine showed lower affinity for dopamine D_2 receptors compared to FGAs. Thus, this has been proposed to be the reason for the lower EPS incidence (Remington and Kapur 2000). Other mechanisms of action that might explain the properties of SGAs are fast dissociation from the D_2 receptor (Kapur and Seeman 2001), blockade of $5HT_{2A}$ receptors (Meltzer and Massey 2011) or preferential limbic D_2 receptor blockade (limbic selectivity) (Skarsfeldt 1995; Mizrahi et al. 2007; Muly et al. 2012).

The continuing introduction of SGAs has revealed other side effects like sedation, glucose intolerance, dyslipidaemia, cardiovascular side effects (orthostatic hypotension, QTc elongation), hyperprolactaemia and sexual dysfunction.

Antipsychotics are still categorized into FGAs and SGAs (Lohr and Braff 2003) though diverse pharmacological properties have questioned this nomenclature (Fischer-Barnicol et al. 2008; Leucht et al. 2009; Kane and Correll 2010). This is supported by findings that different antipsychotics within the same class affect function, (Nejad et al. 2012) metabolism (Leucht et al. 2013) and brain structure (Ebdrup et al. 2013) differently.

Even so, SGAs induced fewer EPS and treated psychotic symptoms. Initially it was also assumed that SGAs would improve cognitive deficits (Minzenberg and Carter 2012), possibly by preserving dopamine transmission in the meso-cortical pathway (Abi-Dargham and Laruelle 2005; Tanda et al. 2014). The effect on cognition was initially supported by smaller trials (Bilder et al. 2002; Harvey et al. 2004) not controlling for the retest/learning effect. As the first, our group showed that when controlling for the re-test effect, changes in cognitive scores were equal to the re-test effect, hence no improvement was found (Fagerlund et al. 2004). This finding is supported by larger and more extensive clinical studies of SGAs suggesting only modest effects on cognition (Keefe et al. 2007; Davidson et al. 2009). Recently, studies have indicated that high level of dopamine blockade, regardless of class, might worsen cognitive deficits such as vigilance (Sakurai et al. 2013) and attention (Uchida et al. 2009) in patients with schizophrenia.

In the study which forms the basis of this thesis, two antipsychotic drugs have been used, an FGA (zuclopenthixol) and an SGA (risperidone). The studies are part of a longitudinal cohort study and when planned it was thought, based on the mesolimbic selectivity theory of second generation of antipsychotics (Abi-Dargham and Laruelle 2005), that there would be differential effects of zuclopenthixol and risperidone with regard to cognitive functions and sensorimotor gating. We also chose the two antipsychotics as they, when the study was initiated, were commonly used as first choice for treating schizophrenia in the clinic in Denmark. Moreover, they represent a first generation antipsychotic (FGA), zuclopenthixol, and a second generation antipsychotic (SGA), risperidone.

However, our initial findings on cognitive functions and sensory motor gating (Mackeprang et al. 2002; Fagerlund et al. 2004) did not show differential effects of first- and second generation antipsychotics. Hence, we did not expect the two compounds to affect cognition, psychopathology or $D_{2/3}$ receptor blockade differently. Thus, the compounds are in the present study seen as tools to examine effects of dopamine $D_{2/3}$ receptor blockade on cognition, grey matter volume and psychopathology.

Zuclopenthixol is still used in the clinic in Denmark, though not as a first choice for first-episode schizophrenia patients. Risperidone is still recommended as a first-line treatment for patients with first-episode psychosis.

Both zuclopenthixol and risperidone have a wide clinical range, similar half-life (approximately 20 hours) and are suitable to test dose-dependent relations with psychopathology, cognition and grey matter volume. Moreover, since both compounds have a high affinity for $D_{2/3}$ receptors they are suitable as tools for examining the effect of extrastriatal dopamine $D_{2/3}$ receptor activity, though influence from other transmitter systems need to be taken into account when interpreting results (Table 1).

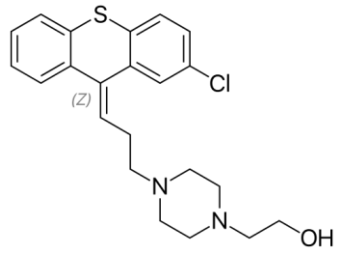

Figure 6. Zuclopenthixol

Zuclopenthixol is a middle dose FGA. Its therapeutic range is 2-40 mg and it has strong D_2 receptor and a modest D_1 receptor affinity (Hyttel et al. 1985). As other FGAs it has a high risk of inducing extrapyramidal side effects.

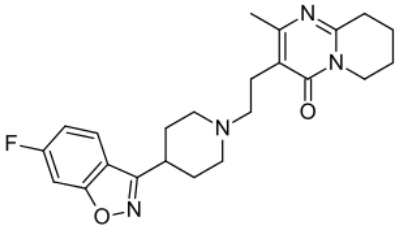

Figure 7. Risperidone

Risperidone is a commonly used antipsychotic compound with SGA properties in the range 1-6 mg with a marked increase in the risk of extrapyramidal side effects above 6 mg, having a range up to 16 mg. It has "classic" atypical properties, with relatively strong D_2 –receptor blockade combined with strong $5HT_{2A}$ –receptor and α1 receptors blockade (Kroeze et al. 2003).

	D_1	**D_2**	**α_1**	**H_1**	**M_1**	**5-HT_{2A}**
Zuclopenthixol	++	+++	(+ / ++)	++	--	--
Risperidone	--	+++	+++	++	--	+++

Table 1. The relative receptor affinities for zuclopenthixol and risperidone. Higher affinity for a specific receptor is indicated by increasing number of + 'es. Receptors not being notably affected by the compound are shown with --. Data is extrapolated from (Kroeze et al. 2003; Hyttel et al. 1985). Abbreviations D_1 = Dopamine D_1 receptor, D_2 = dopamine D_2 receptor, α_1 = Alpha one adrenergic receptor, H_1 = Histamine H_1 receptor, M_1 = muscarine M_1 receptor, 5-HT_{2A} = serotonin 5-HT_{2A} receptor

Cortical Grey Matter Changes in Schizophrenia

Results from Post-Mortem Studies

Since Kraepelin first described the syndrome today known as "schizophrenia" (Kraepelin 1913) structural brain alterations have been suggested to be associated with the disease. Early post-

mortem studies did not show pathological alterations leading to the quote “schizophrenia is the graveyard of neuropathologists” (Plum 1972). However, with improving techniques there is evidence of altered brain structure in patients with schizophrenia. A review conducted by Harrison (Harrison 1999) concluded that overall there was no loss of neurons in the frontal cortex though there were signs of cortical thinning. It was suggested that there might be aberrant cytoarchitecture in the form of dendritic spine loss, termed loss of neuropil, which was also formulated by (Selemon and Goldman-Rakic 1999). This loss of neuropil has since been replicated in more recent studies in different samples (Glantz and Lewis 2000; Selemon et al. 2003; Dorph-Petersen et al. 2009; Sweet et al. 2009; Konopaske et al. 2014). A more recent review by Glausier and Lewis also concludes that there is a loss of dendritic spines in multiple brain regions in patients with schizophrenia and that loss of neuropil has been related to impairment in working memory, attention, sensory-motor processes and sociability (Glausier and Lewis 2013). Other morphological findings such as increased neuronal density (Selemon et al. 1998), mean cell spacing (Casanova et al. 2005; Casanova et al. 2008) or altered shape of pyramidal soma (Rajkowska et al. 1998) have also been related to loss of neuropil (Harrison 1999). Another mechanism suggested to account for the morphological grey matter changes is gliosis (Williams et al. 2013) though most studies do not support this finding (Harrison 1999; Glausier and Lewis 2013). As such, loss of neuropil seems to be the most consistent finding at least in the frontal cortex and Glausier and Lewis conclude that reduced spine density is a common trait in cortical regions of patients with schizophrenia (Glausier and Lewis 2013). It is important to state that alterations in white matter (Hoistad et al. 2009) and ventricular enlargement (Allen et al. 2009) also occur. Moreover, all studies are confounded by antipsychotic medication since studies have been conducted in patients who were medicated.

The neurobiological mechanism is unknown though the dopamine system may be involved. Preclinical studies in rodents have shown that blockade of D_2 receptors - and activation of D_1 receptors - has a positive impact on synaptogenesis where D_2 receptor blockade causes axonal sprouting (Parish et al. 2001; Parish et al. 2002; Li et al. 2014). In the study by Wang et al. D_1 and D_2 receptor knockout mice had reduced dendrite spine density of pyramidal neurons in prefrontal cortex (Wang et al. 2009), a finding also seen in post-mortem studies of patients with schizophrenia (Glausier and Lewis 2013).

Influence of Antipsychotic Medication

The questions if and how antipsychotic drugs affect brain morphology have led to several preclinical studies with conflicting results. Initial findings suggested that FGAs result in an increased volume of the basal ganglia (Benes et al. 1985) whereas SGAs caused no alteration or a decrease in volume (Lee et al. 1999; Andersson et al. 2002). Konopaske and Dorph-Petersen (Konopaske et al. 2008; Konopaske et al. 2007; Dorph-Petersen et al. 2005) have conducted studies where monkeys were exposed to chronic antipsychotic medication and found significant tissue loss in frontal cortex where grey matter reduction was associated to medication. Ultra structural examination showed a significant loss of astrocytes and a non-significant loss of oligodendrocytes in affected areas. In rhe above-mentioned studies the effects of the first generation antipsychotic

haloperidol and the second generation antipsychotic olanzapine on grey matter volume were indistinguishable.

These findings of grey matter volume loss after exposure to antipsychotics were replicated in rats by Vernon et al. (Vernon et al. 2011). However, the same group recently showed that antipsychotic medication did not cause loss of astrocytes but rather loss of neuropil (Vernon et al. 2014), a replication of a previous finding (Selemon et al. 1999). In contrast, Castellano and colleagues did not find that chronic risperidone treatment caused grey matter loss in prefrontal cortex in healthy rats or in an animal model of schizophrenia (Castellano et al. 2009; Castellano et al. 2013).

Studies examining the effect of antipsychotics on grey matter volume in healthy volunteers are sparse though haloperidol exposure has been associated with grey matter volume reduction in putamen one to two hours after administration which reversed after 24 hours (Tost et al. 2010). Long-term studies in healthy volunteers have not been identified.

In patients with schizophrenia grey matter changes have been linked to antipsychotic medication (Ho et al. 2011; Radua et al. 2012; Torres et al. 2013; Andreasen et al. 2013; Fusar-Poli et al. 2013; Vita et al. 2015) though all authors point out it is difficult to separate neurotoxic changes relating to the disorder per se, from neuro-plastic or other potential changes caused by pharmacotherapy. That antipsychotics may affect grey matter volume is supported by Ho and colleagues who found both FGAs and SGAs to be associated with frontal grey matter reduction (Ho et al. 2011). The same group replicated their findings in a later study where treatment (intensity) as well as relapse duration (duration of psychotic episode) was associated with total brain volume loss (Andreasen et al. 2013). Antipsychotic treatment showed a diffuse distribution affecting many areas of the brain whereas relapse duration was more strongly associated with frontal lobe tissue loss. This was supported by two other studies where antipsychotics were associated with cortical grey matter volume decrease (Radua et al. 2012; Fusar-Poli et al. 2013). A mixed result was found by Torres et al. with some areas being smaller in patients compared to controls and some larger and "with no major differences between FGAs and SGAs" (Torres et al. 2013). The most recent meta-analysis relating antipsychotic medication to change in grey matter volume over time in patients with schizophrenia, examined effect of FGAs versus SGAs. Here reduction in grey matter volume over time was related to cumulative exposure to antipsychotics especially for FGAs. This association was not found for patients treated with SGAs but instead higher dose of SGAs during the follow-up period was related to lower the reduction in grey matter volume (Vita et al. 2015).

In line with this, other authors also report an impact of the dose of antipsychotics on volume changes. Our group has previously shown a significant relation between quetiapine dose and striatal and hippocampal volume changes in initially antipsychotic-naïve first-episode patients (Ebdrup et al. 2011b). This is supported by clinical studies relating risperidone treatment to striatal volume changes. Here, high doses of risperidone have shown volume increase (Massana et al. 2005), medium doses showed marginal increase (Glenthoj et al. 2007) and low doses showed no changes in grey matter volume in the striatum (Lang et al. 2001).

Another feature highlighted by Ebdrup et al. (Ebdrup et al. 2013) is that different antipsychotics may not affect grey matter equally in patients. In this review antipsychotic monotherapy affect basal ganglia volumes differently according to compounds. Only clozapine showed consistent results with basal ganglia volume reductions across three studies whereas for other SGAs both reduction, increase and no changes were found.

In summary, antipsychotics affect grey matter volume though the clinical impact of these alterations is still a matter of debate. A key notion is that the underlying mechanism is largely unknown but the dopamine system may be involved in the pathological mechanism seen in patients with schizophrenia and all antipsychotics block dopamine D_2 receptors though differences in receptor profiles, doses and brain areas examined complicate more definite conclusions.

Cognitive Deficits in Schizophrenia

Cognitive deficits are present in schizophrenia and have been a subject of intensive research for the past three decades (Barch and Ceaser 2012). The cognitive deficits were initially suggested to be secondary to psychotic symptoms though it is now clear they represent an independent category of symptoms (Barch and Ceaser 2012). This, since cognitive deficits are present before disease onset (de Paula et al. 2015) and at the beginning of the disease (Addington and Addington 2002; Fagerlund et al. 2004). Moreover, cognitive deficits are continuously present in patients and do not change when positive and negative symptoms improve (Cornblatt et al. 1997).

Most patients experience various cognitive deficits (Palmer et al. 2009) though some cognitive deficits seem to be more impaired than others. One cognitive function that has repeatedly been shown to be affected in patients with schizophrenia is working memory with patients performing worse than healthy controls and is related to the dorsolateral prefrontal cortex (Weinberger et al. 1988; van Veelen et al. 2010; Barch and Ceaser 2012). Other cognitive function impaired in patients with schizophrenia is attention (Keefe et al. 2006; Fagerlund et al. 2004) and executive functions (planning and set shifting) (Fagerlund et al. 2004; Yun et al. 2011). The cognitive deficits have also been linked to outcome and in one study cognitive factors explained as much as 52% of the variance in whether patients would return to work (Nuechterlein et al. 2011). In another study Jakubovski et al. found cognitive deficits to predict worse treatment outcome (Jakubovski et al. 2015) and quality of life has also been linked to severity of cognitive deficits in patients with schizophrenia (Sevy and Davidson 1995). The underlying neural substrate of cognitive functions is still unresolved but the dopamine system is involved in some functions (please see physiology of the dopamine system).

Magnetic Resonance Imaging

Magnetic resonance imaging is a method by which structural high resolution in vivo brain images can be obtained. For MRI acquisition the subject is placed inside the scanner, which generates a strong magnetic field. This static, homogenous magnetic field called B_0 is measured in tesla (T).

Field strength usually starts at 0.5 though development of higher field strength is continuously pursued. Most clinical research is still conducted on 1.5-3 T.

Magnetic resonance imaging takes advantage of the magnetic properties of protons. Protons possess an inherent property called spin, which allows them to interact with an ambient magnetic field. By applying a strong magnetic field spins will tend to align along the direction of the B_0 field creating a small magnetisation. The proton will then spin around the direction of the B_0 field (so called precession) at a specific resonance frequency called the Larmor frequency. By applying a brief radio wave at the Larmor frequency the magnetization is flipped (excitation) and the protons are brought out of equilibrium. After the radio wave has ended the magnetization returns to equilibrium by a process called relaxation. During relaxation protons emit radio signals that can be obtained by a receiver coil, giving the raw MR signal. The relaxation course is exponential and explained by two time constants T1 and T2. T1 represent magnetization recovery along the axis of the B_0 field, and T2 the loss of coherence in the orthogonal xy-plane. The two time constants vary according to different tissue types, and are the main reasons for the good contrast in structural MRI images.

From this, T_1 and T_2 weighted images can be defined. T_1 weighted images are defined by having a short relaxation and echo time hereby maximizing contrast between tissue types. T_1 weighted images are normally used for assessing grey matter. In contrast T_2 weighted images have a long relaxation and echo time and are usually used to assess white matter lesions or inflammation.

The raw MR signal does not contain spatial information. To obtain information of specific slices a slice-selective excitation is imposed perpendicular to the plane of the specific slice. This excitation defines the centre and range for the specific slice. Then, a frequency specific radio frequency pulse is applied simultaneously, which excites protons in the desired slice. By applying gradients (additional magnetic fields) along the axis of the B_0 field the frequency emitted by protons will correspond to their position along this axis. This can also be done along a transverse axis, and when repeated systematically, a grid is generated from which spatial information can be obtained. Image reconstruction is then performed by Fourier transformation of the raw data. Simplified the Fourier transformation converts radio waves into patterns with increasing complexity until they become images. Further details see http://www.cis.rit.edu/htbooks/mri/.

Image Processing

Images of structural brain morphology are usually acquired from T1 weighted 3D high resolution MRI sequences. Since anatomical alterations in patients compared to healthy controls are very subtle they cannot be detected by conventional visual inspection. As such differences are detectable only at group level and by using computational techniques.

There are several freeware programs available (SPM, FSL, Freesufer) using these computational techniques though they all involve more or less the same steps. Today most programs are more or less automated with an optimised default mode set up.

For the current thesis, we used Functional MRI of the brain (FMRIB) software library (FSL) http://www.fmrib.ox.ac.uk/. The procedures we used were brain extraction and tissue segmentation. Brain extraction strips the image of skull and soft tissue components to ensure that further analysis is conducted only on brain tissue. Segmentation into tissue components is done in a voxel wise manner classifying grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) based on intensity in the voxel.

By using regions of interest (ROIs) we can assess morphological properties of a specific region and also extract functional SPECT imaging data. With this approach identical ROIs can be generated for both modalities making comparison across modalities possible. The ROIs also act as a mask including only data present within the ROI. *For MRI voxels within the ROI classified as grey matter would be extracted and a mean density within the ROI calculated*. To examine changes over time difference in morphological properties within the ROIs can be calculated at different time points http://brainimaging.waisman.wisc.edu/~oakes/spam/BrainMaker_Intro.html.

Results from Magnetic Resonance Imaging Studies

With the introduction of magnetic resonance imaging in schizophrenia research (Smith et al. 1984) high resolution quality examinations of grey matter became feasible in vivo. Due to a constantly increasing numbers of studies the nature of structural alterations in schizophrenia has become more faceted. Studies of patients at ultrahigh risk of psychosis (Yung et al. 1998) have revealed that brain abnormalities are present before the onset of psychosis and are especially prominent in the frontal cortex (Fusar-Poli et al. 2011a; Mechelli et al. 2011; Dazzan et al. 2012; Jung et al. 2012).

The importance of assessing structural brain alterations in patients with schizophrenia having their first episode before treatment is initiated has been underscored by the publication of more than 25 studies since 2000 (Radua et al. 2012; Haijma et al. 2013; Scanlon et al. 2014; Rigucci et al. 2013; Smith et al. 2015). Initially samples were small but with an increase in sample size, easier access to MRI scanners and a growing number of studies, meta-analytic approaches are possible.

In the meta-analysis by Haijma and colleagues, more than 700 antipsychotic-naïve first-episode patients were included. Here, total brain volume and grey matter volume loss was present in patients in the antipsychotic-naïve state compared to healthy controls. Moreover, volume loss was also found in hippocampus, thalamus and caudate nucleus. Another recent meta-analysis included a subset of 228 antipsychotic-naïve first-episode schizophrenia patients being structurally MRI scanned. Using a voxel based meta-analytic approach whole brain evaluation of grey matter volume differences between patients and controls was conducted. Their findings supported the previous analysis in that patients had grey matter volume reductions in medial frontal/anterior cingulate cortices as well as insular regions bilateral compared to healthy controls (Radua et al. 2012). Though not identical findings, both studies find decrease in grey matter volume at illness onset.

Frontal and temporal grey matter loss has also been found in medicated chronic schizophrenia patients with grey matter loss located to the superior temporal gyrus and medial frontal regions

(Hulshoff Pol and Kahn 2008; Ho et al. 2011; Andreasen et al. 2011; Cobia et al. 2012; Fusar-Poli et al. 2013). It has been proposed that there is a time course where grey matter volume reduction is more pronounced in early on-set schizophrenia and become more stationary during later stages of the disease (Douaud et al. 2009; Vita et al. 2012).

To summarize, there are cortical grey matter changes in patients with schizophrenia in particular in the frontal and temporal lobe, which may be present before illness onset, and the changes also seem to be present in later stages of the disease. Examining frontal and temporal regions in antipsychotic-naïve patients before and after their first treatment may show the possible relation between antipsychotic exposure in the form of dopamine D_2 receptor blockade and brain alterations in these key regions of interest.

Single Photon Emission Computed Tomography

In vivo measurement of neuroreceptor binding in the living human can be done with single photon emission computed tomography (SPECT) or positron emission tomography (PET). These techniques enable the measurement of receptors, transporters, drug occupancy and synaptic fluctuations of neurotransmitters. The SPECT technique uses single photon emitting isotopes with longer half-life compared to positron emission tomography making it possible to produce the isotopes off site. The resolution of SPECT is lower than PET but due to longer half-life of the tracers steady state examinations are more easily conducted with this type of scans. Numerous ligands have been developed to examine the dopamine system in vivo. For dopamine D_2 receptor quantification both PET and SPECT ligands such as [^{11}C]raclopride, [^{11}C]fallypride, [^{11}C]FLB457 [^{123}I]IBZM and [^{123}I]epidepride have been used (Kegeles et al. 2010c; Tuppurainen et al. 2010; Kegeles et al. 2010b; Corripio et al. 2011; Arakawa et al. 2010). There is a large variability in ligand characteristics for both PET and SPECT with regard to affinity, specificity of receptor binding and metabolites. For examination of cortical dopamine D_2 receptors, high affinity ligands like [^{123}I]epidepride are preferable due to low D_2 -receptor density in these areas. [^{123}I]epidepride has a high affinity and specificity for D_2 and D_3 –receptors (in vitro K_i for D_2 = 0.024 nm and D_3 = 0.020nm) (Kessler et al. 1991; Kessler et al. 1993; Halldin et al. 1995). From autoradiographic data [^{123}I]epidepride binding mainly reflect D_2 receptors in cortical regions (Hall et al. 1996) and as such is suitable to examine cortical dopamine D_2 -receptors.

The size of the binding potential depends on the density of the receptors, affinity of the ligand to the receptor and endogenous dopamine levels. The change in one parameter will influence the size of the binding potential. In general, it is assumed that an increase in endogenous dopamine result in a decrease in the binding potential both due to competition with the ligand but also due to internalization of receptors (Sun et al. 2003; Quelch et al. 2014; Laruelle 2000).

Study Motivation for the PhD Thesis

Antipsychotics dampen dopamine D_2 receptor activity (Seeman et al. 1976) and from the literature we know that there is a relation between blockade of striatal D_2 receptors and the effect on positive symptoms as well as the risk of side effects. Less understood is how extrastriatal dopamine D_2 receptor blockade affects cognitive functions and progressive grey matter loss seen in patients with schizophrenia. Moreover, the association between extrastriatal $D_{2/3}$ receptor BP_{ND} in antipsychotic-naïve patients and the subsequent effect of dopamine D_2 receptor blockade on positive symptoms is unknown.

We have previously shown associations between dopamine $D_{2/3}$ receptor BP_{ND} and attention, flexibility and planning in antipsychotic-naïve schizophrenia patients (Fagerlund et al. 2013). Others have shown that a high level of dopamine blockade might worsen cognitive deficits (Uchida et al. 2009; Sakurai et al. 2013) in patients with schizophrenia. However, no previous studies have examined the effect of dopamine blockade on cognitive deficits in an initially antipsychotic-naïve sample.

By examining patients having their first treatment we avoid confounders such as chronicity, multiple admissions and treatment with several antipsychotic compounds. The longitudinal design using neuroimaging (SPECT and MRI) gives the opportunity to study pathophysiological changes and effects of dopamine D_2 receptor blockade more directly.

The understanding of the dopamine system is constantly increasing but there are still issues that need clarification. The increased dopamine synthesis capacity in associative striatum being linked to psychosis is one of the best validated findings in schizophrenia research. Others have, however, implicated prefrontal dopamine D_2 receptors (Seamans and Yang 2004) as another site involved in psychoses. We have previously shown frontal dopamine D_2 receptors to be associated with psychotic symptoms (Glenthoj et al. 2006); however, whether it can be used to predict treatment response remains unclear.

Finally, no previous study has examined if dopamine D_2 receptor availability is associated with grey matter in patients with schizophrenia, thus despite extensive research this topic remains unsolved.

Objectives and hypothesis

The overall objective of this thesis was to investigate effects of dopamine D_2 receptor blockade in a group of initially antipsychotic-naïve first-episode schizophrenia patients. More specifically, the objectives were to examine the effects of dopamine D_2 receptor blockade on cortical grey matter volume as well as cognitive measures of attention, processing speed and executive functions in patients who had never previously been treated with antipsychotic compounds.

In addition, we wished to examine whether higher baseline frontal D_2 BP_{ND} values can be used to predict outcome with regard to positive symptoms.

In a sample of initially antipsychotic-naïve first-episode schizophrenia patients we hypothesized:

- High extrastriatal blockade will further compromise selected cognitive functions
- Higher frontal baseline BP_{ND} will be associated with more pronounced reductions in positive psychotic symptoms
- Higher cortical dopamine $D_{2/3}$ receptor blockade will be associated with frontal and temporal grey matter volume reductions
- Grey matter reductions will be associated with more negative symptoms at 3 months follow-up
- In secondary analyses, we further explored associations between baseline extrastriatal dopamine $D_{2/3}$ receptor BP_{ND} and grey matter volume.

The main results will be presented and discussed in the above order and is followed by a more general discussion of the main results in relation to the overall objective of the thesis.

Materials and Methods

Study Design

The study has a longitudinal design where antipsychotic-naïve first-episode schizophrenia patients were examined in the naïve state at baseline and again after three months of their first antipsychotic treatment (Figure 8). Since this thesis explores associations with dopamine $D_{2/3}$ receptor availability focus is at baseline and three months follow-up. Previous data on baseline single photon emission computed tomography, magnetic resonance imaging, cognition and psychophysiology has been published (Fagerlund et al. 2004; Glenthoj et al. 2006; Glenthoj et al. 2007; Fagerlund et al. 2013). Moreover, a subgroup of 11 patients and 12 controls were re-examined with magnetic resonance imaging and psychophysiology after 6 years (Hammer et al. 2013).

The present thesis relates dopamine (SPECT), structural MRI, cognitive and psychopathological data to each other.

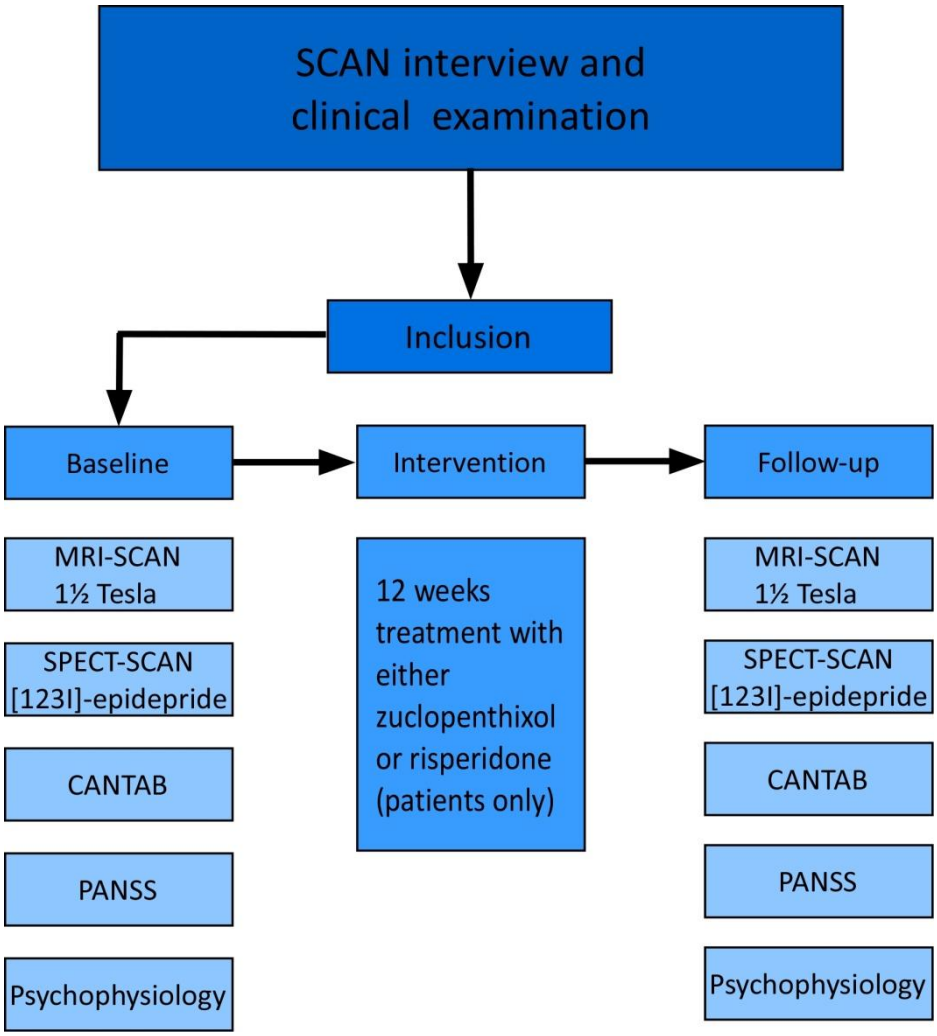

Figure 8.Flowchart of the study.

Participants

Baseline

Thirty-one patients were included in the longitudinal study from the Copenhagen capital region in the period 1998-2001. Inclusion criteria were: An international classification of diseases 10th revision (ICD-10) diagnose of schizophrenia, no prior exposure to antipsychotic medication and age between 18-45 years. Diagnosis was confirmed by using Schedules for Clinical Assessment in Neuropsychiatry, SCAN-2.0 (Wing et al. 1990). Patients with known mental retardation or patients who were compulsorily hospitalized were excluded. Twenty-eight of the 31 patients completed cognitive testing and psychopathology rating, and 25 patients completed all other baseline examinations. At inclusion, all patients were antipsychotic-naïve but three patients were minimally medicated before baseline magnetic resonance imaging was conducted (the last baseline examination) (Glenthoj et al. 2007).

For comparison twenty-five healthy controls were recruited among staff members at the local hospital and students at teaching institutions in the Copenhagen capital region. Twenty healthy controls completed both magnetic resonance imaging and single photon emission computed tomography. Inclusion criteria were age between 18-45 years, physical and mentally healthy. Exclusion criteria were: family history of mental illness (first degree relative), drug abuse of any kind, severe reading impairment (to ensure valid cognitive testing). To rule out severe psychopathology, controls were screened for major psychopathology using SCAN-2.0. Matching variables between patients and controls were age, gender and parental socioeconomic status (P-SES). Socio-economic status was based on combined parental education/occupation and household income parameters and thus, dividing participants into either high (A), middle (B) or low (C) status. By using P-SES we sought to avoid underestimating patients' potential, had illness not occurred, since schizophrenia impacts profoundly on the level of educational and occupational attainment.

After inclusion, participants completed the following baseline examinations.

	MRI	SPECT	Cognition	Psychopathology
Patients	25	25	28	28
Controls	20	20	25	-

Table 2. Showing numbers of participants completing listed examinations at baseline. Abbreviations MRI= magnetic resonance imaging, SPECT = Single photon emission computed tomography.

Intervention

Randomization Procedure

After inclusion each patient was given a consecutive identification number attached to an envelope. The envelope contained information about which medication the patient would be treated with. The treatment was not blinded, but the MRI and SPECT scans were considered "objective" evaluations.

Antipsychotic Medication

Treatment was initiated after completion of baseline examinations. Patients were randomized to treatment with either zuclopenthixol or risperidone. The dose was determined individually based on effect and reported side effects. For zuclopenthixol the dose range was 4-26 mg and for risperidone 1-7 mg. If patients experienced side effects the dose was reduced. Dose of antipsychotic medication was registered and serum concentrations were obtained as part of the SPECT scanning at follow-up. To measure serum concentration, blood was drawn from a vein and was analysed in a standard high performance liquid chromatography (HPLC) procedure. For zuclopenthixol, serum zuclopenthixol was measured since no known active metabolites are produced (http://www.medsafe.govt.nz/profs/datasheet/c/Clopixoltabinj.pdf). For risperidone the active moieties (risperidone + 9-hydroxy-risperidone) were obtained (van Beijsterveldt et al. 1994; van Schaick et al. 2003).

Patients were allowed to use benzodiazepines during the trial to reduce agitation and anxiety, though not on days of examination. Extrapyramidal side effects were regularly assessed with Extrapyramidal Symptom Rating scale (ESRS) (Chouinard and Margolese 2005). If patients experienced extrapyramidal side effects, the dose of antipsychotic was reduced. Substance use was assessed through clinical interviews, and urine was drug screened at baseline and at follow-up.

Clinical Measures

Psychopathology

Patients were assessed with positive and negative syndrome scale (PANSS) (Kay et al. 1987) before and after 3 months of treatment by the same experienced psychiatrist. Duration of untreated psychosis was estimated from interviews and is measured as the time from the first psychotic symptoms measured in months until the time of inclusion in the study.

Neurocognitive Tests and Assessment

Cognitive functions were assessed with the Cambridge Neuropsychological Test Automated Battery (CANTAB) (Lowe and Rabbitt 1998) by the same examiner at baseline and follow-up. CANTAB is a computerized series of standardized non-verbal tests where responses are registered by means of a touch-screen. It is validated for schizophrenia research (Levaux et al. 2007) and for assessing neurobiological substrate for cognitive deficits (Barnett et al. 2010). In the current thesis we wanted to test cognitive domains associated with frontal- striatal pathways.

A priori, we selected domains of attention, executive function, planning and processing speed comprising seven cognitive measures (Fagerlund et al. 2004; Fagerlund et al. 2013).

Selected cognitive measures were:

- CANTAB Rapid visual information processing
- CANTAB Stockings of Cambridge minimal number of moves
- CANTAB Stockings of Cambridge initial thinking time
- CANTAB Stockings of Cambridge subsequent thinking time
- CANTAB intra extra dimensional set shifting task
- Verbal semantic fluency
- Trail making B (minus A)

This test battery has been shown appropriate for assessing cognitive deficits in the fronto-striatal pathways (Elliott et al. 1995).

Single Photon Emission Computerized Tomography Acquisition

SPECT scans were performed using a Tomomatic 232 scanner (Medimatic, Copenhagen), a fast rotating (6 rounds per minute) brain dedicated SPECT scanner that simultaneously recorded radiation from two parallel, trans-axial slices of the brain with a slice thickness of 17 mm and distance between mid-slice levels of 10 mm. Four recording sessions of 15 minutes began approximately 6 hours after the bolus injection. Data were then achieved from 8 orbitomeatal (OM) planes covering the brain from OM +20 to OM +90, generating 8 slices. The spatial resolution in the trans-axial plane was 12 mm (FWHM). The energy window was set to 140-180 KeV. For quantification of $D_{2/3}$ receptors in extrastriatal regions we used [^{123}I]epidepride (Kessler et al. 1991) and a bolus/infusion approach (Pinborg et al. 2000). Tracer steady-state conditions were obtained in extrastriatal regions within 3-4 hours, but the infusion continued for 7 hours in order to minimize individual differences in plasma clearance and binding parameters. A 64x64 filtered back-projection reconstruction matrix was used to reconstruct data. The transaxial slices were corrected with a uniform attenuation coefficient of 0.05 cm^{-1}. Subjects received approximately 150 MBq [^{123}I]epidepride (MAP, Medical Technologies, Inc., Finland) per examination. The radiochemical purity was >99% and the specific activity >1.8x10^{14} Bq/nmol.

Blood Samples Analyses

Three venous blood samples were taken during the SPECT session; the first sample at 30 minutes before the scan, the second at the start of the scan and the third 30 minutes after completion of the scan. Samples were centrifuged and plasma samples counted in a well counter for determining radioactivity (Packard Auto-Gamma 5606, United Technologies Packard). Other plasma samples were examined using high performance liquid chromatography (HPLC) analysis for marked [^{123}I]epidepride and [^{123}I]epidepride metabolites. By using a gradient system the most hydrophilic metabolites come first out of the column and the most lipophilic last. The relative percentage of [^{123}I]epidepride compared to [^{123}I]- metabolites under the curve was calculated as previously described by (Kuikka et al. 1997). Retention time for [^{123}I]epidepride were determined by examination of the passage of pure [^{123}I]epidepride.

Regions of Interest

A set of six anatomical regions of interest (ROIs) (left and right frontal cortex, left and right temporal cortex, and left and right cerebellum) were manually delineated at 2D transverse MRI planes using a locally developed Matlab (Mathworks Inc., Natick, MA, USA) based program (editroi) (Figure 9). ROIs were identified by means of a neuroanatomical atlas (Talairach & Tournaux, 1988, MNI). Subsequently, ROIs were applied to the SPECT images and mean counts of the voxels included in each of the ROIs were extracted. BP_{ND} was calculated for left and right frontal cortex, left and right temporal cortex, and left and right cerebellum. Thalamus, putamen and caudate nucleus were also delineated, but because steady state is not obtainable in the basal ganglia within reasonable time using [^{123}I]epidepride, these ROIs they were not used in the analyses.

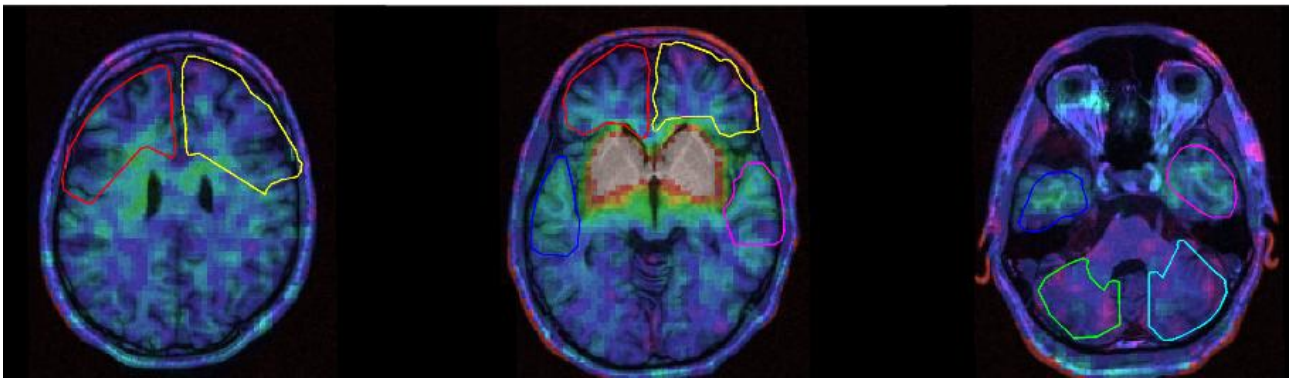

Figure 9. Axial image fusion of SPECT and MR image showing a typical alignment of a set of images. Regions delineated are left (red) and right (yellow) frontal cortex, left (dark blue) and right (purple) temporal cortex, and left (green) and right (light blue) cerebellum. SPECT spatial resolution in the trans-axial plain was 12 mm (FWHM) with a 17 mm slice thickness. MRIs were resliced to the planes defined by the SPECT images giving an in-plane resolution of 1x1 mm in the MRI images and 10 mm between slices. Abbreviations SPECT = single-photon emission computerized tomography, MRI = magnetic resonance imaging.

SPECT Data Analysis/ Quantification of Dopamine D_2 Receptors

The binding potential (BP_{ND}) was used as a measure of regional $D_{2/3}$ receptor availability before and after treatment (Innis et al. 2007).

$BP_{ND} = \frac{VT - VND}{VND}$, where V_T is the total volume of distribution of [^{123}I]epidepride in a ROI and V_{ND} represents the volume of distribution of non-displaceable [^{123}I]epidepride in tissue.

Cerebellum is used as a representation of non-displaceable [^{123}I]epidepride binding (Glenthoj et al. 2006). The regional $D_{2/3}$ receptor occupancy was estimated using the paired distribution volumes before (the unblocked situation) and after treatment (the partially blocked situation) and the Lassen Plot (Lassen et al. 1995). The regions included in the Lassen Plot were left and right frontal cortex,

left and right temporal cortex, and left and right cerebellum (Glenthoj et al. 2006; Norbak-Emig et al. 2016). Occupancy was calculated from the slope of the Lassen plot using linear regression analysis and provides a measure of extrastriatal $D_{2/3}$ occupancy (Figure 10).

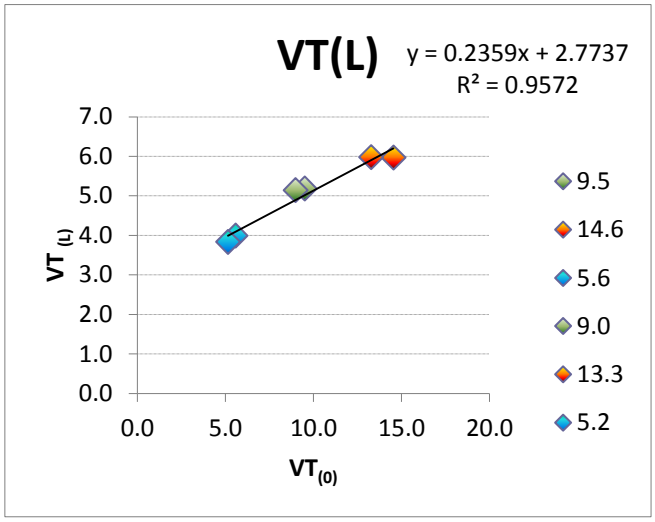

Figure 10.Lassen plot used to calculate extrastriatal occupancy in one patient. The paired distribution volumes in the unblocked situation $VT_{(0)}$ and the partially blocked situation $VT_{(L)}$ were used in a linear regression analysis providing a measure of extrastriatal $D_{2/3}$ occupancy. Occupancy was calculated as ((1-x) x 100%)(.i.e. (1 – 0.2359) x 100% = 76%). Orange markers left and right temporal cortex, green markers left and right frontal cortex and blue markers are left and right cerebellum.

Magnetic Resonance Imaging acquisition

High-resolution 3D T1-weighted sagittal MPRAGE scans of the whole head were acquired for each subject. Magnetic resonance imaging scans were acquired on two different scanners: two patients were scanned on a 1.0 T Siemens Impact scanner (TE= 4.4 ms; TR=11.4 ms; flip angle=15°; matrix=256×256; FOV=250 mm; 0.98×0.98×1 mm voxels; 170 slices). All other participants were scanned on a 1.5 T Siemens Vision scanner (TE=4 ms; TR=9.7 ms; flip angle=12°; matrix=256×256; FOV=250 mm; 0.98×0.98×1 mm voxels; 170 slices).
Patients were re-scanned at follow-up, and controls were not. MRIs were acquired on the same scanner at baseline and at follow-up.

Magnetic Resonance Imaging processing

The T1 weighted images were denoised using prefiltered rotationally invariant nonlocal means 3-dimensional (PRI-NLM3D) filter to reduce Rician noise (Manjon et al. 2012). The Rician noise levels in the images were estimated using an adaptation of the median absolute deviation estimator in the wavelet domain for Rician noise (Coupe et al. 2010). Rician noise estimation and removal was performed using MATLAB® software (version 2014a) (Mathworks Inc., Natick, MA, USA). Mean Rician noise in MRI images was estimated to 2.7%, which is fairly low. To ensure data

quality, all images were visually inspected before and after preprocessing, including assessment of noise maps. Denoised images were processed using functional magnetic imaging of the brain (FMIRB) software library (FSL 5.0.4) (Zhang et al. 2001). Non-brain tissue was removed with brain extraction tool (BET) (Smith 2002). The tissue-type segmentation was performed using FMRIB's Automated Segmentation Tool (FAST) also correcting for bias field (Zhang et al. 2001) generating probabilistic partial volume estimated (PVE) maps corresponding to grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). Each voxel contained a value in the range of 0-1 representing the probability of belonging to a particular tissue class. From the generated PVE maps, total brain volume (TBV) was calculated as the sum of grey matter and WM, and intracranial volume (ICV) as the sum of GM, WM and CSF.

Quantification of Grey Matter Volume

Regional average grey matter volumes were quantified for four of the six anatomical ROIs by averaging GM voxel values within left and right frontal cortex and left and right temporal cortex. A threshold of 0.5 for grey matter was used to increase the probability of voxels included were grey matter. Change in grey matter volume was then calculated as difference in mean intensity and calculated as [grey matter volume$_{\text{follow-up}}$ – grey matter volume$_{\text{baseline}}$].

Multimodal Co-registration

First, the intra subject co-registration between MRI structural T1 scan and SPECT scan was performed using a semi-automatic in-house developed program called ´Interactive Point Selection´ (Willendrup et al., 2004) implemented in Matlab®. At least six anatomical corresponding points were manually identified in SPECT and MR images separately. Two points were located in central putamen, two points in the center of the temporal poles, one point corresponding to incisura cerebelli posterior and one point in the longitudinal fissure cerebri. A rigid transformation matrix between the modalities was then automatically calculated by minimizing the sum of squared errors between the defined points.

The transformation was applied to the T1 weighted MR image and segmentation images (GM, WM and CSF generated using FSL 5.0.4) to enable reslicing to SPECT space (10 mm between slices and an in-plane resolution of 1x1 mm as in the MRI images) (Figure 11). By co-registering baseline and follow-up T1 MR images for each subject rigidly using the automatic method from the AIR sw package (AIR v.5, UCLA) (Woods et al. 1993), baseline ROIs were automatically transferred to follow-up SPECT scans using the linear transformation matrix estimated. Visual inspection was done for each subject to ensure correct transformation.

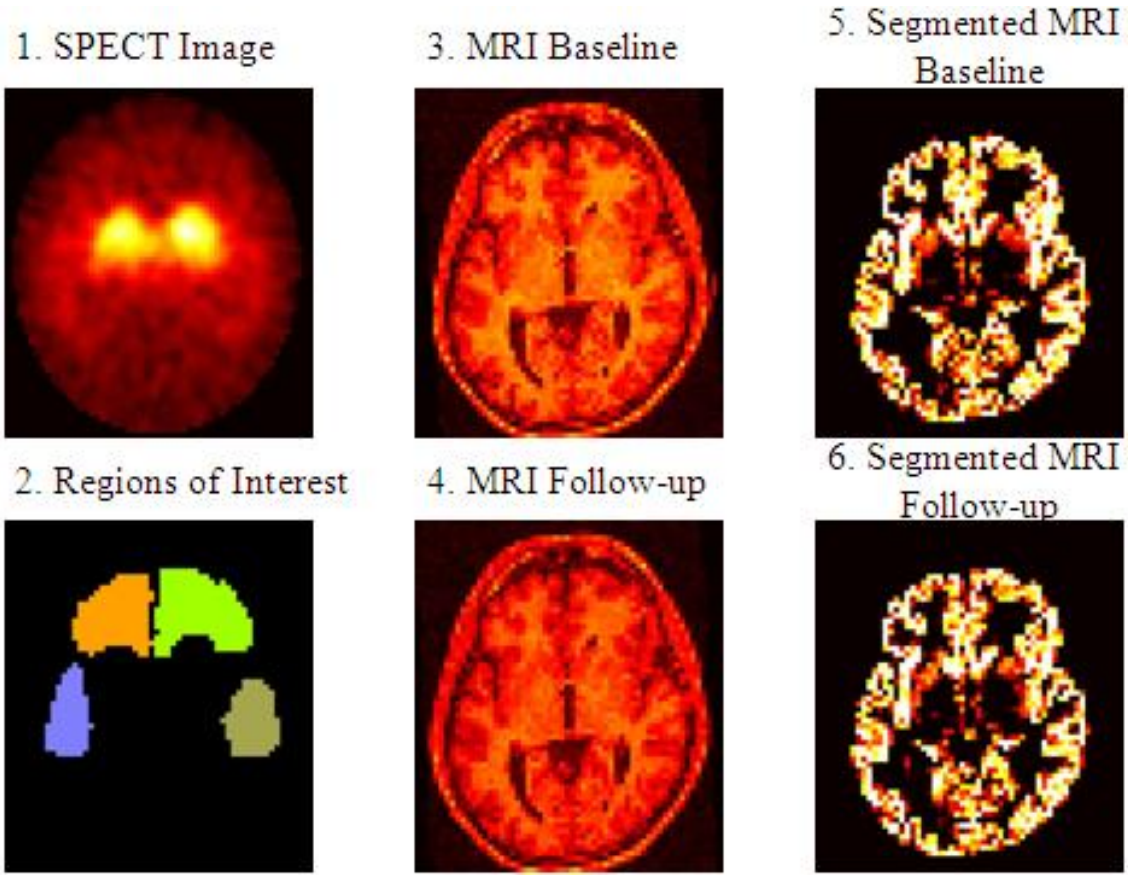

Figure11. The different image modalities used to co-register magnetic resonance and single photon emission computed tomography images. For healthy controls, only baseline magnetic resonance and SPECT images were used.

Image 1; SPECT image at baseline. Image 2; regions of interest applied to partial volume maps, delineated ROIs are: left (orange) and right (green) frontal cortex; left (blue) and right (grey) temporal cortex; and left and right cerebellum (not visualized in ROIs). Images 3 and 4; axial magnetic resonance image at baseline (3) and follow-up (4). Images 5 and 6; segmented partial volume estimated grey matter maps at baseline (5) and follow-up (6).

Statistical Analyses

All data analyses were performed using the Statistical Software System, SPSS 20 (SPSS, Statistics 20, IBM Corporation, Armonk, NY, USA). The Shapiro Wilk test was used to test if continuous variable distributions followed a normal distribution. Duration of psychosis and treatment period became normally distributed after a logarithmic transformation. Group comparisons across gender were tested with Fisher's exact test, and P-SES with Pearson's chi-square test. Possible age differences between controls and patients were tested using the paired samples t-test, and between treatment groups with an independent samples t-test. Differences in duration of psychosis, age and treatment period between the zuclopenthixol and risperidone treatment groups were tested using independent samples t-test.

Both in the complete dataset and in the two treatment groups, separately, occupancy data object variables could be fitted approximately by normal distribution for all ROIs. For BP_{ND} data object variables could also be fitted approximately by normal distribution for all ROIs for the complete dataset. Differences in occupancy between treatment groups were consequently tested using Analysis of covariance ANOVA.

Most of the cognitive measures could be fitted immediately by normal distribution and if not, a log transformation was applied to improve fit. Changes in cognitive function [follow-up - baseline] were calculated for all seven variables. Our measurement of change in cognitive variables were also analysed using the residuals from a regression analysis of endpoint on baseline values, predicting endpoint from baseline. Possible associations between occupancy and cognitive function were tested using correlation analysis in accordance with the statistical properties of the variables. Since previous data have shown the need for extending linear analysis through possible quadratic associations, both occupancy and squared occupancy measures were used in the analyses. Potential [Gender x Occupancy] and [Medication group x Occupancy] interaction effects for both occupancy and occupancy squared measures were tested.

ANCOVA was used to compare grey matter volume between patients and healthy controls at baseline with age, gender, ICV and P-SES as covariates (Andreasen et al. 2013; Glenthoj et al. 2007). Potential hemisphere differences within groups were tested using repeated measures analysis of variance (rmANCOVA) using hemisphere as within-subject factor, group (patients, healthy controls) as between subject factor and age, gender and P-SES as covariates. Next volumetric alterations over time in all patients were analysed with rmANCOVA, using change in volume variables and hemisphere as within subject factor, medication group as between subject factor, and age, gender, ICV and P-SES as covariates.

For rmANCOVA analyses, we first tested frontal cortex and temporal cortex grey matter volume. Subsequently we tested ICV, TBV, GM, WM and CSF. To test for specific changes in regional brain volumes in case of general atrophy frontal and temporal grey matter volume changes were also analysed correcting for TBV.

In *post-hoc* analyses of brain volume and blockade, we tested potential effects of cannabis abuse by excluding the three patients who had a positive urine test for cannabis at the time of inclusion. We

also did *post-hoc* analyses excluding patients (N=3), who were minimally medicated at baseline MRI.

Correlations between dose, serum concentration and occupancy were tested using Pearson's partial correlation coefficient correcting for age, gender, ICV and P-SES for each treatment group. Likewise, correlations between BP_{ND} and volumetric changes, as well as correlations between medication dose, antipsychotic serum concentrations and occupancy on volumetric estimates were tested using partial correlation analysis, correcting for age, gender, ICV and P-SES.
All tests were two-tailed, and the significance level was set to $\alpha = 0.05$. Analyses of the association between extrastriatal dopamine $D_{2/3}$ receptor occupancy, dose and serum concentration and the four ROI grey matter volumes (e.g. left and right frontal cortex and left and right temporal cortex) were corrected for multiple comparisons, correcting for the number of ROIs with a Bonferroni corrected p-value of (0.05/4) 0.01.

PANSS scores were not normally distributed and consequently, group differences were tested using non-parametric techniques. The Mann-Whitney *U* test was used to test differences in PANSS total and PANSS sub-scores between treatment groups. Improvement in psychopathology was calculated separately for PANSS positive, negative, general and total scores [$PANSS_{baseline} - PANSS_{follow\text{-}up}$] and tested using non-parametric Wilcoxon signed rank test. Spearman's rank-correlation coefficient was used to evaluate associations between grey matter volume, BP_{ND}, extrastriatal blockade and PANSS scores.

Results and Discussion

Demographics, Exclusions and Drop-outs

Data from 25 patients (18 males; 7 females) and 20 controls (13 males, 7 females) were included in this thesis (figure 12). The included participants completed the baseline examinations (see method section, participants). After baseline examinations nine patients were randomized to treatment with zuclopenthixol and 16 were allocated to risperidone for 12 weeks. At follow-up examinations were repeated (Figure 12).

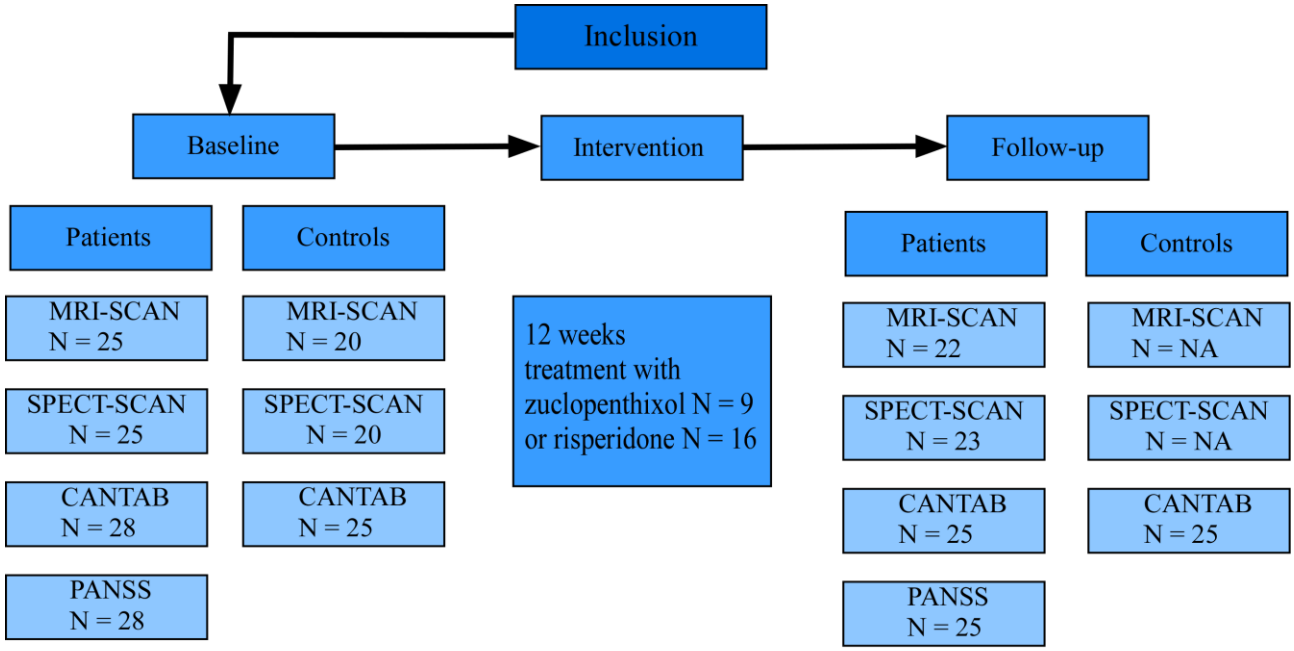

Figure 12. Flowchart of modalities assessed and numbers of participants included.

During the study two patients (one male, one female) were excluded as they became subject to compulsorily hospitalization. The two excluded patients did complete the PANSS assessment before exclusion, one completed the CANTAB test battery and one completed a follow-up SPECT scan. Both patients were in the risperidone group.

At follow-up three patients did not have the MRI scan included in the analyses. One patient had severe motion artefact, two patients did not complete the scan. 23 patients completed the SPECT scans, however, one patient (male) was excluded from follow-up analyses due to incomplete HPLC blood sample analysis (treated with risperidone). The neurocognitive test battery (CANTAB) was completed in 25 patients, one did not complete due to compulsorily hospitalization and two patients did not want to participate. In the healthy control group one (male) was excluded from MRI analysis due to motion artefacts.

By combining the different modalities the following N's were used in the different analyses.

- 25 patients were included in the analysis for SPECT baseline (BP_{ND}) and improvement in psychopathology
- 22 patients were included in the analysis of SPECT at follow-up (blockade) and improvement in psychopathology

- 21 patients were included in the combined analysis of SPECT at follow-up (blockade) and change in cognition
- 22 patients and 19 controls were included in the combined analysis of SPECT (BP_{ND}) and MRI (grey matter volume) at baseline
- 22 patients were included in the combined analysis of MRI at baseline (grey matter volume) and improvement in psychopathology
- 20 patients were included in the analysis of combined SPECT (BP_{ND} and blockade) and MRI at follow-up (change in grey matter volume)
- 20 patients were included in the combined analysis of MRI at follow-up (change in grey matter volume) and psychopathology

Patients and healthy controls included in the analyses were well-matched on age, gender (p-values > 0.49), and initially also on P-SES, but patients had significantly lower P-SES than healthy controls ($p = 0.027$) at follow-up due to patient drop-outs. The two patient groups were comparable regarding age, gender, P-SES, mean duration of psychosis and PANSS scores (p-values > 0.15).

Mean treatment period was 12.6 (SD ±3.4) weeks with no significant difference between the two treatment groups ($t = -0.69$, $p = 0.50$). Patients were moderately ill and psychopathology improved significantly (PANSS total and sub-scores) in the whole group and in the risperidone group. Patients treated with zuclopenthixol also improved in psychopathology (PANSS positive and total) and at trend level in negative symptoms (Table 2).

At inclusion three patients (all males) were tested positive for cannabis, but not at follow-up (two treated with risperidone, one zuclopenthixol). No patients had prior abuse of amphetamine or cocaine. Five patients had a history of alcohol abuse but none were abusing at the time of inclusion. One patient had previously undergone one month of treatment with a selective serotonergic reuptake inhibitor (SSRI) more than four weeks prior to inclusion (treated with risperidone).

	All patients (N = 25)	**Zuclopenthixol** (N = 9)	**Risperidone** (N = 16)	**Controls** (N = 20)
Age	26.5 (5.0)	26.3 (5.2)	27.2	26.0 (4.2)
Sex M/F	18/7	6/3	12/4	13/7
DUP (months)	19.4 (18.2)	15.3 (8.0)	21.7 (21.9)	NA
P-SES (A, B, C)	3, 18, 4	1, 5, 3	2, 13, 1	7, 13, 0
Positive urine test for cannabis (Y/N)	3/25	1/9	2/16	0/20
PANSS Baseline				
Positive	20.2 (3.9)	18.4 (2.3)	21.2 (4.3)	NA
Negative	19.6 (5.3)	17.8 (5.1)	20.6 (5.3)	NA
General	30.2 (6.6)	28.2 (7.6)	31.3 (5.9)	NA
Total	70.0 (12.6)	64.4 (12.3)	73.1 (12.1)	NA
PANSS follow-up				
Positive	10.4 (2.2)	9.7 (1.9)	10.9 (2.3)	NA
Negative	16.5 (3.4)	15.3 (2.9)	17.1 (3.6)	NA
General	20.6 (2.7)	19.8 (2.3)	21.0 (3.0)	NA
Total	47.7 (6.1)	19.8 (2.3)	21.0 (2.9)	NA
PANSS change				
Positive	9.76 (3.2)** [< 0.001]	8.78 (2.2)** [0.008]	10.31 (3.6)** [< 0.001]	NA
Negative	3.12 (4.4)** [0.004]	2.44 (3.5) [0.065]	3.50 (4.9)** [0.021]	NA
General	9.6 (4.9)** [< 0.001]	8.4 (6.2) ** [0.012]	10.3 (4.1)** [< 0.001]	NA
Total	22.24 (10.6)** [< 0.001]	19.67 (10.7)** [0.008]	23.69 (10.6)** [< 0.001]	NA
Treatment period (Weeks)	12.6 (3.4)	12.8 (4.2)	12.5 (3.0)	NA

Table 3. Demographics, clinical ratings and treatment. Mean values are shown. Standard deviations are shown in () and p values in []. Significant differences between groups are marked with, p < 0.05. Significant changes in PANSS score over time are marked with **. There were no other significant differences between groups. Abbreviations DUP = Duration of psychosis, PANSS = Positive and Negative Syndrome Scale.*

Medication and Extrastriatal $D_{2/3}$ Receptor Blockade

Mean extrastriatal $D_{2/3}$ receptor blockade did not differ between medication groups (t = 0.13, p = 0.90). For mean dose in treatment group, please see Table 4 (Norbak-Emig et al. 2016).

In the patient subgroup where SPECT and MRI analyses were combined, correlations between dose, serum concentration and extrastriatal occupancy were co-varied with age, gender, P-SES and ICV. In this subgroup mean extrastriatal dopamine $D_{2/3}$ receptor occupancy was 64.8% (N = 20, range 31-88%, $r^2 = 0.94$) for all patients. 65% (N = 9, range 52-76%, $r^2 = 0.95$) in patients treated with zuclopenthixol (mean dose 9.6 mg, range of 4-26 mg) and 63.9 % (N = 11, range 31-88%, $r^2 = 0.94$) for patients treated with risperidone (mean dose 3.6 mg, range of 1-7 mg). Mean $D_{2/3}$ receptor occupancy did not differ significantly between medication groups (t = 0.29, p = 0.77). Serum concentration and occupancy were not significantly correlated in the patients treated with zuclopenthixol but were for patients treated with risperidone.

For both treatment groups, medication dose and serum concentrations correlated. When uncorrected, dose and extrastriatal occupancy were significantly correlated in the risperidone group (Figure 13A) but not in the zuclopenthixol group (Figure 13B). When correcting for age, gender, P-SES and ICV extrastriatal dopamine $D_{2/3}$ receptor occupancy was not correlated with dose or serum concentration in the zuclopenthixol (dose; r =0.55, p = 0.34, serum; r = 0.66, p = 0.23) or risperidone group (dose; r = 0.63, p = 0.13, serum; r = 0.58, p = 0.17).

	All Patients	**Zuclopenthixol**	**Risperidone**
Number	22	9	13
Mean Dose (mg)	NA	9.6 (4-26)	3.9 (1-7)
Median Dose (mg)	NA	8	3
Mean Serum Concentration	NA	11.8 (4-36)	59.3 (16-192)
Extrastriatal Occupancy (%)	65 (31-88), r = 0.94	66 (52-76) r = 0.95	65 (31-88) r = 0.93
Correlation Dose and Serum Concentration	NA	r = 0.86, p = .003*	r = 0.8, p = 0.002*
Correlation Serum and Extrastriatal Occupancy	NA	r = 0.57, p = 0.04*	r = 0.51, p = 0.16
Correlation Dose and Extrastriatal Occupancy	NA	r = 0.68, p = .01*	r = 0.46 p = 0.21

Table 4. Medication characteristics correlations are not co-varied for age, gender, parental socioeconomic status and intracranial volume. Abbreviations range in (); r = the correlation of the regression slope of the Lassen plot. Significant correlations (p > 0.05) are marked with.*

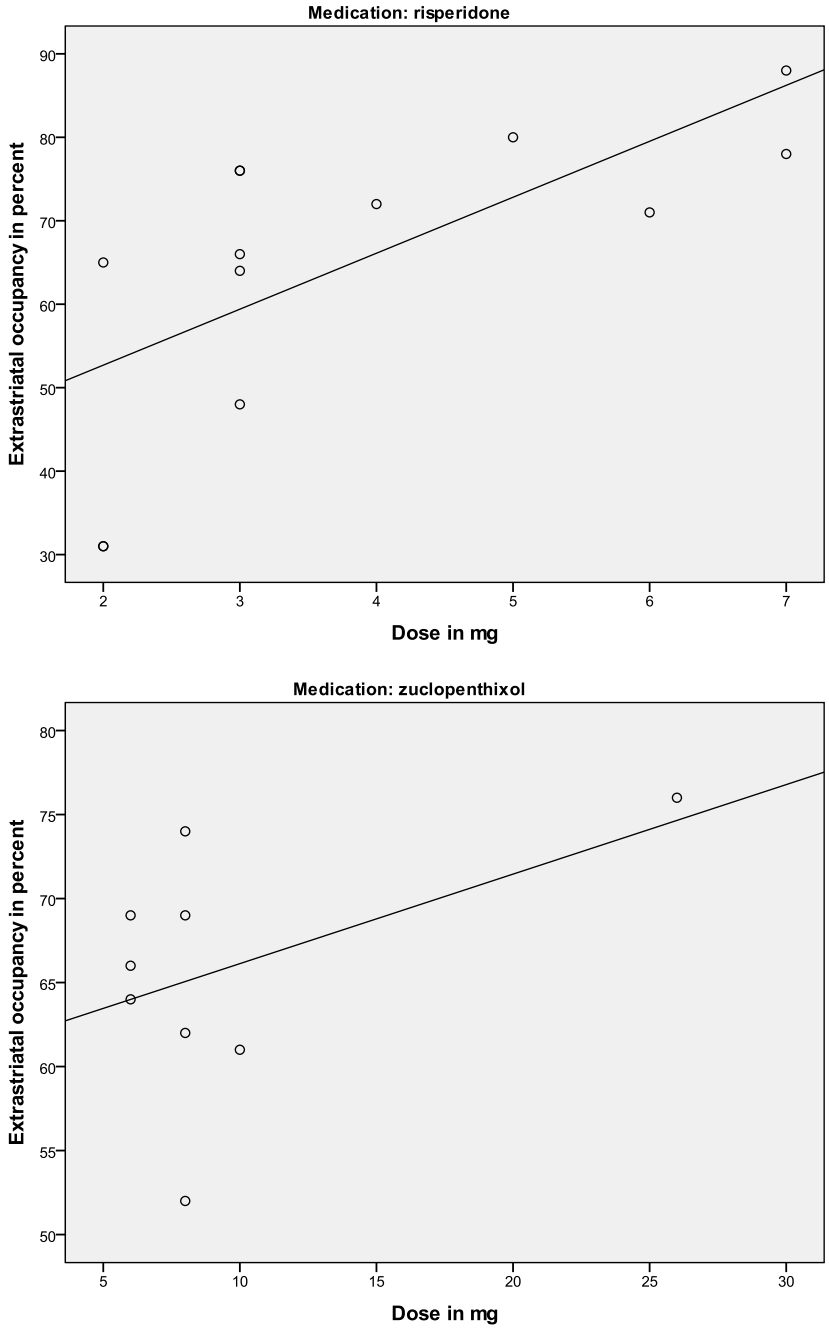

Figure 13: Scatter plots showing correlations between dose and extrastriatal dopamine $D_{2/3}$ receptor occupancy in the two treatment groups.
13A. Risperidone dose and extrastriatal dopamine $D_{2/3}$ receptor occupancy.
13B. Zuclopenthixol dose and extrastriatal dopamine $D_{2/3}$ receptor occupancy.
Patients treated with risperidone had significant correlations between dose and occupancy N = 13, r = 0.68, p = .01. For patients treated with zuclopenthixol no significant correlations were found N = 9, r = 0.46, p = 0.2. (the plots are uncorrected scatter plots). This is most likely due to limited variation in zuclopenthixol dose range with 8 out of 9 patients receiving 6-10 mg.
Note there seems to be only 11 patients depicted in the scatter plot treated with risperidone. This is because two patients received 2 mg risperidone and had extrastriatal occupancy of 31 % and two patients received 3

mg risperidone and had a occupancy of 76 %, hence two patients cannot be seen on the scatter plot as the dots are overlapping.

Extrastriatal Dopamine $D_{2/3}$ Receptor Blockade and Cognition

For the total group of patients (N = 21) in the combined analyses of cognition and extrastriatal $D_{2/3}$ receptor blockade, we showed a significant negative correlation with planning time at follow-up (Stockings of Cambridge initial thinking time, N = 21, r = -0.436, p = 0.048) (Figure 14A). Moreover, blockade and improvement in attention from baseline to follow-up was negatively correlated (signal detection measure A' from the Rapid Visual Information Processing test, N = 21, r = -0.615, p = 0.003) (Figure 14B) (Norbak-Emig et al. 2016).

In the analyses of the separate treatment groups, patients treated with zuclopenthixol showed a significant negative correlation between dopamine blockade and attention at follow-up (signal detection A' from the Rapid Visual Information Processing test, N = 9, rho = -0.795, p= 0.018) and at a trend level with planning latency, likewise at follow-up (Stockings of Cambridge initial thinking time, N = 9, r = -0.70, p = 0.050). For patients treated with risperidone, a significant negative correlation between blockade and improvement in attention was found (signal detection A' from the Rapid Visual Information Processing test, N = 13, r = -0770, p = 0.003). Even when using two different statistical methods (follow-up-baseline and residuals using regression, please see statistical analyses) the overall results remained the same regardless of method. By using the same method (follow-up-baseline) as Fagerlund et al. (Fagerlund et al. 2013; Fagerlund et al. 2004) results are comparable.

The significant negative associations to follow-up scores on planning latency may suggest a beneficial effect of high occupancy levels on specific cognitive functions. However, our group has previously shown the apparent within-group improvement in cognitive scores in patients to be parallel to retest effects in healthy controls and in addition, patients performed worse than controls both at baseline and follow-up regarding planning efficiency (Fagerlund et al. 2004). Taken together our results suggest that faster planning latencies at follow-up were not advantageous, but rather indicate a lack of sufficient planning (Fagerlund et al. 2013).

That antipsychotic medication may affect some specific cognitive functions negatively are in accordance with other findings showing that antipsychotics worsen planning (Harris et al. 2009; Keedy et al. 2014) and attention (vigilance) (Sakurai et al. 2013) in first-episode schizophrenia patients as well as attention (Tost et al. 2006) and decision-making (Eisenegger et al. 2014) in healthy volunteers.

Our results may be explained by the two-state dynamic model of dopamine function in prefrontal cortex (Seamans and Yang 2004) according to which increased frontal D_2 receptor activity allows multiple representations to be presented in the network at the same time. By blocking dopamine D_2 receptors this may switch the D_2 dominated network state into the D_1 dominated state. We speculate this will result in less attention to stimuli due to a weakened D_2 network and possibly an increased D_1 receptor activation hereby decreasing incoming stimuli. Another important feature of prefrontal

functioning is the role of D_1 receptors in the maintenance of information, e.g. in working memory functionally demonstrated to follow an inverted U-curve (Goldman-Rakic 1995). In the study by Nørbak-Emig et al. (Norbak-Emig et al. 2016) the variables significantly affected by blockade of frontal $D_{2/3}$ receptors were the variables that in the baseline data showed quadratic associations with $D_{2/3}$ BP_{ND} in line with an inverted U-curve (Fagerlund et al. 2013).

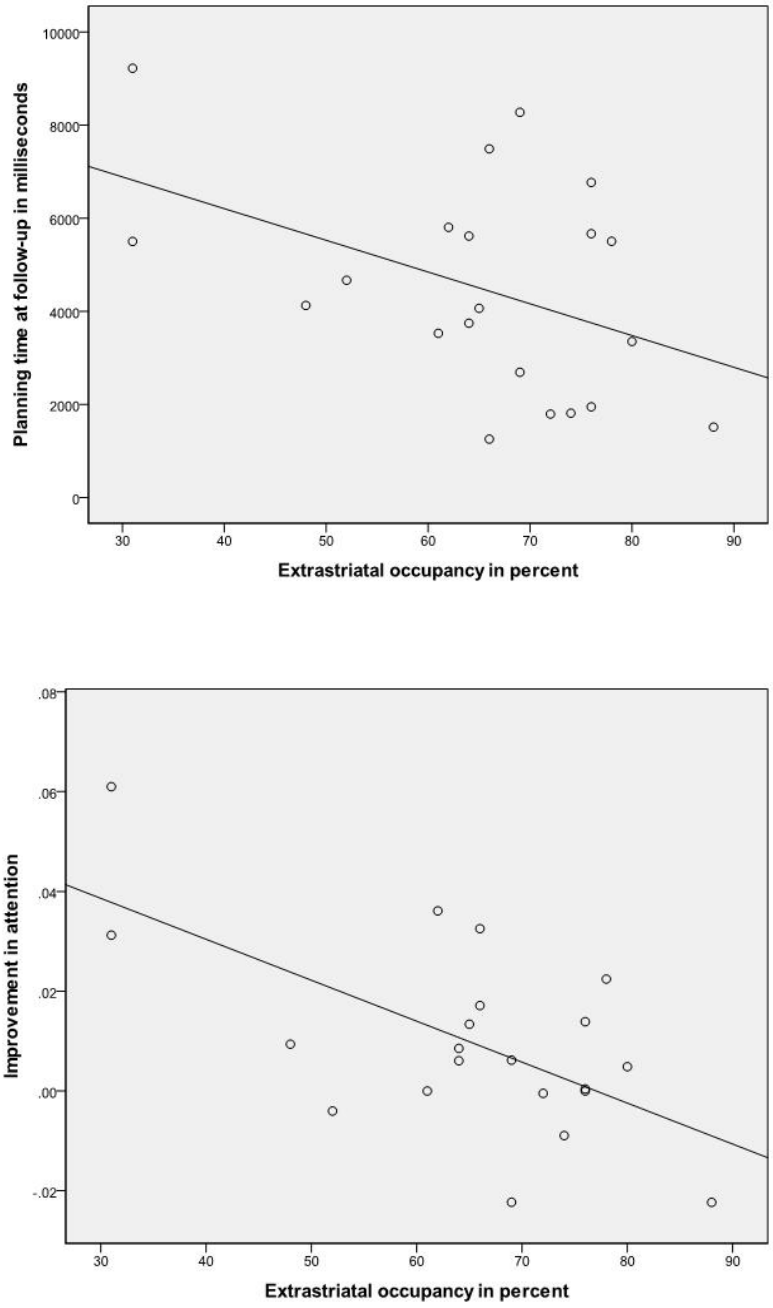

Figure 14. Scatter plots showing correlations between extrastriatal dopamine $D_{2/3}$ receptor occupancy and cognitive measures.

Figure 14A shows planning time at follow-up measured with CANTAB (Stockings of Cambridge initial thinking time), N = 21, r = -0.436, p = 0.048. Extrastriatal occupancy is in per cent, planning time in msek. Figure 14B shows improvement in attention measured with CANTAB (signal detection measure A' from the Rapid Visual Information Processing test), N = 21, r = -0.615, p = 0.003. Occupancy is in per cent, attention scores range from 0-1 with 1 indicating optimal signal detection.

Our results may indicate that blockade of frontal $D_{2/3}$ receptors by antipsychotics may worsen some cognitive domains following an inverted U-curve, possibly by overshooting the optimal window for cognitive processing, perhaps similar to the inverted U-curve involvement of frontal dopamine D_1 receptors' function in working memory (Brozoski et al. 1979; Williams and Goldman-Rakic 1995; Arnsten 2013). Although the cognitive domains examined in this study did not worsen significantly from baseline to follow-up, the strength of the negative correlations between follow-up scores, especially on attention with occupancy, suggests that the impact of occupancy is not negligible. To emphasize this, we have previously demonstrated that the patients treated with zuclopenthixol, occupancy explained as much as 62 % of the variance in attention at follow-up. Therefore, attentional focus and selection may be more affected by dopamine $D_{2/3}$ receptor blockade than any of the other functions (Fagerlund et al. 2013). Our group has also recently shown a negative effect of $D_{2/3}$ receptor blockade on level of function, which is in agreement with the present findings of a negative association between occupancy and certain cognitive functions (Wulff et al. 2015).

However, other parts of the dopamine system may also affect cognitive functions. Blockade of dopamine D_2 receptors in striatum can decrease information flow to prefrontal cortex. By blockade of D_2 receptors this will affect the indirect pathway increasing inhibition of the thalamus (Carlsson 2006) and decreasing dopamine firing in VTA (Surmeier et al. 2007). Dopamine containing neurons in the VTA projects to the prefrontal cortex and decreased firing in VTA may lower dopamine concentration further in prefrontal cortex. Since dopamine D_2 receptors are already blocked in the prefrontal cortex, dopamine would facilitate increased activation of prefrontal D_1 receptors and further push the system toward a D_1 receptor dominated state and worsen already compromised functioning (figure 5). This is clinically relevant since cognitive deficits are most often present at illness onset and may be further compromised by high $D_{2/3}$ receptor blockade (Lepage et al. 2014; Fervaha et al. 2014), which is also indicated by our present data.

Frontal Dopamine $D_{2/3}$ Receptor BP_{ND} and Treatment Response

In the total patient group (N = 25), we found, as expected significant positive correlations between BP_{ND} in frontal cortex bilaterally and improvement in positive symptoms (Figure 15), (BP_{ND} in left frontal cortex rho = 0.56, p = 0.003; BP_{ND} right frontal cortex rho = 0.48, p = 0.016) (Norbak-Emig et al. 2016). This was also found in the risperidone group with significant positive correlations between BP_{ND} in frontal cortex and improvement in positive symptoms (BP_{ND} left frontal cortex p = 0.007, rho = 0.64; BP_{ND} right frontal cortex p = 0.002, rho = 0.71). No significant correlations were found in the zuclopenthixol group (p > 0.4).

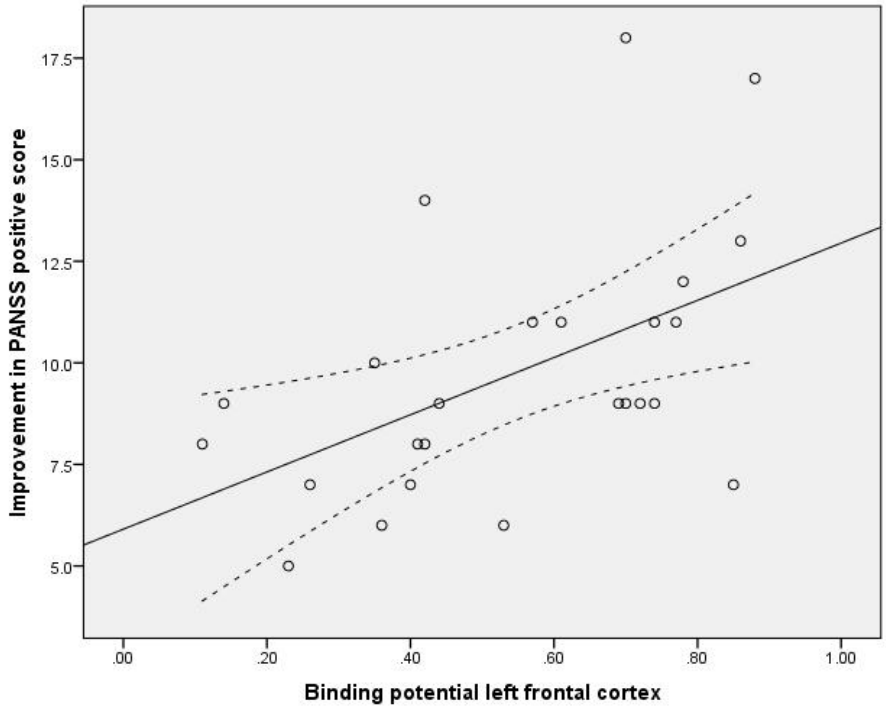

Figure 15. Correlation between left frontal dopamine $D_{2/3}$ receptor BP_{ND} in the antipsychotic-naïve state and treatment-outcome. Improvement in PANSS positive score (follow-up minus baseline) after 3 months of treatment N=25, rho = 0.56, p<0.01. Linear regression shown as fully drawn line, and confidence intervals as dashed lines.

That baseline frontal $D_{2/3}$ receptor BP_{ND} correlates with improvement of positive symptoms supports that high frontal $D_{2/3}$ receptor availability is associated with treatment response in antipsychotic-naïve first-episode schizophrenia patients. Since patients and controls did not differ significantly with regard to BP_{ND} at baseline (Glenthoj et al. 2006), this suggests a receptor-mediated effect which is in accordance with the model of Seamans and Yang (Seamans and Yang 2004). Here, a high state 1 with over-activation of dopamine D_2 receptors will result in reduced inhibition of $GABA_A$ and NMDA receptors resulting in increased glutamategic activation leading to psychotic symptoms. Thus, frontal BP_{ND} may predict treatment response in antipsychotic-naïve patients before the patient's first treatment with a dopamine antagonist.

Although Seamans and Yang's model may explain our results, the question whether there is a hypo-dopaminergic state present in prefrontal cortex is still unanswered. Hypothetically, if so this may compromise the model by Seamans and Yang, since it is suggested that high concentrations of dopamine primarily activate D_2 receptors whereas low levels activate D_1 receptors (Seamans and Yang 2004). Thus, if dopamine levels are low, dopamine D_2 receptors should not come in a state of "over-activation". As of present, one study has shown decreased dopamine synthesis capacity in dorsolateral prefrontal cortex (Slifstein et al. 2015). However, they also found lower dopamine

synthesis capacity in midbrain regions where previous studies (Kumakura et al. 2007; Howes et al. 2013) have found higher dopamine synthesis capacity. Methodological differences in modelling in that both Slifstein et al. and Kumakura et al. use modified and somewhat novel models may account for differences. Another possibility could be that dopamine D_2 receptors in prefrontal cortex are hypersensitive possibly due to an elevated level of D_2^{High} receptors where a small increase in density or dopamine level might be enough to cause psychotic symptoms (Seeman 2013; Seeman and Seeman 2014).

We did not find significant correlations between $D_{2/3}$ receptor BP_{ND} and change in PANSS positive scores in the smaller group of patients treated with zuclopenthixol. This is likely due to the uniform dosing with most patients receiving 8 or 10 mg.

Extrastriatal Dopamine $D_{2/3}$ Receptor Occupancy and Psychopathology

In secondary analyses, we also looked at associations between extrastriatal dopamine $D_{2/3}$ receptor occupancy and improvement in PANSS positive symptoms for the whole group (N = 22). No such associations were found. Separate analyses of the two treatment groups did not alter the results (Norbak-Emig et al. 2016).

This is in apparent opposition to the model of Seamans and Yang (Seamans and Yang 2004) and previous finding where increased presynaptic striatal dopamine activity was associated with treatment response (Demjaha et al. 2012) and associations between striatal dopamine D_2 receptor blockade and treatment response (Howes et al. 2009). Hence, occupancy in general would be expected to decrease positive symptoms. However, an inverse dopaminergic relationship between prefrontal cortex and striatum may account for this discrepancy (Wilkinson 1997; Clarke et al. 2014).

The model by Seamans and Yang suggests that patients with schizophrenia will have increased prefrontal D_2 receptor activity resulting in random, tangential or intrusive thoughts leading to the development of positive symptoms. Blocking dopamine D_2 receptors would “close the gate” and hence reduce positive symptoms. However, frontal striatal interactions may oppose the effect of dopamine blockade. Del and Mora have shown that frontal D_2 receptor activity regulate dopamine levels in nucleus accumbens and by blocking D_2 receptors there will be an increase in dopamine in nucleus accumbens (Del and Mora 2005). This regulatory mechanism has also been demonstrated more recently where dopamine depletion in orbito-frontal cortex caused an increase in the caudate nucleus (Clarke et al. 2014). The authors speculate that this increase is mediated through a dopamine D_1 receptor pathway. If so, blockade of dopamine D_2 receptors could “push” the network towards a higher D_1 state that would increase dopamine in the caudate nucleus. This interaction is also supported by a recent study in an independent sample from our group. Here, low $D_{2/3}$ receptor BP_P in the caudate nucleus at baseline was significantly associated with treatment effect on positive symptoms in initially antipsychotic-naïve first-episode schizophrenia patients (Wulff et al. 2015).

Even so, occupancy studies have repeatedly shown striatal dopamine D_2 receptor blockade being associated with treatment response to psychotic symptoms (Nordstrom et al. 1993; Kapur et al. 2000; Agid et al. 2007; Kegeles et al. 2008; Nord and Farde 2011) and striatum has been proposed to be the target site of therapeutic action of antipsychotic drugs. Still, other studies have not found this association (Wulff et al. 2015; Meisenzahl et al. 2008). In the study by Potkin et al., higher striatal (caudate, putamen) dopamine D_2 receptor blockade was correlated with lower PANSS positive score at follow-up but not with improvement in symptoms (Potkin et al. 2013). Here improvement in positive symptoms was correlated with occupancy in thalamus and amygdala. Possible differences in patient inclusions, sample sizes and methodology in different studies may contribute to this discrepancy. A notion is that some studies use a baseline BP_{ND} estimate (Agid et al. 2007) instead of the patients own BP_{ND} and as such not accounting for inter-individual variability.

Brain Volumes at Baseline and Brain Volume Changes

Patients (N = 22) and healthy controls (N = 19) did not differ significantly in baseline TBV (t = -0.45, p = 0.7), ICV (t = -0.08, p =0.9), GM (t = -0.6, p = 0.6), WM (t = -0.2, p = 0.8), CSF (t = 1.5, p = 0.1), or left (t = -1.4, p = 0.2) and right (t = -0.8, p = 0.4;) frontal as well as left (t = -0.07, p = 0.9) and right (t = 0.6, p = 0.5) temporal ROI grey matter volumes (Table 5) (Norbak-emig et al. 2016). Analyses of treatment groups (13 risperidone, 9 zuclopenthixol) did not reveal significant differences in global brain volumes or in grey matter volume in ROIs (p > 0.1). In patients, duration of untreated illness was not correlated with regional baseline grey matter volume (ROIs) or global volume measures (p > 0.4).

Overall, patients (N = 20) did not have significant changes in ROIs over time (F < 0.6, p > 0.5) with no group x time interaction (F< 1.4, p >0.3). A numerical decrease in grey matter volume in all ROIs over time was observed. Neither was there any significant effect of time in global brain volumes (F < 2.4, p > 0.1) nor significant [group x time] interaction (F < 0.2, p > 0.6). Numerical decreases were observed in all global volumes except CSF (Table 5).

	All patients		**Zuclopenthixol**		**Risperidone**		**Controls**
N	22	20	9	9	13	11	19
Time	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline
ICV	1559 (235)	1514 (181)	1520 (271)	1496 (180)	1586 (215)	1529 (189)	1569 (123)
TBV	1254 (202)	1212 (149)	1226 (239)	1206 (153)	1273 (182)	1218 (154)	1279 (109)
GM	678 (107)	659 (74)	661 (130)	656 (74)	689 (92)	662 (77)	693 (62)
WM	576 (104)	553 (82)	564 (113)	551 (84)	583 (100)	555 (85)	583 (52)

CSF	302 (42)	302 (43)	294(42)	290 (38)	312 (45)	311 (45)	289 (31)
Left FC GM	**52.9* (9.5)**	52.6 (8.7)	56.8 (11.3)	56 (9.9)	50.3 (7.4)	49.7 (6.8)	**57.9* (5.1)**
Right FC GM	**59.5* (11.5)**	59.3 (10.7)	63.1 (13.4)	63.1 (11.4)	56.9 (9.7)	56.2 (9.5)	**63.8* (7.4)**
Left TC GM	**35.8* (6.8)**	35.7 (6.9)	35.7 (8.9)	36.0 (8.1)	35.9 (5.3)	35.5 (6.1)	**36.3* (5.7)**
Right TC GM	**39.1* (6.8)**	38.0 (6.6)	40.2 (8.6)	40.0 (7.3)	38.4 (7.0)	36.3 (5.5)	**38.2* (5.7)**

*Table 5. Brain volumes in patients and controls at baseline and follow-up, values are in mean cm^3, standard deviation in (). Abbreviations: N= number of subjects; TBV= total brain volume; ICV = intracranial volume; GM = grey matter volume; WM = white matter volume; CSF = cerebrospinal fluid; FC = frontal cortex; TC = temporal cortex. Significant differences between left and right hemisphere are marked in bold with *, $p < 0.05$.*

Our finding of comparable brain volumes between patients and controls at baseline are in concordance with findings from an independent cohort from our group (Ebdrup et al. 2010) and findings on basal ganglia volumes in the same patients (Glenthoj et al. 2007). These findings suggest that first-episode patients have very subtle brain volumetric changes. Detecting these changes may still be done using meta-analytic approaches where numbers and therefore power can be considerably higher (Radua et al. 2012; Haijma et al. 2013).

Medication and Brain Volume Changes

In the subgroup (N = 20) where MRI and SPECT scans were combined the extrastriatal dopamine $D_{2/3}$ receptor occupancy did not correlate with changes in frontal or temporal grey matter volume (Norbak-emig et al. 2016). Nor did occupancy correlate with changes in ICV, TBV, GM, WM and CSF volume ($p > 0.7$). To test if treatment impacted the results analyses were repeated with separate analysis of treatment groups though this did not alter the results ($p > 0.2$). In the two treatment groups we also examined associations between brain volume measures medication dose and serum concentration.

First, we did not find associations with ICV, TBV, GM, WM and CSF volume, dose ($p > 0.15$) and serum concentration ($p > 0.13$) in either group. Nor were any significant correlations with ROIs found in the zuclopenthixol group. In the risperidone group, we found positive partial correlations between dose and a relative grey matter volume increase in right frontal cortex ($r = 0.90$, $df = 7$, $p = 0.001$) and left frontal cortex ($r = 0.76$, $df = 7$, $p = 0.017$) (Figures 16A and 16B). Accordingly, positive partial correlations between serum concentration and a relative grey matter volume increase

in the right frontal cortex (r = 0.76, df = 7, p = 0.026) and left frontal cortex (r = 0.72, df = 7, p = 0.035) were found. No significant correlations for the temporal lobes were found. Only the correlation between volume increase and right frontal cortex survived correction for multiple comparisons.

Our finding that patients did not display significant frontal or temporal cortex grey matter volume loss is in contrast to large scale, long-term and more naturalistic MRI studies of more chronic patient samples (Cahn et al. 2006; van Haren et al. 2008; Ho et al. 2011; Vita et al. 2012; Radua et al. 2012; Haijma et al. 2013; Fusar-Poli et al. 2013). The multiple potentially confounding factors that long follow-up periods introduce may complicate interpretation of results. Factors like substance abuse, unhealthy life-style and metabolic disturbances may interfere and veil the direct effect of antipsychotics on brain volume (Van Haren et al. 2013). Moreover, results from meta-analyses investigating effects of antipsychotic medication are not consistent, as mentioned in the introduction, with two studies showing brain volume loss over time (Radua et al. 2012; Fusar-Poli et al. 2013) one mixed results (Torres et al. 2013) and one with differentiated effects of FGAs and SGAs (Vita et al. 2015).

Nevertheless, after 12 weeks we observed subtle numerical volumetric decreases in most ROIs (Table 4). We (Ebdrup et al. 2010), and others e.g. (Molina et al. 2005; Roiz-Santianez et al. 2012) have previously reported similar subtle numerical volumetric changes following initial antipsychotic exposure. Although limited to a three months treatment period, the current design allows for a more accurate estimation of direct associations between specific antipsychotic compounds and brain volume. Interestingly, higher doses of risperidone were associated with a relative frontal grey matter volume increase at three months follow-up in this study (Figures 16A and 16B).

Previous studies examining effect of risperidone treatment on cortical brain volume in schizophrenia patients have shown mixed results. In the studies by Garver and colleagues and Goghari and colleagues (Garver et al. 2005; Goghari et al. 2013), risperidone treatment was associated with increased cortical grey matter volume. No effect on brain volume was shown by Molina et al. and Roiz-Santianez et al. (Molina et al. 2005; Roiz-Santianez et al. 2012) and decrease in frontal volume was found in the study by Girgis and colleagues (Girgis et al. 2006). Methodological differences may explain differences in findings (Table 6). Since two studies did not mention dose parameters, it is difficult to draw conclusions on the dose and grey matter volume relationship. Treatment period and/or chronicity may have an impact on the results since neither of the long-term studies in chronic patients showed any changes (Molina et al. 2005; Roiz-Santianez et al. 2012). Another explanation of the associations between higher doses of risperidone and a relative frontal grey matter volume increase may be pharmacological. In relatively low doses (around 3 mg/day) risperidone induces a substantial frontal serotonin 5-HT_{2A} blockade (around 85%) (Nyberg et al. 1999; Reimold et al. 2007; Ebdrup et al. 2011a) and a combined serotonin and dopamine blockade might be necessary for increase in grey matter volume in first-episode schizophrenia patients.

	Patients	Number	Antipsychotic-naïve	Dose	Treatment period	GM measure-ment	GM change
Garver 2005	Unmedicated Two Months Before Inclusion	7	No	NA	4 weeks	ROI Based Analysis	↑
Girgis 2006	FEP	15	Yes	Mean 2.67 mg/day, Range 1–5 mg.	6 weeks	Voxel Based Morphometry	↓
Goghari 2013	FEP	16	Yes	NA	8 weeks	Cortical Thickness	↑
Molina 2005	FEP Chronic	12 5	Yes	5 mg/day ±2 mg	2 years	ROI Based Analysis	↔
Roiz-Santianez 2012	Chronic	16	No	3-6 mg/day	1 year	Cortical Thickness	↔

Table 6. Methodological differences between studies treating patients with risperidone. Abbreviations: FEP = First-episode schizophrenia, ROI = region of interest, GM = grey matter.

Morphologically, this could be a result of increases in the neuropil. In preclinical studies risperidone treatment is associated with increased neuropil density in the prefrontal cortex (Mackowiak et al. 2009) and risperidone has also been shown to increase the expression levels for proteins implicated in synaptogenesis (Tan et al. 2007). In an animal model for schizophrenia, chronic treatment with risperidone did not cause volume reductions (Castellano et al. 2013). However, in the study by Selemon et al. risperidone caused marked neuron loss in two out of six monkeys though this may have been due to toxic levels of the drug in the two animals (Selemon et al. 1999). Others have also reported volumetric decreases associated with antipsychotic treatment where differences between FGAs and SGAs were indistinguishable (Dorph-Petersen et al. 2005; Konopaske et al. 2007; Konopaske et al. 2008; Vernon et al. 2011; Vernon et al. 2014).

For zuclopenthixol, we found a negative correlation between dose and relative grey matter increase in the right frontal cortex. Although, this finding did not survive correction of multiple comparisons, the directionality is in line with preclinical observations of negative effects of haloperidol, another so-called first-generation antipsychotic, on brain volumes (Dorph-Petersen et

al. 2005; Vernon et al. 2014; Vernon et al. 2011). Moreover, a clinical study trying to separate effects of FGAs and SGAs on brain volume found that patients treated with an FGA (haloperidol) had grey matter volume loss whereas no loss was evident in patients treated with an SGA (olanzapine)(Lieberman et al. 2005). However, the limited variation in zuclopenthixol dose range of 6-10 mg (8 out of 9 patients) compromises more definite conclusions to be drawn from the present dataset.

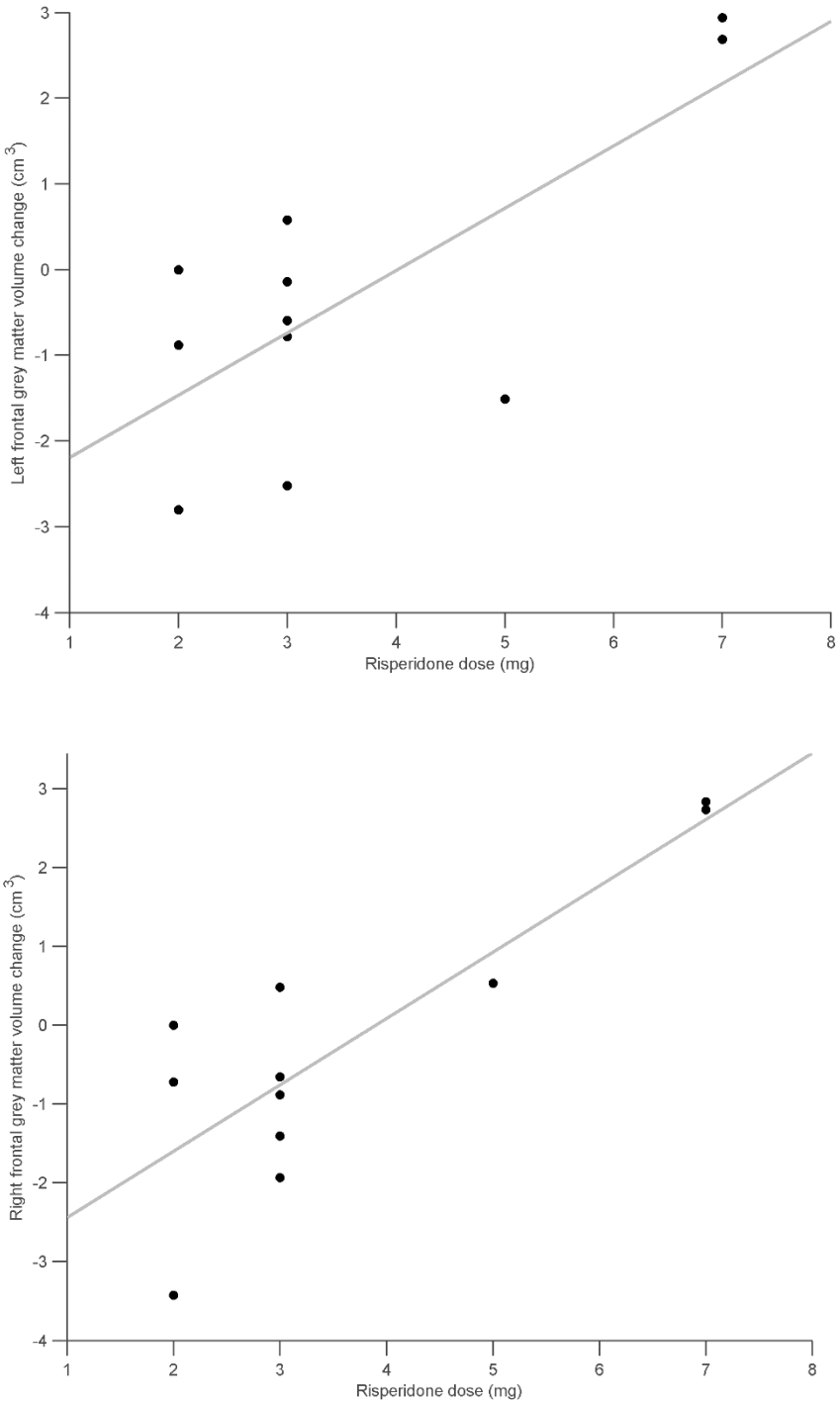

Figure 16. Scatter plots showing significant positive correlations between risperidone dose and grey matter volume change after three months of monotherapy.
16A Left frontal cortex (2A), (N = 11, r^2 = 0.90, p = 0.001).
16B Right frontal cortex (2B), (N =11, r^2 = 0.76 p = 0.017).

Psychopathology and Brain Volumes

We did not observe any significant correlations between psychopathology and global brain measures or grey matter ROI volumes in patients (N = 22) (Norbak-emig et al. 2016). However, when analysing medication groups separately, patients treated with zuclopenthixol showed a positive correlation between improvement in negative symptoms and grey matter volume increase in left temporal cortex (rho = 0.69, df = 9 p = 0.040). In patients treated with risperidone, we found correlations between improvement in negative symptoms and larger baseline grey matter volume in both left (rho = 0.62, df = 13, p = 0.025) and right frontal cortex (rho = 0.69, df = 13, p = 0.010). However, none of these correlations survived correction for multiple comparisons.

Our findings suggest that negative symptoms and grey matter volume are correlated. First, larger baseline grey matter volume in left and right frontal cortex was correlated with improvement in negative symptoms. Second, grey matter volume increase in left temporal cortex was also associated with improvement in negative symptoms. This indicates that grey matter preservation may positively affect improvement in negative symptoms. A study by our group from an independent sample of initially antipsychotic-naïve first-episode schizophrenia patients has shown comparable results where increase in ventricular size was correlated with less improvement in negative symptoms (Ebdrup et al. 2011b). Other studies have also found associations where total brain volume loss and total grey matter volume loss were correlated with more negative symptoms (Cahn et al. 2006) and cortical thinning has been correlated to more negative symptoms (Ansell et al. 2014). That frontal and temporal lobes may be key brain regions affected is also supported by Bodnar et al. where patients with persistent negative symptoms had more cortical thinning in frontal and temporal regions compared to both patients not having persistent negative symptoms and healthy controls (Bodnar et al. 2014). Since negative symptoms are closely linked to functional outcome (Hunter and Barry 2012; Fervaha et al. 2014) our observations encourage identification of efficient treatment regimens for negative symptoms.

Effect of Cannabis on Medication, Cortical Grey Matter and Psychopathology

Post-hoc we re-analysed our data after taking out three patients who were tested positive for cannabis at baseline (Norbak-emig et al. 2016). This did not significantly change observed correlations between baseline BP_{ND} and grey matter volume in patients hence results remained non-significant. In the remaining patients (N = 17), extrastriatal dopamine occupancy correlated positively with a relative volume increase in right frontal GM (r = 0.72, p = 0.005), but not in left frontal GM (p = 0.061), or in the temporal lobe GM volumes (p-values > 0.091).

The positive correlation between occupancy and relative volume increase in right frontal cortex appeared mainly driven by the risperidone group (N = 9, r = 0.96, p = 0.009), whereas in patients treated with zuclopenthixol no significant correlations with occupancy were found (N = 8, p > 0.10). In addition, risperidone dose was positively correlated with a relative right (r = 0.9, p =

0.032), but not left frontal, grey matter volume increase (r = 0.9, p = 0.069). In patients treated with zuclopenthixol, a negative correlation was found between dose and relative grey matter increase in the right frontal cortex (r = -0.97, p = 0.028), but this finding did not survive correction for multiple comparisons. No significant associations were found with temporal cortex grey matter volumes (p > 0.5).

By excluding patients who were tested positive for cannabis use at baseline, extrastriatal dopamine $D_{2/3}$ receptor occupancy appeared positively associated with a relative grey matter volume increase in frontal cortex. These three patients had significantly higher BP_{ND} in both left and right temporal cortex (Glenthoj et al. 2006) though no significant difference in extrastriatal occupancy. Whether cannabis may affect our results is unclear. In cannabis abusers with no psychotic illness cannabis consumption was not associated with dopaminergic alterations (Ghazzaoui and Abi-Dargham 2014), though another study has shown inverse correlations between striatal BP_{ND} and cannabis consumption, where larger intake correlated with lower BP_{ND} in striatum (Albrecht et al. 2013). Our findings underscore the need to further study the influence of cannabis abuse on extrastriatal dopamine receptor availability in schizophrenia patients.

Yet, cannabis consumption could also affect grey matter volume. A study from our group has shown larger hippocampal volume reductions in antipsychotic-naïve first-episode patients with known substance abuse compared to non-abusing patients (Ebdrup et al. 2010). Though possibly coincidental it is interesting that both high dopamine D_2 receptor BP_{ND} occur in temporal regions where abusing patients also have grey matter volume reductions. Others have also related cannabis consumption with grey matter reduction in frontal cortex, anterior cingulate and cerebellum in first-episode schizophrenia patients (Szeszko et al. 2007; Rais et al. 2008; Rapp et al. 2012) though another study found no effect of cannabis on brain volume (Bangalore et al. 2008).

Thus, it remains unsolved whether cannabis consumption could have affected dopamine blockade and possible association with a relative grey matter volume increase.

Extrastriatal Dopamine $D_{2/3}$ Receptor BP_{ND} and Brain Volumes

No significant correlations between BP_{ND} and baseline regional grey matter volume (ROIs) in patients (N = 22, p > 0.3) or in controls (N = 19, p > 0.1) were found (Norbak-emig et al. 2016). Analysing the medication groups separately did not reveal any significant correlations (zuclopenthixol, p > 0.1, risperidone, p > 0.4). No significant correlations between BP_{ND} at baseline and changes in frontal or temporal grey matter volume in patients were found (N = 20, p > 0.3). Nor was BP_{ND} in frontal or temporal ROIs at baseline correlated with change in total grey matter volume or total grey matter volume at follow-up (p > 0.08).

We did not find baseline $D_{2/3}$ receptor BP_{ND} to correlate with grey matter volume in frontal and temporal ROIs in patients nor in controls. Also, baseline BP_{ND} was not associated with grey matter volume changes at follow-up. To date, four studies have examined frontal $D_{2/3}$ receptors in first-episode schizophrenia patients. Three studies did not find frontal $D_{2/3}$ receptor BP_{ND} to be altered

compared to healthy controls (Talvik et al. 2003; Glenthoj et al. 2006; Kessler et al. 2009), and one study found reduced density of D_2 receptors BP_{ND} in the anterior cingulate (Suhara et al. 2002).

Seven studies have examined BP_{ND} in the temporal cortex (Suhara et al. 2002; Tuppurainen et al. 2003; Talvik et al. 2003; Buchsbaum et al. 2006; Glenthoj et al. 2006; Kessler et al. 2009; Kegeles et al. 2010c). A recent meta-analysis including 84 patients and 86 healthy controls from six studies reported unaltered temporal $D_{2/3}$ receptor BP_{ND} in patients compared to controls (Kambeitz et al. 2014). As such, there is no clear indication of altered extrastriatal dopamine $D_{2/3}$ receptor density in patients compared to healthy controls. In healthy subjects higher dopamine $D_{2/3}$ receptor BP_{ND} has been associated with higher grey matter volume in cortical regions (Woodward et al. 2009). Whether larger grey matter volume can support more dopamine $D_{2/3}$ receptors or more receptors generate more grey matter volume remains unsolved.

For patients with schizophrenia aberrations in the dopamine system, but also the other receptor systems, e.g. the cortical serotonin $5HT_{2A}$ receptor system (Rasmussen et al. 2010), glutamate and GABA (Kahn and Sommer 2014) may have affected a relationship between brain volume and $D_{2/3}$ receptor BP_{ND} in our patients.

To our knowledge, no previous studies in antipsychotic-naïve first-episode schizophrenia patients have examined the relationship between extrastriatal dopamine $D_{2/3}$ receptor BP_{ND} and frontal as well as temporal grey matter volume changes, but we did not find such a correlation. Morphologically, the dopamine system is suggested to be important for normal cerebral development and imbalances are suggested to be involved in the development of schizophrenia (Money and Stanwood 2013). Moreover, dopamine receptors have been shown to modulate dendritic cyto-architecture (Wang et al. 2009; Parish et al. 2001) and dopamine D_2 receptor genes have been linked with schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). Thus, an altered function of dopamine D_2 receptors in schizophrenia may disrupt associations present in healthy individuals.

Strengths and Limitations

The inclusion of antipsychotic-naïve first-episode schizophrenia patients enables assessment of biochemical markers before confounding factors like medication and chronicity. By combining assessment of dopamine $D_{2/3}$ receptor activity, cortical grey matter volumes, cognition and psychopathology in a longitudinal design, we have optimised the conditions for relating these potential biomarkers to changes in symptom domains in patients undergoing three months of antipsychotic monotherapy.

Before completion of all follow-up examinations, five of the twenty-five patients were excluded from analyses. However, only three patients dropped out in the analyses conducted in paper one corresponding to a very low attrition rate of 12%. Apart from all patients being in the risperidone group, the dropouts did not differ with regard to clinical and neurochemical values at baseline.

Treatment period and doses were also comparable to the other patients (see table 1) with a mean dose of 3.3 mg. Hence, we judge attrition bias unlikely to have affected our results. In the analyses of grey matter volume and SPECT, five patients were excluded but this was primarily due to low quality of the scans.

Our measurement of change in cognitive measures could favour using a regression model based on the assumption that post-test minus pre-test change scores are contaminated by the regression effect and usually correlate negatively with baseline/pre-test scores. We did use a post-test minus pre-test change measure in this thesis, however, we also analysed the variables using the residuals from a regression analysis of endpoint on baseline values, predicting endpoint from baseline. The results from this analysis did not significantly change the overall results. We do conclude that even when using the two statistical methods the overall picture remains the same.

To minimize the risk for type 2 errors we primarily tested *a priori* hypotheses where we had hypothesised on the relation between dopamine blockade and change in cognition based on our own previous findings (Fagerlund et al. 2004; Fagerlund et al. 2013). For our analyses of receptor density and positive symptoms we also had *a priori* hypothesis based on previous findings (Glenthoj et al. 2006) and hence we did not correct for multiple comparisons.

We found that extrastriatal occupancy affected aspects of cognition. It is, however, important to keep in mind that extrastriatal occupancy is a global measure of cortical dopamine $D_{2/3}$ receptor blockade, and we cannot separate frontal from temporal blockade in this study. Thus, we cannot exclude that direct blockade of temporal $D_{2/3}$ receptors or interactions with temporal $D_{2/3}$ receptors may also have affected cognition (Tregellas et al. 2014).

Moreover, correlation analyses only show associations and not a causal relationship between BP_{ND} and improvement in PANSS scores and other factors like endogenous dopamine and other transmitter systems e.g. serotonin 2A receptors (Rasmussen et al. 2010; Rasmussen et al. 2011; Ebdrup et al. 2011a) may be involved in psychosis and affect our results.

In the analyses of SPECT and cortical grey matter changes, the low spatial resolution of SPECT in itself limits the anatomical detail with which one can assess different sub-regions of the brain. However, ROIs were delineated on MRI images and transferred to SPECT images after careful registration of SPECT and MRI images. Due to the high affinity of epidepride it is impossible to distinguish between D_2- and D_3 receptors, but we note that D_3 receptors are sparsely represented in cortex (Beaulieu and Gainetdinov 2011).

Our sample size is relatively large compared to other studies examining extrastriatal $D_{2/3}$ receptors in antipsychotic-naïve schizophrenia patients. Compared to other MRI studies, the study is, however, small. Although our multimodal design allows for an estimation of potential direct associations between specific antipsychotic compounds and brain volumes, we should not ignore that our small sample size might prevent us from detecting subtle relations between grey matter volume changes and dopamine D_2 receptors in schizophrenia patients.

Psychometrically, the measure of change in the PANSS positive score (calculated by subtraction of the two scores) to assess the effect of blockade on positive symptoms is identical to our previous papers based on the same cohort of patients. This allows us to compare the present with previous data on the same patients. Moreover, our hypothesis was related to the change in the PANSS positive score.

Finally, our study period of only three months limits inferences regarding long-term effects of zuclopentixol and risperidone on brain volumes. This finding is to be considered as supplementing, rather than disavowing the growing evidence of negative volumetric effects of antipsychotics based on large-scale, naturalistic MRI studies.

Conclusion

Overall, our data provide a multifaceted description of the effects of dopamine $D_{2/3}$ receptor blockade after the first antipsychotic treatment in initially antipsychotic-naïve first-episode schizophrenia patients. By combining MRI and SPECT scans with symptomatology and cognitive measures it is possible to bring further insight into what happens when patients have their first treatment.

First, blockade of frontal $D_{2/3}$ receptors by antipsychotics may worsen some specific cognitive domains, possibly by compromising the optimal prefrontal network functioning. Taken together with our previous baseline data (Fagerlund et al. 2013), the present findings further support the notion that frontal dopamine $D_{2/3}$ receptors are involved in specific cognitive processes (attention) in antipsychotic-naïve first-episode schizophrenia patients.

This observation is in contrast to the lack of association between extrastriatal $D_{2/3}$ receptor blockade and extrastriatal grey matter loss, at least not in the early phases of illness. If anything, data suggest a relation between $D_{2/3}$ receptor blockade and a relative frontal grey volume increase. If so, short-term dopamine $D_{2/3}$ receptor blockade does not appear to be neurodegenerative.

Moreover, the data suggest that short-term blockade of dopamine $D_{2/3}$ receptors in contrast to long-term treatment (Ho et al. 2011; Torres et al. 2013; Andreasen et al. 2013; Haijma et al. 2013; Fusar-Poli et al. 2013) does not contribute to cortical grey matter loss and it highlights the importance of supplementing naturalistic observations with longitudinal monotherapy studies of initially antipsychotic-naïve first-episode schizophrenia patients.

We observed positive associations between frontal BP_{ND} at baseline and positive symptoms. This is in line with our previous finding where higher BP_{ND} was associated with more positive symptoms (Glenthoj et al. 2006). In this thesis higher frontal BP_{ND} was related to the effect of their first antipsychotic treatment on positive symptoms suggesting that treatment of positive symptoms may be predicted based on the patient's dopamine activity at baseline. The results on baseline dopamine $D_{2/3}$ receptor BP_{ND} and cortical grey matter volumes did not show significant associations. Thus, the relation between cortical grey matter volumes and dopamine $D_{2/3}$ receptors in first-episode schizophrenia patients remains unsolved.

In summary, our data support that dopamine D_2 receptor blockade has differentiated effects on patient subgroups when assessing different parameters where the therapeutic window for optimal treatment response varies considerably with regard to which parameters one assesses and between patient subgroups.

However, our data do not support treating patients with high doses of antipsychotics. Instead, patients treated with antipsychotics should be monitored closely to assess positive and negative symptoms as well cognitive dysfunction and treated with the lowest effective dose reducing side-effects and not worsening cognitive dysfunction. This treatment regimen may not increase cortical grey matter, though a medium dose might prevent further loss. A previous study has suggested a dose of 4 mg risperidone to be in the optimal range for treating positive symptoms with lowest incidence of side effects (Nyberg et al. 1999).

Perspectives

The studies in this thesis show differentiated effects of dopamine blockade on cognition, psychopathology and grey matter volume in patients and emphasize that finding biomarkers for a personalized treatment instead of the current trial and error approach would to a larger extent tailor the treatment to the individual patient's symptoms and deficits.

Future studies should examine whether partial dopamine D_2 receptor antagonism using a partial dopamine D_2 receptor agonist, like aripiprazole, shows a differentiated effect on cognitive functions in first-episode schizophrenia patients. Also, the findings of increased dopamine synthesis capacity in striatum in patients with schizophrenia, and the shown inverse relationship between prefrontal and striatal dopamine, should be linked with cognitive disturbances. Moreover, investigating relations between dopamine synthesis capacity and other neurochemical abnormalities, not the least glutamatergic, and their association with cognitive functions as well as their predictive value with regard to treatment outcome would be highly valuable. Further, studies relating neurochemical abnormalities to other brain alterations may further elucidate possible neuropathological mechanisms in schizophrenia that might be of potential value for future treatment strategies.

We find that frontal BP_{ND} at baseline could predict treatment outcome on positive symptoms, yet SPECT and PET imaging data are still hard to get but together with the existing literature, the present data contribute with an important insight into the significance of different neurochemical profiles in patients with schizophrenia for treatment outcome. Nevertheless, tracer imaging is still quite expensive and requires the use of radiation and will most likely continue to be a technique preserved for research and not used in clinical psychiatric settings.

However, in research settings by using multimodal approaches including both biological markers (SPECT, PET, EEG and MRI) and clinical assessments (cognitive testing and rating of psychopathology) this may give a broad characterization of the patients. Combined with advanced statistical methods such as unsupervised machine learning this could reveal clinically meaningful patient subgroups at baseline.

Finally, relating baseline findings in antipsychotic-naïve first-episode schizophrenia patients with long-term follow-up data on brain volumes, cognition and functional outcome may help finding stable biomarkers to predict treatment outcome. If we further apply (supervised) machine learning approaches to such data, we might come closer to a more individualized treatment; especially, if the advanced unsupervised approaches applied to the antipsychotic-naïve patients at baseline are combined with the longitudinal data.

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Appendices

Appendix 1: Paper I

Henrik Nørbak-Emig, Bjørn H. Ebdrup, Birgitte Fagerlund, Claus Svarer, Hans Rasmussen, Lars Friberg, Peter N. Allerup, Egill Rostrup, Lars H. Pinborg, Birte Y Glenthøj
Frontal $D_{2/3}$ receptor availability in schizophrenia patients before and after their first antipsychotic treatment: Relation to cognitive functions and psychopathology

Appendix 2: Paper II

Henrik Nørbak-Emig, Lars H. Pinborg, Jayachandra M. Raghava, Claus Svarer, William F.C. Baaré, Peter Allerup, Lars Friberg, Egill Rostrup, Birte Glenthøj and Bjørn H. Ebdrup
Extrastriatal dopamine $D_{2/3}$ receptors and cortical grey matter volumes before and after three months of antipsychotic treatment in antipsychotic-naïve schizophrenia patients

Appendix 3: Declaration of co-authorship paper I+II