Effects of aripiprazole vs. haloperidol on brain activity in healthy volunteers

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LIST OF PAPERS

Paper 1
Bolstad I, Andreassen OA, Reckless GE, Sigvartsen NP, Server A, Jensen J
Aversive event anticipation affects connectivity between the ventral striatum and the orbitofrontal cortex in an fMRI avoidance task

*PLoS ONE 2013 8(6): e68494*

Paper 2
Effects of haloperidol and aripiprazole on the human mesolimbic motivational system: a pharmacological fMRI study

*In review.*

Paper 3
Bolstad I, Andreassen OA, Groote I, Haatveit B, Server A, Jensen J
No difference in frontal cortical activity during an executive functioning task after acute doses of aripiprazole and haloperidol

*Frontiers in Human Neuroscience 2015 9:296*
SUMMARY

Schizophrenia is a disorder characterized by positive symptoms, such as hallucinations and delusions, and negative symptoms, such as affect flatness and social withdrawal, in addition to cognitive impairment. Many of these symptoms have been associated with dopamine system disturbances. Schizophrenia is primarily treated with antipsychotic drugs, which are based on either dopamine D2 receptor antagonism (typicals), or dopamine D2 receptor antagonism in combination with other pharmacological properties (atypicals). Antipsychotics are relatively efficacious in treatment of positive symptoms, but are less effective treating negative and cognitive symptoms, and therefore new strategies are needed in drug development. One such strategy is based on partial agonism to the dopamine D2 receptor, and aripiprazole is a drug that belongs to this category.

The goal of this thesis was to examine possible alterations in behavior and brain activation resulting from single dose treatment of aripiprazole or haloperidol, a typical antipsychotic drug, compared to placebo. Two brain functions shown to be disturbed in psychosis were of particular interest: Handling of incentive salient stimuli by the motivational system (Study 2) and executive functioning (Study 3). Before performing the drug challenge study we aimed to design a reliable task to target the mesolimbic motivational system, and examine cortico-striatal connectivity (Study 1).

First, we designed a task inspired by the conditioned avoidance response (CAR) animal model, for use with functional magnetic resonance imaging (fMRI) with blood-oxygen-level-dependent (BOLD) contrast. Then, this task, targeting incentive motivational salience, was applied without drug intervention. We showed that salient cues predicting aversive events were associated with stronger activations in the mesolimbic system, in particular the ventral striatum (VS), and with stronger connectivity between the VS and the frontal cortex (Study 1). Further, when participants doing this task were given haloperidol, the VS activation was diminished, while after aripiprazole the mesolimbic activation was intermediate of placebo and haloperidol (Study 2). In almost the same sample, when targeting executive functioning with the Tower of London problem-solving task, there were no significant
differences in activation in the frontal cortex between participants given haloperidol and participants given aripiprazole (study 3). These results show that BOLD-fMRI is sensitive to changes from pharmacological manipulation. Further, the group difference in Study 2 implies different effects of haloperidol and aripiprazole, probably related to dopamine transmission.

Taken together, these results indicate that pharmacological fMRI studies add knowledge about current pharmacological treatment strategies, and that pharmacological fMRI may be a useful tool for facilitating drug development for schizophrenia as well as other mental disorders.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (serotonin)</td>
</tr>
<tr>
<td>Aal</td>
<td>Automated anatomical labelling</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood-oxygen-level-dependent</td>
</tr>
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<td>CAR</td>
<td>Conditioned avoidance response</td>
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<tr>
<td>CBF</td>
<td>Cerebral blood flow</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol O-methyltransferase</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>Ctrl</td>
<td>Control condition</td>
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<tr>
<td>DDD</td>
<td>Defined daily dose</td>
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<tr>
<td>DMN</td>
<td>Default mode network</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>EPI</td>
<td>Echo planar imaging</td>
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<tr>
<td>EPS</td>
<td>Extrapyramidal side effects</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FWE</td>
<td>Family wise error</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full-width half maximum</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>HDR</td>
<td>Hemodynamic response</td>
</tr>
<tr>
<td>LSD</td>
<td>Lysergic acid diethylamide</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NA</td>
<td>Nucleus accumbens</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartic acid</td>
</tr>
<tr>
<td>OFC</td>
<td>Orbitofrontal cortex</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PPI</td>
<td>Psychophysiological interaction</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>SPM</td>
<td>Statistical parametric mapping</td>
</tr>
<tr>
<td>SVC</td>
<td>Small volume correction</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>Time at which drug serum concentration reach maximum</td>
</tr>
<tr>
<td>ToL</td>
<td>Tower of London</td>
</tr>
<tr>
<td>VS</td>
<td>Ventral striatum</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
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<td>WFU</td>
<td>Wake Forest University</td>
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1 INTRODUCTION

1.1 Historical preface

In pharmaceutical development, there are many examples where important drug properties have been found by coincident while searching for other effects. This is referred to as serendipity, and has repeatedly been shown to be important in early drug development. One of the most famous examples is the discovery of antipsychotic drugs. In a search for new anti-histamines, promethazine was developed in the 1930s, and noticed for its sedative effect. To further develop this calming effect, chlorpromazine was first synthesized in 1952, and released the year after for clinical investigation as a potential adjunct to surgical anesthesia (Ban, 2007). It was noted that it produced indifference and the patients remained relaxed before and during surgery. Based on this, chlorpromazine was tried out in a psychiatric patient suffering from psychosis and severe agitation. The patient was calmed, and with repeated doses the effect could be maintained. Similar reports soon followed, and by 1955 chlorpromazine treatment of psychosis became common internationally (Ban, 2006). Considering that treatment of psychosis at the time included interventions such as lobotomy, cold baths, and insulin coma, chlorpromazine represented a major breakthrough, and the mental hospital population was dramatically reduced within a couple of decades. Although the mechanism of action of chlorpromazine was not yet known, the quest for other antipsychotic drugs had now started. Also, new methods that could characterize the drugs gave hope for an understanding of the pathophysiology of psychosis. However, it was not before the seminal work of Carlsson and Lindqvist (1963), showing that chlorpromazine altered dopamine metabolism in the mouse brain, the idea of dopamine dysfunction as a cause of psychosis was presented. Prior to this, Carlsson and colleagues (1957) had shown that administration of reserpine, which was also used to alleviate psychotic symptoms, led to blockade of dopamine transport. Further research showed that the clinical efficacy of an antipsychotic drug was directly related to its affinity for dopamine receptors (Seeman and Lee, 1975; Creese et al., 1976).

Much more is known today about the pharmacodynamics of antipsychotic drugs. Nevertheless, the roles that the different neurotransmitter systems play in psychosis are
still in the process of being defined, and how they interact is still largely unknown. Thus, the underlying pathophysiology is still unknown in psychotic disorders. Exploiting the effects of the medication, studies of antipsychotic mechanisms are still used to gain more knowledge about disease mechanisms. However, there is no drug yet that efficiently treats all symptoms associated with psychosis, and the currently available drugs are associated with significant side effects. Thus, there is a large need for better pharmacological agents to treat schizophrenia.

1.2 Schizophrenia

This thesis concerns effects of antipsychotic medication in healthy volunteers. However, in order to understand pharmacology, it is important to understand the basic characteristics of the disease that the medication is intended to treat. Therefore, chapter 1.2 will give an outline of schizophrenia.

1.2.1 Epidemiology & symptomatology

Psychosis is a mental state characterized by loss of contact with reality, most often in the form of delusions or hallucinations. There may be various underlying causes of psychosis. Before a psychotic episode is considered part of a psychotic disorder, other etiological origins such as delirium (toxic psychosis), substance use, other medical illnesses and dementia must be excluded. The most predominant psychiatric cause of psychosis is one of the schizophrenia spectrum disorders, but other psychiatric illnesses, such as bipolar disorder, major depressive disorder and post-traumatic stress disorder, may also cause psychotic episodes (Cardinal and Bullmore, 2011).

Schizophrenia is a severe mental illness with detrimental effects on several important aspects of life, such as social functioning and working life, often with disabling consequences. The mortality for patients with schizophrenia is more than 2 times higher than the for the average population, with the most common causes being suicide and cardiovascular disease, including complications related to antipsychotic treatment (McGrath et al., 2008). The onset of schizophrenia is typically at early adulthood (Messias et al., 2007), and for most patients it is a chronic disease. More than 21 million people worldwide are affected by schizophrenia (World Health Organization, 2014), and the average life-time risk of developing the illness is about 0.7% (Tandon et al., 2008), but the number varies across subpopulations. For instance, the prevalence is
lower in developing than industrialized countries (Saha et al., 2005). In general, the symptomatology seems to be more severe in males, and they more often show premorbid deficits than females (van Os and Kapur, 2009).

Symptoms of schizophrenia are commonly categorized as positive, negative or cognitive (Carpenter and Buchanan, 1994). Positive symptoms include hallucinations, delusions, disorganized speech and disorganized behavior (American Psychiatric Association, 2013). Hallucinations may be visual or tactile, but most common are auditory hallucinations, such as hearing voices. Delusions take many forms, but are characterized by a strong conviction by the patient. As the name indicates, negative symptoms describe the absence of behavior that is normally present, manifesting as emotion flatness, social withdrawal, poverty of speech and reduced motivation (American Psychiatric Association, 2013). A third important characteristic of schizophrenia is cognitive impairment, being present in a high proportion of schizophrenia patients and in high-risk individuals (Schaefer et al., 2013; Fatouros-Bergman et al., 2014). This includes deficits in attention, planning, working memory and executive functioning. Cognitive deficits have been found to be the characteristics that best predicts functional outcome in schizophrenia (Green, 1996). Our cognitive abilities are instrumental in our daily life functioning, and this probably explains their predictive value. But cognitive and negative symptoms are also the most refractory features of the illness, and might therefore persist to cause problems for the patient even if antipsychotic treatment gives relief for positive symptoms.

1.2.2 Etiology

As more sophisticated research methods have emerged, new research has given further insights into the etiology of schizophrenia. This has resulted in several lines of evidence from various branches of medical research. Nevertheless, the understanding of the disease mechanisms is still incomplete, and there is little knowledge about the specific etiology of the illness. However, several risk factors for developing schizophrenia are uncovered, revealing a rather complex etiology for the illness. Schizophrenia has a heritability of about 0.8, meaning that the proportion of variation in risk that is explained by genetic factors is approximately 80% (Cardno and Gottesman, 2000). Studies have identified several of the specific genetic risk factors for developing schizophrenia (Allen et al., 2008; Stefansson et al., 2008; Gejman et
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al., 2011; Schizophrenia Working Group of the Psychiatric Genomics, 2014), many of which are thought to be involved in neurodevelopment and neural migration (Kahler et al., 2008; Saetre et al., 2008). In addition to immune factors and general neurotransmission, some of the genes most strongly associated with schizophrenia are involved in dopamine signaling (Schizophrenia Working Group of the Psychiatric Genomics, 2014). However, the effect size for each genetic risk variant is small and genetic predispositions for schizophrenia may exist as interactions between several risk genes. One study showed that an interaction between a set of four genes coding for proteins regulating synaptic dopamine levels increase risk of schizophrenia (Talkowski et al., 2008). Most recent findings suggest that many genes are involved, each with a small effect (Schizophrenia Working Group of the Psychiatric Genomics, 2014). Also, recent years’ research indicate a genetic overlap between the schizophrenia spectrum disorders and other mental illnesses, bipolar disorder in particular, pointing towards a continuum of psychotic disorders rather than separate conditions (Andreassen et al., 2013; Cross-Disorder Group of the Psychiatric Genomics, 2013), but this notion is still debated.

Although the heritability of schizophrenia is high, various environmental factors are associated with increased risk of schizophrenia. Migration (Cantor-Graae and Selten, 2005) and urban upbringing (Krabben and van Os, 2005) has been associated with increased risk, but the causes underlying these relationships are not clear. One theory links this to social adversity in childhood, suggesting that a set of social risk factors may be combined into a common factor referred to as social defeat (Cantor-Graae and Selten, 2005; Cantor-Graae, 2007). Interestingly, studies in rodents have shown that social defeat increase the baseline level of dopamine in mesocorticolimbic pathways (Tidey and Miczek, 1996), a system that is altered in schizophrenia patients (Laruelle, 1998). In monkeys social rank is correlated with dopaminergic activity (Kaplan et al., 2002; Morgan et al., 2002). Based on these findings, it has been speculated that prolonged experiences of social defeat in humans might increase the dopamine levels and thus sensitize these individuals to development of psychotic symptoms (Cantor-Graae and Selten, 2005). Also in humans, elevated striatal dopamine levels are associated with stress, such as poor maternal care during childhood (Pruessner et al., 2004). There is evidence that cannabis use increase the risk of developing psychosis in a dose-response fashion (Semple et al., 2005; Moore et al., 2007). In the context of this
thesis’s topic, it is interesting to note that endocannabinoid signaling plays an important role in cortico-striatal learning and plasticity, possibly linking the risk conferred by cannabis use to the dopamine system (Kuepper et al., 2010). Obstetric complications have also been related to increased risk (Cannon et al., 2002). Prenatal infection are found to increase the risk for schizophrenia (Fan et al., 2007) and immunological factors are associated through both genetic (Shi et al., 2009) and animal studies (Winter et al., 2009). It has been hypothesized that glutamatergic imbalance may be mediating this association (Miuller and Schwarz, 2007).

In summary, a multifactorial model including both genetic and environmental factors is required to explain the etiology of schizophrenia. Thus, a range of factors seems to interact with each other to produce the illness (Howes and Murray, 2014).

1.2.3 Neuroimaging findings

The introduction of neuroimaging techniques has made it possible to investigate brain alterations on a biological level in persons affected by schizophrenia. Some of the most commonly used methods are structural and functional magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT), and electroencephalography (EEG). PET and SPECT scanners detect signals emitted from an injected radio-tracer substance, and may for example yield information about brain activity or concentrations of specific neurotransmitter receptors. In contrast, functional MRI (fMRI) is a non-invasive technique, but it gives a proxy of neuronal activity. EEG measures electrical activity in the brain.

Structural abnormalities

Evidence for structural brain changes in schizophrenia is convincing. Some of these alterations are observed already in first episode patients, and are shown to progress during the course of the illness (Ellison-Wright et al., 2008), although maybe mostly around illness onset (Pantelis et al., 2005). The alterations include lateral- and third ventricle enlargement and reductions of grey matter in the prefrontal cortex (PFC), insula, anterior cingulate, hippocampus, amygdala and subcortical regions including thalamus and basal ganglia (Shenton et al., 2001). Structural changes have been shown to be associated with cognitive functioning (Antonova et al., 2004). A more
Introduction

widespread cortical thinning has also been reported, and this was found to overlap largely with the brain morphology found in bipolar disorder (Rimol et al., 2010).

Functional abnormalities

A substantial number of functional neuroimaging studies have been conducted in patients with schizophrenia. Functional neuroimaging is grounded on the assumption of association between a brain function and the activation of its neural correlate. Cognitive functioning is impaired in schizophrenia, and numerous functional neuroimaging studies have focused on cognition. This has yielded information about cognitive functions including executive functions, learning and memory, and the associated alterations in brain activations in frontal and temporal cortical regions in patients with schizophrenia (Manoach, 2003). In correspondence with structural findings, the medial temporal lobe, in particular the hippocampus has commonly been implicated in functional imaging studies of patients with schizophrenia (Heckers, 2001). Similarly, decreased activation in the inferior and dorsolateral PFC has been repeatedly described (Achim and Lepage, 2005; Glahn et al., 2005). However, this seems to be accompanied with increased activation in other cortical regions, in particular along the midline, supposedly compensating for impaired prefrontal functioning (Glahn et al., 2005; Minzenberg et al., 2009).

Abnormal activation in emotion processing networks including the amygdala has been linked to social dysfunction in schizophrenia and is thought to be related to problems with identifying affect in others (Poole et al., 2000; Gur and Gur, 2010). Disturbances in reward processing have also been found in patients with schizophrenia. Specifically, studies have shown abnormal activation in the ventral striatum (VS) in patients related to aberrant reward prediction and learning of reward-related associations (Juckel et al., 2006; Jensen et al., 2008). However, some inconsistencies exist regarding some of the functional findings (Achim and Lepage, 2005). A reason for this may be the heterogeneity across patients with schizophrenia rendering demographical characteristics of the study sample an important influential factor for the outcome of the study.

Brain network disturbances have been associated with schizophrenia and constitute an area of increasing attention within neuroimaging (Friston and Frith, 1995; Stephan et
Introduction

_al., 2006_. Recently, the default mode network (DMN) has been investigated in several studies. The DMN is a set of associated brain regions that are active when the person is at rest, and most studies suggest a hyper-connectivity of the DMN in persons with schizophrenia (Karbasforoushan and Woodward, 2012). Further, studies of patients with schizophrenia have found that the anti-correlation between the DMN and task positive networks is disturbed (Wolf _et al._, 2009). In general, brain networks in patients with schizophrenia are found to have higher diversity and less integrated functional connectivity (Lynall _et al._, 2010). One study reported hyper-connectivity between the striatum and a DMN region in the medial frontal cortex, in patients compared to healthy controls, suggesting a disturbance possibly underlying cognitive deficits (Salvador _et al._, 2010).

1.3 **Neurochemical dysfunction in schizophrenia**

Schizophrenia has been found to be associated with certain neurochemical abnormalities. Here follows an overview of the main theories and the evidence supporting these. The findings are based mainly on evidence from patient studies and clinical trials employing a neuroimaging technique, but post-mortem and animal studies have also yielded knowledge.

1.3.1 Dopamine

_Anatomy of the dopaminergic system_

There are three major dopaminergic pathways in the brain. The nigrostriatal projections originate in the substantia nigra and contact the dorsal striatum. These connections are important for control of voluntary movement. Cell death in the substantia nigra gives the motor symptoms seen in Parkinson’s disease. The other two - the mesolimbic- and mesocortical pathways - both originate in the ventral tegmental area (VTA). The mesolimbic projections mainly target the VS, but also contact the hippocampus and the amygdala. The nucleus accumbens (NA) is part of the basal ganglia and makes up the VS together with the olfactory tubercle and ventromedial parts of the putamen and nucleus caudatus (Haber and Fudge, 1997; Ikemoto, 2007). The mesocortical projections’ main contact points are the orbitofrontal, mediolateral, dorsolateral and cingulate cortical areas (Haber and Fudge, 1997).
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The Dopamine Hypothesis

On the background of dopamine involvement in antipsychotic action the Dopamine hypothesis was formulated in the 1970s and suggested that schizophrenia was caused by dopamine hyper-transmission. The focus was on subcortical regions as there was a known density of dopamine neuron terminals and receptors in these areas. However, this first version of the Dopamine hypothesis had obvious shortcomings as it did not account for negative and cognitive symptoms, and it did not concern the dopamine system’s interaction with other neurotransmitter systems. As the research continued a more nuanced framework was required to encompass new knowledge. In contrast to the previous understanding where dopamine transmission was thought to be generally increased, evidence from post mortem, imaging and animal studies now pointed to a regional specificity, emphasizing alterations in prefrontal dopamine transmission and how this could account for the negative and cognitive symptoms.

The current Dopamine hypothesis - the 3rd version postulated by Howes and Kapur in 2009 attempts to accommodate recent findings. Two major shortcomings of the second edition was that it did not suggest an explanation for how the dopaminergic disturbances actually lead to psychosis, or what causes the alterations in dopamine transmission in the first place. Bridging the gap between neurobiology and symptomatology, Howes and Kapur suggested that the increased level of dopamine in the striatum leads directly to hallucinations and indirectly to delusions. This hypothesis is grounded on the evidence that the VS, and in particular the nucleus accumbens, makes up the core of the mesolimbic incentive motivational salience system (Berridge and Robinson, 1998) thought to shape goal-directed behavior. Thus, dopamine signaling has been found to be important for motivational prediction (Schultz, 2002).

Schott et al., 2008 showed in a study combining fMRI and PET while participants were performing a delayed monetary incentive task that dopamine release in the VS was correlated with neural activity in the same area. This suggests that the dopaminergic neurotransmission and the neural response in the mesolimbic motivational system are related in a quantitative fashion during reward processing. Chaotic firing of dopaminergic neurons targeting the VS in patients with schizophrenia is hypothesized to result in aberrant assignment of salience (Kapur, 2003). The salience attribute of an object signifies the behavioral relevance to a person, shaping
that person’s actions. The mesolimbic motivational system has been found to be essential for processing of salient information, for example in reward prediction (Berridge, 2007) and avoidance learning (Jensen et al., 2007). Thus, in schizophrenia, increased activity in the VS would lead to an aberrant assignment of salience to stimuli that should not hold particular relevance to the person (Howes and Kapur, 2009).

Further, within this concept delusions are explained as the person’s attempt to interpret somehow the experienced incomprehensible information, while a hallucination is explained as the direct experience of aberrant salience (Kapur, 2003). An explanation for negative symptoms is also proposed, suggesting that relevant reward signals do not survive among the stimuli aberrantly assigned with salience. This disturbance causes a lack of motivation that in turn induces negative symptoms (Howes and Kapur, 2009).

**Dopamine in the frontal cortex**

Meta-analyses have suggested that hypofrontality - an impaired brain activation response to a cognitive task, is a characteristic of schizophrenia (Davidson and Heinrichs, 2003; Hill et al., 2004). However, the studies included in these reviews differ as they report hypoactivity, no change and even hyperactivity. Studies in schizophrenia patients have shown that frontal activation is related to dopamine levels in the same area (Weinberger et al., 1988), thus indicating that the inconsistent results may be influenced by baseline dopamine levels. It has been shown that a polymorphism at a gene coding for catechol O-methyltransferase (COMT), an enzyme that degrades dopamine and other catecholamines, influence dopamine concentration. Egan et al., 2001 found that schizophrenia patients with one COMT genotype (val/val) coding for a highly active COMT enzyme, and hence yielding a low endogenous dopamine level, has impaired cognition and corresponding PFC activity in comparison with patients with the other homozygous genotype (met/met) coding for a less active COMT. In accordance with this finding, (Mattay et al., 2003) showed in a pharmacological fMRI study that when given amphetamine, healthy individuals with val/val genotype improved both their cognitive performance and PFC efficiency as measured with fMRI, during high loads of working memory. In individuals with met/met genotype, amphetamine was shown to impair performance and PFC activity during high loads. The findings confirmed to an inverted U-curve where too little or too much dopamine results in impaired PFC efficiency.
Introduction

Cortico-striatal connections

Studies have revealed relationships between frontal and striatal dopamine systems, and it has been shown that the frontal cortex is reciprocally connected with mesolimbic and limbic structures, and receives multimodal sensory input (Kringelbach and Rolls, 2004). One study measuring cerebral blood flow and pre-synaptic dopaminergic function in the same session, using PET, showed that decreased cerebral blood flow in the frontal cortex during an executive task was predictive of increase in striatal hyperactivity, suggesting that frontal hypodopaminergia may lead to striatal hyperdopaminergia (Meyer-Lindenberg et al., 2002). However, the direction of this relationship is not yet established (Simpson et al., 2010), and the opposite relationship - that PFC dopamine function is under the influence of the mesolimbic dopamine system has also been found, although these findings are from animal studies (Kellendonk et al., 2006; Bach et al., 2008). Thus, there are indications of a bi-directional relationship.

It is suggested that the cortico-striatal connections are involved in motivation (Morrison and Salzman, 2011), but there is still a lack of knowledge about the content of this interaction and how the striatum influences the PFC.

Dopamine receptors

Dopamine receptors belong to the D1-like family (D1 and D5 receptors) or the D2-like family (D2, D3 and D4 receptors), and they have distributions that differ between neuron types (Missale et al., 1998) and between anatomical brain regions (Seeman, 1992). While D2 receptors are concentrated in the striatum and scarce in the PFC, D1 receptors have a wide spread cortical distribution. Studies have shown that concentration of post-synaptic D2 receptors in the VS is not much elevated in patients with schizophrenia, as was previously thought (Laruelle, 1998; Kestler et al., 2001). Instead, recent reports suggest that presynaptic capacity, i.e. the synthesis and storage of dopamine in presynaptic vesicles, and release of dopamine into the synaptic cleft are elevated (McGowan et al., 2004), and this may underlie the striatal dopaminergic alteration in schizophrenia patients. The current antipsychotic drugs are based on blockage of the postsynaptic D2 receptor. Although this effectively blocks dopaminergic neurotransmission, it has certain detrimental consequences. Through
presynaptic autoreceptors this signals a demand for a compensatory increase in dopamine synthesis and release. It has been shown that presynaptic dopamine capacity is elevated after acute treatment with the antipsychotic drug haloperidol in healthy individuals (Vernaleken et al., 2006), and after therapeutic treatment in schizophrenia patients (McGowan et al., 2004). This insight has important consequences for the knowledge about the current drugs used to treat schizophrenia. If aiming to develop new dopamine-based antipsychotic drugs, presynaptic cell terminals should be emphasized in trying to find ways to manipulate synthesis and release in order to regulate dopamine levels. Another intuitive possibility would be to take one step upstream and look for pharmacotherapeutical targets within the post-synapse of the dopamine neurons that originate in the VTA. Most of the afferents to the VTA are glutamatergic, and the variation of their origin is abundant (Geisler et al., 2007). Thus, investigations of possible new antipsychotics based on modulation of glutamatergic neurotransmission have been performed.

**Treatment resistant patients**

There is a large subgroup of schizophrenia patients that seem to have an underlying pathophysiology that differs from the majority. These patients do not respond to non-clozapine antipsychotic treatment and are hence referred to as treatment-resistant (Mortimer et al., 2010) (See paragraph 1.4.2 about clozapine). This means that D2 receptor blockage, that has been directly linked to alleviation of positive symptoms (Kapur et al., 2000), is not effective in this patient subgroup (Davis et al., 1980). Further, studies have shown that dopamine synthesis capacity is not increased in these patients (Demjaha et al., 2012), maybe suggesting that the pathophysiology of this subgroup mainly exists in other neurotransmitter systems. Yet, the question of what underlies these symptoms, that are similar to symptoms in other schizophrenia patients, remains unanswered. However, there is a growing number of reports connecting schizophrenia to dysfunction in other neurotransmitter systems. This may reveal the pathophysiology of this patient group in the future, and possibly explain the treatment refraction of cognitive symptoms in people with schizophrenia.

The current Dopamine hypothesis incorporates a range of factors that have been associated with increased risk of developing schizophrenia (section 1.2.2), and suggests an interaction between genes and environment resulting in a vulnerability
underlying the dopamine disturbance. Importantly, this theory also gives room for evidence showing that other neurotransmitter systems are essential to understanding this disease.

1.3.2 Glutamate

Glutamate physiology

The amino acid glutamate is the primary excitatory neurotransmitter of the brain. It acts on metabotropic and ionotropic receptor types, the three major kinds of the latter type being the N-methyl-D-aspartate (NMDA) receptor, the α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptor and the kainite receptor (Kew and Kemp, 2005). The NMDA receptor is a cation channel that is essential for the excitatory postsynaptic potential as it allows sodium (Na⁺) and calcium (Ca⁺) ions to pass into the cell and potassium (K⁺) to pass out of the cell. Because the NMDA receptor does not only require glutamate to bind to it, but also the postsynaptic cell to be depolarized for it to open the ion channel, it is the signal of a cluster of nearby neurons that is summarized and conveyed. The NMDA receptor has been found to be of particular importance for long-term potentiation and synaptic plasticity, and thus for memory formation (Bliss and Collingridge, 1993; Li and Tsien, 2009).

The Glutamate hypothesis

The first evidence of glutamate implication in schizophrenia was findings of lowered levels of glutamate in the cerebrospinal fluid (CSF) in patients (Kim et al., 1980). Subsequent reports regarding this issue were inconsistent (McCullumsmith et al., 2004), but several more recent lines of evidence point to a role for glutamate involvement in schizophrenia. Studies have shown that NMDA antagonists can induce disturbances in healthy volunteers that resemble cognitive and behavioral symptoms seen in people with schizophrenia, and that enhancement of NMDA receptor activity in these patients may lead to symptom alleviation (Krystal et al., 2003). Hence, a hypothesis of NMDA receptor hypofunction has been proposed. In addition to the evidence mentioned, genetic studies have shown that some of the genes that are most strongly associated with risk of schizophrenia are implicated in glutamate neurotransmission (Coyle, 2006). Most compelling is perhaps the evidence from
ketamine challenge studies in healthy volunteers, demonstrating that NMDA receptor stimulation induce characteristics that resemble negative and cognitive symptoms of schizophrenia (Adler et al., 1999; Newcomer et al., 1999).

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the adult brain, and it reduces excitability in the post-synaptic cell. There is evidence for selective reduction of GABA transmission in the dorsolateral PFC, cingulate cortex and hippocampus in schizophrenia and this disturbance is related to dopamine and glutamate transmission (Benes and Berretta, 2001; Lewis et al., 2005). Specifically, it is suggested that NMDA receptor hypofunction may be causing the reduced GABAergic function found in patients with schizophrenia (Coyle, 2006). In addition, glutamate is a precursor for GABA.

What underlies the disturbance in dopaminergic transmission is not clear. Laruelle proposed that the answer may be related to dysfunctional NMDA transmission (Abi-Dargham and Laruelle, 2005; Laruelle et al., 2005). This theory was put forward based on observations of psychotomimetic effects of NMDA antagonists, and to evidence from animal studies showing that long-term treatment with an NMDA antagonist induce decreased dopaminergic activity in mesocortical pathway and increased activity in the mesolimbic system (Jentsch and Roth, 1999), resembling the alterations found in schizophrenia patients. Thus, there are important interactions between the neurotransmitter systems, and good reason to believe that dopamine transmission may, at least in part, be regulated by glutamate. Thus, the Glutamate hypothesis does not contradict the Dopamine hypothesis, rather they seem to complement each other.

1.3.4 Serotonin

In the central nervous system, the amino acid neurotransmitter serotonin, or 5-hydroxytryptamine (5-HT), is involved in various functions, such as sleep, mood and emotional behavior. In the 1950s it was discovered that lysergic acid diethylamide (LSD), that was known to have a psychotomimetic effect, had strong affinity for the serotonin receptors (Gaddum and Hameed, 1954). This led to the idea that serotonin disturbance might be involved in psychotic disorders and that serotonergic hallucinogens might provide a model of psychosis. In contrast to classical antipsychotic drugs, most of the newer (atypical) antipsychotic drugs have stronger
antagonistic action on the 5-HT2A receptor than they do for the D2 receptor (Meltzer and Huang, 2008). Still, they have comparable efficacy when it comes to alleviating positive symptoms, and possibly superior effect on cognitive impairment. This suggests that serotonin is an important treatment target, particularly for cognitive impairment, and that serotonin constitutes a considerable role in the pathophysiology of schizophrenia.

1.4 Antipsychotic medication

On a global level less than 50% of the people with schizophrenia receive appropriate care (World Health Organization, 2014). The majority of these are treated with antipsychotic medication, often in combination with other medication and psychotherapeutic intervention. Antipsychotic drugs are used to treat and prevent psychotic symptoms such as hallucinations and delusions, especially in schizophrenia and bipolar disorder. Although there is some debate about whether antipsychotics can be categorized into two distinct groups (Tyrer and Kendall, 2009), they are commonly classified as either typical or atypical. These are also referred to as first- and second-generation, respectively. There is a four-to-one preference in the literature for the typical/ atypical categorization (Meltzer, 2013) and this nomenclature is used in this thesis.

1.4.1 Typical antipsychotic drugs

Typical antipsychotics include the first drugs that were discovered to have an effect on psychotic symptoms, and the ones with similar pharmacodynamic profiles that were subsequently developed. In 1952 chlorpromazine was discovered to have an ameliorating effect on psychotic positive symptoms. This was the beginning of the era of pharmacological treatment of psychosis, and triggered the development of other antipsychotic drugs (Ban, 2007). All antipsychotic drugs have affinity for the dopamine D2 receptor, and receptor binding is linked to clinical potency (Creese et al., 1976). The majority are antagonists to this receptor. For a typical antipsychotic drug to be clinically efficacious, it has to occupy minimum ~65% of the postsynaptic D2 receptors in the striatum (Wadenberg et al., 2001). However, clinical efficacy in this context concerns curbing of positive symptoms, but treatment with typical
Introduction

Antipsychotics has minimal or even negative effect on negative symptoms and cognitive dysfunction.

Haloperidol (Haldol\textsuperscript{®}) is a high potency typical antipsychotic drug (< 2 mg needs to be administered in order to achieve the dose equivalent of 100 mg chlorpromazine), and is often regarded as a “model drug” in research context because there exists a broad literature concerning the drug. The typical antipsychotic drugs are usually not the first choice of medication for a patient with schizophrenia or bipolar disorder. However, they are commonly used in the clinic to treat psychosis, but some also other conditions. Typical antipsychotics are effective, but one of the main reasons for the decreased use in psychosis treatment is that they are associated with severe adverse effects, in particular extrapyramidal side effects (EPS) (Kane, 2001). EPS appear when approaching the upper limit of the therapeutic window, at about 80% striatal D2 receptor occupancy. EPS may occur as acute movement disorders including dystonia (spams), akathisia (restlessness), bradykinesia (slow movement execution) and dyskinesia (jerky movements) and tardive dyskinesia, all representing major discomfort for the patients.

1.4.2 Atypical antipsychotic drugs

An atypical antipsychotic drug is, by its simplest definition, one that gives minimal EPS at clinically effective doses (Meltzer, 2000). Another way of making the distinction is based on pharmacology, attributing the effect of chlorpromazine-like drugs exclusively to dopamine D2 receptor blockade. As atypical antipsychotics include drugs with various pharmacodynamic profiles, a common pharmacological mechanism that can explain their action of treatment has not been identified. Rather, it seems like simultaneous effects on dopamine receptors, various serotonin (5-HT) receptors, muscarinic cholinergic receptors and histamine receptors are involved. Unlike the typical antipsychotic drugs, most atypical antipsychotics have much higher affinity for 5-HT2A receptors than for dopamine D2 receptors (Meltzer \textit{et al.}, 1989; Meltzer and Huang, 2008). Clozapine was recognized to be effective in treatment of schizophrenia in 1988 (Kane \textit{et al.}, 1988), and has later been recognized as a prototypical atypical antipsychotic drug that has superior treatment results (McEvoy \textit{et al.}, 2006) and is effective in preventing suicide attempts (Meltzer \textit{et al.}, 2003). However, clozapine is associated with agranulocytosis and the patients prescribed with
the drug have to be blood-monitored (Idanpaanheikkila et al., 1977). Because of its serious side effects it is only used in patients with treatment-resistant psychosis (after two other failed antipsychotics), where in most cases it is effective against positive symptoms. The largest group of atypical antipsychotics has pharmacodynamic profiles that bear resemblance with clozapine. Atypical antipsychotics are often preferred because of their advantageous motor side effect profile (Kahn et al., 2008). However, they are associated with several metabolic side effects such as weight gain, glucose dysregulation, increase in lipid levels and type 2 diabetes mellitus (Amiel et al., 2008).

The clinical antipsychotic trials for intervention effectiveness (CATIE) (Lieberman et al., 2005) and the Cost utility of the latest antipsychotic drugs in schizophrenia study (CUtLASS) (Jones et al., 2006) – two large multicenter randomized clinical trials did not find any difference in effectiveness or tolerability between typical or atypical antipsychotic drugs. These studies have later been criticized because of methodological issues; in particular, the dosing scheme has been questioned. In addition, the drug representing the “typical” category was pherphenazine (Trilafon®), a drug that by some is suggested to behave more like an atypical drug and thus do not provide a valid comparison. Haloperidol is often considered a standard drug and is typically used in clinical trials. Still, recent meta-analyses indicate that there is little difference in efficacy between typical and atypical agents, while the side effect profile plays a bigger role (Leucht et al., 2013).

Atypical drugs are generally considered advantageous relative to typical drugs regarding cognitive impairment, (Keefe et al., 1999; Woodward et al., 2005), although no difference in effect between the categories was reported in the CATIE study (Keefe et al., 2007). The drugs vary as to how beneficial they are across the different cognitive domains and this probably relates to their differential pharmacodynamics (Keefe et al., 1999; Woodward et al., 2005).

Aripiprazole

Aripiprazole (Abilify®) is a relatively new antipsychotic drug and is categorized as an atypical. However, there are important dissimilarities distinguishing it from all other antipsychotics. Although aripiprazole, like most antipsychotics, has high affinity for the D2 receptor, its antagonism on the 5-HT2A receptor is lower than for most other atypical antipsychotics. In general, atypical antipsychotics are believed to exert some
of their symptom alleviating effects through 5-HT2A antagonism (Meltzer and Huang, 2008), in combination with D2 antagonism. Aripiprazole is a partial agonist to the 5-HT1A receptor, and has been suggested to be a dopamine-serotonin stabilizer (Burris et al., 2002). But perhaps most important, unlike other antipsychotics it is a partial agonist to the D2 receptor, exerting some intrinsic (stimulating) effect on this receptor. D2 receptor occupancy during therapeutic treatment is at least 90% (Yokoi et al., 2002), in contrast to other atypical antipsychotic drugs of which the occupancies are about 40 - 70% (Kapur et al., 2000; Frankle et al., 2004). According to theory this means that in a dopamine-rich environment it will display a net inhibitory effect because its stimulating effect will be lower than the effect from endogenous dopamine. However, in a dopamine-deficient environment aripiprazole will display agonism, because the stimulating effect on D2 receptors will exceed the net effect exhibited by endogenous dopamine (Burris et al., 2002). Aripiprazole has been shown to be a potent agonist at D2 autoreceptors (Kikuchi et al., 1995). If the dopamine autoreceptors are blocked, as is the case with most antipsychotic drugs, this will tell the presynaptic neuron to release more dopamine into the synaptic cleft. Thus, in contrast to autoreceptor blockade by an antagonist, autoreceptor agonism will lead to decreased dopamine release. In vivo studies have shown that aripiprazole binds to D2 autoreceptors, inducing a decrease in dopamine release and thus it may lower synaptic dopamine levels (Burris et al., 2002).

Based on its unique pharmacological profile, aripiprazole holds promise for a more complete treatment for schizophrenia, including alleviation of negative and cognitive symptoms. This has been supported in several studies showing cognitive improvement (Fleischhacker, 2005; Kern et al., 2006; Schlagenhauf et al., 2010; Suzuki et al., 2011). For many patients, aripiprazole have been shown to have a favorable side effect profile (Argo et al., 2004; Fleischhacker, 2005). As with most atypicals, there is lower risk of EPS than what is associated with the use of typicals (Yokoi et al., 2002). In addition, it is shown to cause minimal weight gain (Bak et al., 2014) and it does not increase prolactin levels in the blood (Kane et al., 2002; Peuskens et al., 2014), problems commonly associated with other atypical antipsychotic drugs.
1.4.3 Conditioned avoidance response

Drugs that have antipsychotic effects have the unique ability to selectively suppress a certain behavior in a classical animal model referred to as the conditioned avoidance response (CAR) task. During the CAR task the animal is conditioned to respond to a stimulus by moving from one place to another in order to avoid an aversive event (avoidance behavior). Interestingly, when given an antipsychotic drug the avoidance behavior is suppressed whereas the animal is still able to escape when the aversive event is presented. Suppression of CAR is only achieved within a specific dosage window, and only with drugs that have antipsychotic effect (Wadenberg, 2010). It is suggested that the CAR behavior is dependent on striatal dopaminergic neurotransmission (Davies et al., 1973; Wadenberg et al., 1990; Nicola, 2007). The dose range at which the CAR model is sensitive largely overlaps/coinide with the doses that show efficacy in patient treatment (Wadenberg et al., 2001). The CAR test has been around for more than 60 years and is widely used in characterization of potential new antipsychotic drugs. All current licensed antipsychotic drugs suppress avoidance during the CAR test, and drugs that have failed clinical trials also failed the CAR test (Wadenberg, 2010). Obviously, in the animal model the CAR test holds very good predictive validity and has also shown to be reliable. Nevertheless, the underlying mechanisms of the specific sensitivity and ability of the CAR test to identify antipsychotic drugs is not yet understood. One tentative explanation relates to the notion of aberrant incentive motivational salience in psychosis (Kapur, 2003; Wadenberg, 2010). The altered dopaminergic neurotransmission resulting from D2 receptor blockade is thought to disturb incentive motivation. On this background, the theory suggests that the increased response to non-salient stimuli proposed to underlie psychosis is the mechanism modelled with CAR. Supporting this hypothesis, one animal study employed a design resembling the CAR test, but where the conditioned stimulus was assigned either high or low salience. It was shown that administration of amphetamine resulted in enhanced avoidance behavior associated with the low-salient stimulus, but not the high-salient stimulus (Li et al., 2008). Other theories of what underlies the mechanism of CAR suppression also exists. For example, the dominant traditional theory has been that disruption of CAR represents an inability to initiate motor response (Aguilar et al., 2000), but this has been contradicted by studies showing that CAR inhibition is present even if the animal was given an antipsychotic
drug only in the acquisition phase of the trial (Li et al., 2004). Another hypothesis focuses of the aspect of threat in the CAR test and in persecutory delusions in patients with psychosis and this has been suggested as a common denominator (Moutoussis et al., 2007).

Based on the specificity of the CAR animal model and its predictability of efficacious antipsychotic drugs, it is likely that the mechanism of the CAR model holds a clue to the mechanism of schizophrenia pathology. Possibly, by studying processes similar to the CAR in humans, more knowledge can be generated about the underlying neuropathology of schizophrenia, and consequently about possible approaches for pharmacological treatment.

1.5 Pharmacological fMRI

All studies of medical interventions intended for use in humans, such as a drug, device or change in behavior are defined as a clinical trial. Clinical trials are categorized based on how far developed the intervention is, and on the purpose they serve. Phases I – III studies screen for safety and measure efficacy with increasing sample sizes (from about 20 participants to > 1000 participants), while phase IV studies aim to gather information about effect, risk and benefit on drugs that are already on the market. Neuroimaging is currently not a tool commonly used in drug development. Thus, most current human pharmacological neuroimaging studies are phase IV studies.

There is a growing number of reports where psychopharmacology is studied with fMRI, a field known as pharmacological fMRI or pharmacological MRI. Initially the technique was used mainly in preclinical studies, but many reports now exist where pharmacological fMRI have been applied in humans (Honey and Bullmore, 2004). Animal studies have obvious advantages in terms of intervention and yield information that cannot be obtained in human pharmacological fMRI. However, the results may be precluded by anesthetic effects (Haensel et al., 2015), and extrapolation to the human brain is not straight-forward. Human pharmacological fMRI is an emerging field, and the technique is used to study neurotransmission in the normal and in the diseased brain, for example in studies of psychiatric conditions and addiction. Brain activations linked to various neurotransmitters, including dopamine, serotonin, glutamate, GABA and acetylcholine have been studied (Honey and Bullmore, 2004). The dopamine
system innervates key brain regions implicated in motor and neurocognitive functions, making it an interesting target of pharmacological fMRI studies. Several studies have been performed using amphetamine or methylphenidate, both substances that increase the concentration of dopamine in the synaptic cleft, to study effects of dopamine modulation. For example, based on an fMRI task involving anticipation of gains and losses performed by healthy volunteers, one study showed that amphetamine lead to reduction of differences between gains and losses regarding both VS activation and positive arousal. This indicates that amphetamine may modulate tonic endogenous VS activation, also during loss, and this may enable the organism to maintain motivation when facing adversity (Knutson et al., 2004). Another study found activations in the brain reward system and the orbitofrontal cortex (OFC) after infusion of methamphetamine in healthy individuals, and linked these findings to subjective ratings of “mind-racing” (Vollm et al., 2004). Using an aversive conditioning fMRI paradigm Menon et al., 2007 showed that amphetamine increases a learning related signal in striatal regions to cues signaling aversive events. In the same study it was shown that haloperidol eliminates this signal thus suggesting that dopamine modulates the brain responses to aversive stimuli in an aversive conditioning paradigm. One study revealed dampening of activation in the middle occipital and the fusiform gyri after haloperidol challenge in healthy individuals. These changes were found to normalize within 24 hours (Brassen et al., 2003). Honey et al., 2003 showed that the dopamine D2 receptor antagonist sulpiride increased the connectivity from the ventral tegmental area to the caudate nucleus, while methylphenidate (that blocks dopamine reuptake) decreased this connectivity, indicating that dopamine modulates connectivity within the basal ganglia.

The CAR animal model detects efficacy in potential antipsychotic drugs, possibly targeting mechanisms of reward processing. Translating this model to a human pharmacological fMRI task would enable examination of the mechanism of action for differential current antipsychotic drugs.

A few studies have examined effects of typical and atypical antipsychotic drugs on striatal activation in healthy individuals, but cognitive functions have rarely been examined in this manner. However, cognitive, psychomotor and affect impairments after administration of antipsychotics have been reported in behavioral studies
(Ramaekers et al., 1999; Saeedi et al., 2006; Vernaleken et al., 2006). One fMRI study reported no differences in prefrontal activation or behavior after administration of the atypical antipsychotic drug sulpiride, however, differences were observed in the putamen (Dodds et al., 2009). Pharmacological antipsychotic treatment affects cognition, at least in some patients, and more studies of antipsychotic drugs in healthy volunteers would help mapping the effects of antipsychotic drugs on activations related to cognitive functions and memory.

To summarize, early studies suggest that pharmacological fMRI is a tool that is sensitive enough to pick up pharmacologically modulated activations in the brain. However, there may be region- and process specific differences as to where this method may be successfully applied. As in all fMRI-studies it is important to acknowledge that the fMRI signal is an indirect measure (based on regional blood supply) of neural activation. In addition, when studying dopamine agonists or antagonists using fMRI, as in the current project, one should keep in mind that these substances can affect regional blood flow, as can dopamine itself (Mandeville et al., 2013). Nevertheless, pharmacological fMRI has a potential for testing transmitter models of disorder, predicting treatment response and supporting the development of novel compounds in neuropsychiatry.
Introduction

1.6 Aims

The main object of the present work was to gain more knowledge about antipsychotic drugs by studying the effects that these drugs have on the mesolimbic motivational system and on executive functioning. Using fMRI, we aimed at determining the effects on brain activation in healthy individuals.

Specifically, the aims were as follows:

1. Develop an fMRI task to robustly target mesolimbic motivational system, and examine the effects of incentive motivational salience on activation in mesolimbic structures and on connectivity between the OFC and the mesolimbic system (Study 1).

2. Using the task from Study 1, examine the effects of the two antipsychotic drugs, aripiprazole and haloperidol, on brain activation related to incentive motivational salience (Study 2).

3. Examine the difference in effects of aripiprazole and haloperidol on activation in brain areas involved in executive function (Study 3).
2 MATERIAL AND METHODS

2.1 Participants

All data were collected from healthy volunteers that gave written informed consent. Studies 2 and 3 were based on largely overlapping samples, with 46 of the persons participating in both studies. Study 1 is based on a separate sample. Participants were recruited by advertisement posted at the university and they were financially compensated for their participation. All three studies were approved by the Regional Committee for Medical and Health Research Ethics for South-Eastern Norwegian Health Authority. In addition, Studies 2 and 3 were approved by The Norwegian Medicines Agency and registered in ClinicalTrials.gov.

Persons were excluded if they had a psychiatric diagnosis as assessed with a standardized psychiatric diagnostic interview (Mini International Neuropsychiatric Interview (MINI) (Lecrubier et al., 1997), reported any somatic or psychiatric health issues when asked a standardized set of questions, had taken psychotropic medication the last year or any drugs or medications the last two weeks, or had brain abnormalities as shown by a high resolution structural MRI scan.

In addition, the participants in Studies 2 and 3 underwent a somatic health examination by a physician, were screened for contraindications for aripiprazole and haloperidol (identified by haematology, clinical chemistry and electrocardiography). They were screened for drug usage and females were tested for pregnancy. Inclusion criteria for Studies 2 and 3 were weight between 56 and 94 kg and age between 18 and 56 years.

In Study 1, three datasets were excluded because of excessive movement in the scanner. In Studies 2 and 3, five participants were excluded before randomization and one data set was lost due to technical problems. In Studies 2 and 3 two and three participants respectively, were unable to be scanned due to side effects. Further, three and two data sets, respectively, could not be used due to excessive head movement in the scanner. Information about the included data-sets are given in Table 1.
Methods

Table 1. Participant overview.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age</th>
<th>Sex (males)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>16</td>
<td>26 (6)</td>
<td>8</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>15</td>
<td>26 (8)</td>
<td>8</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>17</td>
<td>25 (7)</td>
<td>8</td>
</tr>
<tr>
<td>Placebo</td>
<td>16</td>
<td>23 (3)</td>
<td>7</td>
</tr>
<tr>
<td>Study 2 (n = 48)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>14</td>
<td>26 (8)</td>
<td>7</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>16</td>
<td>25 (7)</td>
<td>7</td>
</tr>
<tr>
<td>Placebo</td>
<td>18</td>
<td>24 (3)</td>
<td>8</td>
</tr>
<tr>
<td>Study 3 (n = 48)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Studies 2 and 3 are largely based on the same sample: 14, 16 and 16 of the participants in the aripiprazole, haloperidol and placebo groups, respectively, are present in both these studies. Study 1 is based on a separate sample.

2.2 Study design

2.2.1 Procedure

All three studies were approved by the Norwegian Regional Committees for Medical and Health Research Ethics. In addition, Studies 2 and 3 were approved by The Norwegian Medicines Agency and registered as a Phase IV clinical trial in ClinicalTrials.gov (identifier: 2009-016222-14)\(^1\), and was monitored by the central monitoring group at Oslo University Hospital, Ullevål.

All participants were healthy individuals. Study 1 was conducted on a separate sample, and did not involve any intervention. Studies 2 and 3 were based on the same sample, and data were collected during the same session with two different fMRI paradigms. The sample sizes were determined based on considerations regarding statistical power and stringency.

\(^1\) https://clinicaltrials.gov/
The experimental design is depicted in Figure 1. The drug study was a single-blinded three-armed randomized non-crossover clinical trial, where participants received an acute dose of aripiprazole, haloperidol or placebo. After they had passed telephone screening, they had three visits at the hospital. During the first visit they gave written informed consent and were screened for inclusion. The second visit included a structural MR scan that, in addition to screening, was used in the processing of the functional MR images. Participants that were included in the study came back for a third visit where they were randomized into one of the three treatment groups. They received one dose of the assigned drug / placebo at arrival in the morning, and were scanned with fMRI approximately 4.5 hours later. For safety reasons they were closely monitored during the day and remained at the hospital for a total of 12 hours after drug administration.

Figure 1. Procedure flow chart.

2.2.2 Administration of drugs

The antipsychotic drugs were given in their original tablet forms. All participants belonged to either a lower (56-75 kg) or higher (>75-94 kg) weight group. Participants randomized to the haloperidol group were given either 2 or 3 mg of haloperidol (average 0.03 mg/kg; administered as 1 mg tablets) and those randomized to the aripiprazole group were given either 10 or 15 mg of aripiprazole (average 0.16 mg/kg; administered as 5 mg tablets) depending on weight group. Participants randomized to the placebo group received either two or three placebo pills. However, because of observed side effects the dose regime was lowered after four participants in each group
had been scanned, so that the remaining 14 participants in each group received either 1 or 2 mg of haloperidol (average 0.02 mg/kg) or 5 or 10 mg of aripiprazole (average 0.10 mg/kg), dependent on weight group. The doses are described in Table 2.

Table 2. Dose regime.

<table>
<thead>
<tr>
<th>Administered dose (mg)</th>
<th>Resulting dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56 – 75 kg</td>
<td>&gt; 75 – 94 kg</td>
</tr>
<tr>
<td>Aripiprazole</td>
<td>5 / 10</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>1 / 2</td>
</tr>
</tbody>
</table>

Resulting dose given in means and standard deviations. Designation kg refers to subject body weight in kilograms. Mg = milligrams.

2.3 Experimental tasks

2.3.1 Motivational salience task (Studies 1 & 2)

In Study 1 an experimental task inspired by conditioned avoidance was designed to target and study motivational salience, and the same task was used to study antipsychotic drug effects in the motivational system in Study 2. During one trial of the task, the participants would first see either a turquoise or an orange circle against a black background, for two seconds. The circle preceded a target (a yellow star) at which they were instructed to respond by pushing a button as quickly as possible in order to avoid a subsequent sound. One colored circle predicted a loud aversive sound and the other colored circle predicted a low-volume neutral tone. The participants were instructed to act on every target and told that if they were able to press sufficiently quickly they could avoid the sound (both in aversive and non-aversive trials). After the response, there was either 1.5 seconds of sound or silence depending on their response times. The task contained 40 aversive and 40 neutral trials and a sound was presented in approximately 20% of the trials within each condition (the paradigm adapted to individual response times). The participants were told beforehand which color predicted which sound, and that an aversive or a non-aversive sound would be heard in some trials depending on their response quickness, but they were not informed about the outcome schedule. The task design was event-related and the trial sequence was
Methods

randomized across the participants. The inter-trial-interval was jittered between $3 - 7$ s with an average duration of 5 s.

2.3.2 Tower of London task (Study 3)

Study 3 aimed to investigate the effects of antipsychotic drugs on cognition and a task targeting executive functioning was used. The Tower of London (ToL) task (Shallice, 1982) is commonly used in clinical neuropsychology for assessment of executive function. In the current version, designed for fMRI, the participants were presented with a screen depicting two pegboards each with three differently colored balls mounted onto three pegs. The object of the task was to mentally calculate how many moves ($2, 3, 4$ or $5$) were needed to manipulate the balls on the lower pegboard to reach the goal configuration depicted in the upper pegboard, moving only one ball at a time. Answers were indicated by button presses. The task was presented in a blocked fashion. During an experiment block the participants solved as many ToL problems as they could within 32 seconds. Control blocks were comprised of four tasks of eight seconds duration. Before they entered the scanner, all participants had completed a ToL training version to make sure they understood the task.

2.4 fMRI

In the present project we were interested in studying neuronal activity in the human brain. Functional MRI (fMRI) with blood-oxygen-level-dependent (BOLD) contrast is a technique that is widely used to study brain activity in humans. By using BOLD fMRI an indirect measure of neuronal activity can be derived. When a brain region engages in a neuronal process, the activation increases the neurons’ demand for oxygen and energy. The increased demand is accommodated by an increase in local blood supply. For reasons that are not fully understood, there is an overcompensation, so that the active brain region is supplied with more oxygen than it consumes (Logothetis, 2008). Hence, the ratio of oxygenated/ deoxygenated blood is higher in active than resting brain tissue. The change in MR signal that is triggered by neuronal activity is referred to as the hemodynamic response (HDR) (Huettel et al., 2009). Importantly, the oxygenated hemoglobin and deoxygenated hemoglobin have different magnetic properties, and this forms the basis for the signal that is detectable with MRI. Because it lacks one of four possible oxygen molecules deoxygenated hemoglobin
holds a significant magnetic moment. This property creates distortions in its surrounding magnetic field, causing nearby protons to precess at a different frequency, in turn leading to less BOLD-fMRI signal\(^2\) (Thulborn et al., 1982). As active regions have higher ratios of oxygenated/ deoxygenated blood these areas manifest as increased signal in an fMRI image. The signal changes resulting from neuronal activation are very small, usually less than 5\% (Raichle and Mintun, 2006), but nevertheless enable researchers to create maps of neural activation. However, it is important to bear in mind that fMRI is only an indirect measure of neuronal activity based on minute alterations in blood oxygenation, suggesting that conclusions based on fMRI data should be drawn with care.

In comparison with other functional neuroimaging techniques, the spatial resolution of fMRI is good. The spatial resolution refers to the ability to discriminate between details in an image. The minimum volume element in a three-dimensional image is called a voxel. Several issues, such as size of the area of interest (e.g. the entire brain or a smaller region) and the maximum time of image acquisition tolerated by the subject, are considered when the dimensions of the voxels are decided. Typically, 3-5 mm cubes are used for current fMRI studies examining the entire adult human brain. It is worth noting that a typical fMRI voxel (55 mm\(^3\)) in the cerebral cortex may contain as much as 5.5 million neurons and 2.2 – 5.5 × 10\(^{10}\) synapses (Logothetis, 2008).

The temporal resolution of fMRI is poor in comparison to certain other techniques, such as electroencephalography (EEG) which measures the brain’s spontaneous electrical activity during a time interval. Temporal resolution of fMRI is limited by sampling rate as well as to characteristics of the BOLD response. The sampling rate (repetition time; TR) is typically around 2 seconds. In addition, the HDR, reflecting the neuronal metabolic demand, is slow compared to the neuronal activity. It lasts for about 10 s, peaking at around 5 seconds after stimulus onset. Thus, the neuronal activity is estimated based on relatively sluggish responses in the vascular system.

\(^2\) The decay of transversal magnetization as proton spins get out of phase after an MR excitation pulse is described by the T\(_2\) time constant. T\(_2\) decay in addition to local inhomogeneities resulting from local differences in magnetization is described by the time constant T\(_2^*\). It is T\(_2^*\) decay that is exploited during fMRI.
2.5 Image acquisition

All fMRI data were acquired on a 3T General Electric Signa HDxt scanner (GE Healthcare, Milwaukee, WI, USA). The BOLD-fMRI protocol consisted of a T2*-weighted sequence in the transverse plane with the following parameters: repetition time (TR) = 2000 ms; echo time (TE) = 25 ms and flip angle = 78 degrees; matrix = 64; field-of-view (FOV) = 256 mm; slice thickness = 3.5 mm; slice gap = 0.5 mm; slices = 36. One run in Study 1 consisted of 454 (±3) volumes; in Study 2 456 (±6) volumes and in Study 3 292 volumes. Three dummy acquisitions were acquired initially during each scan and discarded. A T1-weighted image series was collected for each participant and used for radiological screening to identify anatomical abnormalities. In addition, in Studies 2 and 3 the structural images were used in the co-registration and spatial normalization of the fMRI time series. This T1-weighted image series had the following parameters: TR = 7.7 s; TE = 3.0 s; flip angle = 12 degrees; slices = 172; slice thickness = 1.2 mm; FOV = 256 mm; matrix = 256; sagittal acquisition. Cushions were placed around the participants’ head to minimize head movement, and they were given earplugs to reduce noise.

The task stimuli were presented through VisualSystem goggles, sound stimuli were delivered through AudioSystem headphones (Studies 1 and 2) and responses collected by ResponseGrips (Nordic Imaging Lab, Bergen, Norway). The E-Prime software (Psychology Software Tools Inc., Pittsburgh, Pennsylvania, USA) was used to program the tasks and control the experiments. Task presentation and MRI uptake was synchronized using SyncBox hardware (Nordic Imaging Lab, Bergen, Norway).

2.6 Image preprocessing

Of particular interest in the present studies were the VS and the PFC. As imaging in these areas are especially prone distortions due to magnetic susceptibility, the data were visually inspected to secure image quality. No data sets were excluded for these reasons. Data pre-processing and analyses was performed using the Statistical Parametric Mapping software 8 (SPM8). Within each volume series, the functional image volumes were aligned to the first volume, and then spatially normalized to the Montreal Neurological Institute (MNI) standard stereotactic space. This was done either directly to a standard echo planar imaging (EPI) template (Study 1) or by using
parameters obtained from the MNI normalization of the individual’s structural image (Studies 2 and 3). The images resampled at a resolution of 3 mm isotropic voxels. Finally, to improve the signal-to-noise ratio and minimize the impact of anatomical differences between participants, functional images were smoothed using an 8 mm Full-Width at Half Maximum (FWHM) Gaussian isotropic kernel. Possible slow signal drift was removed with a high-pass filter with a 128 s cut-off.

2.7 Behavior analysis

The behavioral data were analyzed using Statistical Package for the Social Sciences (version 14 (Studies 1) and 21 (Studies 2 and 3), SPSS Inc., Chicago, Illinois, USA).

2.8 Image analysis

Paper 1

A general linear model (GLM) was built where stick functions representing onset times for Aversive and Neutral trials, were convolved with a canonical hemodynamic response function. To minimize potential signal contamination from movement, trials where sound was delivered were modelled as regressors of no interest, but not used in any contrasts. Individual Aversive > Neutral contrast images were moved to a second-level random-effects group analysis and used in one-sample t-test analyses. As we had an a-priori hypothesis regarding the motivational salience system, voxel-wise small volume corrections (SVC)s were applied within bilateral regions-of-interest (ROI)s. Anatomically defined masks for the VS (Fox and Lancaster, 1994; Nielsen and Hansen, 2004), amygdala, anterior insula and anterior cingulate cortex (three latter: automated anatomical labelling (aal) SPM8 Wake Forest University (WFU) PickAtlas toolbox (Maldjian et al., 2003) were employed. Subsequently, a whole brain analysis with the same contrast (Aversive > Neutral) was performed. All analyses were corrected for multiple comparisons with a stringent threshold of $p_{\text{Family Wise Error (FWE)}} < .05$.

To test our hypothesis regarding alterations in functional connectivity between the mesolimbic system and the OFC related to stimulus salience level, psycho-physiological interaction analyses (PPI) was performed. A PPI analysis can reveal how the correlations in neuronal activity between two brain areas are influenced by a
psychological variable. The left VS and the right amygdala were chosen as seeds in two parallel analyses (anatomically defined as described above). Hence, the psychological variable’s (Aversive vs. Neutral) effect on the seed regions’ correlations with the PFC was examined. In brief, the neuronal time series for the seed region was combined with the psychological variable to derive an interaction term that was then used as the explanatory variable in a GLM. The resulting t-contrasts were entered into a random effects group analysis and tested for statistical significance. A SVC was applied within the left PCF, using a mask including the superior, middle and medial parts of the OFC as defined in the aal WFU PickAtlas toolbox provided through SPM8.

**Paper 2**

To target effects of antipsychotic drugs on motivational salience the experimental task developed in Study 1 was employed, and the within-group image analysis in Study 2 was performed in the same manner as the initial analysis in Study 1. In addition, to explore possible interaction and main effects a 2 (Aversive, Neutral) x 3 (aripiprazole, haloperidol, placebo) flexible factorial model was set up, and corrected values at threshold $p_{FWE} < 0.005$ were used. Possible group effects were explored performing two sample t-tests within the aforementioned ROIs, corrected at peak level threshold $p_{FWE} < 0.05$.

**Paper 3**

This study targeted executive functioning and employed a different experimental task than Studies 1 and 2. The GLM model was built by convolving box-car functions for block onsets in experiment (ToL) and control (Ctrl) conditions, with a canonical hemodynamic response function. Individual contrast images (ToL > Ctrl) were moved to second-level random-effects within-group analyses. To test for task effects one sample t-tests were performed within each group. As we had a specific hypothesis regarding haloperidol vs. aripiprazole an ROI analysis in the PFC was performed, followed by a whole-brain analysis. Activation clusters in the PFC in the placebo group were used to create a mask employed in the ROI group analysis between aripiprazole and haloperidol, ensuring that the mask contained areas specifically targeted by the task employed. In the placebo group, significant (after correcting for
multiple comparison at whole brain level, threshold $p_{FWE} < .05$) task-related activations for the in the OFC were extracted and combined into a mask used in the analysis of effects between the drug groups. The mask, consisting of 971 voxels, was constructed using five clusters in the frontal and cingulate gyri and the insula.
3 RESULTS

Paper 1

Classically, the mesolimbic system has been considered solely as a reward system. However, recent research as reviewed above has shown that it might be better conceptualized as a system processing incentive motivational salience, including both positive and negative stimuli. The OFC has been implicated in motivation, but most studies have been performed with appetitive stimuli. Thus, similar studies have been conducted previously but with other stimulus modalities. The goal of Study 1 was twofold. One aim was to develop further an fMRI paradigm targeting incentive motivational salience by using aversive auditory stimuli, and replicate previous findings. Then, employing this avoidance-based paradigm we aimed to investigate the effect of salience on connectivity between the mesolimbic system and the OFC.

Analysis of mesolimbic brain responses to the different salience conditions yielded findings of increased activation during aversive versus neutral conditions. This corroborates the previously suggested concept of incentive motivational salience, and supports the involvement of this system in the processing of aversive stimuli. Further, the results of a PPI analysis showed an increase in connectivity between the VS and the OFC during aversive conditions compared to neutral conditions. This finding implicates the OFC in motivation in relation to VS function, and this may represent a role for the OFC in associating a cue with the motivational significance of its outcome.

Paper 2

Typical and atypical antipsychotic drugs have different pharmacodynamic profiles, and may induce different behavioral effects, but much is still unknown about their effects on the brain. Study 2 aimed to examine the effects of the dopamine D2 receptor antagonist haloperidol and the dopamine D2 receptor partial agonist aripiprazole on brain activation in the mesolimbic motivational system. To target this system the fMRI paradigm developed in Study 1 was employed. The sample consisted of healthy volunteers randomized into one of the two drug groups or a placebo group.
Results

The behavioral results indicated that the participants given haloperidol were more indifferent towards the salient stimuli (the aversive sound) than the other participants, possibly bearing resemblance to the behavior observed in the CAR animal model. To analyze brain activations, a priori ROIs in the VS, amygdala, anterior insula and anterior cingulate cortex were investigated. Within-group analyses contrasting aversive vs neutral conditions yielded robust activations for all three groups, but with weaker findings in the haloperidol group. Analyses of between group differences revealed a significant difference in activations between the placebo and the haloperidol groups in the right VS.

In summary, the results suggested that the activation in the mesolimbic motivational system was significantly attenuated by haloperidol as compared to placebo, and that aripiprazole seemed to result in activation strengths that were intermediate of haloperidol and placebo. This implies drug-induced alterations in brain activation that are independent of pathology and related to altered dopamine transmission. In addition, this indicates that the method is sensitive and supports the application of pharmacological fMRI to study properties of antipsychotic drugs in humans.

**Paper 3**

Cognitive impairment is a symptom of schizophrenia that is difficult to treat with antipsychotic medication, and such treatment may even lead to decreased cognitive functioning. While Study 2 focused on the mesolimbic motivational system, Study 3 targeted the effects of haloperidol and aripiprazole on executive functioning. Study 3 is based on an almost identical sample, but now the Tower of London planning task was used during the MRI scanning, and comparisons between the two drug groups were performed during analyses. The image analyses of task effects yielded a strong response within each group in the bilateral middle, medial and superior frontal gyri. To examine differences between the drug groups a mask based on task-related activation in the placebo group was used in an ROI analysis in the frontal cortex. An uncorrected whole-brain analysis of effects between the haloperidol and the aripiprazole groups yielded three small clusters in the frontal gyrus, the temporal gyrus and the putamen. However, when correcting for multiple comparisons there was no significant
difference. The lack of difference in Study 3, in contrast to the difference found in Study 2, may reflect the drugs’ differential pharmacodynamic profiles, and how they differ in their effects on specific brain regions.
4 DISCUSSION

4.1 Findings and interpretations

4.1.1 Summary
The three studies that form the basis of this thesis are fMRI studies conducted in samples of healthy subjects. The first study examines normal brain function, while the latter two studies are placebo-controlled intervention studies with acute doses of aripiprazole or haloperidol. The fMRI task designed in Study 1, successfully targeted brain regions involved in motivational salience and it was shown that cortico-striatal connectivity was modulated by incentive motivational salience. With the same task applied in Study 2, we detected brain activation alterations in the VS resulting from treatment with the typical antipsychotic haloperidol. Aripiprazole challenge yielded an activation magnitude in the mesolimbic system that was intermediate of placebo and haloperidol. In addition, using the same experimental set-up and largely overlapping sample, but a different task targeting executive functioning, Study 3 yielded a tendency to poorer performance in the aripiprazole group, but no significant difference in brain activation between the drug groups.

4.1.2 Pharmacological fMRI
There is an increasing number of studies employing pharmacological fMRI (Stein, 2001; Honey and Bullmore, 2004), and as a potential tool in drug discovery and development, this method has gained a lot of interest from the pharmaceutical industry (Borsook et al., 2008). Several pharmacological fMRI studies have examined the dopamine system, and many of these have studied effects of transmission-enhancing substances such as amphetamine or methylphenidate. Others have studied the effect of antipsychotics in patient population or in healthy subjects. By including healthy subjects as participants in a pharmacological fMRI study the possible effect of pathology may be omitted. However, the findings may be difficult to generalize to a patient population.
**Conditioned avoidance response (CAR)**

In **Study 1**, an fMRI paradigm inspired by the CAR task was developed. In the animal model rats are taught to avoid a foot shock by moving to a different chamber of the cage when for example a lamp is turned on. Paralleling this set-up, the current participants were given the opportunity to avoid an aversive event (an unpleasant loud noise) by quickly pushing a button. In the animal model antipsychotic drugs at certain dose range specifically suppress the avoidance behavior while the animal is still able to escape foot shock. The CAR task does not hold any face validity, that is – there is no phenomenological resemblance with antipsychotic treatment in patients, and thus no intuitive explanation for CAR’s predictive ability. However, one proposed theory is grounded in the concept where psychosis is thought to be caused by aberrant incentive motivational salience (Jensen *et al.*, 2008; Heinz and Schlagenauf, 2010). Thus, the selective suppression of CAR by antipsychotics may represent dampened salience assignment. The bold intention behind the paradigm used in **Studies 1 and 2** was to design a procedure where neuronal activity associated with similar behavior could be studied in humans, possibly contributing to bridge the gap between the animal model and the salience model of psychosis. In line with previous literature (Jensen *et al.*, 2003; Vernaleken *et al.*, 2006; Delgado *et al.*, 2009; Pohlack *et al.*, 2012), the results from Study 1 showed that by using aversive stimuli the mesolimbic motivational system, in particular the VS could be targeted. This was replicated in **Study 2**. Interestingly, the behavioral analysis in **Study 2** showed that, in contrast to subjects given placebo or aripiprazole, subjects given haloperidol did not avoid more aversive than neutral events. This may indicate that these subjects perceived the aversive and neutral stimuli as of more similar salience relative to the other two groups. It is possible to speculate that this attitude represents an indifference to the two stimulus types. If that is the case it is possible to draw a parallel to the CAR model where, when given an antipsychotic, rats demonstrate indiscrimination to whether a cue predicts an aversive or neutral event.

The CAR test is a sensitive and specific indicator of antipsychotic efficacy, no matter whether the drug has a typical or atypical profile (Wadenberg and Hicks, 1999; Wadenberg, 2010). Aripiprazole, even though having a pharmacological profile that differs from almost all other antipsychotics, disrupt CAR in the animal model (Natesan *et al.*, 2011). Therefore, it could be argued that if the current fMRI study aims to
imitate/parallel the CAR test, one could expect that haloperidol and aripiprazole would have similar brain response and behavioral outcome. However, this was not the case, although there was a trend towards a difference in BOLD activation between the placebo and the aripiprazole groups. Some have argued that disruption of avoidance by dopaminergic antagonists arise because of motor side effects (Aguilar et al., 2000). This could explain our results. However, a study by Smith et al., 2004 employing computational modeling reported evidence that this explanation is unlikely, and concludes that this rather relates to motivation and incentive salience. Rather, one possibility is that the difference in outcome between the two groups could be biased due to doses not being equipotent. Another probable reason is that the two drugs have different pharmacodynamic profiles, and it is not unlikely that an antagonist and an agonist would impact fMRI measures differently. Nevertheless, the group difference in behavior and brain activation shows that this method is sensitive to drug-induced alterations, supporting that pharmacological fMRI may be a valuable tool for characterizing antipsychotic drugs.

**Cortico-striatal connections**

Abnormalities in fronto-striatal interactions have been found in persons with schizophrenia, possibly identifying a dysfunction in the communication between these two brain regions that have long been implicated in psychosis (Salvador et al., 2010). A recent study reported disturbance in cortico-striatal connectivity to be associated with negative symptoms (Reckless et al., 2015). **Study 1** examines normal functional connectivity between the VS and the OFC and how it is affected by motivational salience. The OFC is reciprocally connected with areas that are important for motivation such as the VS and the amygdala, and it receives multimodal sensory input (Kringelbach and Rolls, 2004). Neurons in the OFC have been found to represent the intensity of a conditioned stimulus in a graded fashion (Morrison and Salzman, 2011). The OFC is thought to play an important role in emotion and motivation, because it is strongly interconnected with the different sensory modalities. Thus, it represents the goals for an individual’s actions (Rolls and Grabenhorst, 2008). Although the VS traditionally has been thought of as a reward center, there is now growing evidence for an implication of this system also during negative conditions (Jensen et al., 2003; Menon et al., 2007; Delgado et al., 2008; Pohlack et al., 2012). The anticipation phase is shown to induce a stronger response, measured as dopamine release in the VS than
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the outcome phase (Schultz et al., 1992; Berridge and Robinson, 1998; Cardinal and Bullmore, 2011). On this background, we investigated whether a cue for an aversive outcome (anticipating a salient event) would have a different impact on cortico-striatal connectivity than a neutral cue. The analysis showed that anticipation of aversive outcome evoked increased connectivity between the two regions maybe indicating that this connection represents the association between a cue (conditioned stimulus) and the motivational significance of this cue.

Mapping of effects

The sample used in Study 3 was largely overlapping with the one used in Study 2, but here they performed the Tower of London planning task. In addition, the statistical approach was slightly different, comparing the two drug groups directly by using a task-activation cluster from the placebo group as ROI. This study did not yield any difference in brain activation within the ROI between the two groups. In the CAR-based paradigm in Study 2 there was a trend difference in the direct comparison between the drug groups in the mesolimbic system. The difference between the two studies’ results is likely to reflect the pharmacodynamic profiles of the two drugs. However, it cannot be ruled out that the lack of control of baseline dopamine tone, or mis-specification of the ROI in Study 3 could have biased the result. A recent study, examining the same drugs in healthy subjects, but using an n-back working memory paradigm, identified group differences in behavior as well as in BOLD activation patterns in frontal and parietal regions (Goozee et al., 2015). The behavioral results in the same study showed that the response time for the aripiprazole group were longer. This is similar to what was found in Study 3 in the current PhD thesis, where the participants in the aripiprazole were unable to respond to as many problems as the haloperidol and the placebo groups. This might be related to the broad pharmacologic profile of aripiprazole that targets several neurotransmitter systems.

Using multiple paradigms within the same sample of participants and within the same fMRI session, as in this thesis, may be beneficial as it promotes the possibility to compare a drug’s effect on multiple cognitive processes and brain functions. Detailed mapping of antipsychotic drugs’ neurocognitive profiles may be beneficial to patients because it may ultimately enable a more tailored pharmacotherapeutic treatment.
4.1.3 Effects of haloperidol and aripiprazole

**Brain activity in the motivational system**

**Studies 2 and 3** are among the first to investigate the effects of aripiprazole on brain activity with fMRI in healthy individuals, while more literature exists on the effects of haloperidol. In study 2 we found that the activations in the mesolimbic motivational system in subjects given haloperidol were reduced relative to placebo. Most evidence of haloperidol action in the VS comes from PET studies, although, some studies have investigated effects of haloperidol in the motivational system in healthy persons using fMRI. Menon et al., 2007 found that while amphetamine increased prediction error signal, it was depleted by haloperidol, indicating that both physiological responses and brain activation are dopamine modulated. This is in line with our results. Another study reported that amphetamine and haloperidol increased functional connectivity within differential networks (Diaconescu et al., 2010). The mesolimbic activations in the aripiprazole group did not differ significantly from those in the placebo group or the haloperidol group. However, regarding statistical strength and number of anatomical structures remaining significant after correcting for multiple comparison, it seemed like the activity was dampened in the aripiprazole group relative to the placebo group, but less so within the haloperidol group. This was expected based on the pharmacodynamics of the two drugs. The potent dopamine D2 receptor antagonist haloperidol reduces dopamine transmission in the mesolimbic system more than the partial agonist aripiprazole. Aripiprazole has some intrinsic effect on the D2 receptor and hence allow for more dopamine transmission than haloperidol (Burris et al., 2002; Shapiro et al., 2003). In addition, aripiprazole is known to have relatively high affinity for presynaptic D2 autoreceptors (Kikuchi et al., 1995). The effect of this on VS neurotransmission would be a possible decreased release of dopamine into the synapse. This effect, together with the relatively moderate antagonistic effect on D2 receptors, suggests that the brain response after aripiprazole should be closer to placebo, as compared to haloperidol. However, the effect on cognition of aripiprazole’s affinity for VS autoreceptors is more complicated.
Discussion

**Brain activity in the frontal cortex**

Study 3 revealed no significant difference in activation, although there were small differences on a nominal level. The samples in Studies 2 and 3 were largely overlapping. This is an obvious advantage when comparing the two studies. However, the two studies used slightly different statistical approaches; Study 2 comparing all three groups, and Study 3 comparing the difference in brain response directly between the two drugs group. The latter approach was chosen based on the idea that between drug-group effects on brain activation might be easier to identify if we were able to examine the region specifically activated by the employed task. We had results in the motivational salience paradigm targeting the mesolimbic system, but not in the executive paradigm targeting PFC. There may be several explanations for this. This may well be a result of the two drugs’ differential pharmacodynamic effects. Different brain regions have different compositions of dopamine receptor types (Missale et al., 1998). The predominant dopamine receptor in the frontal cortex is D1, whereas D2 receptors are rare within the same region. D1 receptors have been found to be essential for cognitive functioning (Goldman-Rakic et al., 2004). Nevertheless, antipsychotic drugs with dominant D2 antagonism may be detrimental for frontal metabolism, while atypicals with a broader profile may be more beneficial (Schlagenhauf et al., 2008; Schlagenhauf et al., 2010). One study suggested that the signaling pathway underlying this is the striato-thalamo-cortical feedback loop. Further, it is theorized that antagonist action on inhibitory D2 receptors increase activation of inhibitory GABAergic neurons projecting to the frontal cortex (Tamminga and Holcomb, 2001). Another explanation for lack of group difference in the frontal cortex in Study 3 relates to baseline dopamine levels. Whether a partial agonist displays antagonistic or agonistic properties under certain conditions, depend on the basal level of receptor activation. Although this has been extensively studied in vitro, molecular properties and detection methods may constitute important issues influencing the ability to accurately map the pharmacodynamic characteristics. (Hoyer and Boddeke, 1993). For Study 3, considering the baseline-dependence of the dopamine effects is of particular importance. In 2003, Mattay and colleagues elegantly showed that the efficiency of the PFC during working memory is dependent on synaptic dopamine levels. It was shown that a polymorphism on a gene that is implicated in endogenous dopamine tone is determinative of whether subjects gain advantage in cognitive performance from
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amphetamine (Mattay et al., 2003). If baseline dopamine level is low, stimulation by amphetamine is positive, but if endogenous levels are high, more dopamine stimulation impairs performance and reduces related BOLD activation. This association has also been shown for attentional processing (Blasi et al., 2005), emotional processing (Smolka et al., 2005) and for executive functioning (Barnett et al., 2007). Thus, in Study 3, the absence of significant differences in frontal activation between the two drug groups might in theory be caused by unequal baseline dopaminergic levels. This is relevant in two respects: One of course being that greater variance in frontal cortical efficiency would yield less chance detecting differences in general. However, this is the case for all cognition studies in healthy subjects. Second, when dealing with a partial agonist, endogenous dopamine levels influences its pharmacodynamic properties. In the frontal cortex, this might result in differential effects of the drug between subjects, depending on their genotype. However, these are speculations, and such a relationship may only be revealed by genotyping the participants. In addition, when interpreting absence of study effect methodological issues must be carefully considered. In Study 3 potential caveats relate in particular to doses and study design (see 4.2.3). A recent study examining the same drugs in healthy subjects, but employing an n-back working memory task reported differences in BOLD activation between the haloperidol and placebo groups, and between the haloperidol and aripiprazole groups (Goozee et al., 2015). Although using a similar procedure this study had a cross-over design and slightly higher doses of haloperidol.

Although affinity for the dopamine D2 receptor seems to be a necessary property of all current efficacious antipsychotic drugs they all have affinities to a range of other neurotransmitter systems in addition, the atypicals maybe more abundant than typicals. Although aripiprazole is an “atypical” atypical, in that its D2 affinity is higher than its 5-HT2A affinity, its pharmacological profile is very different from haloperidol. Whereas the effect of haloperidol is thought to be primarily related to its D2 antagonism, studies have shown that the 5-HT2A antagonism is necessary for a D2 partial agonist to be efficient and tolerable (Swainston Harrison and Perry, 2004). Although, studies in patients are inconsistent (CATIE-, Cutlass-studies), primate studies have brought evidence that atypicals are beneficial for cognitive functioning relative to typicals. Further, it has been shown that the 5-HT2A affinity of atypicals is essential for their low EPS profile (Meltzer and Huang, 2008).
4.2 Methodological considerations

4.2.1 Drugs and dosing

The dosing scheme was designed aiming for doses of haloperidol and aripiprazole in the lower range of therapeutic doses used in patients with psychosis. When performing a psychopharmacological challenge study, using single doses, it is important to aim for study doses that are close to what is used in clinical treatment, but at the same time it is necessary to minimize side effects. In a clinical setting drugs are usually introduced slowly, starting off at a low dose and increasing dosage until reaching the therapeutic window. Thus, in studies with single doses, finding the optimal level might be challenging. During the current experiment, the doses had to be lowered because some of the participants in the aripiprazole group experienced discomfort, and a few were not fit for scanning due to this.

The doses used in Studies 2 and 3 may not have been equipotent. Conversion methods to calculate equivalent doses have been suggested, but are not formally established. Defined daily dose (DDD) calculations might serve a useful purpose in patient studies, as it indicates the medication load put on the patient and level of symptom relief. However, different types of drugs, or combinations of drugs with different pharmacological profiles, may result in identical DDds, and this might not be the optimal measure when performing drug challenge study in healthy subjects. A recent paper with similar set-up that reported differences in BOLD activation used haloperidol doses slightly higher than in the current studies (Goozee et al., 2015).

Knowing the T\textsubscript{max} (the time at which drug serum concentration reach maximum) of the studied drugs is important when planning a clinical trial. The average T\textsubscript{max} for the current drugs are known, but when drugs are orally administered, there are great individual differences due to inter-subject absorption differences. Therefore, collecting data on pharmacokinetics is an advantage, enabling the researcher to interpret the results more reliably. In the current study, in order to identify the T\textsubscript{max} of the two drugs, respectively, three blood samples were drawn from the subjects at 3, 5 and 7 hours after the drugs were administrated. Unfortunately, the analyses of these did not yield results for most of the subjects. Nevertheless, the usefulness of such a measure in
relation to effects in the brain is questionable because the concentration of active substance is not necessarily accurately reflected in the serum concentration.

Even though the current study was single blinded, and the participants received the drugs as original tablets, there is no indication that the participants were aware of the content of the tablet they received. On the contrary, when asked to guess whether they had received drug or placebo, the percentage of participants answering that they had been given drug was equal in the haloperidol and the placebo group (~58%). However, significantly more participants correctly guessed that they had received a drug in the aripiprazole group, probably because they experienced more discomfort.

In contrast to the current study, many clinical trials consist of males only. In general, there is a desire for homogenous samples in order to minimize individual variance. In addition, there is a risk of unknowingly including a pregnant women, and the fluctuating hormone levels in females may in some contexts interfere with the results. Nevertheless, even if mixed samples bring about certain challenges, research relevant for the female population is equally important.

4.2.2 Generalizability

Although it is a great advantage that the current studies are performed in a healthy sample, enabling an examination of drug effects without interfering pathophysiology, some important issues should be considered. During clinical treatment, antipsychotic drugs are administered in a long-term manner, while in the current drug challenge single doses were used. In addition, as in most cognitive studies performed in healthy subjects, the current samples consisted mainly of undergraduate students. In other words, these studies are based on relatively homogenous samples that may not be demographically representative for patients. However, findings from healthy populations are essential in all new drug development as clinical trials are based on results from phase I studies which include healthy participants only. Nevertheless, one should be cautious when generalizing the current results to a patient population, and thus, they should be combined with studies in clinical populations order to provide us with a more accurate understanding of how these drugs affect behavior and the corresponding neural correlates.
Traditionally, specific antipsychotic effects have been thought to have an onset delay by some weeks after initiating treatment. Newer research has challenged this notion showing that antipsychotic effect, which cannot be attributed to secondary nonspecific behavioral effects, is actually observed within hours within 24 hours and then grows with time (Kapur et al., 2005). This has important clinical implications, but it also indicates that the use of single doses in pharmacological challenge studies like the current, may yield information about underlying mechanisms that coincide with antipsychotic effects in patients.

4.2.3 Statistical power

The issue of sample sizes is debated in fMRI research. Although the current studies fulfilled the criteria recommended by Desmond and Glover (2002), larger sample sizes might have been more optimal to detect differences, and type 2 errors in the current studies cannot be ruled out. Further, to reduce inter-subject variability and thus increase power, a within-subject cross-over design would have been advantageous. Specifically, it can be argued that Study 3 would benefit from a within-subject design, as executive functioning is affected by baseline dopamine levels. However, as performance of the Tower of London task is improved by practice, such training effects could have interfered with drug effects.

4.2.4 fMRI method

It is important to remember that the BOLD signal is merely a reflection of the influence neuronal activity has on the cerebral blood flow (CBF). In relation to this, one issue that is of particular importance when performing dopamine manipulation studies and using fMRI, is the fact that dopamine, and dopamine antagonists and agonists may have properties yielding vasoconstriction and vasodilation of brain vessels (Mandeville et al., 2013). Thus, care should be taken when interpreting findings. Further, factors like aging and disease may also affect the cerebral blood flow and thus influence how reliably the BOLD signal reflects neuronal activity (D'Esposito et al., 2003).

When using an ROI approach selecting the region for which to test for statistical effect within is crucial. There are several ways of defining an ROI, but commonly regions are selected from public fMRI software or toolboxes, and described in terms of MNI...
coordinates. This was the approach chosen in Studies 1 and 2. In Study 3 the ROI was defined on the background of activation clusters from the task contrast in the placebo group, with the aim of using an ROI that reflected this specific ToL task. However, by using this approach there is a risk of mis-specifying the ROI, and inducing a bias in the group analysis.

4.3 Conclusions and future directions

There is a need for more studies to further characterize the mechanism of actions of the antipsychotic drugs on market to optimize treatment, and to develop new treatments. Current pharmacological treatment is not optimal, in particular in regard to the efficacy concerning cognition and negative symptoms. Many aspects of these drugs’ effects are still unexplained, and one way to gain more knowledge may be to investigate and compare these effects in healthy samples.

Some suggestions based on this work may be made for future studies. There are certain methodological considerations that may improve quality and statistical power. Firstly, if studying cognitive function it may be beneficial to use a cross-over trial design if the fMRI paradigm allows this. Secondly, haloperidol dose may be possible to increase, without inducing side effects, in order to increase the effect.

In line with previous reports, our Study 2 revealed differential effects of haloperidol and aripiprazole in the motivational mesolimbic system in healthy subjects. Study 3 did not reveal any significant difference in frontal activation between the drugs. This finding is in line with several studies in patients that have shown no difference regarding cognitive impairment between typical and atypical drugs. However, this finding remains inconclusive, as certain methodological issues may have influenced the results. Study 1 and Study 2 corroborate the notion that the mesolimbic system is involved in processing of aversive stimuli, and cannot be conceptualized merely as a reward system, as previously thought. Further, Study 1 showed increased cortico-striatal connectivity related to salience.

Taken together, the results of this thesis suggest that pharmacological fMRI may be a useful tool in the characterization of current antipsychotic drugs and the development of new treatment strategies.
Discussion
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