PhD thesis

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The Connection between Dopamine D_2 Activity, Reward Disturbances and Psychopathology in Antipsychotic-Naïve First-Episode Patients with Schizophrenia

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Preface

The present PhD study is based on data collected during my appointment as PhD student at the Center for Neuropsychiatric Schizophrenia Research (CNSR) and the Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS), Psychiatric Center Glostrup, University of Copenhagen.

Recruitment of patients and healthy controls as well as clinical examinations and treatment took place at CNSR and CINS. The structural and functional magnetic resonance imaging (fMRI) scans and single photon emission computed tomography (SPECT) examinations, including concomitant plasma analyses, were carried out at the Diagnostic Department, Section of Functional Imaging Unit (FIUnit) and Section of Physiology and Nuclear Medicine, Glostrup Hospital, University of Copenhagen. The plasma analyses for SPECT were also performed during a validation period at the Neurobiology Research Unit (NRU), Rigshospitalet, University of Copenhagen. The estimations of serum amisulpride concentrations were performed at the Department of Clinical Biochemistry and Pharmacology, Odense University Hospital.

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Across the parking lot from CNSR at the Department of Clinical Physiology and Nuclear Medicine, everyone was incredibly helpful and made not only me but also our patients feel most welcome. A special thanks to you, Annette Foldager, for making the day run smoothly.

I am very grateful for the opportunity I was given to work at CNSR as a PhD student. I would like to thank my marvellous colleagues for our fruitful discussions at journal club, at PANSS ratings and for spontaneous talks in the hallway. Also, the way frustrations sometimes easily could be turned into a laugh is truly invaluable.
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List of Publications

This thesis is based on the following two manuscripts:

Paper One

Wulff S, Pinborg LH, Svarer C, Jensen LT, Nielsen MØ, Allerup P, Bak N, Rasmussen H, Frandsen E, Rostrup E, Glenthøj BY. Striatal $D_{2/3}$ Binding Potential Values in Drug-Naïve First-Episode Schizophrenia Patients Correlate with Treatment Outcome. 2014. In review

Paper Two

Wulff S, Rostrup E, Nielsen MØ, Jensen LT, Pinborg LH, Svarer C, Glenthøj BY. Normalisation of Brain Reward Abnormalities Correlates with Dopamine $D_{2/3}$ Receptor Blockade - a Longitudinal Study of First-Episode Schizophrenia Patients. 2014

Summary

Schizophrenia is a complex, severe chronic brain disorder that often manifests itself in early adulthood. The pathophysiology and underlying pathogenesis are not fully understood. The neurotransmitter dopamine is known to play an essential role in the neurobiological mechanisms, and in recent years increased dopamine activity in the striatum has been a consistent finding in patients with schizophrenia at onset. All antipsychotic medication shares a blocking effect on the dopamine receptors in the striatum. The treatment response, however, varies and 20-30% of all patients are considered resistant to the treatment. This heterogeneous response to treatment is in line with the hypothesis of schizophrenia being not one unique disorder, but rather, a heterogeneous group of disorders. During the last decades, an imbalance in other neurotransmitter systems in the brain and the interplay between them have been suggested as being important to the pathophysiology. Recently, it has been hypothesised that patients not responding to treatment belong to subgroup(s) of patients without dopaminergic hyperactivity, but perhaps disturbances in other neurotransmitter systems.

Dopamine also plays a key role in brain reward processing. There are consistent findings of reward disturbances in patients with schizophrenia, which are suggested to associate with psychotic symptoms, such as delusions and hallucinations. Evidence indicating, that dopamine plays a direct role in this relationship, however, is still lacking.

Today, treatment is based on trial and error. The ability to place patients in subgroups and to predict the treatment response would spare the patients from unnecessary treatment trials and adverse effects. The literature emphasises the need for predictive markers for treatment outcome as well as for the development of side effects. In the search for such predictive markers, it is crucial to examine the patients before their brains have been modified by antipsychotic medication and repeated relapses. Longitudinal studies in antipsychotic-naïve first-episode patients are therefore of great importance.

This thesis intends to investigate the association between the initial binding to dopamine $D_{2/3}$ receptors and the treatment effect from the blockade of these receptors, as well as the proposed relationship between the blockade of dopamine $D_{2/3}$ receptors and normalisation of brain reward disturbances in antipsychotic-naïve first-episode schizophrenia patients.

The study is based on data from a longitudinal study of 28 antipsychotic-naïve first-episode schizophrenia patients and 26 healthy controls. The participants were examined with psychopathology measures, single photon emission computed tomography using [^{123}I]-iodbenzamide as the radioligand to explore the dopamine $D_{2/3}$ receptor binding potential (BP_p), and a functional magnetic resonance imaging paradigm to explore reward abnormalities. Examinations were performed before and after six weeks of treatment with the selective dopamine $D_{2/3}$ antagonist amisulpride.

At baseline we found that a lower BP_p in the striatum, which is believed to reflect dopamine hyperactivity, was related to a better treatment response. Patients showed alteration of brain activity in the striatum during the anticipation phase of reward processing compared to healthy controls. This alteration in reward processing normalised after treatment with amisulpride. In the group of patients responding to treatment, the dopamine blockade was significantly correlated with the normalisation of brain activity.

Together, these findings might reflect a heterogeneous treatment response based on different neurochemical profiles. They support the hypothesis of a subgroup of patients with dopamine hyperactivity who show a good treatment response compared to patients with normo or low dopaminergic activity, who might have disturbances in a different neurotransmitter system. They might suggest BP_p as a possible predictive marker for the treatment response. In addition to confirming brain reward disturbances in schizophrenia patients, the findings also supported the role of dopamine in reward processing in the illness, not to mention a direct link between the dampening of the dopaminergic activity via $D_{2/3}$ receptor blockade and a normalisation of these reward disturbances. We believe these data provide additional insights into the biology of schizophrenia regarding treatment outcome and the heterogeneity of the disorder.

Dansk Resumé (Danish Summary)

Sammenhængen mellem Dopamin D_2 aktivitet, Forstyrrelser i Belønningssystemet og Psykopatologi hos Antipsykotika-Naïve Patienter med Debuterende Skizofreni

Skizofreni er en kompleks og alvorlig hjernelidelse, som ofte manifesterer sig i den første del af voksenalderen. På nuværende tidspunkt er hverken den bagvedliggende patogenese eller patofysiologi klarlagt. Neurobiologisk vides dopamin i hjernen at spille en afgørende rolle, og indenfor de senere år har et af de konsistente fund været øget dopaminaktivitet i striatum (en del af de basale hjernekerner) hos patienter med debuterende skizofreni. Al antipsykotika i dag blokerer dopaminreceptorerne i striatum. Behandlingsresponset varierer dog betydeligt, og studier peger på, at 20-30 % af patienterne er resistente overfor den nuværende medicinske behandling. Det meget heterogene respons stemmer overens med at skizofreni kan forstås som mere end én sygdom. Igennem de senere årtier har forstyrrelser i andre neurotransmittere, og samspillet imellem disse og dopamin, som tilgrundliggende årsag vundet frem. I nyere tid er det foreslået at patienter med skizofreni, der ikke har effekt af den nuværende behandling hører til en undergruppe indenfor skizofreni uden øget dopaminaktivitet men med mulig forstyrrelser i et andet neurotransmitter system.

Dopamin spiller desuden en afgørende rolle i hjernens belønningssystem. Enslydende fund har vist at patienter med skizofreni har forstyrrelser i belønningssystemet. Det er foreslået at disse forstyrrelser danner ophav til psykotiske symptomer såsom vrangforestillinger og hallucinationer. Der mangler dog direkte bevis for dopamins rolle ved disse forstyrrelser.

Det er i dag ikke muligt at forudsige, hvilke patienter, der vil opleve god effekt af den medicinske behandling. Patienterne må ofte igennem adskillige behandlingsforsøg og risikerer ubehagelige bivirkninger før den optimale behandling findes. Litteraturen understøtter et nødvendigt behov for prædiktive markører, der kan forudsige enten behandlingseffekt eller risiko for udvikling af bivirkninger. I forsøg på at finde sådanne markører er opfølgende studier af patienter, der ikke tidligere har været udsat for antipsykotisk behandling, yderst vigtige. Optimalt bør hjernen derfor undersøges inden eventuel påvirkning af medicin eller recidiv af psykosen.

Med dette PhD studium undersøges sammenhængen imellem dopaminforstyrrelser og behandlingseffekt af dopaminblokade hos patienter med skizofreni, der ikke tidligere har været i

behandling med antipsykotika. Endvidere undersøges relationen imellem dopaminblokaden og forstyrrelser i belønningssystemet.

Resultaterne bygger på data fra et opfølgende studium, hvor 28 antipsykotika-naïve patienter med debuterende skizofreni samt 26 raske kontrolpersoner er inkluderet. Patienternes er vurderet ved psykopatologiske ratings, og samtlige deltagere er undersøgt med single photon emission computed tomography med [^{123}I]-iodbenzamide som radioaktivt sporstof, samt funktionel magnetisk resonans skanning med et spilparadigme. Ved skanninger beregnes dopaminereceptorens bindingspotentiale (BP_p) samt forstyrrelserne i belønningssystemet. BP_p benyttes som et indirekte mål for antallet af dopamin receptorer, hvortil der ikke er bundet dopamin. Undersøgelserne blev foretaget før og efter seks ugers behandling med amisulprid, en relativ selektiv dopamin $D_{2/3}$ antagonist.

Data påviste en sammenhæng imellem et lavere BP_p, hvilket afspejler høj dopaminaktivitet, inden behandling med amisulprid og bedre behandlingsrespons. Patienterne udviste forstyrrelser i belønningssystemet svarende til forventningsfasen sammenlignet med de raske kontroller. Forstyrrelserne normaliseredes efter behandling. I gruppen af patienter, der havde effekt af behandlingen, var normaliseringen i hjerneaktiviteten relateret til dopaminblokaden.

Resultaterne afspejler mulige undergrupper af patienter med skizofreni. De støtter tidligere fund og hypotesen omkring en undergruppe af patienter med øget dopaminaktivitet, der oplever god effekt af medicin grundet dopaminblokaden. Patienter, der oplever dårligere effekt af behandlingen har derimod et højere BP_p, og kunne tænkes at have forstyrrelser i et af de øvrige transmittersystemer. Resultaterne antyder BP_p som en mulig prædiktiv markør for behandlingsrespons. Data bekræfter endvidere forstyrrelser i belønningssystemet hos patienter med skizofreni, og støtter dopamins rolle i disse forstyrrelser og i sammenhængen med de psykotiske symptomer. Vi mener, resultaterne bidrager med betydningsfuld viden til mulig undergruppering af skizofreni og på sigt mulig stratificeret behandling.

List of Abbreviations

Alpha-MPT	Alpha-methylparatyrosine
ACC	Anterior cingulate cortex
Amy	Amygdala
ANOVA	Analyses of variance
BNST	Bed nucleus of the stria terminalis
BOLD	Blood oxygen level dependent
B_{max}	Density of receptors
BP	Binding potential
cAMP	Cyclic adenosine monophosphate
CT	Computed tomography
dACC	Dorsal anterior cingulate cortex
DAT	Dopamine transporter
[^{18}F]-DOPA	6-[18F]Fluoro-L-DOPA
DSM-IV	Diagnostic and statistical manual of mental disorders, fourth edition
DTI	Diffusion tensor imaging
DUI	Duration of untreated illness
EC_{50}	Plasma concentration predicted to provide 50% receptor occupancy
E_{Max}	Maximum attainable receptor occupancy
EPS	Extrapyramidal side effects
FGA	First-generation antipsychotic
fMRI	Functional magnetic resonance imaging
FSL	FMRIB Software Library
GABA	Gamma-aminobutyric acid
GAF	Global Assessment of Functioning
GAF-F	Global Assessment of Functioning, functioning
GAF-S	Global Assessment of Functioning, symptoms
HC	Healthy controls
Hipp	Hippocampus
Hypo	Hypothalamus
[^{123}I]-IBZM	123-Iodbenzamide
ICD-10	International Classification of Diseases, 10th revision

K_d	The dissociation constant
MID	Monetary incentive delay
MNI	Montreal Neurological Institute
^{1}H-MRS	Proton magnetic resonance spectroscopy
NAcc	Nucleus accumbens
NB	Nucleus basalis
OFC	Orbital frontal cortex
PANSS	Positive and Negative Syndrome Scale
PECANS	Pan European collaboration on antipsychotic naïve schizophrenia
PET	Positron emission tomography
PKA	Protein kinase A
Post-CA	Postcommissural caudate
Post-PU	Postcommissural putamen
Pre-DCA	Precommissural dorsal caudate
Pre-DPU	Precommissural dorsal putamen
ROI	Region of interest
SCAN	Schedules for Clinical Assessment in Neuropsychiatry
Schz.p	Schizophrenia patients
(S)-amisulpride	Serum amisulpride
SGA	Second-generation antipsychotic
SPECT	Single photon emission computed tomography
SD	Standard deviation
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SPM	Statistical Parametric Mapping
SPSS	Statistical package for the social sciences
SWN	Subjective Well-Being Under Neuroleptics Scale
SWN-K	Subjective Well-Being Under Neuroleptics Scale, short form
UHR	Ultra-high risk
vmPFC	Ventral medial prefrontal cortex
VP	Ventral pallidum
VS	Ventral striatum
VTA	Ventral tegmental area

Background

The Pathophysiology of Schizophrenia

Schizophrenia is a complex and severe chronic mental illness. The median lifetime prevalence is estimated to be 0.4%, though prevalence estimates show prominent variation (McGrath et al.2008). The basic neurobiological mechanisms and the underlying pathophysiology and pathogenesis are not fully understood. Multifactorial models including genetic, developmental and environmental factors have been proposed as the aetiology for the disorder, and it seems likely that schizophrenia is not one unique disorder, but a heterogeneous group of disorders.

Schizophrenia usually manifests itself in early adulthood, earlier among males than females, and the typical age at onset in males is the early to mid-20s. The course of the disease is typically characterised by alternating episodes of illness exacerbation and partial remission. The psychopathology is multidimensional with primary symptoms divided in positive and negative symptoms, and cognitive deficits. Positive psychotic symptoms can be thought of as an add-on to normal conditions and comprise, e.g. delusions, hallucinations and disorganised thinking. Negative symptoms, in contrast, are thought of as reduced, or a lack of, conditions and comprise symptoms reflecting two factors: 1) diminished expression such as alogia (poverty of speech) and affective flattening, and 2) symptoms expressing volitional pathology, e.g. avolition (lack of drive or motivation), anhedonia (inability to anticipate pleasure) and social withdrawal (Strauss et al.2013;Millan et al.2014). Negative symptoms can be difficult to differentiate from depressive symptoms, and patients with an inefficient treatment trial from an antidepressant prior to the manifestation of the illness are not uncommon. Finally, cognitive deficits comprise, e.g. attention, working memory, problem solving, planning and social cognition.

The course of disease often initiates with a prodromal phase, where the aforementioned symptoms are present in a light version, where they most likely impact daily functioning. In some individuals, the prodromal phase is followed by a deterioration of functioning before psychotic symptoms turn into a full-blown psychosis. In a recent meta-analysis, the mean transition risk in high-risk individuals was 29% at the one-year follow-up, and most of these individuals developed a schizophrenia spectrum disorder (Fusar-Poli et al.2013).

Treatment and Treatment Response

For decades, the cornerstone in schizophrenia treatment has involved the administration of antipsychotic compounds, which all share the blockade of striatal dopamine D_2 receptors. The treatment primarily affects positive symptoms. Some patients experience a total remission of positive symptoms, but most patients only experience an amelioration of these symptoms. Hallucinations, for example still occur, but they are dampened and therefore less disturbing. Unfortunately, existing treatment methods show little effect on negative symptoms and cognitive deficits, which can be just as agonising for patients as positive symptoms. Research shows that negative symptoms and cognitive deficits play a key role in the course of the disease, since patients with these symptoms have a poorer treatment outcome and prognosis (Tamminga et al.1998;Green et al.2000;Strauss et al.2013;Fervaha et al.2014;Corigliano et al.2014). In addition, antipsychotic treatment often induces adverse effects such as extrapyramidal side effects (EPS), weight gain, and increased prolactin hormone, which in some patients leads to sexual dysfunction and galacthorrhoea. Furthermore, the dopamine receptor blockade is associated with secondary negative symptoms, and finding a balance between treating the positive symptoms without inducing side effects is a clinical challenge.

In daily clinical practice, the response of individual patients to antipsychotic treatment differs considerably, and approximately 20-30% of schizophrenia patients do not respond appropriately to either first-generation antipsychotics (FGAs) or second-generation antipsychotics (SGAs) and are considered treatment resistant, presenting persistent psychotic symptoms and chronic disability (Lieberman1993;Levine and Leucht2010;Schennach-Wolff et al.2011). The reason for the heterogeneous treatment response is not clear. Two forms of treatment resistance have been proposed, one present at onset and throughout the illness and the other evolving as the illness progresses (Sheitman and Lieberman1998). An earlier study (Kolakowska et al.1985) found that treatment resistance to antipsychotics in most non-responders is unchanged throughout the illness, indicating that the treatment response is linked to a type of illness and not the stage of illness. This suggests that responders and non-responders are separate subtypes. It has recently been hypothesised that the existence of subtypes is based on different neurochemical profiles which are due to differences in endogenous dopamine activity (Howes and Kapur2014). Earlier, Stone et al., from the same group, also suggested that responders and non-responders represent

separate subtypes and thus benefit from different treatment approaches (Stone et al.2010). This is consistent with findings from our group at CNSR, implying that there are subtypes with either serotonergic or dopaminergic disturbances (Ebdrup et al.2011;Rasmussen et al.2011).

Demjaha et al. found that striatal dopamine synthesis capacity is significantly higher in good responders compared to patients with treatment-resistant illness and healthy controls (HC) (Demjaha et al.2012). This may indicate that patients with a higher dopamine elevation are more likely to respond to antipsychotics with dopamine receptor blockade. A potential limitation of this study is the inclusion of chronically medicated patients, which may influence the presynaptic dopamine synthesis capacity.

Growing evidence shows that glutaminergic abnormalities might also play a role in schizophrenia, particularly in treatment-resistant patients (Egerton and Stone2012;Egerton et al.2012). In these studies, glutamate levels were measured with separate proton magnetic resonance spectroscopy (^{1}H-MRS) in the anterior cingulate cortex (ACC). It was found that first-episode patients with persistent psychotic symptoms, despite antipsychotic treatment, had an elevated level of glutamate compared to patients with a good treatment response. To explore whether treatment resistance was due to underlying glutaminergic and not dopaminergic disturbances, a subgroup of the patients (n=14) included in the previously mentioned study by Demjaha et al. (2012) were examined with ^{1}H-MRS. Glutamate in the anterior cingulate was elevated in non-responders (n=6) compared to HC. The study further indicated a possible lower level of glutamate in responders versus non-responders; however, this was not significant (Demjaha et al.2014). Together, these studies support the hypothesis of distinct subtypes based on different neurochemical profiles, one of which is believed to evolve from dopamine hyperactivity.

The dopamine receptor blockade from antipsychotics is known to be closely related with the antipsychotic response (described in detail later). However, in line with the hypothesised normodopaminergic subtypes, previous findings show that some patients have a poor treatment response, despite high striatal dopamine receptor occupancy (Pilowsky et al.1993;Wolkin et al.1989). At this point, treatment is still based on trial and error and only few predictive markers of the treatment response have been suggested (Abi-Dargham et al.2000;Crespo-Facorro et al.2013). In the study by Abi-Dargham et al. (Abi-Dargham et al.2000), a significant correlation was observed between a high level of striatal dopamine and greater improvement of positive symptoms. Early

intervention programmes that shorten the initial psychotic episode may alter the outcome in a positive direction (Perkins et al.2005).

The blockade of dopamine receptors has also been found to be related to drug-induced side effects, such as EPS and increased plasma prolactin levels in unmedicated patients (Kapur et al.2000). It has further been found to be associated with (secondary) negative symptoms, such as avolition, apathy and affective flattening (Heinz et al.1998;Bressan et al.2002;Kapur2003), and also with subjective experience (de Haan et al.2004;Marder2005). Other data report an increased risk of developing dysphoria, akathisia and EPS in a subgroup of patients believed to have a lower dopamine function (Voruganti et al.2001). This study indicates that patients with a normal or lower level of dopamine are in greater risk of developing adverse effects. The Subjective Well-Being Under Neuroleptics Scale (SWN) (Naber1995) has been used in studies that relate subjective experiences with the dopaminergic changes. Two studies (de Haan et al.2000;Mizrahi et al.2007) that used the SWN reported a negative association between striatal D_2 receptor occupancy and subjective well-being in patients treated with D_2 antagonists. De Haan et al. (de Haan et al.2000) also suggested that negative subjective experience is more sensitive to D_2 receptor occupancy than EPS. Both studies, however, included previously medicated patients.

In the literature subjective experiences are suggested as key factors for quality of life, medication adherence and recovery (Marder2005). In a short-term twelve-week trial involving 727 schizophrenia patients treated with amisulpride, an early improvement of subjective well-being within the first four weeks was found to be the most important predictor for improvement of psychopathology and social functioning (Lambert et al.2007). Another study revealed that improvement in subjective well-being within the first two weeks of treatment was a significant predictor for symptomatic response (Schennach-Wolff et al.2010). Thus, as Marder notes, the components of remission should include not only symptoms improvement, but also focus on the subjective quality of life and functional remission (Marder2005).

There is still a substantial need for more knowledge on the neurobiological mechanisms responsible for the treatment response in individual patients as well as for predictive markers for the treatment outcome. At this point, treatment is still based on trial and error. The literature suggests different neurochemical profiles in responders versus non-responders to present treatments, but only few predictive markers of the treatment response have been suggested.

Finding predictive markers for treatment outcome would spare patients from long, unnecessary treatment trials and adverse effects. Such markers are a crucial step toward stratified medication and thus important to early intervention, compliance and the course of the disease.

The Brain Dopamine System

Dopamine is similar to adrenalin and noradrenalin a catecholamine. It has an inotropic effect on blood vessels, leading to a vasoconstriction and regulation of blood pressure, and works as a modulating neurotransmitter in the brain. Released from neurons into synapses and via dopamine receptors, dopamine influences the activity of other neurons. For decades the dopamine system in the brain has been the subject of investigation concerning its involvement in various behavioural disorders, e.g. Parkinson's disease, drug abuse and schizophrenia.

One of the most enduring hypotheses in schizophrenia research is the dopamine hypothesis that links exceeded levels of dopamine with psychosis. Several individuals have been called the father of this hypothesis, including J.M Van Rossum, but consensus exists that the hypothesis originally is based on findings by A. Carlsson, who discovered that antipsychotic compounds exert their effect via dopamine receptors (Carlsson and Lindqvist1963). Evidence that antipsychotics block dopamine receptors did not appear until 1974 when Seeman and his group (Seeman et al.1974;Seeman and Lee1975) demonstrated that the antipsychotic potency of FGAs strongly correlates with the blockade of dopamine receptors in vitro (later known as D_2). This discovery and the demonstration that dopamine-mimetic drugs (such as amphetamine and cocaine) induce or exacerbate psychotic symptoms are highly consistent with the dopamine hypothesis. In vivo studies have further shown that 60 to 70% occupancy of striatal D_2 receptors is required to achieve the antipsychotic effect of FGAs (Farde et al.1992;Kapur et al.2000;Catafau et al.2006).

Dopamine Receptors

In the late 1970s, a suggestion was made to divide dopamine receptors into two groups, D_1 and D_2 (Kebabian and Calne1979), but later gene cloning procedures revealed an even higher degree of complexity. Two dopamine receptor subfamilies, termed D_1-like and D_2-like receptors, were

defined, the D_1-like receptors consisting of two subtypes, D_1 and D_5, and the D_2-like receptors consisting of D_2, D_3 and D_4.

All receptors are classified as G protein-coupled receptors, but the helices forming the ligand binding site in the cellular membrane differentiate the two subfamilies and are likely to cause the functional difference between them. The five subtypes exhibit different characteristics and are distributed differently across the brain.

Of the two D_1-like receptors, the D_1 receptor is found in high levels, whereas the D_5 receptor is found in much lower levels in regions such as the caudate, putamen, nucleus accumbens (NAcc), olfactory tubercle, hypothalamus, thalamus and frontal cortex. Both subtypes stimulate the production of second messenger cyclic adenosine monophosphate (cAMP) via stimulation of adenylyl cyklase and result in increased activity of protein kinase A (PKA).

Of the D_2-like receptors, the D_2 subtypes are the most studied. Similar to the D_1 receptor, they are found in regions such as the caudate, putamen, NAcc and olfactory tubercle, but at low levels in the cerebral cortex. Less is known about the D_3 and D_4 receptors, but their distribution is more restricted and predominately found in limbic regions. In contrast to D_1-like receptors, D_2-like receptors do not stimulate adenylyl cyklase in the cells. This results in decreased PKA activity. The D_2 receptor has also been found to exist in two states – high and low. Determining whether there is an imbalance between these two states in schizophrenia is difficult, however, since the ligands in neuroreceptor imaging studies are shown to bind to both states (Seeman2006). The intracellular signalling networks regulated by dopamine in the receptors are complex, and abnormalities in these networks are seen as a potential cause of the pathological processes. There is also the potential that more receptors will be discovered.

D_1, D_2 and D_3 receptors in the ventral striatum (VS) are well known to mediate motor activity in different ways. The mediation through D_2 receptors involves both presynaptic autoreceptors and postsynaptic receptors, which decrease or increase motor activity, respectively. The D_1 receptors show a synergistic effect on the D_2 receptor, leading to an increase in motor activity. The D_3 receptors, mainly located in the NAcc, mediate a decrease in motor activity through postsynaptic receptors. The D_1 and D_2 receptors also play a role in reward and reinforcement mechanisms in the mesolimbic area. Notable studies, initially in non-human primates, by Goldman-Rakic and collaborators (Sawaguchi and Goldman-Rakic1991;Goldman-Rakic1994) demonstrate the

involvement of prefrontal D_1 receptors in working memory, and our group has recently found evidence that frontal D_2 receptors are involved in cognitive functions in antipsychotic-naïve first-episode schizophrenia patients (Fagerlund et al.2013). D_3, D_4 and D_5 receptors are thought to play a role in learning and memory in the hippocampal areas of the brain. Knowledge about D_4 and D_5 receptors is still limited, although developing antipsychotic compounds targeting D_3 and D_4 receptors has been in focus in recent years since they are involved in cognition. In addition, antagonists of these receptors are proposed to have a lower incidence of EPS (Strange2000;Beaulieu and Gainetdinov2011).

In schizophrenia, previous post-mortem studies show an increased density of D_2 dopamine receptors in the basal ganglia of schizophrenia patients. Most of the patients, however, had been treated with antipsychotics for most of their lives, and the exposure of long-term medication has been shown to increase the density of D_2 dopamine receptors (Mackay et al.1982). This evidence supports the importance of including anti-psychotic naïve patients in studies.

Dopamine Activity

Dopamine releases in two different modes: baseline tonic and reactive phasic. An imbalance between these two modes has been suggested to result in the pathological disturbances in schizophrenia (Grace1991;Grace1993). The tonic mode is a relatively continuous (background) dopamine release in the extracellular space. The phasic mode, on the other hand, releases dopamine in a brief high-amplitude pulse (a burst firing) in the synaptic cleft. Here it activates the postsynaptic dopamine receptors and is then rapidly removed via presynaptic reuptake and autoreceptors. The activation of the phasic dopamine release takes place in response to behaviourally relevant stimuli (Schultz1986), but the intensity of the phasic dopamine release is dependent on the tonic background level. Burst firing of dopamine neurons is influenced by glutamatergic afferents and the striatal tonic dopamine level is modulated by gamma-aminobutyric acid (GABA) inhibition, which prevents some dopamine neurons from spontaneous firing. Thus, an interplay between the GABA and glutamatergic afferents are necessary to induce burst firing of dopamine neurons (Grace et al.2007). Other transmitter systems, e.g. serotonergic and acetylcholine, are also known to modulate both dopamine and GABA neurons (Cachope and Cheer2014;Faure et al.2014). Growing knowledge about the interactions between

neurotransmitters and projections strongly indicates that a primary disturbance in one system influences the other systems, and most likely involves more than one distinct part of the brain (Carlsson et al.2001;Carlsson2006).

The dopamine hypothesis, as described previously, is still thought of as a main hypothesis in psychosis, and dopamine has been referred to as "the final common pathway" leading to psychosis (Howes and Kapur2009). The original dopamine hypothesis, however, has been extended and modified throughout the years. Besides the previously mentioned hypothesis of an imbalance between tonic and phasic dopamine as the cause of the pathology in schizophrenia, other notable hypotheses are the neurodevelopmental hypothesis and the co-existence of hypo and hyper-dopaminergic activity in the prefrontal and subcortical part of the brain. Frontal lobe dysfunction and low prefrontal dopamine activity, as seen in post-stroke patients, were found to associate with greater subcortical dopamine activity, which led to the emergence of the neurodevelopmental hypothesis in schizophrenia (Weinberger1987). This hypothesis suggests that neurodevelopmental dysfunction of the more vulnerable prefrontal cortex at an early age would result in striatal dopamine hyperactivity, causing positive symptoms. Preclinical studies further indicated that dopamine deficits in the prefrontal cortex co-existed with an excess of subcortical dopamine (Davis et al.1991). This was later supported by a PET study of schizophrenia patients (Meyer-Lindenberg et al.2002). Also, negative and cognitive symptoms have been suggested to associate with prefrontal hypoactivity (Andreasen et al.1992;Goldman-Rakic et al.2004). It has also been suggested that two prefrontal network states exist. Network state 1, where D_2 activity dominates and network state 2, where D_1 activity dominates (Seamans and Yang2004). State 2 is thought of as a robust network, where weak inputs are left out. Behaviourally, this means the brain is able to focus on a few inputs to guide the action. The response in state 2, however, is less flexible than state 1, where the brain is subjected to several inputs to act on. A balance between the two states is assumed in the normal brain. In schizophrenia, it is suggested that an increase of network state 1 leads to positive symptoms, whereas an increase of network state 2 causes negative symptoms. A previous study from our group supports this model. In antipsychotic-naïve first-episode schizophrenia patients, a correlation was demonstrated between the D_2 binding potential in the frontal cortex and positive symptoms in male patients (Glenthoj et al.2006).

Dopamine Pathways in the Brain

Dopamine exhibits both homeostatic and regulatory roles, and the dopamine neurons are embedded in ascending and descending tracts through which they communicate.

Pathways run between the midbrain and striatum in loops. Dopamine cells in the midbrain primarily arise from the ventral tegmental area (VTA) and substantia nigra (SN). Projections from the midbrain to the striatum and back are arranged in an inverse dorsal–ventral topography. The VTA and the medial part of the SN are associated with the limbic system, whereas the lateral and ventral SN are related to the associative and motor part of the striatum (the division of the striatum is described in detail in Methods in the Region of Interest (ROI) section). The VS (limbic system) receives limited input from the midbrain, but projects to a wide range of dopamine neurons (Haber2003).

The nigrostriatal loops from the VTA and SN have been shown to overlap, but in some literature, the pathway between SN and the dorsal striatum, involved in motor control, is referred to as the nigrostriatal pathway. This is highly relevant in Parkinson's disease, but also relevant with regard to the development of EPS from the D_2 receptor blockade of antipsychotics.

Five parallel cortico-striatal-thalamo-cortical circuits have been described previously by Alexander and colleagues (Alexander et al.1990). These circuits were distinguished from their separate functional area of the cortex and described as closed loops between the cortex, striatum and back to the cortex through the thalamus. The circuits, or loops, which are reflected in the functional subdivisions of the striatum, can be divided into three loops: limbic, associative and sensorimortor. The limbic loops, include the VTA and the medial part of the SN with projection to the limbic striatum (VS, including the NAcc), as mentioned above, and projection to or input from several parts of the mesolimbic system, e.g. the olfactory bulb, hippocampus, amygdala, ACC, medial prefrontal cortex and the orbital prefrontal cortex. The associative loops include projections from the SN to the associative striatum (the more central part of the striatum) and forward projection to or input from the associative areas of the cortex, including the dorsolateral prefrontal cortex. Finally, the sensorimotor loops project from the SN to the sensorimotor striatum (dorsal part of striatum) and include the forward projection to or from the primary motor, premotor cortex and supplementary motor areas (Martinez et al.2003a). See Figure 1.

The limbic loops play an important but complex role in motivation, reward, emotions and affect (orbital and medial prefrontal cortex), whereas the associative loops are thought to be relevant with regard to cognition and executive function (dorsolateral prefrontal cortex). Hypofunction of the associative loops might be related to the cognitive deficits and negative symptoms in schizophrenia. The sensorimotor loops execute motor actions and planning of movements.

More recently, it has been demonstrated that the basal ganglia (comprising the striatum and midbrain) also play a role in adaptive behaviours that require associative learning (e.g. adaption of past experiences in the prediction of future outcomes) and reward evaluation. Integration with brain regions involved in cognition and motor control are therefore essential. This led to the hypothesis of integration between the functional circuits (Haber and Knutson2010).

The reward circuit is an example of this integrative processing. Wise and colleagues have previously described the complexity of the reward circuit, where projections from the ventromedial part of the prefrontal cortex, VTA and NAcc play key roles (Wise2004). More recent connectivity studies on the reward circuit have further expanded to focus on the ACC and insula (Palaniyappan and Liddle2012;Gradin et al.2013).

Finally, the tuberoinfundibular pathway runs separately from the hypothalamus to the pituitary gland and is involved in endocrine functions, e.g. influencing the prolactin level. Dopamine receptor blockade from antipsychotics in this pathway may lead to hyperprolactinemia, as observed in treatment with dopamine antagonists, including amisulpride.

Although the extensiveness and intricacy of neurocircuits in the brain mean that fully understanding their complexity is a long way off, fMRI-based neuroimaging techniques, such as diffusion tensor imaging (DTI) and fibre tracking, which explore the cytoarchitecture of the different brain structures, and more recently optogenetics, are emerging approaches to expanding current knowledge in this field of research.

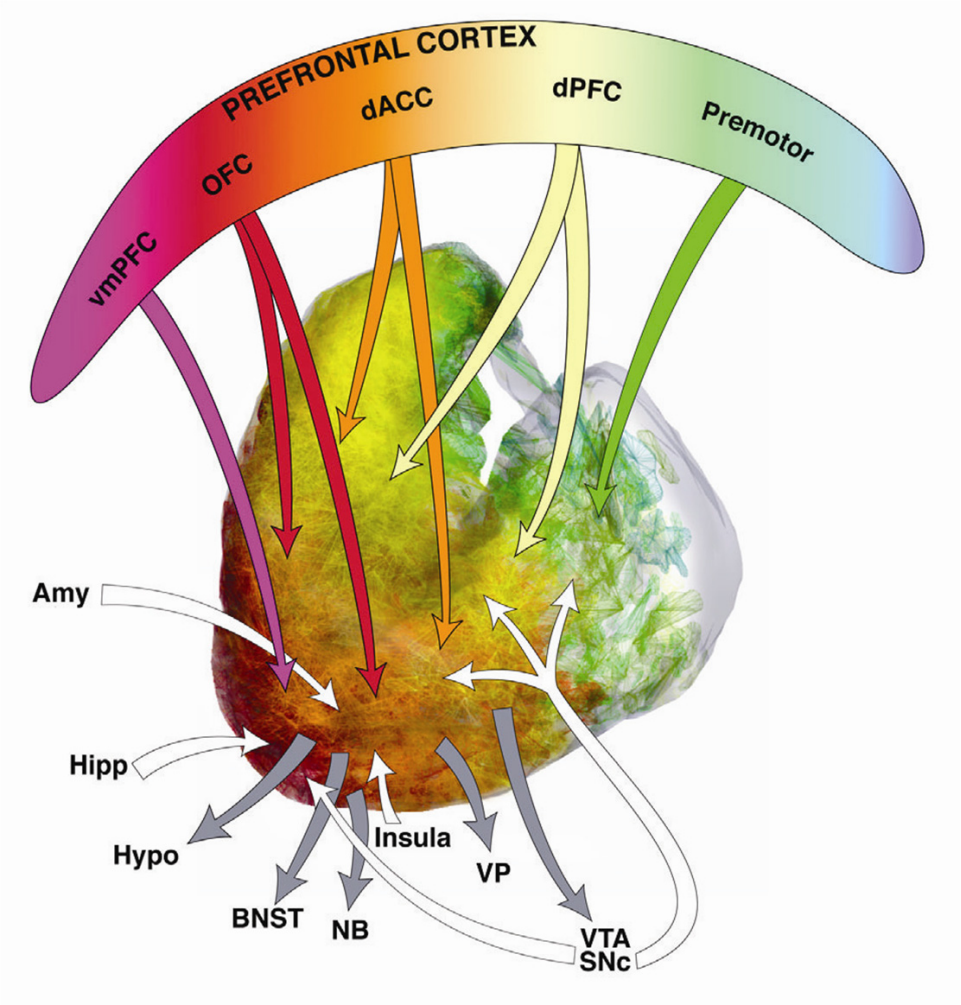


Figure 1. Schematic illustration of ventral striatal connections

Fuchsia: ventral medial prefrontal cortex (vmPFC); red: inputs from the orbital frontal cortex (OCF); orange: inputs from the dorsal anterior cingulate cortex (dACC); yellow: inputs from the dorsal prefrontal cortex (dPFC); green: inputs from premotor areas; white arrows: other inputs; gray arrows: outputs; Amy: amydala; BNST: bed nucleus of the stria terminalis; Hipp: hippocampus; Hyp: hypothalamus; NB: nucleus basalis; SNc: substantia nigra pars compacta; VP: ventral pallidum; VTA: ventral tegmental area. BNST and VP are beyond the scope of this thesis.

Dopamine and Reward

One of the first pioneering studies drawing attention to the reward system involved observations from rat studies in 1954 by Olds and Milner (Olds and Milner1954), who discovered that stimulation with electrodes in certain areas of a rat's brain had an impact on the rat's behavioural response. In addition, the rat would continuously self-stimulate the brain areas (using a lever connected to the implanted electrode) and refrain from carrying out vital activities such as eating. Based on findings from the experiments, it was concluded that stimulation in these areas led to pleasure.

Several studies investigating the reward system in both rodents and monkeys subsequently followed. Electrophysiological studies carried out by Schultz and his group contributed substantially to the field. They showed that phasic dopamine release in the striatum (burst firing of the dopamine neurons from the VTA and SN) is associated with goal-directed behaviour and occurs in response to primary rewards, such as food or liquid or through an auditorily or visually (conditioned) rewarding stimuli.

Familiar from Pavlov's experiments with dogs, classical conditioning is learning that occurs when a conditioned stimulus (a previously neutral stimulus, which in Pavlov's case was a bell ringing) is paired with an unconditioned stimulus (i.e. a reward, such as food). In Pavlov's experiment, a dog began to anticipate food after a period of learning that when a bell rang it would receive food. Thus, the conditioned stimulus becomes important (salient) when associated with a reward as its outcome. Schultz found and extensively described how dopamine firing increases in response to an unexpected reward. After a learning period with a repetitive (otherwise neutral) stimulus prior to the reward, however, the increase in dopamine firing moved from the time of the reward itself to time of the (now conditioned) stimulus occurred. Thus, unexpected rewards elicit strong increases in the firing rate, whereas anticipated rewards produce little or no change. Moreover, Schultz showed that if the reward was larger than expected from the conditioned stimulus, dopamine firing also occurred at the time of the reward (positive prediction error response). Conversely, if a stimulus-predicting reward was followed by a reward smaller than expected or failed to appear, dopamine firing was depressed at the time of the reward (negative prediction error response) (Schultz1998). Thus, dopamine firing has been proposed to mediate the prediction

error for the anticipated reward, which has been demonstrated in more recent studies in mice with the use of optogenetics (Ilango et al.2014;Tsai et al.2009).

Research has targeted various components of the reward process. Initially, dopamine was considered a pleasure drug and later an association was proposed between dysfunction of the mesolimbic pathway and anhedonia (as described in the anhedonia hypothesis by Wise 1982). Berridge and Robinson (Berridge and Robinson1998) found that dopamine is associated with wanting and motivation rather than liking and pleasure. The conditioned stimulus was suggested as an incentive or motivational cue leading to action (goal-directed behaviour). They noted that associative learning and liking could not alone lead to goal-directed behaviour; an incentive salience attribution was needed. This incentive salience attribution was thought to make the neutral stimulus an incentive signal leading to the behavioural change/goal-directed behaviour for reward. Hence, the proposal was made that dopamine firing was closely linked to incentive salience and the motivation to obtain a reward.

The link between pleasure and dopamine, and in line with the anhedonia hypothesis, was particularly questioned due to studies on rodents, where orofacial movement linked with liking (from sucrose) did not change after dopaminergic lesions or administration of antipsychotic compounds (Berridge and Robinson1998;Wise2004). The self-stimulation behaviour observed in previous rat studies was then suggested to be linked to addiction rather than pleasure (Berridge2012).

The (associative) learning (often referred to as reinforcement learning) is thought to be an important contributor to motivation and salience. Learning is believed to bring the incentive value to a neutral cue, which after a learning period becomes associated with the reward (Bayer and Glimcher2005). A recent rat study using optogenetics demonstrated that a link like this exists between phasic dopamine prediction error signalling and cue-reward learning (Steinberg et al.2013).

The Salience Hypothesis

In an attempt to explain the pathophysiology of psychosis from dopamine disturbances, Berridge and Robinson's incentive/motivational salience hypothesis has been linked to the development of delusions and hallucinations in the salience hypothesis (Heinz2002;Kapur2003). The salience

hypothesis proposes that the increased dopamine activity observed in schizophrenia leads to an aberrant assignment of salience, i.e. the dopamine firing is elicited not only at relevant cues, but due to dopamine hyperactivity, it also fires in response to otherwise irrelevant cues. It further suggests that antipsychotics, due to dopamine blockade, dampen aberrant salience and thereby ameliorate psychotic symptoms.

As Kapur further suggests, delusions can be explained by secondary cognitive processes, where the individual attempts to make sense of aberrant salient cues. The hallucinations, on the other hand, "reflect a direct experience of the aberrant salience of internal representations" (Kapur2003).

Results from PET and SPECT Studies

In vivo neuroreceptor imaging techniques such as positron emission tomography (PET) and SPECT are used to measure the synaptic fluctuations of neurotransmitters. These techniques have been widely used in schizophrenia research, since they provide (more or less) direct evidence of dopaminergic transmission in the brain. The competition between endogenous transmitters and radioligands for specific receptors has been demonstrated in rodent, nonhuman primate and human studies, and is a crucial principle underlying neuroreceptor imaging techniques.

The interpretation of PET and SPECT data on D_2 receptor BP is complicated, since the receptor BP is a measure reflecting both the affinity and the density of the receptors. An increase in endogenous dopamine synthesis and release is believed to lead to a decrease in D_2 receptor BP, both via direct competition with some but not all ligands (the occupancy model) and via agonist-induced internalisation of the receptors (Laruelle2000;Sun et al.2003;Quelch et al.2014). Thus, the literature supports that increased dopamine levels lead to decreased availability of the receptors, which is reflected in low receptor BP of the more commonly used radioligands such as raclopride and iodbenzamide (consistent with the occupancy model), whereas in vivo binding of other ligands, e.g. butyrophenones and benzazepines show either no change or change opposite to what is expected with the occupancy model (Laruelle2000).

Early PET and SPECT studies on psychosis, which focused on striatal post-synaptic D_2 dopamine receptors, have shown inconsistent results that can partially be explained by the different

methodologies used, the variance of the radioligands, the different methods used for applying the ligand, and the heterogeneous groups of patients investigated. Most studies of unmedicated schizophrenia patients have failed, however, to demonstrate significant differences in the striatal D_2 receptor BP in patients compared to HC (Laruelle1998;Howes et al.2012).

In line with this, two later dopamine depletion studies in unmedicated schizophrenia patients did not demonstrate significant differences in baseline BP compared to HC (Abi-Dargham et al.2000;Kegeles et al.2010). The primary aim of these two studies was to indirectly investigate the striatal dopamine levels in schizophrenia patients compared to HC. Abi-Dargham et al.'s study examined a group of eight antipsychotic-naive first-episode schizophrenia patients and ten previously treated chronic patients experiencing an episode of illness exacerbation with the use of SPECT 123-Iodbenzamide ([^{123}I]-IBZM). In the other study, which was by the same group, Kegeles et al. examined 18 previously treated schizophrenia patients with use of PET [^{11}C]Raclopride. Striatal dopamine BP was measured prior to and after acute dopamine depletion with alpha-methylparatyrosine (alpha-MPT). In both studies, the acute dopamine depletion resulted in a larger increase in D_2 receptor availability in patients compared to HC, indicating a higher dopamine activity in patients. In the study by Kegeles et al., this was found to be more pronounced in the associative striatum, thus suggesting higher synaptic dopamine concentration in this area.

Indices of elevated dopamine release can be examined with the use of amphetamine in amphetamine challenge studies (Laruelle et al.1995). Studies in schizophrenia patients have shown that an amphetamine-induced increase in dopamine is larger in schizophrenia patients compared to HC, and related to exacerbation of positive symptoms and to the response of antipsychotic treatment (Laruelle et al.1996;Breier et al.1997;Abi-Dargham et al.1998;Laruelle et al.1999). These studies mainly included medicated patients. One SPECT study examined 13 patients with schizotypal personality disorder (Abi-Dargham et al.2004) and found a similar decrease in [^{123}I]-IBZM binding after amphetamine administration compared to HC. These patients were unmedicated, but not necessarily antipsychotic drug naïve. The decrease in [^{123}I]-IBZM binding was lower than the decrease observed in patients with schizophrenia studied during episodes of illness exacerbation, suggesting that a possible increase in dopamine level is a state in psychotic exacerbation phases.

The amphetamine challenge studies, however, do not provide information about baseline dopamine levels (without psychopharmacologic intervention). The previously mentioned dopamine depletion studies, on the other hand, might indirectly provide knowledge about baseline dopamine levels, which are thought to reflect tonic conditions. These studies are, however, restricted by the difficulty in knowing whether or not the depletion of dopamine is actually complete (Fujita and Innis2002).

Imaging studies of the striatal dopamine transporter (DAT) are essential in Parkinson's disease, and several studies have also looked at possible changes in DAT in patients with schizophrenia. DAT, a protein located on the membrane of the dopamine presynaptic terminal, provides the reuptake of dopamine from the synapse into the cell cytoplasm and is considered to be an indirect marker of dopamine activity. Different radioligands have been used in vivo: [^{123}I]b-CIT with high affinity for both DAT and 5-HT transporters, [^{18}F]-CFT, and a technetium labelled cocaine analogue [99mTc]TRODAT-1. No significant changes in DAT in the striatum have been found in either medicated or antipsychotic-naïve schizophrenia patients compared to HC (Laakso et al.2000;Laruelle et al.2000;Lavalaye et al.2001;Hsiao et al.2003;Schmitt et al.2005). In addition, later dual-isotope imaging did not find any changes in DAT either (Yang et al.2004;Schmitt et al.2008). Schmitt et al.'s study found no significant differences in ligand binding in an entire group of 20 acutely ill patients compared to HC; however, in a subgroup of patients with predominant positive symptoms (n=12), a significantly higher TRODAT-1 binding was found compared to both HC and patients with a higher negative symptom score. This could perhaps reflect an increase in DAT availability and thereby an indirect indication of an elevated dopamine level or hyperdopaminergic state in this subgroup of patients.

It has been suggested that the major dopaminergic abnormality in schizophrenia is presynaptic and thus affects dopamine synthesis capacity and release (Howes et al.2012). To estimate the presynaptic function of the striatal dopaminergic system, the rate of endogenous dopamine synthesis has been measured with PET using primarily 6-[18F]Fluoro-L-DOPA ([^{18}F]-DOPA) as the ligand. The fluorodopa influx constant (expressed as K_i) reflects the conversion and subsequent storage of 18F-flourodopamine in synaptic vesicles, which is found to correlate with the presynaptic dopamine synthesis capacity. Studies using F-DOPA ligands have focused on the

striatal dopamine synthesis capacity, since it is difficult to assess cortical dopamine function due to the low signal-to-noise ratio in the cortex (McGowan et al. 2004). The majority of studies in schizophrenia have found dopamine synthesis capacity to be elevated in patients compared to HC. The first three studies done (Reith et al.1994;Hietala et al.1995;Dao-Castellana et al.1997) included small groups with five to seven patients – though only one group of drug naïve. Dao-Castellana et al.'s study found no difference between patients and HC, but found the variability of [^{18}F]-DOPA uptake values to be higher in the caudate and in the putamen in patients, which might reflect heterogeneity in the patients for striatal presynaptic dopaminergic function. Hietela et al.'s study reported that the increase was higher in the putamen than in the caudate, and the increase in the caudate was only statistically significantly lateralised to the left hemisphere. This could indicate that patients lacked the asymmetry of controls. In one study (Elkashef et al.2000), which reported conflicting results, a subgroup (n=9) of medication-free patients had a significant decrease in [^{18}F]-DOPA uptake in the VS, but non-significant in the caudate and putamen, as well as a significant increase in the posterior cingulate compared with HC. The subgroup of patients was unmedicated but not drug naïve and had a mean illness duration of 15 years. The difference in patient groups might have an impact on the results and might explain the conflicting results compared to other studies.

Later studies found consistent results with elevated striatal DOPA uptake in schizophrenia patients (Lindstrom et al.1999;Meyer-Lindenberg et al.2002;McGowan et al.2004). Also, in a more recent study by Demjaha et al. (Demjaha et al.2012), schizophrenia patients responding to treatment had significantly higher dopamine synthesis capacity compared to HC and a group of patients resistant to treatment, as previously described above.

To determine whether presynaptic dopaminergic dysfunction precedes the onset of schizophrenia or is secondary to its development additional studies have examined patients in the prodromal phase of the disease or cohorts of individuals at ultra-high risk (UHR) for psychosis. In a prodromal study (Howes et al.2009), striatal ^{18}F-DOPA uptake was found to be elevated to an intermediate degree in patients with prodromal symptoms of schizophrenia compared to patients with schizophrenia. In a later study, the dopamine synthesis capacity was found more pronounced in patients who later became psychotic compared to non-transition patients (Howes et al.2011). In both studies and in the previously mentioned study by Demjaha et al., the elevation was most

pronounced in the associative striatum. Similarly, one study (Egerton et al.2013) reported an elevated capacity in a larger cohort of UHR individuals. Yet another study indicated that vulnerability to psychosis exists in first-degree relatives of patients with schizophrenia (Huttunen et al.2008). In this study, the first-degree relatives showed changes similar to those seen in studies of antipsychotic-naïve patients.

Combined, these studies provide robust evidence for presynaptic dopaminergic dysfunction in schizophrenia, where dopamine hyperactivity is found not only in schizophrenia patients, but also is associated with transition and vulnerability to psychosis.

Functional Magnetic Resonance Imaging Method in brief

Since its development in the early 1990s, fMRI has been a useful research method, also with regard to schizophrenia.

Primarily hydrogen nuclei in water are necessary to obtain a magnetic resonance signal. The strong magnetic field leads to an alignment of the nuclei similar to how small magnets align. Radiofrequency pulses into the brain tissue to cause the nuclei to excite away from this alignment (resting state), and the rate in which the nuclei return to the resting state provides information about the nearby tissue. Blood oxygen level dependent (BOLD) contrast is based on changes in oxygenated and deoxygenated blood, and the magnetic properties of deoxyhemoglobin. It is believed that neuronal activation leads to an increase in deoxyhemoglobin and that cerebral blood flow increases to overcompensate for the decrease in oxygen. A subtle increase in the BOLD response is the result. Moreover, regions with sparse vascularisation have a lower hemodynamic response and therefore a weaker or absent BOLD response.

The BOLD response can be measured in certain areas of the brain during or in relation to e.g. specific stimuli or tasks performed in the scanner. With a temporal resolution in the order of seconds, the BOLD response only represents a surrogate signal for neuronal activity. It cannot provide a direct measure of, for example phasic dopamine release to an important cue, since this phasic dopamine burst firing happens within milliseconds. Being a non-invasive method with a spatial resolution in the order of mm, fMRI remains a highly useful method and is used extensively in research. (Buxton2013;Logothetis and Wandell2004)

Reward and fMRI

fMRI with event-related tasks is a useful method to investigate the reward processing in humans. One of the most common tasks or paradigms used to elicit anticipatory activation in reward processing is the monetary incentive delay (MID) task developed by Knutson and colleagues (Knutson et al.2000). This reward task was modelled on the task used in preclinical studies by Schultz et al., when dopamine firing was elicited in monkeys. With the use of money as a secondary reward, it is possible to investigate the BOLD response when anticipating either a gain or a loss of money (reward or punishment).

In a study in healthy subjects Knutson et al. (Knutson et al.2001b) examined whether reward outcome and reward anticipation could be dissected using an MID task. The study showed that NAcc was primarily activated during reward anticipation and that the ventromedial frontal cortex was activated during the reward outcome phase. Similar to the preclinical studies, the activation of NAcc was attenuated during the outcome of the reward, and was additionally suppressed when an anticipated reward was not obtained.

In a another study, Knutson et al. (Knutson et al.2001a) observed an increase of NAcc activation during the anticipation of a reward gain, but not during anticipation of a reward loss. This was further examined differentiating between certain and uncertain outcome. It was then demonstrated that in trials with uncertain outcomes, NAcc activation increased in anticipation of both gain and loss (Cooper and Knutson2008). Thus the NAcc activity in uncertain trials was driven by motivational salience only, and not by the value of the reward (valence). It was further observed that varying the outcome uncertainty had a significant impact on arousal, while the strongest response was observed when the reward was unpredicted.

Whether or not NAcc activity is linked to motivational salience only and not to valence has been debated. Using a different conditioning paradigm, Kirsch et al. found NAcc activity during reward anticipation more pronounced when the reward was money, compared to verbal feedback (Kirsch et al.2003). In contrast, subsequent studies have implicated that valence has no impact on NAcc activity, which has been found to be driven solely by motivational salience (Zink et al.2004;Jensen et al.2007). In line with the hypothesis from preclinical studies (Berrigde and Robinson) that dopamine fires due to motivational cues rather than pleasure, Zink et al. found, using a modified MID task, that VS activity was dependent on a subject's performance (motivational salience)

rather than the feeling of pleasure. This was supported by a study by Jensen et al., who concluded that VS is important to the learning process for motivationally salient stimuli (the reward prediction error), despite the valence of the stimuli, and their study also indicated that the orbitofrontal cortex might code for the valence.
Consequently, various studies support the idea that motivational salience rather than the value of the reward has an impact on VS activity. Furthermore, uncertainty has a significant impact on arousal, and the strongest response is observed when the reward is unpredicted.

Another view on the reward process is the theory of associative learning, which entails that the learning process is dependent on the predictive cue linked to the reward outcome, i.e. learning involving the discrepancy between what is expected and what is received (the reward prediction error). Numerous studies have examined the associative learning component linked to the cognitive processes in reward and in schizophrenia and to dopamine disturbances (Deserno et al.2013). With this cognitive approach, it has also been suggested that associative learning is linked to the development of delusions in schizophrenia (Corlett et al.2007). Since the focus of the present study is the motivational component of the reward process, associative learning is only briefly mentioned.

Reward Abnormalities in Schizophrenia

An attenuated BOLD response in the VS during reward anticipation has been a consistent finding in fMRI studies of partly medicated and drug-naïve UHR individuals (Juckel et al.2012;Roiser et al.2013) and in antipsychotic-naïve (Nielsen et al.2012b;Esslinger et al.2012) and unmedicated schizophrenia patients (Juckel et al.2006;Schlagenhauf et al.2009;Schlagenhauf et al.2014). Even in healthy first-degree relatives of schizophrenia patients reward alterations are found, which is comparable to findings in patients (Grimm et al.2014). In their study, the reward abnormalities were described as a core deficit in schizophrenia and also suggested to be linked to a candidate gene variant.
In line with the salience hypothesis, reward abnormalities in the anticipation phase were found to correlate with psychotic symptoms in antipsychotic-naïve patients (Nielsen et al.2012b;Esslinger et

al.2012). Also in the UHR group, a correlation was observed between the BOLD response to irrelevant stimuli in the VS and to positive symptoms (Roiser et al.2013).
While the degree of positive symptoms is found to be related to aberrant salience, supporting the salience hypothesis, an explanation for the negative symptoms is still a puzzle. One of the simpler explanations for negative symptoms is the excessive amount of stimuli that increase noise in the system and drown out salient stimuli. Thus, if all stimuli were salient the cognitive processes would not be able to differentiate between them, which could lead to drive reduction and additional negative symptoms (Howes and Kapur2009).
Correlations between attenuated activity in the VS and negative symptoms have been reported in the previously mentioned studies on unmedicated patients (Juckel et al.2006) and in patients treated with typical neuroleptics (Schlagenhauf et al.2008), but similar correlations have not been observed in antipsychotic-naïve patients.

Only few longitudinal studies exist that examine medication effects on reward processing. The previously mentioned study by Schlagenhauf et al. (Schlagenhauf et al.2008) indicated that only SGAs normalise reward disturbances. Patients in the study were not antipsychotic naïve, but they were examined before and after switching medication from FGAs to olanzapine, which led to a normalisation of reward abnormalities. A partial normalisation of brain reward abnormalities following treatment has been demonstrated in a previous study from our group, and the increase in the BOLD response was especially observed in patients with a treatment effect on positive psychotic symptoms (Nielsen et al.2012a). As in the present study, these patients were all initially antipsychotic naïve and were treated with the relatively selective dopamine $D_{2/3}$ receptor antagonist amisulpride.

Multimodal Studies in Reward Processing

As mentioned previously, fMRI studies do not measure the direct influence of dopamine or other transmitters in reward processing. To provide a more direct measurement, multimodal studies are needed. Until now, few studies have combined fMRI with either PET or SPECT, but these studies have mainly been in HC.

In studies on healthy volunteers, the findings are inconsistent. For example, in a pharmacological study using a MID task, amphetamine was found to reduce the signal in the VS during anticipation of reward (Knutson et al.2004). Another study used an aversive conditioning task and found the BOLD response that occurred during reward learning could be modulated and either increased or decreased with amphetamine or haloperidol, respectively (Menon et al.2007). Schott et al. (Schott et al.2008) demonstrated significant correlations between reward-related mesolimbic fMRI activation and dopamine release (expressed as a significant decrease of [11C]-Raclopride BP_{ND}) in the left VS using a variant of the MID task. Urban et al. (Urban et al.2012) also used a variant of the MID task but did not observe any changes in [11C]-Raclopride binding across conditions. Finally, a recent study demonstrated an inverse correlation between dopamine synthesis capacity (measured with F-DOPA) and striatal prediction error signal (Schlagenhauf et al.2013). The inconsistent findings could perhaps be caused by a difference in the chosen methodologies, but taken together they seem to support a role for dopamine in reward processing.

Multimodal studies in UHR or schizophrenia patients are sparse. A study by Roiser et al. (Roiser et al.2013) found aberrant salience in UHR patients, but observed no correlation with the presynaptic dopamine activity using [^{18}F]-fluorodopa PET. No previous studies have directly examined the influence of striatal dopamine $D_{2/3}$ receptor blockade on the striatal BOLD response during reward anticipation in schizophrenia patients, and direct proof of the role of dopamine in the aberrant salience found in schizophrenia is still lacking.

Study Motivation of the PhD Thesis

One of the best-validated findings in schizophrenia research is the association between increased presynaptic dopamine synthesis capacity and release in the striatum and positive psychotic symptoms. Dopamine plays an important role in brain reward processing. In recent years, reward disturbances in unmedicated schizophrenia patients have not only been a consistent finding, but they have even been proposed as a core deficit. Reward disturbances have been linked to the striatal dopamine hyperactivity in the salience hypothesis, thus explaining the development of psychotic symptoms (Heinz2002;Kapur2003). Direct proof of such a link is, however, still absent.

It is a fact that antipsychotic compounds all share the striatal dopamine blockade, but not all patients experience symptom relief with existing treatments. Moreover, treatment tends to ameliorate positive symptoms only. At this point, no biomarkers or predictive markers of the treatment response have been found. Treatment is still based on trial and error, which for many patients includes long, unnecessary treatment trials and adverse effects.

Other neurotransmitters, e.g. the serotonergic GABAergic and glutamatergic systems, are also known to play a role in the pathophysiology of schizophrenia. It has been proposed that separate subtypes exist based on the disturbances in neurotransmitter systems and that these subtypes may benefit from different treatment approaches.

There is a substantial need to find biomarkers and predictive markers for the treatment response, e.g. by constructing a functional model that reflects the abnormalities in the disorder and is able to subdivide the patients. In order to characterise these markers and to study the effects of specific interventions, investigating potential disturbances before they are modified by antipsychotic medication and repeated relapses is crucial. Longitudinal studies of antipsychotic-naïve first-episode schizophrenia patients are therefore of great importance in schizophrenia research.

The overall aim of the present PhD thesis is to contribute to a better understanding of the mechanisms underlying the heterogeneous response to antipsychotic treatment in schizophrenia patients, and in the long term to contribute to a model that subdivides patients and stratifies schizophrenia treatment.

Objectives and Hypotheses

The primary objectives of this thesis were to investigate the association between dopamine D_2 receptor binding and treatment outcome, as well as the proposed link between dopamine and brain reward disturbances in antipsychotic-naïve first-episode schizophrenia patients.
The overall hypotheses were:

- The effect of $D_{2/3}$ receptor blockade on positive symptoms is associated with baseline striatal $D_{2/3}$ receptor BP_p in antipsychotic-naïve first-episode schizophrenia patients
- The effect of antipsychotic medication on positive symptoms and salience disturbances are associated with each other and linked to the degree of $D_{2/3}$ receptor blockade

Two papers are included in the thesis:

Paper one explores the association between striatal dopamine $D_{2/3}$ receptor binding potential and treatment outcome.
Hypotheses: 1) First-episode schizophrenia patients with low striatal dopamine $D_{2/3}$ receptor BP_p in the antipsychotic-naïve state achieve a better treatment response from striatal $D_{2/3}$ receptor blockade than patients with a high BP_p, particularly regarding positive symptoms. 2) High striatal dopamine $D_{2/3}$ receptor occupancy is associated with deterioration of a patient's functioning and subjective well-being.

Paper two examines the association between striatal dopamine $D_{2/3}$ receptor blockade, alterations in reward processing and psychopathology.
Hypotheses: 1) Blockade of striatal dopamine $D_{2/3}$ receptors improves the abnormalities in salience; i.e. we expect to find a positive correlation between $D_{2/3}$ receptor occupancy and a normalisation of the BOLD response at follow-up. 2) The normalisation of the salience disturbance associates with the improvement of positive symptoms.

Methods

Study Design

Patients meeting the International Classification of Diseases, 10th revision (ICD-10) criteria for either schizophrenia or schizoaffective psychoses and HC matched for age, gender and parental socio-economic status were included as part of a large multimodal, longitudinal study investigating antipsychotic-naïve first-episode schizophrenia patients – the Pan European Collaboration on Antipsychotic Naïve Schizophrenia (PECANS) – from August 2009 to September 2013. Figure 2 provides an overview of the PECANS study.

The present thesis focuses on the first six weeks of the study programme, which involved assessing patients with psychopathology measures and examining patients and HC with SPECT and fMRI. In addition to being part of the recruitment process and psychopathological ratings, the author was involved in the treatment for a six-month period, in the assessment and in the SPECT data analyses, and to a minor extent in the analysis of the fMRI data.

Data on structural and fMRI, psychophysiology and neuropsychology are part of other PhD theses and will be published elsewhere. There is a partial overlap between the participants in the present study and participants in previous publications from the PECANS study on psychophysiology (During et al.2014) and reward processing (Nielsen et al.2012b;Nielsen et al.2012a). The overlap between the present study and the two studies on reward processing was nine patients and six controls, and eight patients and six controls, respectively.

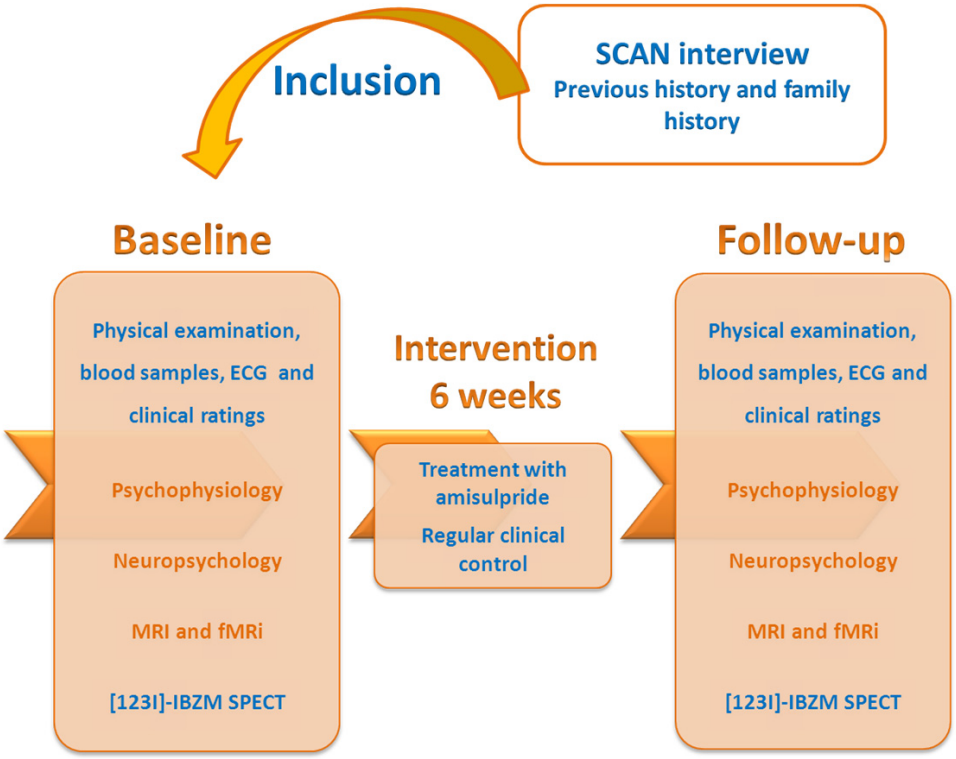


Figure 2. Overview of the PECANS study

Author involvement in the various stages of the PECANS study are highlighted in blue.

Participants

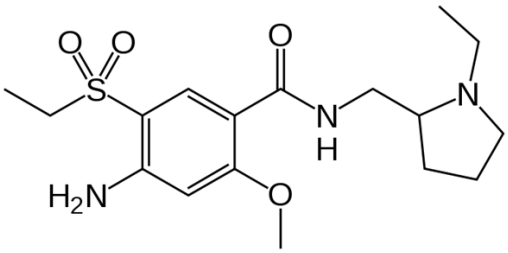
Thirty-two antipsychotic-naïve first-episode schizophrenia patients 18-45 years of age were recruited from both in and out-patients psychiatric centres in the Capital Region of Denmark. After referral, patients went through a structured diagnostic interview (Schedules for Clinical Assessment in Neuropsychiatry (SCAN) version 2.1 (Wing et al.1990)). The final diagnosis was based on the SCAN interview and clinical observations. Patients included in the present study all fulfilled the ICD-10 criteria for schizophrenia, and no patients were diagnosed with schizoaffective psychoses.

Exclusion criteria were previous treatment with an antipsychotic compound or methylphenidate. Patients receiving antidepressants were excluded or went through a minimum one-month wash-

out period before inclusion. Other reasons for exclusion were drug or alcohol dependence (according to ICD-10), pregnancy, neurological disorders, serious head trauma and claustrophobia. Previous diagnoses of dependence or a current non-regular use of cannabis were not a reason for exclusion. Patients were allowed to receive benzodiazepine during the study period, though no later than 12 hours prior to the examinations.

Twenty-eight HC were recruited using advertisements, e.g. the web-based contact forum forsøgsperson.dk, and matched for age, gender and parental socio-economic status. Introductory chapters from the SCAN were used to assess and exclude earlier or present psychiatric illnesses. HC with psychiatric illnesses among relatives were not accepted. Otherwise, the exclusion criteria were the same as for the patient group.

Both patients and HC were tested for drug use prior to the examinations with urine tests (Rapid Response, Jepsen HealthCare, Tune, Denmark), and female participants were also tested for pregnancy prior to the scans with a urine human chorionic gonadotropin test. HC received money for participating in the study and both groups were given the amount of money they won in the fMRI examinations. Patients, though, received the winnings on a gift certificate.



Intervention

After baseline examinations patients were treated with amisulpride. The dosage was slowly increased and individually adjusted according to the clinical impression of symptoms and complaints of side effects. Medical treatment against side effects or any supplementary medication was not allowed during the study period. Follow-up examinations were performed after six weeks of treatment. To ensure amisulpride steady-state conditions between brain and plasma at the time of examinations, dose adjustment in the week prior to follow-up examinations was not allowed.

Amisulpride was chosen due to its selective $D_{2/3}$ receptor antagonistic effects. It resembles the structure of [^{123}I]-IBZM as it is a substituted benzamide derivative with an elimination half-life of approximately 12 hours (summary product for Solian®), and proven effective for treating positive and negative symptoms in schizophrenia (Puech et al.1998;Leucht et al.2002). Amisulpride acts as a selective antagonist with a high affinity for D_2 and D_3 dopamine receptors* and shows negligible affinities for other receptors, including 5-HT_2 and D_4 receptors (Schoemaker et al.1997).

Low binding affinity for D_2 receptors driven by fast dissociation from the receptor has been suggested as a characteristic of SGAs (Kapur and Seeman2001). Even though amisulpride is categorised as a SGA, it shows a high affinity for the $D_{2/3}$ dopamine receptors and a lower incidence of EPS compared to FGAs. The latter has been explained by a preferential affinity for corticolimbic $D_{2/3}$ receptors (Perrault et al.1997;Schoemaker et al.1997).

This preference for corticolimbic over striatal $D_{2/3}$ receptors has been investigated in PET and SPECT studies of previous medicated schizophrenia patients treated with amisulpride. These results have been disputed due to an inconsistent use of methods between the studies (Olsson and Farde 2001). For example, preferential corticolimbic occupancy was demonstrated in a PET study using the high affinity tracer [^{76}Br]-FLB 457 (Xiberas et al.2001), but it has been argued that these results might be influenced by an underestimation of the occupancy due to pre-equilibrium conditions in the striatum. Another study using SPECT with the high affinity tracer [^{123}I]-Epidepride (Bressan et al.2003) supported the findings of preferential corticolimbic occupancy at low amisulpride plasma levels. However, also with use of [^{123}I]-Epidepride, striatal and extrastrital occupancy measurements are incomparable (Pinborg et al.2000). Two later SPECT studies examined only the striatal receptors, with use of [^{123}I]-IBZM, and observed a wide range of striatal occupancies from optimal dose effects (la Fougere et al.2005;Meisenzahl et al.2008). These findings, which show an effect from lower plasma levels and lower striatal binding, are suggested to support a preferential extrastriatal blockade at low amisulpride plasma levels.

Moreover, the effect on negative and positive symptoms has been suggested to depend on the administered dose range, where a low dose of amisulpride associates with effect on negative symptoms (Boyer et al.1995;Leucht et al.2002). The explanations for amisulpride's supposed effect on negative symptoms are not resolved, but one hypothesis concerns its selectivity towards presynaptic $D_{2/3}$ autoreceptors in the striatum when given in low dose (Schoemaker et

al.1997;Perrault et al.1997). The corticolimbic binding at lower doses might be another explanation.

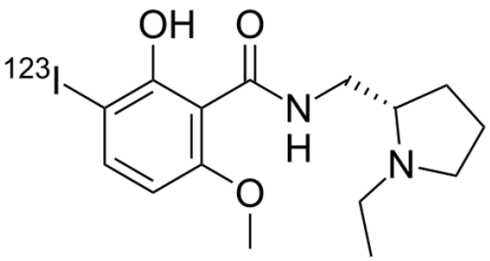
Amisulpride can induce substantial prolactin increase and minor weight gain, although these endocrine side effects are not proven to be higher than what is seen in other antipsychotics (Leucht et al.2002).

* The dissociations constant (K_d) is 2.8 and 3.2 nm for D_2 and D_3 receptors, respectively (the smaller K_d, the greater affinity). For comparison, K_d for the D_1 receptor is above 1000 nm.

Clinical Measures

Psychopathology in patients was assessed with the Positive and Negative Syndrome Scale (PANSS) (Kay et al.1987). For subjective experience of well-being and functioning outcome measures, the short form of the Subjective Well-Being Under Neuroleptic Treatment Scale (SWN-K) (Naber et al.2001) and Global Assessment of Functioning (GAF) (Endicott et al.1976) were used for assessment.

PANSS ratings were performed by trained investigators and recorded on DVD with the acceptance of the patient. Part of these ratings was used for persistent evaluation of inter-rater reliability. Comprised of 20 items, SWN-K is a self-rating Likert scale divided into five subscales, each of which contains two positive and two negative statements. All statements refer to the past seven days and scores can range from 20 to 120 points, with higher scores indicating greater well-being. Interpretation of the scale has been debated and a recent study emphasised that the five subscales are highly correlated, which indicates that the total score is more reliable than the subscores (Pazvantoglu et al.2013). The total score was used and the response to treatment defined as a percentage change. A numeric scale (0 through 100), GAF comprises two scores, a symptom score (GAF-S) and a functioning score (GAF-F), where the lower a score the worse the symptoms/functioning. The duration of untreated illness (DUI) was also estimated from the point in time a patient experienced a continuous invasive deterioration of functioning due to psychosis-related symptoms (Crespo-Facorro et al.2007). Handedness was assessed in all participants with the Edinburgh Handedness Inventory (Oldfield1971). Extrapyramidal symptoms were assessed with the Extrapyramidal Symptom Rating Scale, both at baseline and follow-up, in the patient group (Chouinard and Margolese2005).



SPECT Acquisition

SPECT examinations were performed in the morning and lasted approximately five hours. To reduce the radiation dose, the HC were only scanned at baseline.

Data were obtained with a Siemens Symbia™ T2 series SPECT•CT scanner with low energy high-resolution collimators and two-slice computed tomography (CT). The ligand (S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-3-iodo-6-methoxybenzamide ([^{123}I]-IBZM) was used and chosen due to its selectivity for striatal $D_{2/3}$ receptors (Kung et al.1990;Seibyl et al.1992). The scanning was performed using a bolus plus constant infusion technique (Carson et al.1993;Seibyl et al.1996). The ligand arrived in the morning from the Netherlands (GE Healthcare, Eindhoven). All participants received 185mBq [^{123}I]-IBZM per scanning, one half administered as a bolus injection 180 min prior to the scanning and the other half as an infusion for 240 min during the entire session. The bolus injection and the infusion were administered in one of two peripheral cubical venous catheters. Prior to the bolus administration 200 mg perchlorate mixture i.v. was given to block thyroid uptake of free radioactive iodide. After 180 min of rest, a CT scout and 2x30 min tomography were performed. To minimise movement in the scanner, the participants had their head fixated with foam pads and a strap across the forehead.

CT images were acquired with 5 mm slices at 130 keV using Siemens CARE Dose 4D dose optimisation. SPECT images were acquired with 64 views spanning 180°, 26 seconds per projection and a matrix of 128x128. The scanner's NM extrinsic spatial resolution was 7.4 mm.

At follow-up, patients were told to skip their dose of amisulpride the evening prior to the scan to reduce the effect of individual differences in administration-timing on the examination day. They instead received their individual dose of amisulpride three hours prior to the scanning along with the [^{123}I]-IBZM bolus injection. Serum amisulpride ((S)-amisulpride) was measured prior to the

dose of amisulpride and at 60, 120, 150, 180, 210, 240 min after the administered dose. The mean value during the one-hour scanning period was used in the analyses.

Blood Compound Analyses

Tracer steady state is assumed after 180 min of constant infusion following the bolus injection with [^{123}I]-IBZM (Laruelle et al.1995;Laruelle et al.1996). The dopamine $D_{2/3}$ receptor BP_p was calculated directly from the ratio of activity in the brain (obtained from the SPECT scan) to the activity in the plasma (obtained from the free un-metabolised/intact [^{123}I]-IBZM in plasma), with the activity in the cerebellum used as a reference.

The plasma analyses were performed on the same day as the SPECT acquisition. Venous blood samples were collected during the SPECT scan. One 10 ml blood sample was collected prior to the bolus injection of [^{123}I]-IBZM. The initial sample was used to determine the plasma free fraction of [^{123}I]-IBZM. The free [^{123}I]-IBZM was separated from the protein-bound [^{123}I]-IBZM by ultrafiltration (Centrifree, 30,000 MW) (Zea-Ponce and Laruelle1999).

Radiolabelled metabolites and native [^{123}I]-IBZM were determined using Oasis® WCX solid phase extraction units (Waters, U.S.A.) and stepwise elution with water, 40% acetonitrile and acidified 95% methanol. The native compound was eluted in the water phase and the metabolites in the subsequent elution.* One 10 ml blood sample was collected three times during the one-hour scanning period for the plasma metabolite analyses and activity measures. The plasma activity was measured using a gamma counter (Wizard2, PerkinElmer), while decay was corrected to the bolus injection time.

* The plasma metabolite analysis of [^{123}I]-IBZM involved a manual method developed by Erik Frandsen at the Diagnostic Department, Section of Clinical Physiology and Nuclear Medicine, Glostrup Hospital, University of Copenhagen, Denmark that has not been published, but was well validated from February 2010 to May 2011. Within this period, blood samples were analysed at the Diagnostic Department, Glostrup Hospital using the method described above and at NRU using the BioTrap system (Bio Trap 500 MS).

MRI Acquisition

Structural and fMRI scans were carried out on a Philips Achieva 3.0 T whole-body MRI scanner (Philips Healthcare) with an eight channel SENSE Head Coil. Whole-brain three-dimensional high-resolution T1-weighted structural images were acquired for anatomical reference (repetition time=10 ms, echo time=4.6 ms, flip angle=8° and voxel size=0.79x0.79x0.80 mm). The fMRI included an event-related study performed with a reward paradigm. For the fMRI, 1080 (540/run) whole brain functional echo-planar images were acquired (repetition time=2 seconds, echo time=25 ms, flip angle=75°, 38 slices and voxel size= of 2.4 mm in thickness and 2.9 x 2.9 mm in plane resolution). The acquired matrix size 80 x 80 was interpolated to 128x128. The fMRI and MRI scans were obtained on a different day than the SPECT scan and the mean interval was 4.4 days at baseline and 5.8 days at follow-up in the patient group, and 7.8 days in the HC group.

The Reward Paradigm

A variant of the MID task described by Knutson (Knutson et al.2000;Knutson et al.2001a;Knutson et al.2001b) and modified by Cooper and Knutson (Cooper and Knutson2008) was used to elicit VS activation in response to cues indicating monetary gain and loss.

The task consisted of two runs and the total time was 36 min (18 min/run). Participants were presented initially with a cue indicating the conditions of the trial. After a short delay, a visual target appeared briefly on the screen and participants were instructed to press a button while the target was on the screen. After another delay, participants received feedback on the money outcome. Prior to the scanning participants were instructed about the meaning of the cues and were given 10 minutes to practice the task outside the scanner to minimise later learning effects in the scanner. Initial target duration for all participants and trial conditions was 300 ms. To maintain a hit rate of approximately 66% in the experiment, an automated adaptive timing algorithm adjusted the time of the target cue.

There were six different trial conditions representing two levels of uncertainty (certain and uncertain), crossed with three levels of value expectation (gain, neutral and loss). Duration of one trial was 15 seconds. Each of the six trial conditions was presented in a block randomised order 12 times in each of the two runs. The contrast showing the most pronounced alterations in patients was the overall effect of salience during reward anticipation (Nielsen et al.2012b). This BOLD

contrast was therefore chosen for the analyses and was modelled by uncertain gain and loss (salient) cues versus neutral cues, i.e. a subject's BOLD response on salient cues minus the BOLD response on neutral cues. Within this chosen contrast, the outcome depended on whether the button was pressed in time. In uncertain-gain trials, participants earned €7 on a hit and €0 on a miss. In uncertain-loss trials, participants earned €0 on a hit and lost €7 on a miss. In the two neutral trials, participants knew the outcome would be €0, regardless of whether they hit the button in time. The participants were instructed to respond rapidly in all trial conditions but were not informed about the adaptive timing algorithm. After the entire scanning was over, participants received the amount of money they had won, typically €45–85 per run.

Subanalyses in our previous studies (Nielsen et al.2012a;Nielsen et al.2012b) showed that the attenuation of this BOLD contrast in the patients was caused by a lower BOLD response to salient cues in the patient group compared to the HC group. There was no significant difference in the BOLD response to the neutral cues between groups. The term BOLD response in the present study refers to the difference between salient and neutral cues.

When comparing the first and second run, the BOLD response was stronger in the first run than in the second run, and a larger group difference was observed. A possible explanation for this observation is that the participant adapted to the paradigm. Accordingly, only data from the first run were used in the analyses.

Image Analyses

SPECT images were reconstructed using syngo® MI software (version VA60B) Flash 3D, 4 subsets and 8 iterations with a 9 mm gaussian filter. Attenuation correction using CT images and scatter correction was applied. The two IBZM tomographies were added together and activity measurements were decay corrected to the time of the radioligand injection. The CT image from the SPECT scan and the structural MRI were co-registered using the Statistical Parametric Mapping (SPM) 8 method. The result of the SPM co-registration was inspected in all three planes and, if needed, was adjusted manually using local implementation of an image overlay method (Willendrup et al. 2004). The information from the co-registration between the CT and MR images was used for co-registration between SPECT and MRI. Inspection and manual adjustments were repeated, if needed.

The high-resolution structural MRI was used for the definition of ROI, which were automatically applied to the co-registered SPECT image using a volume-of-interest brain template (Svarer et al.2005).

For the fMRI analyses, the FMRIB Software Library (FSL) version 5.0 was used. Motion correction and spatial smoothing with a 5 mm gaussian filter and a temporal high pass filter with a 200-second cut-off were used initially. Initially, statistical analyses were performed in FSL FEAT using the individual paradigm information from each subject. Relevant contrasts between various stimulus types (salient, non-salient) were calculated at individual subject level. Next, the statistical images were spatially normalised to Montreal Neurological Institute (MNI) standard space using the brain-extracted T1 MRI. The defined ROI applied to the SPECT image was defined in MNI standard space and via the T1 MRI applied to the functional images. The mean value of the statistical parameter (the z-score) was extracted from the ROI, and used in the analyses.

Region of Interest

We wanted our ROI automatically applied and used a brain template to delineate the volumes-of-interest. The anatomically defined caudate was chosen as ROI; see Figure 3.

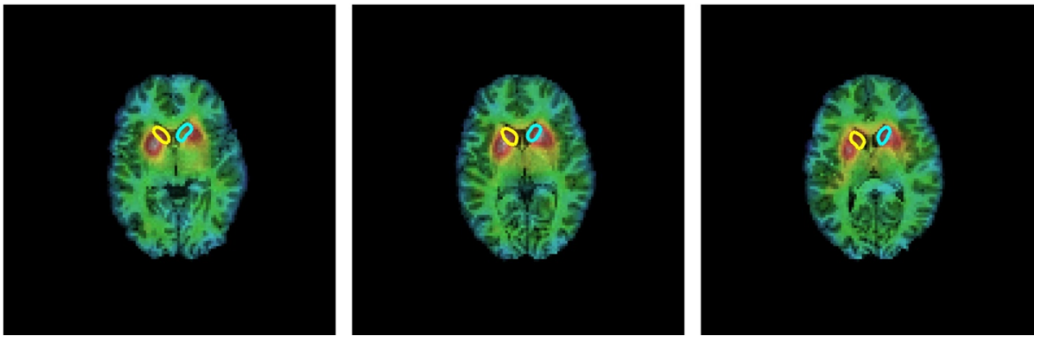


Figure 3. The caudate (region of interest) visualised on a T1 weighted MRI and [^{123}I]-IBZM background

In previous high-resolution PET studies, dopamine hyperactivity has been found to be more pronounced in the associative part of the striatum in schizophrenia patients and prodromal patients compared to HC (Howes et al.2009;Kegeles et al.2010). We wanted to relate these dopamine disturbances with our previous findings of salience abnormalities during reward

anticipation in the limbic striatum (Nielsen et al.2012b;Nielsen et al.2012a). The brain template used for the automatically applied ROIs divides striatum in caudate and putamen. With a spatial resolution of 7.4 mm in the present SPECT system, it was assumed, similar to another SPECT study (Stone et al.2005), that the caudate was distinct enough to differentiate from the putamen. The associative striatum equals the anatomically defined main part of the caudate and a part of the putamen, and the limbic striatum equals VS, including NAcc and the ventral part of the caudate (Martinez et al.2003b); see Table 1.

Since the associative striatum (defined as functional subdivision of the striatum) is better presented in the caudate than the putamen*, the caudate was considered the most appropriate ROI. The cerebellum was chosen as the reference region in the SPECT data analysis (Farde et al.1990).

Functional Subdivision	Anatomic Subdivision
Limbic striatum	Ventral striatum: Nucleus accumbens, precommissural ventral putamen and precommissural ventral caudate
Associative striatum	Precommissural dorsal putamen (pre-DPU), precommissural dorsal caudate (pre-DCA) and postcommissural caudate (post-CA)
Sensorimotor striatum	Postcommissural putamen (post-PU)

Table 1. Striatal subdivision. Precommissural refers to rostral and postcommissural refers to caudal anterior commissure. Modulated from Martinez et al. 2003

*In the template, the caudate includes the precommissural dorsal caudate (pre-DCA) and postcommissural caudate (post-CA), whereas the putamen includes part of the globus pallidus, the Precommissural dorsal putamen (pre-DPU) and the Postcommissural putamen (post-PU). It was not possible to differentiate the pre-DPU.

Data Analysis

The BP reflects the potential of a specific binding site to interact with either a specific radioligand or a specific endogen neurotransmitter (e.g. dopamine) and is defined in vitro as the ratio of B_{max} (density of receptors) to K_d (radioligand equilibrium dissociation constant=k_{off}/k_{on}) (Mintun et al.1984).

We used a bolus injection plus constant infusion of radioligand to obtain tracer steady state in the plasma and brain water. It was assumed that the total parent radioligand (free plus protein-bound radioligand) in plasma at steady-state equals the concentration in the tissue (which is not directly measurable).

In contrast to BP_F, the BP_P is not corrected for the fraction of radioligand bound to plasma proteins (f_P), assuming that correcting for protein binding differences could add more variability to the data (Innis et al.2007). Determination of the free fraction of [^{123}I]-IBZM was included in the method as an additional analysis, but it was found stable with a mean value of 5.4 (SD 0.9)% and therefore not corrected for in the analyses.

Thus, BP_p was used as a measure of the regional dopamine $D_{2/3}$ receptor density available for [^{123}I]-IBZM binding. BP_p refers to the ratio at steady state of specifically bound radioligand to that of total parent radioligand in plasma (Innis et al.2007). The occupancy was calculated as:

$$Occupancy(\%) = \left(1 - \frac{BP_p(treatment)}{BP_p(baseline)}\right) \times 100\%$$

Statistical Methods

IBM SPSS Statistics 20 was used for the statistical analyses. BP_p and BOLD activity were tested for normality using the Shapiro-Wilk test. For the between group comparison, an independent t-test was used when appropriate, and a Mann-Whitney test when there was evidence of non-normal distribution. Repeated measures ANOVA (analyses of variance) were carried out with an approximately normal distribution and with group and gender used as between-subjects factors when the mean of BP_p and BOLD activity was compared. A paired t-test was used when baseline measurements were compared with follow-up data. For correlations, a nonparametric Spearman's correlation coefficient was used. The relationship between BP_p and change in PANSS scores, GAF and SWN-K were analysed using general linear modelling techniques. The change in GAF, SWN-K and PANSS score over time was calculated as a percentage delta score from baseline to follow-up. Patients responding to treatment were defined as having an improved PANSS positive score of more than 30%, which is similar to a previous study.(Meisenzahl et al.2008) In general, a conventional significance level of 0.05 was used.

Results

Excluded Participants and Dropouts

Thirty-two patients went through baseline examinations that included SPECT and fMRI. After baseline examinations, one patient had his diagnosis changed to severe major depressive disorder with psychotic symptoms. One patient turned out to have a regular use of cannabis during the study period and was tested positive for cannabis prior to the scans. These two patients were excluded completely from all analyses while two other patients were excluded due to a technical problem with the SPECT image acquisitions.

From the 28 patients included at baseline, four patients discontinued the study during the six-week treatment period. Three of them experienced an illness exacerbation during hospitalisation and one patient did not wish to participate at follow-up. In addition, one patient had a panic attack at the follow-up scans, one patient was not PANSS rated at follow-up and the blood analyses for one patient were not performed at the SPECT examination.

In the HC group, two subjects received antidepressants at the six-month follow-up examinations and were thus excluded from all analyses.

As a result, the complete sample consisted of 28 patients and 26 HC at baseline, and 21 patients and 26 HC at follow-up; see Figure 4.

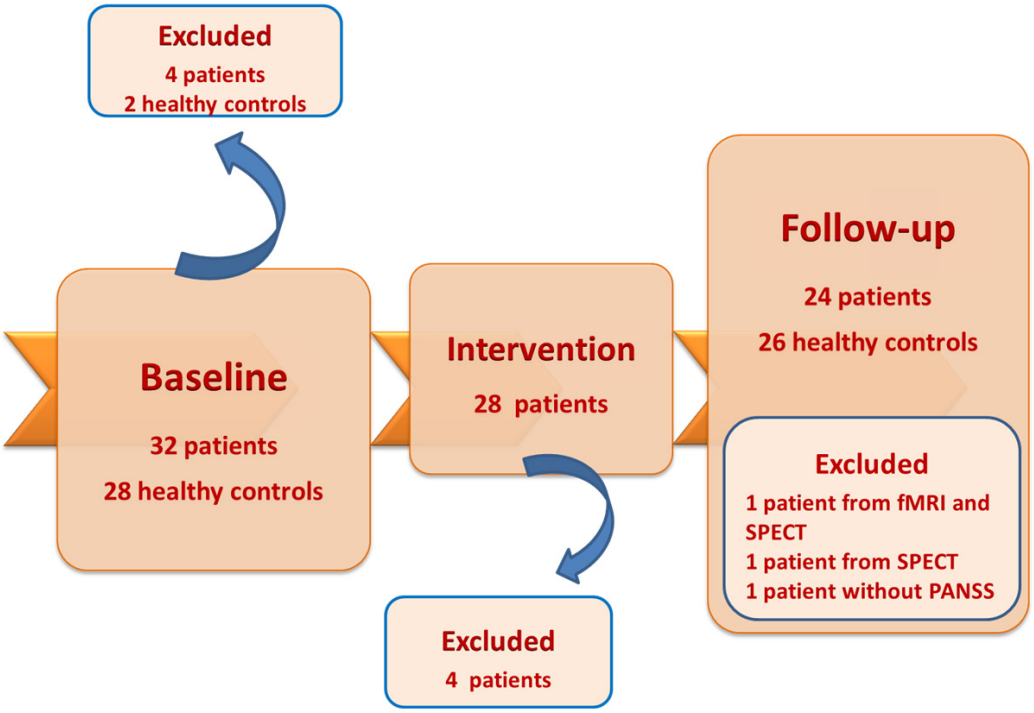


Figure 4. Dropouts

Demographic and Clinical Data

The patient group did not differ significantly by gender, age, or handedness from the HC group. The two groups did, though, differ on tobacco intake. 61 % of the patients were smokers compared to 25 % in the HC group.

Patients were moderately ill with a baseline total PANSS score of 81, and a functioning on GAF-F at 42. Patients, who discontinued the study during the treatment period showed higher PANSS scores and lower GAF and SWN-K at baseline, but the difference was not significant (group comparison). Table 2 presents the demographic data and psychopathology measures in more detail.

Table 2. Demographic data and psychopathology

		N	Mean	SD	Range
Females/males	Schz.p	14/14			
	HC	13/13			
Age (years)	Schz.p	28	23	4.4	18 – 37
	HC	26	23	4.7	18 – 38
Hand score	Schz.p	28	64	57	-88 – 100
	HC	24	61	62	-100 – 100
Diagnosis no.		28			
DF.20.0 Paranoid		19			
DF.20.1 Disorganised		3			
DF.20.3 Undifferentiated		3			
DF.20.9 Unspecified		3			
Baseline		28			
DUI (weeks)			69	88	2 – 312
GAF-S			40	9.3	25 – 61
GAF-F			42	11.9	30 – 75
SWN-K total		24	67	13.7	41 – 88
PANSS positive			20	3.6	10 – 29
PANSS negative			20	7.7	7 – 38
PANSS general			41	8.4	22 – 56
PANSS total			81	15.3	39 – 102
Follow-up		24			
Female/male		12/12			
GAF-S		23	57*	8.6	37 – 80
GAF-F		23	56*	12.6	32 – 75
SWN-K total			76*	12.7	44 – 99
PANSS positive			14*	3.5	7 – 20
PANSS negative			20	6.1	9 – 33
PANSS general			31*	7.3	18 – 48
PANSS total			65*	13.8	40 – 100
Dose (mg)			238	120	50 – 500
(S)-amisulpride (ng/ml)			392	290	15 – 1013

DUI: duration of untreated illness; GAF-F: Global Assessment of Functioning, functioning; GAF-S: Global Assessment of Functioning, symptoms; HC: healthy controls; PANSS: Positive and Negative Syndrome Scale; Schz.p: schizophrenia patients; SD: standard deviation; SWN-K: Subjective Well-Being Under Neuroleptics Scale short form. *Significant difference from baseline

The patients were treated with a mean daily dose of amisulpride of 238 mg/day (SD 120 mg) with the range 50 - 500 mg/day. The dose of amisulpride was individually adjusted according to the

clinical impression of symptoms and no medical treatment against adverse effects was allowed. At baseline, none of the patients scored on the clinical global impression of EPS. At follow-up, two patients scored mildly on the clinical global impression of parkinsonism due to definite slowness in movements.

At group level, patients responded well to treatment and ended up with a total PANSS score of 65, and an improvement on functioning with a GAF-F of 56. The difference between baseline and follow-up measures of PANSS, GAF and SWN-K in patients was significant. PANSS, GAF and SWN-K scores all improved; however, the PANSS negative score did not change significantly. See Table 2.

SPECT Data

At baseline there were no significant differences in BP_p of the caudate between patients and HC. As expected, due to the intervention with amisulpride, the BP_p in the group of patients had decreased significantly at follow-up compared to baseline BP_p. See Table 3 and also visualised in Figure 5. The BP_p did not correlate with DUI or any psychopathology measures (PANSS, SWN-K and GAF scores) at baseline.

Table 3. SPECT data

Baseline		**N**	**Mean**	**SD**	**Range**
BP_p total caudate	Schz.p	28	2.9	1.1	1.5 – 5.6
	HC	26	2.7	0.7	1.6 – 4.3
BP_p left caudate	Schz.p		2.9	1.1	1.6 – 5.8
	HC		2.9	0.7	1.6 – 4.5
BP_p right caudate	Schz.p		2.9	1.2	1.3 – 5.6
	HC		2.6	0.8	1.5 – 4.3
Follow-up					
BP_p total caudate		22	1.3*	0.7	0.3 – 2.5
BP_p left caudate			1.3*	0.7	0.4 – 3.0
BP_p right caudate			1.2*	0.7	0.3 – 2.6
Occupancy total caudate (%)			52	19	15 – 84
Occupancy left caudate (%)			50	20	7 – 85
Occupancy right caudate (%)			55	21	17 – 83

BP_p: binding potential; HC: healthy controls; Schz.p: schizophrenia patients; SD: standard deviation. *Significant difference from baseline

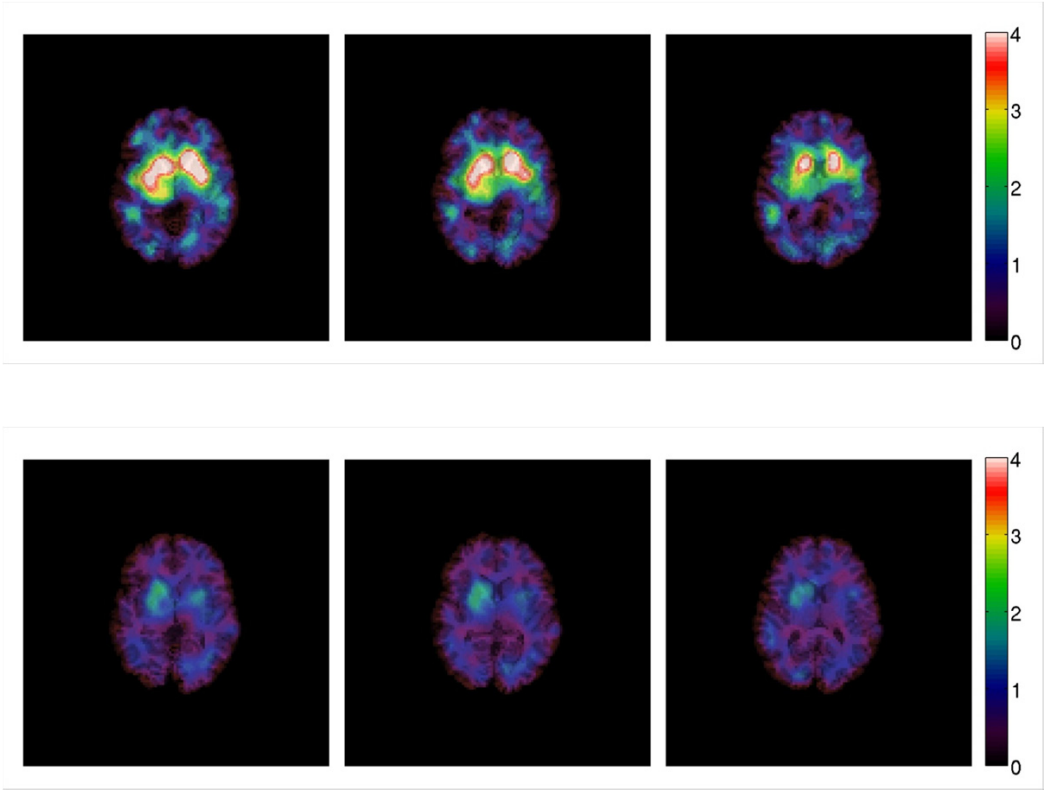


Figure 5. SPECT [^{123}I]-IBZM of a patient before (top) and after (bottom) six weeks of treatment with the relatively selective dopamine $D_{2/3}$ receptor antagonsist amisulpride. The BP_p decreased significantly from baseline to follow-up.

Paper One

Correlations between striatal dopamine $D_{2/3}$ receptor BP_p and treatment outcome

Since clinical data were available for a few patients who did not go through the SPECT scan at follow-up, 24 patients were included in these analyses.

Significant positive correlations were found between baseline BP_p of the caudate and the change in PANSS score in the total patient group. Patients with a low BP_p had a better treatment response. The correlations were significant for PANSS positive ($p=0.048$, $r^2=0.166$)*; PANSS general ($p=0.011$, $r^2=0.257$); and PANSS total ($p=0.003$, $r^2=0.342$) but non-significant for PANSS negative

scores (p=0.328); see Figure 6. There were no significant correlations between BP_p at baseline and the percentage change in either GAF or SWN-K.

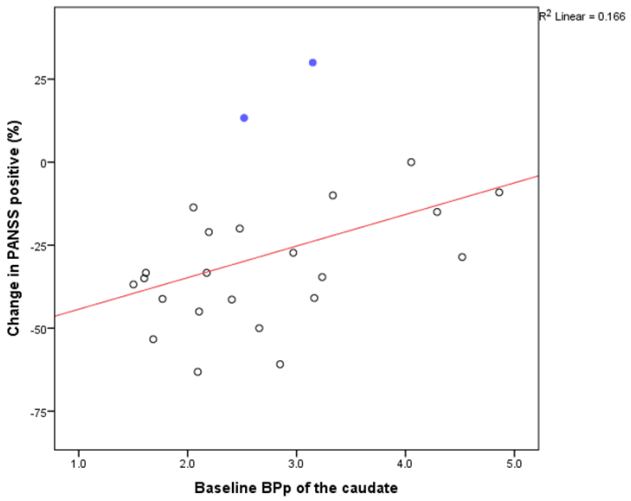


Figure 6. Relationship between BP_p of the caudate and the change in PANSS positive score in the patient group. The blue dots represent the two possible outliers

BP_p: binding potential

* Two patients with a worsening of positive symptoms (marked with blue dots in Figure 6) had very low PANSS positive scores at baseline. One was also treated with a low dose of amisulpride (50 mg). Omitting them from the regression strengthened the results (p=0.014, r^2=0.268).

The group of patients who responded to treatment (defined as an improvement of PANSS positive >=30%) (n=13, f=5, m=8) was further compared to patients not responding (n=11, f=7, m=4). At baseline, responders had a significantly lower BP_p (mean BP_p 2.2 vs. 3.3 in the caudate, p=0.003); a lower GAF-F (mean 37 vs. 51, p=0.002); and a lower GAF-S (mean 37 vs. 46, p=0.028). The responders had, per definition, a significantly higher improvement on the PANSS positive, but also on the PANSS general and total score compared to non-responders. They improved more on the GAF-S compared to non-responders; however, they did not improve more on the GAF-F and SWN-

K. Furthermore, responders and non-responders were found to have similar baseline PANSS scores.

In the secondary analyses, it was further tested whether the groups and results changed if the rescaled PANSS positive scale ($PANSS_{0-6}$) was used instead. Responders were then defined as having an improvement on $PANSS_{0-6}$ positive >=50% (Obermeier et al.2011;Leucht2014). One male patient moved to the non-responder group; the results were otherwise similar.

Correlations at follow-up (BP_p, occupancy and psychopathology)

The dose of amisulpride was well correlated with (S)-amisulpride ($p<0.001$). The amisulpride and (S)-amisulpride doses were both significantly correlated with the occupancy ($p=0.003$ and $p<0.001$ respectively); see the dose-occupancy relationship below.

There were no correlations between caudate BP_p at baseline and the optimal dose needed to obtain a clinical effect. Thus, it was not possible to predict which dose the patient would benefit from based on the baseline BP_p. Furthermore, the dose (or (S)-amisulpride) did not correlate with the change in PANSS, change in SWN-K or change in GAF. Consistent with these findings, no significant correlations between the occupancy and change in PANSS, SWN-K or GAF were found. The occupancy, however, was significantly negatively correlated with GAF-F at follow-up ($p=0.049$; Spearman's rho=-0.446). Since we did not have any hypothesis on the specific GAF-F score, this finding is more explorative, but it may reflect that the more the dopamine receptors are blocked, the worse the functioning becomes; see Figure 7.

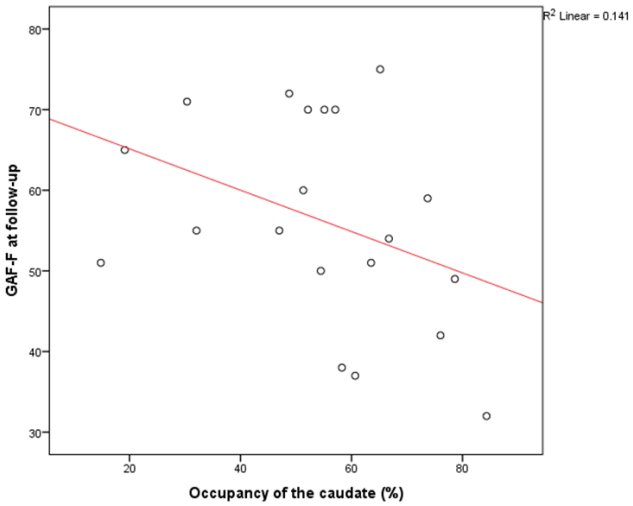


Figure 7. Correlation between occupancy and functioning by GAF-F. A first-order linear approximation is included in the figure solely to illustrate the correlation sign

GAF-F: Global Assessment of Functioning, functioning

The dose-occupancy relationship

The optimal dose of amisulpride for the individual patient (as established by the treating physician) resulted in a highly varying occupancy between patients (mean 52% (SD 19.1)). This wide range of striatal occupancy is consistent with earlier [^{123}I]-IBZM SPECT studies of amisulpride (la Fougere et al.2005;Meisenzahl et al.2008).

The dose-occupancy or concentration-occupancy curve can be described as a curvilinear function (Farde et al.1988;Farde et al.1992). Fitting a Michaelis-Menten curve to the present occupancy data, the plasma concentration predicted to provide 50% occupancy of the maximum attainable receptor occupancy (EC_{50}) is 265 ng/ml; see Figure 8. With a maximum attainable receptor occupancy of 100 % (E_{max}) and C_{ami} as the concentration of (S)-amisulpride, the equation is:

$$\text{Occupancy (\%)} = \frac{\text{Emax} \times \text{Cami}}{\text{Cami} + 264.75}$$

In general, lower EC_{50} values have been reported in previous studies compared to the present study. Meisenzahl et al., for example, reported that the EC_{50} was 30ng/ml, but in this case, E_{max}

was treated as a free parameter and fitted to 87%. The methodologies used are generally inconsistent and the occupancy is often calculated from the baseline BP_p from another group of patients or HC. In the present study, (S)-amisulpride was measured near the t_{max} of amisulpride, the curve was fitted to an E_{max} of 100%, and the occupancy was calculated from baseline and follow-up BP_p in the same patients.

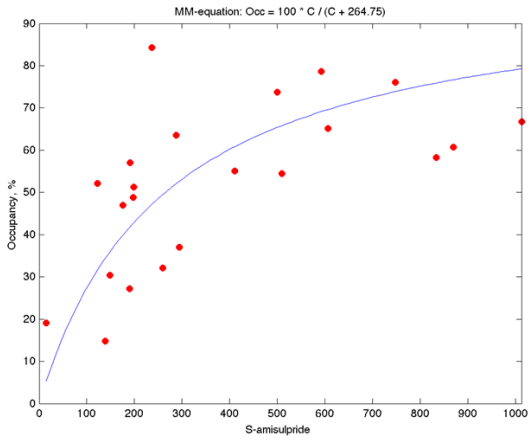


Figure 8. Concentration-occupancy relationship illustrated by (S)-amisulpride and the occupancy of caudate
MM: Michaelis-Menten; Occ: occupancy

fMRI Data

At baseline, patients showed a significantly lower BOLD response than HC in the caudate. The BOLD response was normally distributed in the HC group only and there was a significant difference in the variation of the BOLD response between the two groups; see Table 4.
There was no significant difference between left and right side, tested with a repeated measures ANOVA (approximation of normal distribution). Handedness was found to be non-significant as the covariate and there was no effect of gender, group or group*gender interaction.

At follow-up, the significant difference of the caudate BOLD response between the groups disappeared. Furthermore, the BOLD response was normally distributed in both the HC group and

the patient group. The patients showed a trend-wise increase (normalisation) in the BOLD response (p=0.082) compared to HC, who showed a non-significant decrease (p=0.764); see Table 4.

	N	Schizophrenia Patients			N	Healthy Controls		
Baseline (females/males)	**14/14**	**Mean**	**SD**	**Range**	**13/13**	**Mean**	**SD**	**Range**
BOLD response total		0.287	0.679	-0.672 – 2.616		0.664	0.586	-0.423 – 2.173
BOLD response left caudate		0.334	0.700	-0.853 – 3.625		0.725	0.675	-0.871 – 2.616
BOLD response caudate		0.239	0.567	-0.919 – 1.606		0.603	0.610	-1.002 – 1.729
Follow-up (females/males)	**13/11**				**11/12**			
BOLD response total		0.453*	0.597	-0.842 – 1.543		0.643	0.713	-0.450 – 2.229
BOLD response left caudate		0.460*	0.700	-1.019 – 1.897		0.679	0.833	-0.827 – 2.433
BOLD response right caudate		0.446*	0.567	-0.665 – 1.585		0.607	0.686	-0.568 – 2.262
Change BOLD response total caudate		0.207	0.558	-0.783 – 1.260		-0.046	0.719	-1.481 – 1.663
Change BOLD response left caudate		0.201	0.637	-0.893 – 1.442		-0.090	0.897	-1.971 – 1.524
Change BOLD response right caudate		0.213	0.541	-0.673 – 1.331		-0.001	0.778	-1.509 – 1.802

Table 4. Functional magnetic resonance imaging data

*significant different from baseline

Correlations at baseline (secondary analyses)

There were no significant correlations between the BOLD response at baseline and the psychopathology expressed as PANSS, or SWN-K and GAF.

In addition, no correlations were found between BP_p and the BOLD response in the patient group or the HC group, analysed by group and together.

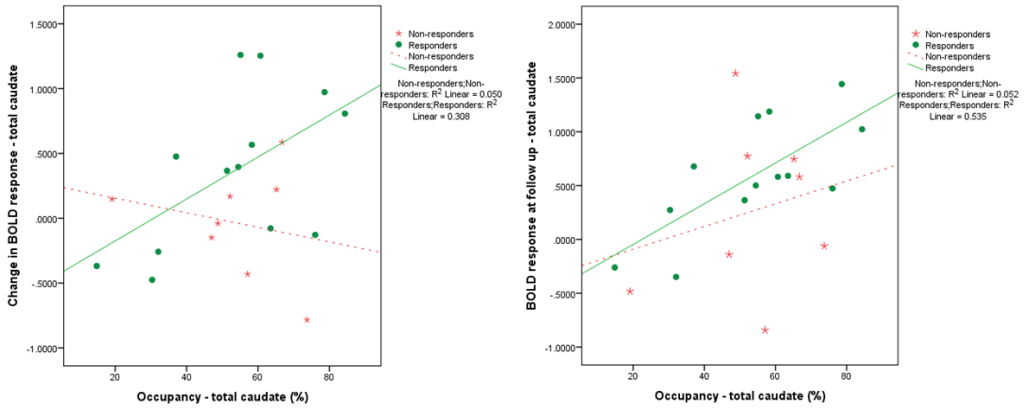
Paper Two

Correlations between striatal $D_{2/3}$ receptor occupancy, alterations in reward processing and psychopathology

There was no significant correlation between the occupancy and the change in BOLD response in the total group of patients (n=22; p=0.138; rho=0.327). However, when the analysis in the separate groups of responders and non-responders was repeated, there was a significant correlation between occupancy and change (normalisation) in the BOLD response from baseline to

follow-up in the patients responding to $D_{2/3}$ receptor blockade (n=13; p=0.035; rho=0.588), whereas no correlations were found in the non-responders (n=8; p=0.955); see Figure 9(A).

In line with this, a significant positive correlation between occupancy and the BOLD response at follow-up in the whole group was observed (n=22; p=0.026; rho=0.475). In the group of responders, the correlation was significant (n=13; p=0.011; rho=0.676), whereas this was not the case in the group of non-responders (n=8; p=0.736); see Figure 9(B).



(A) (B)

Figure 9. (A) Correlation between the occupancy and change in BOLD response from baseline to follow-up. (B) Correlation between the occupancy and BOLD response at follow-up.

The correlations were significant in the group of responders (green dots), and non-significant in the group of non-responders (red stars) in both figures. Note: one patient was excluded in the division because there was no PANSS rating at follow-up, which is why n=21 in the figures.

Furthermore, there was a significant correlation between (S)-amisulpride and change in BOLD response in the whole group of patients (n=23; p=0.044; rho=0.424), as well as between (S)-amisulpride and the BOLD response at follow-up (n=23; p=0.030; rho=0.453). Analysed separately, these correlations were only significant in the group of responders and not in the group of non-responders; see Figure 10. There were no significant correlations between the dose of amisulpride and the change in BOLD response or the BOLD response at follow-up.

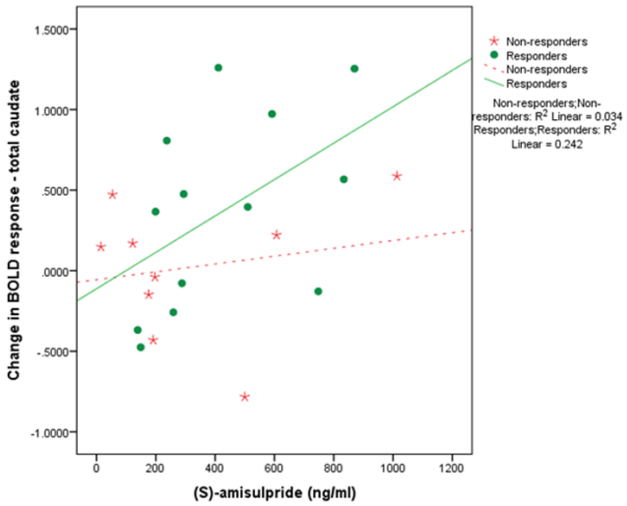


Figure 10: Correlation between (S)-amisulpride and change in BOLD response. The correlations were significant in the group of responders (green dots), and non-significant in the group of non-responders (red stars). Note: one patient was excluded in the division because there was no PANSS rating at follow-up, which is why n=22.

Correlations at follow-up (BOLD response and psychopathology)

There was a nearby significant positive correlation between the change in the BOLD response and the change in the PANSS positive score (n=23; p=0.050; rho= -0.413); see Figure 11.

Excluding the two possible outliers mentioned earlier*, the correlation became highly significant (n=21; p=0.008, rho= -0.562). Thus, the more the BOLD response increased the more the positive symptoms tended to improve.

*The two possible outliers (marked as blue dots in Figure 11) both had low PANSS positive scores at baseline and a worsening of positive symptoms. Furthermore, at the utmost, patients received a low dose of amisulpride and as an exception, the fMRI was performed 23 days after the PANSS rating and SPECT scan.

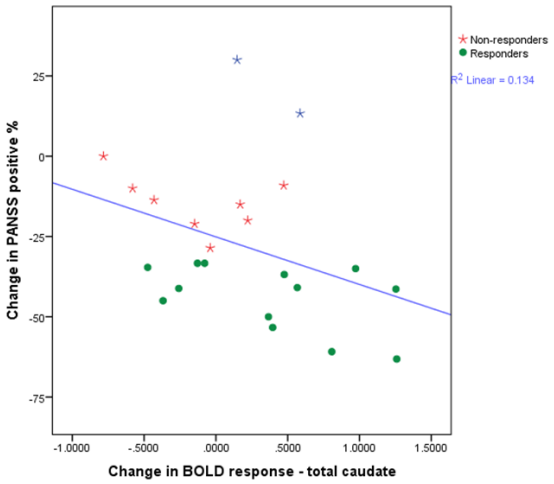


Figure 11. Correlation between the change in BOLD response and change in PANSS positive. The correlation is shown for the whole group of patients. Responders (green dots), and non-responders (red stars). The two possible outliers (blue stars).

Discussion

Paper One

Paper one, where the primary aim was to explore a possible association between striatal dopamine $D_{2/3}$ receptor BP_p and treatment outcome, outlines the first part of the results.

In the group of 24 initially antipsychotic-naïve first-episode schizophrenia patients, a significant negative correlation was observed between baseline BP_p of the caudate and clinical improvement of positive symptoms after six weeks of treatment with the relatively selective $D_{2/3}$ antagonist amisulpride. This was in agreement with our hypothesis. The data might suggest baseline $D_{2/3}$ receptor BP_p as a potential predictive marker for treatment response on positive symptoms. Patients with good treatment response had a significantly lower baseline $D_{2/3}$ BP_p compared to non-responders, even though the PANSS scores at baseline did not differ between the two groups. This underlines the inability to differentiate good and poor responders based on baseline PANSS scores.

With a low striatal BP_p interpreted as an indication of high dopamine activity, the correlation between BP_p and treatment response on positive symptoms is consistent with the depletion study by Abi-Dargham et al. (Abi-Dargham et al.2000), who reported an association between the effect of dopamine depletion on D_2 receptor BP (interpreted as a high dopamine activity) and greater improvement of positive symptoms in unmedicated schizophrenia patients after treatment with different antipsychotics. It is further in line with a study by Demjaha et al. (Demjaha et al.2012) who reported a higher dopamine synthesis capacity in a group of 12 responders versus 12 non-responders to antipsychotic treatment.

Responders in the present study were defined as patients whose PANSS positive score improved more than 30%. This is in accordance with a study by Meisenzahl et al.(Meisenzahl et al.2008). A similar division of the patients and the same results were obtained if responders were defined with a 50% improvement on the rescaled $PANSS_{0\text{-}6}$ positive score (Obermeier et al.2011;Leucht2014). The group of responders improved significantly more on both the PANSS positive, total and general scales, although neither of the groups had an improvement in negative symptoms. Not surprisingly, the responders also improved significantly more on GAF-S than non-responders, although no significant differences were found regarding the change in GAF-F and

SWN-K. This could further suggest that even though patients experience a relief of positive symptoms, their level of functioning and well-being might not improve. A longer follow-up period would of course be necessary to make firm conclusions regarding final remission and recovery, but the present data emphasise the importance of considering improvement of positive symptoms versus adverse effects from the antipsychotic treatment.

The mean dose of amisulpride was relatively low (238 mg/day) since the patients were all drug naïve. Dose and (S)-amisulpride were both well correlated with striatal occupancy, and in line with the expected dose-occupancy curve. A maximum occupancy of 80% was observed. Even if the individually adjusted dose of amisulpride was administered to each patient simultaneously (three hours prior to the SPECT scan), no associations between the striatal occupancy and improvement on the PANSS, SWN-K or GAF were observed. This is consistent with the previously mentioned [^{123}I]-IBZM SPECT study of amisulpride (Meisenzahl et al.2008). Significant correlations between striatal occupancy and outcome have been supported by other PET and SPECT studies (Nordstrom et al.1993;Kapur et al.2000;Catafau et al.2006;Agid et al.2007;Kegeles et al.2008) correlating striatal occupancy with improvement on the Brief Psychiatric Rating Scale, the PANSS positive and the Clinical Global Impression scale. A negative correlation between striatal occupancy and SWN-K at follow-up has also been reported in one of these studies (Agid et al.2007). This finding was not replicated in the present data. A negative correlation between striatal occupancy and the GAF-F at follow-up was found, which could reflect that the more the dopamine receptors are blocked, the worse the functioning. This also supports the precautions clinicians need to be aware of when treating the patients.

Possible explanations for the conflicting results regarding the occupancy and treatment outcome are the different compounds and methodology used in the studies, the small sample sizes, the inclusion of a mix of schizophrenia patients and patients with schizophreniform disorder, and also partly because patients were previously medicated. Moreover, the mentioned studies included patients with motor adverse effects and striatal occupancies above threshold, i.e. the studies have a broader range of occupancy. One major limitation in several of the studies is the calculation of the occupancy. This is often calculated based on the baseline BP obtained from different subjects or from a subgroup of patients. In the present study, BP_p is measured at baseline in the antipsychotic-naïve state and at follow-up, which due to inter-individual differences gives a more

precise estimate. Furthermore, the present sample size is rather large, homogeneous and includes only first-episode schizophrenia patients.

Paper Two

Paper two, where the primary aim was to explore the associations between striatal dopamine $D_{2/3}$ receptor blockade, alterations in reward processing and the psychopathology, outlines the second part of the results.

In the group of 22 patients who underwent both fMRI and SPECT scans, a significant positive correlation was observed between the striatal $D_{2/3}$ receptor blockade and BOLD response during the anticipation phase of reward processing at follow-up. Additionally, there was a significant positive correlation between the occupancy of dopamine $D_{2/3}$ receptors and the normalisation of the BOLD response in the group of patients responding to treatment with the relative selective $D_{2/3}$ receptor antagonist amisulpride. Also, a significant correlation between (S)-amisulpride and the change in BOLD response was observed. These findings are consistent with the main hypothesis that dopamine $D_{2/3}$ receptor occupancy normalises the BOLD response during the anticipation phase of reward.

Furthermore, a correlation between the normalisation of the BOLD response and improvement of positive symptoms was observed, which is in agreement with previous results (Nielsen et al.2012a). Even though the caudate was chosen as the ROI, in contrast to the VS in earlier studies by Nielsen et al. (Nielsen et al.2012b;Nielsen et al.2012a), the present data confirm the attenuated activation of the BOLD response during the anticipation phase in antipsychotic-naïve schizophrenia patients compared to HC before antipsychotic treatment. The findings are also consistent with the literature (Juckel et al.2006;Schlagenhauf et al.2009;Esslinger et al.2012;Juckel et al.2012;Roiser et al.2013;Schlagenhauf et al.2014) and underline the importance of striatal dopamine activity for the aberrant incentive salience found in schizophrenia patients. They further confirm reward disturbances as suggested core deficits in schizophrenia.

Regarding the association between the BOLD response and psychopathology at baseline, it was not possible with the present sample of patients and the present ROI to confirm a (negative) correlation between positive symptoms and the BOLD response at baseline, which has been reported in the VS in earlier studies (Nielsen et al.2012b;Esslinger et al.2012). The patient groups

in previous studies have a similar PANSS positive score, but changing the ROI to include the total caudate might have attenuated possible correlations between psychopathology and reward abnormalities (see Limitations). Moreover, previous studies included a majority of male patients and gender differences combined with the previously discussed complexity of schizophrenia could have also weakened the baseline relations in the present study. When males and females were analysed separately, however, the correlations were still non-significant, but the sample sizes were small. In line with this, no correlation was found between a lower BP_p and attenuated BOLD response in the patient group at baseline.

Even though the data support dopamine having a key role in reward anticipation in schizophrenia, it should be taken into consideration that other neurotransmitter systems – e.g. the serotonergic, GABAergic and/or glutamatergic systems – could be involved in the observed reward processing disturbances (Cohen et al.2012), which is concordant with the present findings; see below. A recent study reporting a similar attenuated BOLD response in the anticipation phase in not only schizophrenia but also alcohol dependence and major depressive disorders indirectly supports the involvement of more than one neurotransmitter system in reward processing (Hagele et al.2014). This perspective is well in line with the literature on the heterogeneous treatment response and suggested schizophrenia subgroups.

Heterogeneity and a Possible U-curve

This study supports the hypothesis of subgroups of patients occurring due to different neurochemical profiles. A heterogeneous treatment response in schizophrenia is well known (Levine and Leucht2010) and possible subgroups of patients with and without striatal hyperdopaminergic activity, reflected in good and poor treatment response to dopamine blockade, has previously been proposed (Rasmussen et al.2011;Ebdrup et al.2011;Demjaha et al.2012;Howes and Kapur2014). The negative correlation between BP_p and response to treatment, even though it has to be seen as a continuum, is in line with the suggestions of subtypes in schizophrenia that might respond differently to a D_2 receptor blockade. Patients in the present study not responding to the dopamine blockade from amisulpride showed higher baseline BP_p.

This subgroup of patients might correspond with the supposed subgroup of patients without dopamine disturbances, as proposed in previous studies.

A non-normalised distribution of the BOLD response in the patient group at baseline could reflect the heterogeneity. The finding of a significant association between the occupancy and the change in the BOLD response in the group of only responders in particular supports the heterogeneity of the disease. It seems likely that non-responders belong to a subgroup of patients without hyperdopaminergic activity, since they either show a small worsening or no change in BOLD response despite high dopamine occupancy, as illustrated in Figure 9A.

This supports the idea of an inverted U-curve, where the top of the U-curve reflects the optimal level of dopamine linked with the optimal BOLD response in the striatum. The hypothesis is adopted from findings in the frontal cortex for D_1 receptors and cognition and suggested as a possible link in the striatum (Cohen et al.2007). Patients with dopamine hyperactivity – placed on the right side of the curve – respond to treatment with a dopamine blocker and their positive symptoms dampen in line with normalisation of the BOLD response as they move to the left towards the top of the curve. Patients with lower dopamine activity or even hypoactivity – placed on the top or to the left of the curve – get worse from the dopamine blockade and thus move down the curve. Patients on the right side of the curve are similarly at risk of moving too far to the left (down the curve) due to an excessive dopamine blockade, thereby increasing the risk of side effects such as negative symptoms. In the present study, the latter is not observed, since the treatment was individualised and no treatment against side effects was allowed. It is of great importance in the clinic to find this optimal treatment window (top of the U-curve).

The heterogeneity of the disease combined with the size of the cohort is at the same time a possible explanation for the lack of a significant relationship between the occupancy and the change in the BOLD response between baseline and follow-up examinations in the total group of patients (responders and non-responders).

Limitations and Strengths

The strengths of the study are in the inclusion of solely antipsychotic-naïve first-episode schizophrenia patients who have never been exposed to antipsychotic compounds. This means the brain has not been modified by antipsychotics and the changes observed at baseline can be attributed to the disease and not to modulation from medication. The relatively large sample size, the longitudinal study design with antipsychotic monotherapy, and the combination of several modalities are also important strengths in the study. Given that antipsychotic-naïve patients had to go through the extensive examination programme (the patients had to stay unmedicated for a mean of five to seven days), the risk of selection bias is unavoidable, i.e. severely ill patients are likely to be excluded. Even so, the patients included in the study were moderately to severely ill, with a total PANSS score of 81. Also, inclusion of severely ill patients would probably have strengthened the correlations as opposed to weakening them.

The spatial resolution of the SPECT system is limited and the activity measured in the ROI is influenced by the activity in the adjacent areas. One of the strengths of the study was the use of the bolus/infusion technique and the inclusion of only drug-naïve patients, which provides a more precise estimate of the occupancy. Total activity in the brain may be lower due to the division and decay of the administered dose, causing statistical counting errors.

The salience disturbances are believed to be more pronounced in the ventral part (limbic striatum) as compared to the rest of striatum (Knutson et al.2001a), whereas high-resolution PET studies have found dopamine hyperactivity to be predominately localised in the associative striatum in schizophrenia patients and in the prodromal state (Howes et al.2009;Kegeles et al.2010). In order to relate fMRI and SPECT data, the caudate was chosen as the ROI. Choosing the entire caudate as the ROI, however, might have diminished the fMRI signal and thereby the findings.

The last dose of amisulpride was administered at two different times before the SPECT and fMRI scans. The dose was administered the evening prior to the fMRI scan, while the other dose was administered three hours prior to the SPECT scan. (S)-amisulpride was measured as a mean during the SPECT scan, presuming a steady-state level. Even considering that antipsychotics generally have a slower wash-out period from brain tissue than from plasma (Tauscher et al.2002), the

discrepancy of administration between the fMRI and SPECT scans might have weakened the associations between occupancy and changes in the BOLD signal, whereas the opposite is unlikely.

Not matching for smoking is one of the limitations of this study. Nicotine use has been found to affect the BOLD response in the reward anticipation phase (Peters et al.2011). Previous studies have reported a relationship between chronic nicotine intake and enhancement of dopamine transmission (Jasinska et al.2014); however, changes are modest in human studies (5-10%) and in test-retest variability (Thomsen et al.2013). In agreement with this, a recent PET study did not find any significant effect of moderate smoking on synthesis capacity in the striatum (Bloomfield et al.2014b). Moreover, we were primarily interested in within-group findings. Assuming the participants did not change their smoking habits, not controlling for nicotine intake is less likely to have a significant impact on the findings. Furthermore, since schizophrenia patients have a higher use of tobacco than HC, matching for smoking might have added bias in the HC group.

A few patients received a small amount of benzodiazepines during hospitalisation, but none did so regularly and benzodiazepines were not allowed 12 hours prior to any of the examinations. Two patients, however, tested positive for benzodiazepine prior to the baseline SPECT scan. This was not taken into account in the analyses, since the positive tests were due to a minimum dose intake the prior evening due to insomnia.

Drug abuse, including cannabis, is known to affect striatal dopamine transmission and the reward activity either directly or indirectly via dopamine neurons, although the mechanisms and relationships are still being investigated (Gardner2005;Glenthoj et al.2006;Nestor et al.2010;van Hell et al.2010;Loewinger et al.2013;Bloomfield et al.2014a). None of the patients included had a current misuse during the study period. One patient, though, was excluded from all analyses due suspicion of abuse and positive tests for cannabis prior to the scans.

General Conclusion & Future Perspectives

The data presented in this thesis are believed to bring additional insights to the biology of schizophrenia regarding treatment outcome and the heterogeneity of the disorder. The data supports a heterogeneous treatment response in schizophrenia, which associates with possible subtypes based on different neurochemical profiles. After six weeks of monotherapy with the relatively selective dopamine D_2 receptor antagonist, amisulpride, patients with a low striatal BP_p showed an improvement of positive symptoms, whereas patients with a high baseline BP_p experienced a poor treatment response. These findings are consistent with previously suggested subtypes, where patients with striatal dopamine hyperactivity are one subtype and patients not responding to treatment with a dopamine blocker have disturbances in other neurotransmitter systems, e.g. the glutamatergic transmitter system. Today, treatment is based on trial and error and finding possible predictive markers of treatment response would alleviate our patients' ineffective treatment trials and unnessecary adverse effects. In this cohort of antipsychotic-naïve first-episode schizophrenia patients, data suggest BP_p as a predictive marker of treatment response with respect to the positive symptoms, whereas psychopathology at baseline did not predict treatment response. Multimodal imaging studies investigating more than one transmitter system in drug-naïve patients are important from a future perspective to bring further knowledge and perhaps support the hypotheses of subtypes in schizophrenia due to underlying disturbances of the different neurotransmitters.

Reward disturbances have been a consistent finding in schizophrenia and are found to relate with positive symptoms, supporting the salience hypothesis. A direct association between dopamine and salience abnormalities has, however, not been verified. Data presented in this project are, to our knowledge, the first to demonstrate a positive correlation between occupancy of dopamine $D_{2/3}$ receptors and a normalisation of the BOLD response during the anticipation phase of reward processing in initially antipsychotic-naïve first-episode schizophrenia patients who respond to treatment with a dopamine $D_{2/3}$ receptor antagonist. This further supports the hypothesis of distinct subtypes in schizophrenia with different neurochemical profiles. Reward disturbances are not specific for schizophrenia, thus a model of reward processing might not be useful as a

diagnostic tool, but perhaps in the long term such a model might contribute to the prediction of treatment response and thus be used as a tool for stratified medication.

In recent years, machine learning has become an exciting approach to pattern recognition and discovery of potential biomarkers for schizophrenia or subtypes. Large cohort studies and international cooperation between research units are essential in this regard, in particular carrying out longitudinal multimodal studies in the prodromal phase and in drug-naïve first-episode patients. Moreover, advanced imaging techniques, such as functional connectivity MRI with diffusion tensor tractography, keep evolving.

Despite these emerging tools and research approaches, focus on psychopathology is still important. With the many facets of schizophrenia and overlapping symptomatology with other disorders, investigating more specific symptoms and bringing together the research fields of e.g. bipolar affective disease, attention deficit hyperactivity disorder, autism and some neurological disorders are relevant approaches in future research as well. The more recent discovery of anti-NMDA receptor encephalitis is an example of overlapping symptomatology, which could also support autoimmunity as an etiologic factor in schizophrenia.

Finally, within preclinical neuroscience, the neuromodulation technique – optogentics – should be mentioned. This field of research is both an interesting and promising approach to basic neuroscience.

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Appendix

Paper One

Wulff S, Pinborg LH, Svarer C, Jensen LT, Nielsen MØ, Allerup P, Bak N, Rasmussen H, Frandsen E, Rostrup E, Glenthøj BY. Striatal $D_{2/3}$ Binding Potential Values in Drug-Naïve First-Episode Schizophrenia Patients Correlate with Treatment Outcome. 2014. In review

Paper Two

Wulff S, Rostrup E, Nielsen MØ, Jensen LT, Pinborg LH, Svarer C, Glenthøj BY. Normalisation of Brain Reward Abnormalities Correlates with Dopamine $D_{2/3}$ Receptor Blockade - a Longitudinal Study of First-Episode Schizophrenia Patients. 2014

Paper One

Striatal $D_{2/3}$ Binding Potential Values in Drug-Naïve First-Episode Schizophrenia Patients Correlate with Treatment Outcome

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Abstract

One of best validated findings in schizophrenia research is the association between blockade of dopamine D_2 receptors and the effects of antipsychotics on positive psychotic symptoms. The aim of the present study was to examine correlations between baseline striatal $D_{2/3}$ receptor binding potential (BP_p) values and treatment outcome in a cohort of antipsychotic-naïve first-episode schizophrenia patients. Additionally, we wished to investigate associations between striatal dopamine $D_{2/3}$ receptor blockade and alterations of negative symptoms as well as functioning and subjective well-being.

Twenty-eight antipsychotic-naïve schizophrenia patients and 26 controls were included in the study. Single photon emission computed tomography (SPECT) with [^{123}I]iodobenzamide ([^{123}I]-IBZM) was used to examine striatal $D_{2/3}$ receptor BP_p. Patients were examined before and after six weeks of treatment with the $D_{2/3}$ receptor antagonist amisulpride.

There was a significant negative correlation between striatal $D_{2/3}$ receptor BP_p at baseline and improvement of positive symptoms in the total group of patients. Comparing patients responding to treatment to non-responders further showed significantly lower baseline BP_p in the responders. At follow-up, the patients demonstrated a negative correlation between the blockade and functioning, whereas no associations between blockade and negative symptoms or subjective well-being were observed.

The results show an association between striatal BP_p of dopamine $D_{2/3}$ receptors in antipsychotic-naïve first-episode patients with schizophrenia and treatment response. Patients with a low BP_p have a better treatment response than patients with a high BP_p. The results further suggest that functioning may decline at high levels of dopamine receptor blockade.

Key words: [^{123}I]iodobenzamide, SPECT, occupancy, amisulpride, subjective well-being

Introduction

Schizophrenia is a complex brain disorder with multifactorial disease mechanisms. In spite of great advances in the understanding of the pathophysiological mechanisms during the last decades,

progress in treatment strategies has been hindered by e.g. the complexity of the illness and the absence of biologically valid diagnostic criteria.

Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) data generally support an increase in striatal dopamine release and synthesis capacity in psychotic schizophrenia patients as well as in patients at ultra-high risk for psychosis.[1-12] In recent studies, this is found more pronounced in the associative striatum.[8-10,12] Antipsychotic compounds suppress dopamine activity via blockade of striatal D_2 receptors, and the correlation between antipsychotic potency of first generation antipsychotics (FGAs) in vitro and blockade of the D_2 receptor is well validated.[13,14] In line with this, in vivo studies have found that 60% to 70% occupancy of striatal D_2 receptors is required to achieve an antipsychotic effect of FGAs.[15-17]

Most studies of unmedicated schizophrenia patients have failed to demonstrate significant differences of striatal D_2 receptor BP in patients compared to healthy controls (HC) (for references and review, see Laruelle 1998).[18] This meta-analysis did show a modest (around 12%) elevation in baseline striatal D_2 receptor BP in patients. In a newer meta-analysis, however, no difference between patients and controls was observed, when analyses included only drug-naïve patients.[19]

In unmedicated patients, no differences in baseline D_2 receptor BP compared to control were apparent.[9,20] However, patients showed a greater change in D_2 BP following pharmacological dopamine depletion than controls, indicating that baseline extracellular dopamine concentrations and D_2 receptor occupancy by dopamine are elevated in schizophrenia.[9,20] The interpretation of PET and SPECT data on D_2 receptor BP in schizophrenia patients is complicated by the fact that increases in endogenous dopamine release and synthesis are likely to decrease D_2 receptor BP both by direct competition with the ligand and via agonist-induced internalisation of the receptors.[21-23] Consequently, the literature tends to support increased dopamine stimulation and decreased availability of striatal D_2 receptors in psychotic patients.

In daily clinical practice, the response of individual patients to antipsychotic treatment differs considerably,[24-26] possibly due to differences in endogenous dopamine activity between patients.[27] In a recent study dopamine synthesis capacity was found to be significantly higher in 12 good responders compared to 12 patients with treatment resistant illness.[11] This may suggest that patients with greater dopamine elevation may be more likely to respond to dopamine antipsychotics. Earlier data from the same group suggest that responders and non-responders

represent separate subtypes that may benefit from different treatment approaches.[28] This is consistent with findings from our group, implying subtypes with either serotonergic or dopaminergic disturbances.[29,30] Moreover, growing evidence has shown that glutamatergic disturbances might play a role in schizophrenia, particularly in treatment-resistant patients.[31,32]

In line with the subdivision of schizophrenia patients based on baseline dopamine activity is the finding that some patients have a poor treatment response despite high striatal D_2 receptor occupancy.[33,34] Other data suggest that lower baseline dopamine activity is associated with an increased risk of developing dysphoria, akathisia and extrapyramidal side effects (EPS).[35] In addition to EPS, dopamine receptor occupancy by antipsychotics has also been associated with (secondary) negative symptoms such as avolition, apathy and affective flattening[36-38] and with subjective experience.[39,40] The Subjective Well-Being under Neuroleptics Scale (SWN)[41] has been used in studies relating subjective experiences with dopaminergic changes. Two studies[42,43] have found a negative association between striatal D_2 receptor occupancy and subjective well-being in patients treated with D_2 antagonists, and de Haan et al.[42] suggest that negative subjective experience might be more sensitive to D_2 receptor occupancy than EPS. Both studies, however, included previously medicated patients.

In general, the literature emphasises the need for predictive markers for treatment response as well as for development of side effects, thereby sparing patients unnecessary treatment trials and adverse effects. In the search for such markers, it is critical to examine the patients before their brains have been modified by antipsychotic medication and repeated relapses – and to follow the patients over time to study the effects of specific interventions.

In the present study, SPECT [^{123}I]-IBZM was used to examine the relationship between baseline $D_{2/3}$ receptor BP_P and treatment outcome in a relatively large cohort of antipsychotic-naïve first-episode schizophrenia patients. Additionally, we related $D_{2/3}$ receptor occupancy following six weeks of monotherapy with the relatively selective $D_{2/3}$ antagonist amisulpride to functioning and subjective well-being. High resolution PET studies have found dopamine activity more pronounced in the functional defined associative striatum.[8,9,44] In order to use an automatically application of regions, we chose the anatomic subdivision of the striatum. With the spatial resolution of our SPECT system (7.4 mm) we assumed similar to Stone et al.'s[45] SPECT study, that part of the

associative striatum was within the caudate,[44] and the caudate was distinct enough to differentiate from the putamen.

We hypothesised: 1) first-episode schizophrenia patients with low striatal dopamine $D_{2/3}$ receptor BP_p in the antipsychotic-naïve state achieve a better treatment response from striatal $D_{2/3}$ receptor blockade than patients with a high BP_p, particularly regarding positive symptoms; and 2) high striatal dopamine $D_{2/3}$ receptor occupancy is associated with deterioration of a patient's functioning and subjective well-being.

Methods

The study was conducted in accordance with the Declaration of Helsinki II and approved by the Danish National Committee on Biomedical Research Ethics (H-D-2008-088). Written informed consent was obtained from all participants.

Participants

Thirty-two first-episode patients, age 18-45 years, meeting the International Classification of Diseases, 10th revision (ICD-10) criteria for schizophrenia were recruited from the Capital Region of Denmark as part of a large, multimodal longitudinal study on antipsychotic-naïve first-episode schizophrenia patients (the Pan European Collaboration Antipsychotic-Naive Studies (PECANS)). There is a partial overlap between the participants in the present study and participants in previously published papers from the PECANS study on different modalities regarding reward processing[46,47] and psychophysiology[48].

A structured diagnostic interview (Schedule of Clinical Assessment in Neuropsychiatry (SCAN), version 2.1) was performed to verify the diagnosis. None of the patients included in the study had ever been treated with antipsychotic medications or methylphenidate. Patients in antidepressant treatment were either excluded or taken out of their medication one month prior to baseline examinations. Other exclusion criteria were serious head traumas, neurological diseases, developmental disorders, pregnancy and drug dependency (according to ICD-10). Twenty-eight HC matched by gender, age and parental socioeconomic status were recruited through advertisement. The exclusion criteria were the same as for the patients, and also included former

or current psychiatric illnesses, drug abuse or psychiatric diagnosis among first-degree relatives. Urine samples were used for drug screening (Rapid Response, Jepsen HealthCare, Tune, Denmark) for all participants prior to the SPECT scanning.

Four patients and two HC were excluded from all analyses. Two patients were excluded due to technical problems with the SPECT image acquisition and one was diagnosed with severe major depressive disorder with psychotic features (DF 32.3) just after baseline examinations. Since conflicting results exist for the association between $D_{2/3}$ receptor availability and cannabis,[49,50] we also excluded one patient due to positive cannabis screenings before the SPECT scans. The excluded HC both received antidepressants at a later six-month follow-up examination. Four patients discontinued the study and an additional three patients were not included at follow-up; see supplementary material. The patients who discontinued the study were not significantly more ill based on the Positive and Negative Syndrome Scale (PANSS). Thus, the complete dataset consisted of 28 patients, 26 HC at baseline and 21 patients at follow-up.

Medication

Treatment with amisulpride was started up in the patients after the baseline examinations. Amisulpride was chosen as the "tool compound" due to its relatively selective $D_{2/3}$ receptor antagonistic effects.[51] The dosage was slowly increased and individually adjusted according to the clinical impression of symptoms and complaints of adverse effects. No medical treatment against adverse effects was allowed. Follow-up examinations were done after six weeks of treatment. No adjustment to the dose was allowed in the last week prior to follow-up examinations.

Clinical measures

In the patient group, psychopathology was assessed with PANSS.[52] Subjective experience of well-being and functioning were assessed with the short form of the SWN (SWN-K)[53] and Global Assessment of Functioning (GAF), respectively. We used both the GAF symptom score (GAF-S) and the GAF functioning score (GAF-F). Adverse effects were rated with the Extrapyramidal Symptom Rating Scale[54] (see supplementary material).

The change in PANSS scores was calculated as a percentage change between scores at follow-up and baseline. Patients responding to treatment were defined as having an improvement of PANSS positive score of more than 30% similar to a previous study.[55]

SPECT acquisition

SPECT data were obtained with a Siemens Symbia™ T2 series SPECT•CT scanner with low energy high-resolution collimators (full width at half-maximum 7.4 mm) and two-slice CT. The ligand, (S)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-3-iodo-6-methoxybenzamide ([^{123}I]-IBZM) was chosen due to its selectivity for striatal $D_{2/3}$ receptors.[56,57] All participants received 185mBq [^{123}I]-IBZM per scanning (GE Healthcare, Eindhoven, Holland). The SPECT scanning was performed using the constant infusion technique.[58,59] A CT scout and 2 x 30 min. tomography were performed. The individually adjusted dose of amisulpride was administered to all patients at same time, three hours prior to the SPECT scanning at follow-up.

Plasma free fraction of [^{123}I]-IBZM was determined using ultrafiltration (Centrifree, 30,000 MW).[60] The plasma metabolite analysis of [^{123}I]-IBZM was performed using Oasis® WCX (Waters, U.S.A.) solid phase extraction units and stepwise elution with water, 40% acetonitrile and acidified 95% methanol. The native compound was eluted in the water phase and the metabolites in the subsequent elution.

All participants had a structural MRI scan performed for co-registration. The HC were only scanned at baseline to reduce the radiation dose.

Note that the supplementary material contains the details of the SPECT and MRI acquisitions.

Image analyses

SPECT images were reconstructed with scatter correction and CT-based attenuation correction using Flash 3D iterative reconstruction (4 subsets, 8 iterations, Gaussian filter 9 mm) on a Siemens syngo® workstation (software version VA60B). The two [^{123}I]-IBZM tomographies were summed and activity measurements were decay-corrected to the time of the radioligand injection. The CT image from the SPECT scanning and the MRI image were co-registered using the Statistical Parametric Mapping (SPM8) method. The result of the SPM co-registration was then carefully inspected in all three planes and, if needed, adjusted manually using a local implementation of an

image overlay method.[61] The information from the co-registration between CT and MRI images was used for co-registration between SPECT and MRI. Inspection and manually adjustments were repeated if needed.

Regions of interest were defined using the high resolution structural MR images and automatically applied to the co-registered SPECT image using a volume-of-interest brain template.[62] We focused on the caudate and chose the cerebellum as the reference region.[63]

Data analysis and statistics

BP_p was used as a measure of the regional dopamine $D_{2/3}$ receptor density available for [^{123}I]-IBZM binding. BP_p refers to the steady-state ratio of specifically bound radioligand to that of total parent radioligand in plasma.[64] The occupancy was calculated as:

$$Occupancy(\%) = \left(1 - \frac{BP_p(treatment)}{BP_P(baseline)}\right) \times 100\%$$

IBM SPSS version 20 statistics was used for the statistical analyses. For the between group comparison, an independent t-test was used when appropriate, and Mann-Whitney U test when there was evidence of non-normal distribution. The repeated measures ANOVA analyses were carried out with an approximate normal distribution and with group and gender used as between-subjects factors when means of the BP_p were compared. Paired t-test was used when baseline measurements were compared with follow-up data. Spearman's correlation coefficient was used in the analyses, though the correlations between BP_p and change in PANSS scores, GAF and SWN-K were analysed using general linear modelling techniques.

Results

Demographic and clinical data

The patient group did not differ significantly by gender, age or handedness from the HC group. The two groups did, though, differ on smoking habits; see Table 1.

Table 1. Demographic data and psychopathology

Baseline		**N**	**Mean**	**SD**	**Range**
Female/male	Schz.p	14/14			
	HC	13/13			
Age (years)	Schz.p	28	23	4.4	18 – 37
	HC	26	23	4.7	18 – 38
Hand-score	Schz.p	28	64	57	-88 - 100
	HC	24	61	62	-100 - 100
SES	Schz.p	28	A 4	B 12	C 8
	HC	25	A 5	B 16	C 4
Tobacco	Schz.p	28	61 % smokers		
	HC	24	25 % smokers		
DUI (weeks)			69	88	2 - 312
GAF-S			40	9.3	25 - 61
GAF-F			42	11.9	30 - 75
SWN-K total		24	67	13.7	41 - 88
PANSS positive			20	3.6	10 - 29
PANSS negative			20	7.7	7 - 38
PANSS general			41	8.4	22 – 56
PANSS total			81	15.3	39 – 102
Follow-up		24			
Female/male		12/12			
GAF-S		23	57*	8.6	37 – 80
GAF-F		23	56*	12.6	32 – 75
SWN-K total			76*	12.7	44 – 99
PANSS positive			14*	3.5	7 – 20
PANSS negative			20	6.1	9 – 33
PANSS general			31*	7.3	18 – 48
PANSS total			65*	13.8	40 – 100
Dose (mg)			238	120	50 – 500
(S)-amisulpride (ng/ml)			392	290	15 – 1013
Diagnosis no.		28			
DF.20.0 Paranoid		19			
DF.20.1 Disorganised		3			
DF.20.3 Undifferentiated		3			
DF.20.9 Unspecified		3			

DUI: duration of untreated illness; GAF-F: Global Assessment of Functioning – functioning score; GAF-S: Global Assessment of Functioning – symptom score; HC: healthy controls; PANSS: positive and negative syndrome scale; Sch.p: schizophrenia patients; SD: standard deviation; SES: Parental socioeconomic status (A:high/B:moderate/C:low); SWN-K: Subjective Well-Being under Neuroleptics Scale, short version; *significant difference from baseline. DUI was calculated from the time a patient experienced a continuous invasive deterioration of functioning due to psychosis-related symptoms [65]

SPECT data, baseline

There were no significant differences in BP_p (total, left or right caudate) between patients and HC at baseline; see Table 2.

Table 2. SPECT data

Baseline		N	Mean	SD	Range
BP_p total caudate	Schz.p	28	2.9	1.1	1.5 - 5.6
	HC	26	2.7	0.7	1.6 - 4.3
BP_p left caudate	Schz.p		2.9	1.1	1.6 - 5.8
	HC		2.9	0.7	1.6 - 4.5
BP_p right caudate	Schz.p		2.9	1.2	1.3 - 5.6
	HC		2.6	0.8	1.5 - 4.3
Follow-up					
BP_p total caudate		22	1.3*	0.7	0.3 - 2.5
BP_p left caudate			1.3*	0.7	0.4 - 3.0
BP_p right caudate			1.2*	0.7	0.3 - 2.6
Occupancy total caudate (%)			52	19	15 - 84
Occupancy left caudate (%)			50	20	7 - 85
Occupancy right caudate (%)			55	21	17 - 83

BP_p: binding potential; HC: healthy controls; Schz.p: schizophrenia patients; SD: standard deviation; *significant difference from baseline

Follow-up data

The difference between baseline and follow-up measures of BP_p, GAF, SWN-K and PANSS in patients was all significant. BP_p decreased. PANSS, GAF and SWN-K scores all improved. The PANSS negative score did not change significantly; see Table 1.

Correlations between BP_p and treatment response

Since clinical follow-up was available for a few patients without follow-up SPECT, 24 patients were included in the analyses. follow-up

Significant positive correlations were found between baseline BP_p of the caudate and the change in PANSS score in the total patient group. Patients with a low BP_p had a better treatment response. The correlations were significant for PANSS positive ($p=0.048$, $r^2=0.166$); PANSS general

(p=0.011, r^2=0.257); and PANSS total (p=0.003, r^2=0.342) but non-significant for PANSS negative scores (p=0.328); see Figure 1. The two patients with a worsening of positive symptoms (green circles in Figure 1) had very low PANSS positive scores at baseline. One was also treated with a low dose of amisulpride (50 mg). Omitting them from the regression strengthened the results (p=0.014, r^2=0.268).

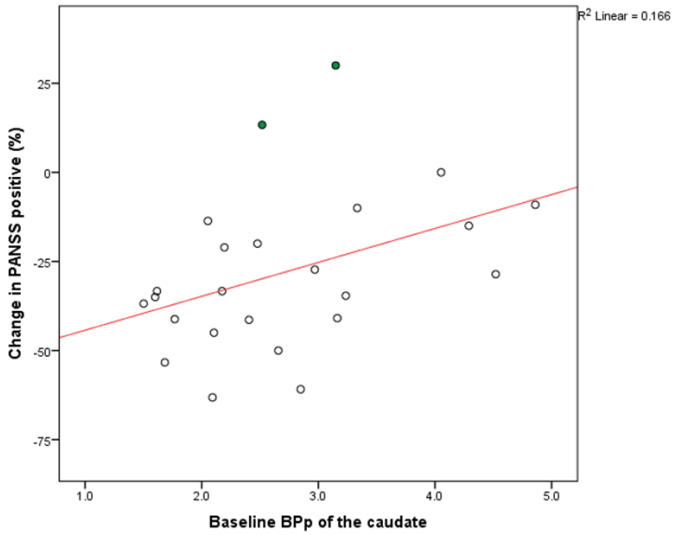

Figure 1. Relationship between BP_p of the caudate and change in PANSS positive score in the patient group

No significant correlations between BP_p at baseline and percentage change in either GAF or SWN-K were found.

We also compared patients responding to treatment (defined as an improvement of PANSS positive >=30%) (n=13, f=5, m=8) to patients not responding (n=11, f=7, m=4). Responders had a significantly lower BP_p (mean BP_p 2.2 vs. 3.3 in the caudate, p=0.003); a lower GAF-F (mean 37 vs. 51, p=0.002); and a lower GAF-S score (mean 37 vs. 46, p=0.028) at baseline. The responders had, per definition, a significantly higher improvement on the PANSS positive, but also on the PANSS general and total score compared to non-responders. Furthermore, responders and non-responders were found to have similar baseline PANSS scores. The responders improved more on the GAF-S compared to non-responders; however, they did not improve more on the GAF-F and

SWN-K. In secondary analyses, we also tested whether the groups or results changed if the rescaled PANSS positive scale ($PANSS_{0-6}$) was used. Responders were then defined as having an improvement on $PANSS_{0-6}$ positive >=50%.[66,67] One male patient moved to the non-responder group; the results were otherwise similar.

Correlations at follow-up

The mean daily dose of amisulpride was 238 (SD 120) mg and was well correlated with the serum level of amisulpride ((S)-amisulpride) ($p<0.001$). We did not find any correlations between caudate BP_p at baseline and the optimal dose needed to obtain clinical effect. The dose of amisulpride and (S)-amisulpride were significantly correlated with the occupancy ($p=0.003$ and $p<0.001$); see supplementary material. There were no significant correlations between the dose (or (S)-amisulpride) and change in PANSS, change in SWN-K or change in GAF. Consistent with these findings we did not find any significant correlations between the occupancy and change in PANSS, SWN-K, or GAF. The occupancy was significantly negatively correlated with GAF-F, however, at follow-up ($p=0.049$; Spearman's rho=-0.446). Since we did not have any hypothesis on the specific GAF-F score, this finding is more explorative, but may suggest that the more the dopamine receptors were blocked, the worse the functioning; see Figure 2.

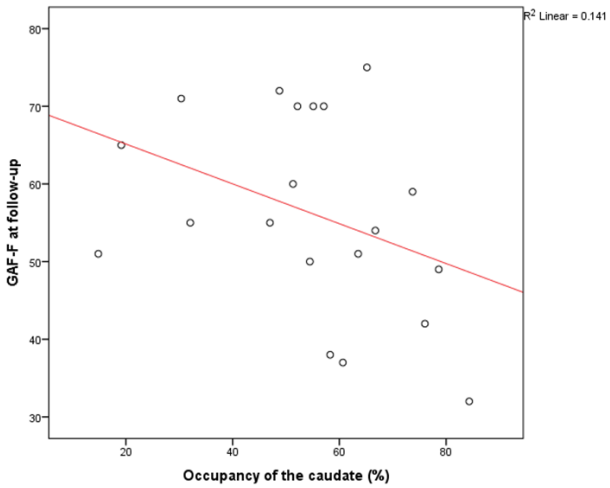

Figure 2. Correlation between occupancy and functioning by GAF-F. A first-order linear approximation is included in the figure solely to illustrate the sign of correlation

Supplementary analyses

There was no significant correlation between BP_p and PANSS, SWN-K, GAF scores and DUI at baseline.

Discussion

As expected, we found a significant negative correlation between the BP_p of the caudate at baseline and clinical improvement of positive symptoms in 24 antipsychotic-naïve first-episode schizophrenia patients after six weeks of treatment with the relatively selective $D_{2/3}$ antagonist amisulpride. Additionally, the fact that the patients with a good response had significantly lower baseline $D_{2/3}$ BP_p compared to non-responders, even though the groups' PANSS baseline scores did not differ, supports baseline $D_{2/3}$ BP_p as a potential marker for treatment outcome. The latter finding mentioned underlines the inability to differentiate good and poor responders based on baseline PANSS scores.

Our findings of a lower baseline BP_p, interpreted as higher synaptic dopamine, that correlates with better six weeks medication response on positive symptoms are consistent with the previously mentioned study by Abi-Dargham et al,[20] where an association between elevated synaptic dopamine and greater improvement of positive symptoms was demonstrated in 14 unmedicated patients after treatment with different antipsychotics. The present data are also in line with Demjaha et al.'s[11] study that shows a higher dopamine synthesis capacity in 12 responders versus 12 non-responders to antipsychotic treatment. Together these studies indicate a hyperdopaminergic subtype in schizophrenia.

We chose to define responders based on an improvement on the PANSS positive score of more than 30%. This was in accordance with a study of Meisenzahl et al.[55] However, we obtained a similar division in our patients – and the same results – if we defined the responders with a 50% improvement on the $PANSS_{0\text{-}6}$.[66,67] The responders improved significantly more on both the PANSS positive, total and general scales, although neither responders nor non-responders had any improvement of the negative symptoms. Not surprisingly, the responders also improved significantly more on GAF-S than non-responders, although no significant differences were found regarding the change in GAF-F and SWN-K. These findings suggest that even if patients experience a relief of positive symptoms, their level of functioning and well-being might not improve. A longer

follow-up period would of course be necessary to make firm conclusions regarding final remission and recovery, but the present data emphasise the importance of considering improvement of positive symptoms versus adverse effects, particularly secondary negative symptoms from antipsychotic treatment.

Since the patients were all drug naïve a relatively low mean dose of amisulpride (238 mg) was used in the present study. Dose and (S)-amisulpride were, as expected, well correlated with striatal occupancy. We observed a maximum occupancy of 80%. Even if the individually adjusted dose of amisulpride was administered to all patients at same time, we did not find any associations between the striatal occupancy and improvement on the PANSS, SWN-K or GAF. This is consistent with another [^{123}I]-IBZM SPECT study of amisulpride.[55]

Significant correlations between striatal occupancy and outcome have, however, been supported by other PET and SPECT studies[16,17,68-71] correlating striatal occupancy with improvement on the Brief Psychiatric Rating Scale, the PANSS positive and the Clinical Global Impression scale. Possible explanations for the conflicting results are the different compounds and methodology used in the studies, small sample sizes, inclusion of a mix of schizophrenia patients and patients with schizophreniform disorder, and partly previously medicated patients. Moreover, these studies included patients with motor adverse effects and striatal occupancies above threshold, i.e. the studies had a broader range in occupancy. Moreover, in several of the studies occupancy was calculated from baseline BP obtained from different subjects or from a subgroup of the patients. In the present study, BP_p was measured at baseline in the antipsychotic-naïve state and at follow-up, which due to inter-individual differences gives a more precise estimate. Furthermore, our sample size was rather large, homogeneous and included only first-episode schizophrenia patients.

The present data did not replicate the previously mentioned findings of a negative correlation between the striatal occupancy and SWN-K at follow-up. We did, however, find a negative correlation between striatal occupancy and functioning on the GAF-F score at follow-up. This could suggest that the more the dopamine receptors are blocked, the worse the functioning, which support the precaution we need to be aware of in treating patients.

Limitations and strengths of the data

Given that the antipsychotic-naïve patients had to go through an extensive examination programme we risk ending up with only the mildest cases. Even so, the patients included were moderately to severely ill with a total PANSS score of 81. Patients and controls in our study were not matched for smoking. The relationship between tobacco and enhancement of dopamine transmission has been reported, however changes in human studies are modest (5-10%) and comparable to test-retest variability.[72] A recent PET study did not find any significant effect of moderate smoking synthesis capacity in the striatum.[73] Moreover, schizophrenia patients have a higher use of tobacco than HC, which is why matching for smoking would have added bias to either the patient or the HC group. Also, our primary outcomes were analyses within the patient group and these analyses were not affected by the lack of matching for smoking.

A few patients received a small amount of benzodiazepines during hospitalisation, but none did so regularly and benzodiazepines were not allowed 12 hours prior to any of the examinations. Three patients, though, received benzodiazepine the evening prior to the baseline SPECT scan, and two of them had positive urine tests prior to the scan. Another limitation in the study was the spatial resolution of the SPECT images. Chosing an anatomic subdivision of striatum precludes identification of the total associative striatum. We focused on the caudate rather than the entire striatum since the majority of the associative striatum is represented in the caudate, and we reasoned that the spatial resolution was sufficient to make this simpler subdivision (see introduction).

The strengths of the study are: inclusion of solely antipsychotic-naïve schizophrenia patients never previously exposed to antipsychotic compounds, the relatively large sample size, the longitudinal study design and the fact that the patients all went through a standardised treatment using antipsychotic monotherapy with a relatively selective $D_{2/3}$ receptor antagonist (amisulpride).

Conlusion

The present data demonstrate a significant correlation between caudate $D_{2/3}$ BP_p and treatment response in a relatively large group of antipsychotic-naïve schizophrenia patients where low BP_p associates with a better treatment response.

Psychopathology at baseline did not predict treatment response and no associations between striatal $D_{2/3}$ occupancy and clinical improvement were found; on the contrary, the results suggest that the more the dopamine receptors were blocked, the worse the patient's functioning became as measured with GAF.

The findings support the suggestions of subtypes in schizophrenia that might respond differently to D_2 receptor blockade, possibly due to different neurochemical profiles. Finding possible prediction markers of treatment response would spare patients ineffective treatment and unnessecary adverse effects.

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Paper One - Supplementary Material

Discontinuation of the study

Seven out of the remaining 28 patients were not included at follow-up. Four patients discontinued the study before the follow-up examinations at six weeks, three of who were given olanzapine due to clinical worsening or lack of improvement during hospitalisation in the follow-up period and one patient did not wish to participate in the follow-up examinations. One patient had a panic attack in the scanner at follow-up and the examination was stopped. For one patient, we did not receive blood analyses at the follow-up scan and another patient was not PANSS rated at follow-up.

Medication and adverse effects

The dose of amisulpride was individually adjusted according to the clinical impression of symptoms and complaints of adverse effects. At baseline, none of the patients scored on the clinical global impression of EPS. At follow-up, two patients scored very mild on the clinical global impression of parkinsonism due to definite slowness in movements.

SPECT acquisition

SPECT acquisition was performed with the following parameters: step and shoot acquisition mode with contour; matrix, 128x128; angular range, 180; 64 views; 26 seconds per step. CT images were acquired with 5 mm slices at 130 keV using Siemens CARE Dose 4D dose optimisation.

One half of the 185mBq [^{123}I]-IBZM was given as bolus, and the other half as a constant infusion over 240 min. Prior to the [^{123}I]-IBZM bolus administration, 200 mg perchlorate mixture i.v. was given to block thyroid uptake of free radioactive iodide. After 180 min. of rest a CT scout and 2 x 30 min. tomography were performed. Blood samples were collected prior to the bolus and every 30 min. during the scanning period.

No adjustment to the dose of amisulpride was allowed in the last week prior to follow-up examinations to ensure amisulpride steady-state conditions in the brain and blood. In addition, all patients received their individual dose of amisulpride along with the [^{123}I]-IBZM bolus injection to

reduce the effect of individual differences in timing of amisulpride administration on the day of the SPECT examinations. (S)-amisulpride was measured prior and 60, 120, 150, 180, 210, 240 min. after receiving the dose, and the mean value during the one hour scanning period was used in the analyses.

MRI acquisition

All participants had a structural MRI scanning performed with Philips Achieva 3.0 T whole-body MRI scanner (Philips Healthcare, Best, The Netherlands) with an eight channel SENSE Head Coil. Whole-brain 3-dimensional high-resolution T1-weighted structural images were acquired for anatomical reference (repetition time=10 milliseconds, echo time=4.6 milliseconds, flip angle=8°, and voxel size=0.79x0.79x0.80 mm).

The concentration-occupancy relationship

The optimal dose of amisulpride for the individual patient (as established by the treating physician) resulted in highly varying occupancy between patients (mean 52% (SD 19.1)). This wide range of striatal occupancy is consistent with earlier [^{123}I]-IBZM SPECT studies of amisulpride.[1,2]

The dose-occupancy curve can be described as a curvilinear function.[3,4] Fitting a Michaelis-Menten curve to the present occupancy data, the plasma concentration predicted to provide 50% occupancy of the maximum attainable receptor occupancy (EC_{50}) is 265 ng/ml (see Figure 1SM). With maximum attainable receptor occupancy of 100 % (E_{max}) and C_{ami} as the concentration of (S)-amisulpride, the equation is: $\text{Occupancy}\ (\%) = \frac{\text{Emax} \times \text{Cami}}{\text{Cami} + 264.75}$

In general lower values of EC_{50} have been reported in previous studies compared to the present study. E.g. in the study by Meisenzahl et al. the reported EC_{50} is (30ng/ml), but in this case E_{max} was treated as a free parameter, and fitted to 87%. The methodologies used are in general inconsistent, and the occupancy often calculated from baseline BP_p from another group of patients or HC. In the present study (S)-amisulpride was measured near t_{max} of amisulpride, we fitted the curve to an E_{max} of 100%, and the occupancy was calculated from baseline and follow-up BP_p of the same patients.

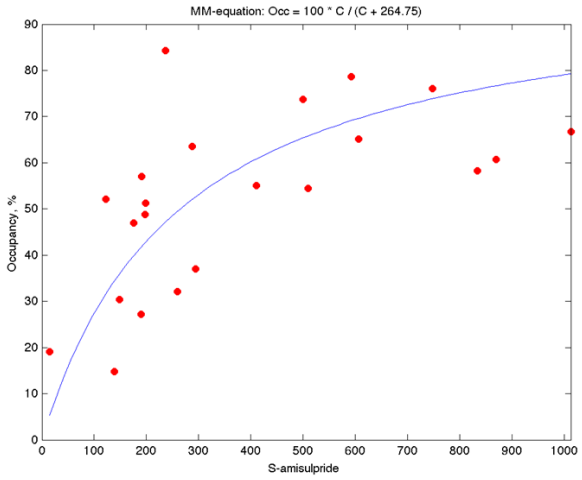

Figure 1SM. Concentration-occupancy relationship illustrated by serum (S)-amisulpride and the occupancy of caudate

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Paper Two

Normalisation of Brain Reward Abnormalities Correlate with Dopamine $D_{2/3}$ Receptor Blockade – A Longitudinal Study of First-Episode Schizophrenia Patients

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Abstract

Background

Attenuated striatal blood oxygen level dependent (BOLD) response during reward anticipation has been a consistent finding in unmedicated patients with schizophrenia. We have previously demonstrated a partial normalisation of these disturbances following treatment with the relatively selective $D_{2/3}$ receptor antagonist amisulpride. However, a direct link between striatal $D_{2/3}$ blockade and reward processing abnormalities has not been demonstrated. In the present study, we examined the association between striatal $D_{2/3}$ receptor blockade, alterations in reward processing and psychopathology in a longitudinal study of antipsychotic-naïve first-episode schizophrenia patients.

Method

Twenty-eight antipsychotic-naïve first-episode schizophrenia patients and 26 matched healthy controls were examined with Single Photon Emission Computed Tomography using ^{123}I-labeled-iodobenzamide. Reward disturbances were measured with functional magnetic resonance imaging using a variant of the monetary-incentive-delay task. Patients were assessed before and after six weeks of treatment with flexible doses of amisulpride.

Results

In line with our previous data, we found an attenuated striatal BOLD response in the patients at baseline that was no longer significant at follow-up. This normalisation of the BOLD response correlated positively with the improvement of positive symptoms, and in patients who responded to dopamine blockade, the correlation between occupancy and change in BOLD response was found to be significant. In accordance with this, $D_{2/3}$ receptor occupancy was associated with the BOLD response at follow-up.

Conclusion

To our knowledge, the present data are the first to demonstrate a direct influence of striatal $D_{2/3}$ receptor blockade on striatal brain activity during reward anticipation in a longitudinal study on antipsychotic-naïve first-episode schizophrenia patients.

Key words: [^{123}I] -IBZM, SPECT, fMRI, amisulpride, occupancy

Introduction

An attenuated blood oxygen level dependent (BOLD) response in the ventral striatum (VS) during reward anticipation has been a consistent finding in functional magnetic resonance imaging (fMRI) studies of ultra-high risk (UHR) (1;2), antipsychotic-naïve (3-5), and unmedicated schizophrenia patients (6-8). Preclinical data have implicated a distinct role for subcortical dopamine systems in incentive salience and reward prediction (9;10). Consistent with these data, it has been suggested that subcortical dopamine disturbances lead to aberrant assignment of salience, causing delusions in schizophrenia (the salience hypothesis) (11;12). In line with this, we have previously demonstrated a partial normalisation of brain reward abnormalities following treatment of antipsychotic-naïve schizophrenia patients with the relatively selective dopamine $D_{2/3}$ receptor antagonist amisulpride (13). The increase in the BOLD response was especially observed in patients with a clear treatment effect on positive psychotic symptoms (4). The BOLD response is, however, an indirect measure of dopaminergic neural activity.

To our knowledge, no previous studies have directly examined the influence of striatal dopamine $D_{2/3}$ receptor blockade on the striatal BOLD response during reward anticipation in schizophrenia patients and direct proof of the role of dopamine in the aberrant salience found in schizophrenia is still lacking. Roiser et al. (2013) found no correlation between presynaptic dopamine activity using [^{18}F]fluorodopa positron emission tomography (PET) and aberrant salience in UHR patients. Findings in studies on healthy volunteers have been inconsistent, but over all they indicate that dopamine plays a role in the reward-related BOLD response using the Monetary Incentive Delay (MID) task (14-17). The inconsistencies are perhaps caused by methodological differences.

In the present study, we examined the association between striatal dopamine $D_{2/3}$ receptor blockade, the striatal BOLD response during reward anticipation, and the psychopathology of schizophrenia patients who had never been exposed to antipsychotic medication at baseline examinations. These initially antipsychotic-naïve first-episode patients were examined with single photon emission computed tomography (SPECT) [^{123}I]-IBZM and a variant of the MID task during fMRI before and after six weeks of monotherapy with amisulpride. The secondary analyses explored the relationship between baseline dopamine $D_{2/3}$ receptor binding potential (BP_p) values, fMRI changes during salience assignment, and the psychopathology.

Based on the literature and previous data from our group, our two hypotheses were: 1) Blockade of striatal dopamine $D_{2/3}$ receptors improves abnormalities during reward anticipation; i.e. we expect to find a positive correlation between $D_{2/3}$ receptor occupancy and an increase in the striatal BOLD response during reward anticipation between baseline and follow-up. Correspondingly, we also expect to see a positive association between $D_{2/3}$ receptor blockade and the striatal bold response at follow-up; and 2) Normalisation of the BOLD response during salience assignment correlates with the improvement of positive symptoms.

Methods

The study, conducted in accordance with the Declaration of Helsinki II, was approved by the Danish National Committee on Biomedical Research Ethics (H-D-2008-088). Written informed consent was obtained from all participants.

Participants

Patients were recruited from in- and outpatient psychiatric centres in the Capital Region of Denmark as part of the Pan European Collaboration on Antipsychotic Naive Schizophrenia (PECANS) project, a large multimodal longitudinal study on antipsychotic-naïve first-episode schizophrenia patients. The participants in the present study were presented in a previous study on the association between striatal dopamine $D_{2/3}$ receptor BP_p and treatment outcome in schizophrenia that included detailed information on baseline BP_p and detailed clinical information (Wulff et al. Paper One). There is also a partial overlap between the participants in the present study and participants in previous publications from a PECANS study on psychophysiology (18) and reward processing (3;4). The overlap between the present study and the two studies just mentioned on reward processing comprised, respectively, nine patients and six controls, and eight patients and six controls. Structural and other fMRI data, data on cognitive disturbances, genetic data, and data on the hypothalamic-pituitary-adrenal axis and oxidative stress related to the PECANS cohort will also be published elsewhere.

Thirty-two antipsychotic-naïve patients, age 18-45 years, meeting the International Classification of Diseases, 10^{th} revision (ICD-10) criteria for schizophrenia were included. A structured diagnostic

interview (Schedule of Clinical Assessment in Neuropsychiatry (SCAN), version 2.1) was performed to verify the diagnosis. None of the patients had ever been treated with antipsychotic medications or methylphenidate at inclusion. Patients receiving antidepressants were either excluded or taken out of their medication one month prior to baseline examinations. Other exclusion criteria were: severe head traumas, neurological diseases, developmental disorders, pregnancy and current drug dependency (according to ICD-10).

Twenty-eight healthy controls (HC) matched by gender, age, and parental socioeconomic status were recruited through advertisement. Exclusion criteria were the same as for patients, but additionally included former or current psychiatric illnesses, drug abuse, or psychiatric diagnosis among first-degree relatives.

All participants were asked about present substance use and urine samples were obtained for drug screening (Rapid Response; Jepsen HealthCare, Tune, Denmark). Females were also given a urine pregnancy test (serum HCG) prior to magnetic resonance imaging (MRI) and SPECT scans.

Four patients and two HC were excluded completely from all analyses, four patients discontinued the study and an additional three patients were not included at follow-up. Thus, the complete data set consisted of 28 patients and 26 HC at baseline, and 21 patients and 23 HC at follow-up. The supplementary material describes the reasons for exclusion in further detail.

Study design and psychopathology

At baseline all participants underwent MRI, fMRI and SPECT scans. In the patient group, psychopathology was assessed with the Positive and Negative Syndrome Scale (PANSS) (19) within the same week as the fMRI and SPECT scans. Follow-up examinations were performed after six weeks of treatment. The HC did not receive medication and only had one SPECT scan performed to minimise the radiation dose.

Medication

Amisulpride was chosen due to its relatively selective binding to and high affinity for dopamine D_2 and D_3 receptors (20). Treatment was initiated after the baseline examinations and the dosage was slowly increased and individually adjusted. Medical treatment against side effects was not permitted. In order to ensure amisulpride steady-state conditions in the brain and blood at follow-

up examinations, the dose of amisulpride was not allowed to be adjusted in the last week prior to the examinations.

SPECT acquisition

The ligand (S)-*N*-[(1-ethyl-2-pyrrolidinyl)]-methyl-2-hydroxy-3-iodo-6-methoxybenzamide ([^{123}I]-IBZM) was chosen due to its selectivity for striatal $D_{2/3}$ receptors (21;22). The participants received 185mBq of [^{123}I]-IBZM (GE Healthcare, Eindhoven, Holland), with half of the dose given as bolus injection and the other half given as a constant infusion during the entire 240-minute session (23). In addition, all patients received their individual dose of amisulpride along with the [^{123}I]-IBZM bolus injection to reduce the effect of individual differences in timing of amisulpride administration on the day of the SPECT scans. The mean value of (S)-amisulpride during the one-hour scanning period was used in the analyses. Further details concerning the SPECT procedure and image analyses can be found in our previous study (Wulff et al. Paper One) and supplementary material.

MRI and fMRI acquisition

All participants had a structural MRI scan performed with Philips Achieva 3.0 T whole-body MRI scanner (Philips Healthcare; Best, The Netherlands) with an eight channel SENSE Head Coil. Whole-brain 3-dimensional high-resolution T1-weighted structural images were acquired for anatomical reference (repetition time=10 milliseconds, echo time=4.6 milliseconds, flip angle 8°, and voxel size=0.79x0.79x0.80 mm). For the fMRI, 1080 (540/run) whole-brain functional echo-planar images were acquired (repetition time=2 seconds, echo time=25 milliseconds, flip angle 75°, 38 slices, thickness=2.4 mm and voxel size=2.4x2.9x2.9 mm).

The MRI and fMRI were obtained prior to the SPECT scanning on a separate day. The mean interval was 4.4 days at baseline and 5.8 days at follow-up in the patient group, and 7.8 days in the group of HC.

The reward paradigm

The reward paradigm has previously been described in detail in Nielsen et al. 2012 (13;24). In brief, a variant of the MID task described by Knutson (25-27) and modified by Cooper and Knutson (28) was used to elicit VS activation in response to cues indicating monetary gain and loss.

The task consisted of two runs and the total time was 36 min (18 min/run). Participants were presented initially with a cue indicating the conditions of the trial. After a short delay, a visual target appeared briefly on the screen and participants were instructed to press a button while the target was on screen. After another delay, participants received feedback on the monetary outcome.

Preliminary analyses showed a higher response in the first run compared to the second one as well as a larger group difference, possibly due to adaptation effects. As a result, the present study only considers data from the first run.

In the present study, the chosen BOLD contrast was, similar to our previous study (4), the overall effect of salience during reward anticipation. This was estimated by calculating the difference between uncertain or salient cues (the response to conditions in which outcome was dependent on subject performance) vs. the control or neutral condition (where outcome is zero regardless of performance). Subanalyses in our previous studies (13;24) showed that the attenuation of this BOLD contrast in the patients was caused by a lower BOLD response to salient cues in the patient group compared to HC. There was no significant difference of the BOLD response to the neutral cues between groups. The term BOLD response in the present study refers to the difference between salient and neutral cues. The paradigm timing was automatically adjusted to obtain an average hit rate of 66%, depending on individual subject performance.

Regions of interest (ROI)

The caudate was chosen as our ROI. Compared to our previous studies on reward, where we looked at the VS, the somewhat larger ROI was chosen due to the SPECT system's limited resolution. The salience disturbances are believed to be more pronounced in the ventral part (limbic striatum) as compared to the rest of the striatum (26), whereas high-resolution PET studies have found dopamine hyperactivity to be predominately localised in the associative striatum in schizophrenia patients and in patients in the prodromal state (29;30). We wanted an anatomic

defined region, due to the automatically applied brain template (see below). Since our goal was to relate fMRI and SPECT data, the caudate was chosen as the ROI. Similar to another SPECT study (31), we assumed that the caudate comprises the main part of the associative and limbic striatum (32); see Figure 1. The ROI was automatically applied to the co-registered SPECT image using a volume-of-interest brain template (33). The same ROI was defined in Montreal Neurological Institute (MNI) space and applied to the fMRI analyses. The cerebellum was chosen as the reference region in the SPECT data analysis (34).

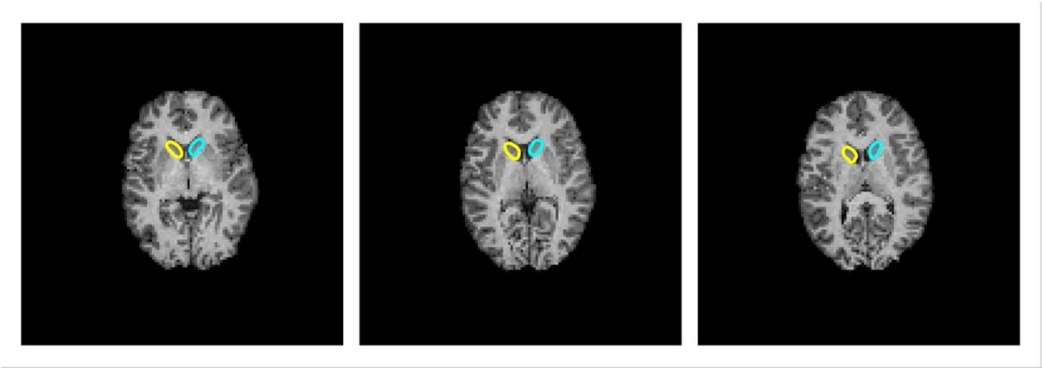


Figure 1. Region of interest: the caudate

Statistical analyses

IBM SPSS statistics 20 was used for the statistical analyses. Binding potential and BOLD activity were tested for normality using the Shapiro-Wilk test. Repeated measures ANOVA analyses with group and gender as between-subjects factors were used for the comparison of the means of BP_p and BOLD activity. For the between group comparison, an independent t-test was used when appropriate, and Mann-Whitney U test when there was evidence of a non-normal distribution. A paired t-test was used when baseline measurements were compared with follow-up data. For correlations, Spearman's correlation coefficient was used. The change in the PANSS score at follow-up was calculated as a percentage delta score from baseline to follow-up.

Results

Detailed information on the demographics, dopamine $D_{2/3}$ receptor BP_p, and psychopathology are available in Wulff et al. Paper One. Table 1, below, provides a clear summary of the data for the matter of greater legibility.

There was no significant difference between patients and controls regarding gender, age, and handedness. Nor was there any difference in BP_p between the groups at baseline. The mean dose of amisulpride was 238 mg/day (range 50-500 mg/day). After the six weeks of treatment, the patients improved significantly regarding symptoms; however, the negative symptom score was unchanged.

	Schizophrenia Patients		**HC**
	Baseline	Follow-up	Baseline
N (female/male)	14/14	13/11	13/13
Age (years)	23 (4.4)		23 (4.7)
BP_p total caudate	2.9 (1.1)	1.3 (0.7)	2.7 (0.7)
BP_p left caudate	2.9 (1.1)	1.3 (0.7)	2.9 (0.7)
BP_p right caudate	2.9 (1.2)	1.2 (0.7)	2.6 (0.8)
PANSS positive	20 (3.6)	14 (3.5)	
PANSS negative	20 (7.7)	20 (6.1)	
PANSS general	41 (8.4)	31 (7.3)	
PANSS total	81 (15.3)	65 (13.8)	

Table 1. Demographics, BP_p and psychopathology
Data are specified as mean, and standard deviation in parentheses. BP_p: binding potential; HC: healthy controls; PANSS: Positive and Negative Syndrome Scale

BOLD response at baseline

Patients showed a significantly lower BOLD response than HC in the caudate. The BOLD response was normally distributed in the HC group only and there was a significant difference in the variance of the BOLD response between the two groups; see Table 2.

Tested with repeated measures ANOVA (approximation of normal distribution), there was no significant difference between left and right side. Handedness was found to be non-significant as a covariate and there was no effect of gender, group, or group*gender interaction.

		N (f/m)	Mean	SD	Range
Baseline					
BOLD response total	Schz.P.	14/14	0.287	0.679	-0.672 – 2.616
	HC	13/13	0.664	0.586	-0.423 – 2.173
BOLD response left caudate	Schz.P.		0.334	0.700	-0.853 – 3.625
	HC		0.725	0.675	-0.871 – 2.616
BOLD response caudate	Schz.P		0.239	0.567	-0.919 – 1.606
	HC		0.603	0.610	-1.002 – 1.729
Follow-up					
BOLD response total	Schz.P	13/11	0.453*	0.597	-0.842 – 1.543
	HC	11/12	0.643	0.713	-0.450 – 2.229
BOLD response left caudate	Schz.P		0.460*	0.700	-1.019 – 1.897
	HC		0.679	0.833	-0.827 – 2.433
BOLD response right caudate	Schz.P		0.446*	0.567	-0.665 – 1.585
	HC		0.607	0.686	-0.568 – 2.262
Change BOLD response total caudate	Schz.P	13/11	0.207	0.558	-0.783 – 1.260
	HC	11/12	-0.046	0.719	-1.481 – 1.663
Change BOLD response left caudate	Schz.P		0.201	0.637	-0.893 – 1.442
	HC		-0.090	0.897	-1.971 – 1.524
Change BOLD response right caudate	Schz.P		0.213	0.541	-0.673 – 1.331
	HC		-0.001	0.778	-1.509 – 1.802

Table 2. fMRI data
f/m: females/males; BOLD: blood oxygen level dependent; HC: healthy controls; SD: standard deviation; *significantly different from baseline

BOLD response at follow-up

At follow-up, there were no longer any significant differences in the BOLD response in the caudate nucleus between the groups. The BOLD response was now normally distributed in both the HC group and patient group.

The patients had a trend-wise increase in the BOLD response between baseline and follow-up (p=0.082) compared to HC, who showed a non-significant decrease (p=0.764); see Table 2.

Correlations at baseline (secondary analyses)

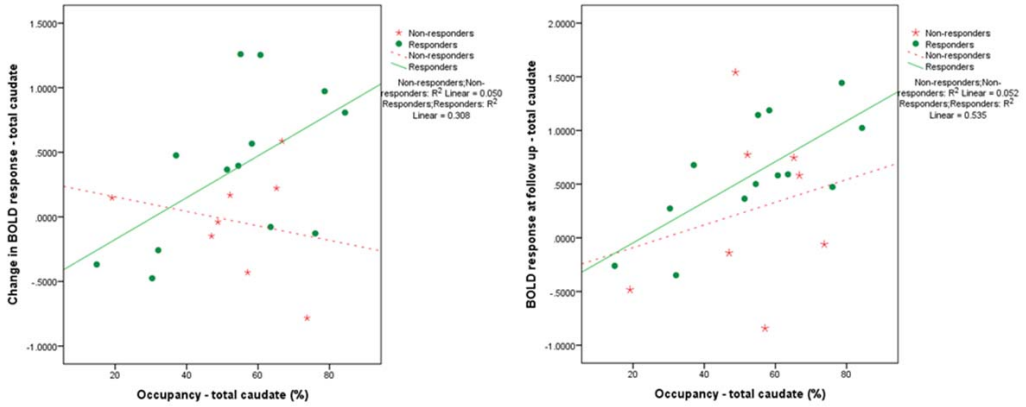
There were no significant correlations between the BOLD response and the psychopathology expressed as PANSS. In addition, no correlations between BP_p and the BOLD response in the patient group or the HC group were found when analysed by group or together.

Correlations at follow-up

There was no significant correlation between the occupancy and the change in BOLD response in the total group of patients (n=22; p=0.138; rho=0.327). However, looking at the separate groups of responders and non-responders, we found a significant correlation between occupancy and change (normalisation) in the BOLD response from baseline to follow-up in the patients responding to $D_{2/3}$ receptor blockade (n=13; p=0.035; rho=0.588), whereas no correlations were found in the non-responders (n=8; p=0.955); see Figure 2(A). Responders were defined as having an improvement in positive symptoms above 30%; see Wulff et al. Paper One.

Also in accordance with this, we found a significant positive correlation between occupancy and the BOLD response at follow-up in the whole group of patients (n=22; p=0.026; rho=0.475). In the group of responders, the correlation was significant (n=13; p=0.011; rho=0.676), which was not the case in the group of non-responders (n=8; 0=0.736); see Figure 2(B).

Furthermore, there was a significant correlation between the change in the BOLD response in the whole group of patients and (S)-amisulpride (n=23; p=0.044; rho=0.424), as well as between the BOLD response at follow-up and (S)-amisulpride (n=23; p=0.030; rho=0.453). Analysed separately, these correlations were only significant in the group of responders and not in the group of non-responders; see Figure 2SM in the supplementary material. No correlations were seen with the dose of amisulpride.



(A) (B)

Figure 2. (A) Correlation between the occupancy and change in BOLD response from baseline to follow-up. (B) Correlation between the occupancy and BOLD response at follow-up. The correlations were significant in the group of responders (green) and non-significant in the group of non-responders (red) in both the figures. Note: one patient was excluded in the division due to the lack of a PANSS rating at follow-up, which is why n=21 instead of n=22.

There was a nearly significant positive correlation between the change in the BOLD response and the change in the PANSS positive score (n=23; p=0.050; rho= -0.413); see Figure 3.

Excluding two possible outliers* this correlation became highly significant (n=21; p=0.008, rho=-0.562). Thus, the more the BOLD response increased, the more the positive symptoms tended to improve.

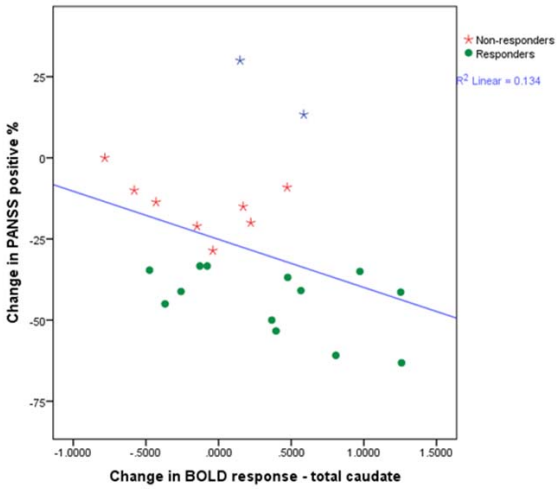


Figure 3. Correlation between the change in BOLD response and change in the PANSS positive score. The correlation is shown for the whole group of patients. Non-responders: red; responders: green; possible outliers: blue

*The two possible outliers marked in blue in Figure 3 both had a low PANSS positive score at baseline and a worsening of positive symptoms. The outermost of these two received a very low dose of amisulpride and as an exception, the fMRI was performed 23 days after the PANSS rating and SPECT scan.

Discussion

To our knowledge, this is the first study of its kind to demonstrate a positive correlation between occupancy of dopamine $D_{2/3}$ receptors and a normalisation of the BOLD response during the anticipation phase of reward processing in initially antipsychotic-naïve first-episode schizophrenia patients responding to treatment with a dopamine $D_{2/3}$ receptor antagonist.

Consistent with this, and with our main hypothesis, we also found a significant correlation between $D_{2/3}$ receptor blockade and the striatal BOLD response at follow-up in the whole group of patients, as well as a significant correlation between (S)-amisulpride and the change in the BOLD response between baseline and follow-up. Furthermore, we observed a correlation between the normalisation of the BOLD response and improvement of the positive symptoms, which is in

agreement with our previous results (4). Thus, these findings underline the importance of striatal dopamine activity for the aberrant incentive salience found in schizophrenia patients.

The study further supports the hypothesis that subgroups of patients exist due to different neurochemical profiles. A heterogeneous treatment response in schizophrenia is well known (35) and the existence of possible subgroups of patients with or without striatal hyperdopaminergic activity, reflected in good and poor treatment response to dopamine blockade, has previously been proposed (36-38). The non-normalised distribution of the BOLD response in the patient group at baseline might reflect this heterogeneity, but particularly the finding of a significant association between the occupancy and the change in the BOLD response in only the group of responders reflects the heterogeneity. It seems likely that non-responders could belong to a subgroup of patients without hyperdopaminergic activity, since they either show a small worsening or no change in the BOLD response despite a high occupancy, as illustrated in Figure 2A.

The heterogeneity of the disease combined with the relatively small size of the cohort could also be the primary explanation for the lack of a significant relationship between the occupancy and the change in the BOLD response between baseline and follow-up examinations in the total group of patients (responders as well as non-responders).

In spite of the change of ROI, as compared with our earlier studies (3;4), the present data confirmed an attenuated activation of the BOLD response during the anticipation phase in antipsychotic-naïve schizophrenia patients compared to HC before antipsychotic treatment. The findings are also consistent with the literature (1;2;5-8), thus, confirming reward disturbances as core deficits in schizophrenia. Furthermore, we found that the alterations normalised after six weeks of treatment with amisulpride, likewise confirming our previous data.

Regarding the association between the BOLD response and psychopathology at baseline, we were unable to confirm in this sample of patients and with the present ROI a (negative) correlation between positive symptoms and the BOLD response at baseline, which has been reported earlier by ourselves and others in VS (3;5). The patient groups in the previous studies had a similar PANSS positive score, but changing the ROI to include the total caudate could very well have attenuated possible correlations between psychopathology and reward abnormalities (see Limitations and Strengths). Moreover, the previous studies included a majority of male patients and gender

differences combined with the previously discussed complexity of schizophrenia may also have weakened the baseline relationships in the present study. When the females and males were analysed separately, however, the correlations were still non-significant, but the sample sizes were small.

Even if our data indicate that dopamine plays a key role in reward anticipation in schizophrenia, it should be taken into consideration that other neurotransmitter systems, e.g. the serotonergic, GABAergic and/or glutamatergic systems, could also be involved in the observed reward processing disturbances (39). A recent study reporting a similar attenuated BOLD response in the anticipation phase in not only schizophrenia but also alcohol dependence and major depressive disorders indirectly supports the involvement of more than one neurotransmitter system in reward processing (40).

Limitations and Strengths

One of the particular strengths of this study is the inclusion of initially antipsychotic-naïve first-episode schizophrenia patients, which means that the brain has not been modified by antipsychotics at baseline examinations. In addition, the longitudinal study design, the monotherapy with a relatively selective $D_{2/3}$ receptor antagonist, and the combination of several modalities are all also significant strengths of this study. The examination programme was extensive (the patients had to stay unmedicated for a mean of five to seven days), which could lead to selection bias, where more severely ill patients were excluded from the study. If severely ill patients had been included, it would probably have strengthened the correlations as opposed to weakening them.

One of the weaknesses is that choosing the entire caudate as the ROI might have diminished the fMRI signal and thereby our findings. In addition, the patients and controls in our study were not matched for smoking. Nicotine use has been found to affect the BOLD response in the reward anticipation phase (41). Previous studies have reported a relationship between chronic nicotine intake and enhancement of dopamine transmission (42). These changes, however, are modest and in the order of test-retest variability (43). In agreement with this, a recent PET study did not find any significant relationships in the striatum (44). In addition, we were primarily interested in within-group findings and assumed that participants did not change their smoking habits during

the examination period, which is why not controlling for nicotine intake is not likely to have had a significant impact on our findings. Furthermore, since schizophrenia patients have a higher use of tobacco than HC, matching for smoking would have added a bias to the HC group.

Drug abuse, including cannabis, is known to affect striatal dopamine transmission and the reward activity either directly or indirectly via dopamine neurons, although the mechanisms and relationships are still being investigated (45-50). One patient was excluded from all analyses due to misuse of cannabis and testing positively for cannabis prior to the scans. Two patients tested positive for benzodiazepine prior the baseline SPECT scan. The latter two patients were not excluded since neither of them used benzodiazepine regularly and because the positive test was due to a minimum dose taken the evening prior to the scan due to insomnia.

Conclusion

The present study is, to our knowledge, the first to confirm a direct link between dopamine blockade and normalisation of reward disturbances in a follow-up study of antipsychotic-naïve first-episode schizophrenia patients. Combined, our data strongly indicate that salience abnormalities and psychopathology are linked to each other as well as to dopamine activity in schizophrenia patients.

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Paper Two - Supplementary Material

Participants

Four patients and two HC were excluded completely from all analyses. Two patients were excluded due to technical difficulties regarding SPECT image acquisition, one was diagnosed with severe major depressive disorder with psychotic features (DF 32.3), and the last one tested positive for cannabis on the day of the scan. The two healthy controls were both taking antidepressants at the six month follow-up examination.

Four patients discontinued the study and an additional three patients were not included at follow-up. Three patients were given olanzapine due to clinical worsening or lack of improvement during hospitalisation in the follow-up period and one patient did not wish to participate in the follow-up examinations. Additionally, one patient experienced a panic attack in the scanner at follow-up and the examination was stopped. For one patient, we did not receive the blood analyses at the follow-up scan and another patient was not PANSS rated at follow-up.

SPECT acquisition

The SPECT and computerised tomography (CT) data were obtained with a Siemens Symbia™ T2 series SPECT•CT scanner with low energy high resolution (LEHR) collimators (full width at half maximum 7.5 mm) and two slice CT. The ligand, ([^{123}I]-IBZM), was chosen due to its selectivity for striatal $D_{2/3}$ receptors (1;2), and the SPECT scanning was performed using the constant infusion technique (3;4).

The participants received 185mBq [^{123}I]-IBZM (from GE Healthcare, Eindhoven, Holland), – one half given as bolus injection, one half given as infusion during the entire 240-minute session. Prior to administration of the [^{123}I]-IBZM bolus, 200 mg perchlorate mixture i.v. was given to block thyroid uptake of free radioactive iodine. After 180 min., a CT scout and 2 x 30 min. SPECT scans were performed. Between the two scans, a low-dose CT scan was acquired for attenuation correction. Blood samples were collected prior to administration of the bolus and every 30 min. during the scanning period. The plasma-free fraction of [^{123}I]-IBZM was determined using ultrafiltration (Centrifree, 30,000 MW) (5). The plasma metabolite analysis of [^{123}I]-IBZM was performed

using Oasis WCX solid-phase extraction units, Waters, and stepwise elution with water, 40% acetonitrile and acidified 95% methanol. The native compound was eluted in the water phase and the metabolites in the subsequent elution.

S-amisulpride was measured prior and 60, 120, 150, 180, 210, 240 min. after receiving the dose. The mean value during the one-hour scanning period was used in the analyses.

Image analyses

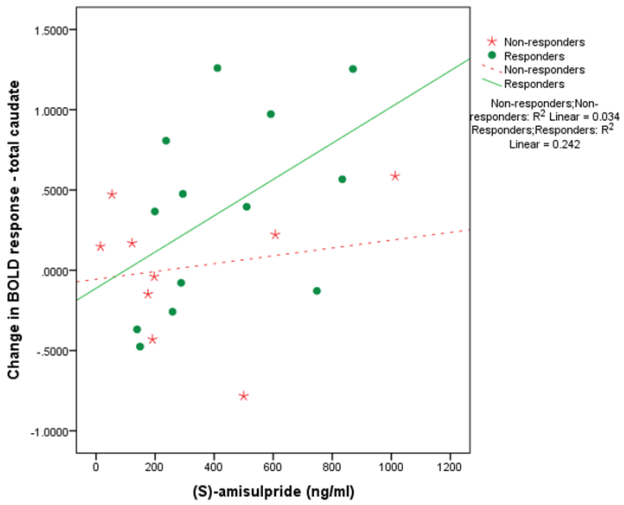
SPECT images were reconstructed with scatter correction and attenuation correction using Flash 3D iterative reconstruction (4 subsets, 8 iterations, Gaussian filter 9 mm) on a Siemens Syngo workstation (software version VA 60 B).

The two obtained IBZM tomographies were summed and activity measurements were decay-corrected to the time of the radioligand injection. The CT image from the SPECT scanning and the MRI image were co-registered using the Statistical Parametric Mapping (SPM) 8 method. The result of the SPM co-registration was then inspected in all three planes and if needed adjusted manually using a local implementation of an image overlay method (6).

The information from the co-registration between CT and MRI images was used for co-registration between SPECT and MRI. Inspection and manually adjustments were repeated if needed.

Additional figure concerning results – correlations at follow-up

Figure 2SM: Correlation between (S)-amisulpride and change in BOLD response. The correlations were significant in the group of responders (green) and non-significant in the group of non-responders (red). Note: one patient was excluded in the division due to the lack of PANSS rating at follow-up, which is why n=22.



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