Acute anti psychotic modulation of the structure and function of the brain

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Acute antipsychotic modulation of the structure and function of the brain

Peter Hawkins

A thesis submitted to King’s College London for the degree of Doctor of Philosophy in Neuroimaging

2017
Declaration

I, Peter Hawkins, confirm the work in this thesis is my own. Any material derived from other sources has been appropriately referenced.
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Abstract

Antipsychotic medication remains the primary treatment for schizophrenia. MRI research has been instrumental in developing our understanding of the therapeutic mechanism of these drugs and how they modulate the structure and function of the brain. However, imaging drug effects is known to be affected by several potential confounds which can alter interpretation of changes measured with MRI.

In addition to studies in patients, single dose investigations in healthy volunteers show changes in brain structure and function. Alterations in standard structural measurement have been observed within a few hours using MRI. These same doses are also capable of causing significant physiological changes such as cerebral blood flow (CBF) which may potentially influence T1-weighted images. Similarly, observed functional changes due to exposure to these compounds may be partly due to indirect effects, such as drug influence on the vasculature. Although these issues are well known, their effect on our ability to accurately image antipsychotic drug effect remains poorly characterised.

This thesis will attempt to bring increased precision to this area by addressing these confounds using single dose administrations of three different antipsychotics (risperidone, olanzapine and haloperidol) in placebo-controlled, double-blind studies in healthy volunteers.

First, quantitative relaxometry was employed to gain a precise measure of T1 within each voxel in addition to standard volumetric analysis. Quantitative measures of blood flow were also collected and assessed alongside structural metrics. A supplementary preclinical study also examined the effect of a single dose of one of the drugs on T1 maps in rats. While dose and drug-dependent effects on CBF were seen, there was no indication of T1 or structural alterations.

Second, the effects of these drugs were assessed on task based activation, specifically within the reward network (which has consistently shown to be dysfunctional in schizophrenia and reliably shown to be manipulated by dopaminergic modulation). The monetary incentive delay task showed clear drug-related differences on both anticipatory and consummatory reward related activation. Importantly these analyses controlled for measures of blood flow and vascular reactivity (by means of a breath hold task), which were also differentially altered by the drugs.
This thesis brings increased knowledge, clarity and precision to our understanding of the effect of these drugs on imaging parameters in both the functional and structural domain, and their effect across different phases of reward processing.
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Chapter 1  Introduction

1.1 Overview and rationale

Antipsychotics have been in widespread use for some 50 years, yet a systems level mechanism of action has not been established. This inhibits our ability to understand the neuropsychological functions antipsychotics can affect and limits our ability to assay novel compounds for their antipsychotic potential. Importantly, these limitations restrict our understanding of the differences between the individual antipsychotics currently in use.

One way to address many of these questions is the simple application of placebo controlled experiments. The aim of this thesis is to apply such experiments to answer questions around the effect these drugs assert on brain structure and function.

Chapter 1 provides a context for this work. It summarises the development of antipsychotic medication with a focus on its interaction with dopamine. Aspects of dopamine dependant function are reviewed and related back to a prevalent theory of the symptomology of schizophrenia. MRI based research of the action of antipsychotics is then briefly reviewed, with specific issues concerning the MR imaging of pharmacological compounds explored. The aims and hypotheses are then presented.

Chapter 2 provides a detailed explanation of the MR methods employed in this thesis and describes the collection of the data used to test the hypotheses put forward.

Chapter 3 is concerned primarily with the influence of the drugs on brain structure. A comprehensive assessment of both quantitative and standard structural images is undertaken, while also assessing the effect of these compounds on cerebral blood flow.

Chapters 4 & 5 move into the functional domain. Chapter 4 explores the effect of these drugs on the anticipation of reward while additionally addressing the impact of any changes in cerebral reactivity and blood flow. Chapter 5 repeats this process for BOLD activation related to reward outcome.

Chapter 6 summarises the major points crossing chapters 3-5 and addresses the hypotheses made. It discusses the findings in the context of the wider literature, highlights limitations of the studies and analysis conducted, and makes suggestions for future work.
1.2 Background

Schizophrenia is a highly debilitating disorder with an estimated lifetime prevalence of around 0.5% - 1% of the population (McGrath et al., 2008, Simeone et al., 2015), rising to 3% for all psychotic disorders (Perala et al., 2007). It is characterised by two main symptom dimensions that appear to follow independent trajectories over time: i) positive or psychotic symptoms, such as delusions and hallucinations; and ii) negative symptoms, such as poverty of speech and blunted affect (van Os and Kapur, 2009). Cognitive dysfunction in the form of memory and attention deficiencies are also common, although the presence of these are highly correlated with the negative symptoms of the disorder (Dominguez Mde et al., 2009).

Schizophrenia carries with it a significantly increased comorbidity and mortality risk (Saha et al., 2007), and the disorder has a profound effect on social functioning. In England alone, schizophrenia has been estimated to cost society £11.8 billion per year (Mangalore and Knapp, 2007, Andrews, 2012), highlighting its extreme human and economic cost. Despite this heavy socioeconomic burden, effective treatment is often elusive and around a third of sufferers remain treatment resistant (defined as failing to respond to separate treatment trials of two or more different antipsychotic compounds). Considerable adverse effects of antipsychotics are also common, and the development of newer, more efficacious pharmacological therapies has stalled in recent years. Various psychosocial interventions have also since been developed with some success (Turner et al., 2014) and a combination of psychological and pharmacological approaches have particular promise (Guo et al., 2010). Nevertheless, the relative efficacy and accessibility of pharmacological treatment preserves its status as the primary treatment for both first episode psychosis and chronic cases of the disorder. Some 9.5 million items classified as antipsychotics in the British National Formulary (BNF) were dispensed at a cost of £140 million by the NHS in England alone in 2015 (this figure not including private prescriptions (NHS, 2017)). Their use off-label for non-psychotic related conditions such as dementia (Marston et al., 2014) or learning disabilities (Sheehan et al., 2015) is becoming more common, with treatment for symptoms of aggression and irritability in a range disorders being one of the largest growth areas (Bachmann et al., 2014, Kalverdijk et al., 2008, Zito et al., 2013).
Despite their enduring use and ubiquitous nature, there are still significant gaps in our knowledge about the exact mechanism by which these compounds provide their therapeutic benefit - even though much of what we know about the illness has been derived from studies observing the action of these pharmacological agents. These studies provide us with important information regarding the mechanisms underpinning reduced symptoms, as well as lack of response to treatment and side effects. This can be described at the molecular and cellular level in terms of targets or cascades, or at the systems level in terms of networks of brain regions – and these studies can therefore contribute to our understanding of the disorder itself at these different levels of detail.

The application of magnetic resonance imaging has been crucial in this respect, offering a non-invasive, in vivo method to observe both the changes that occur in the brains of people with schizophrenia, and the therapeutic and non-therapeutic action of these drugs. There are, however, several caveats to this approach. Accurately observing the effect of these drugs in patients (as is the case in the clear majority of studies) is potentially confounded by changes to brain structure or function that result from other factors, such as illness status. Schizophrenia is a notoriously heterogeneous disorder (Takahashi, 2013, Tsuang and Faraone, 1995) which can make comparisons between different cohorts problematic, and patients will often have differing levels of disease severity, duration and previous exposure to medication (both in terms of dose and subtype). Placebo control or dose/subtype manipulation are also very difficult within these populations due to ethical considerations regarding the need to provide the appropriate drug and dose for the clinical presentation, making tight experimental control of these studies elusive.

Additionally, and more specifically to MRI, observing the action of pharmacological agents using MRI is open to several confounds. The BOLD signal is reliant on intact neurovascular coupling to provide an accurate, albeit indirect, measure of neuronal activity (Hillman, 2014, Buxton et al., 2004). Pharmacological MRI studies examining drug effects will typically interpret changes in the BOLD signal as a drug influence on neuronal circuits – however, any changes could also be reflective of a drug influence on other components of the signal, such as synaptic signalling to blood vessels or the reactivity of the vasculature, and may not be purely neuronal in nature (Iannetti and Wise, 2007). Several studies have indicated that pharmacological manipulation of the physiology of the brain can alter the BOLD signal in the absence of neuronal activity (Choi et al., 2006), while others have
indicated that structural imaging using MR may also be influenced by transient drug induced physiological changes, such as cerebral blood flow (Franklin et al., 2013).

This thesis will explore the influence of three of the most commonly prescribed antipsychotic drugs (each with complementary and contrasting pharmacological profiles) on a range of MRI methods, while attempting to take into account the indirect effects of the drug on this method of measurement. There follows a summary on the current understanding of the mechanism of these drugs, how they interact with the systems believed to be involved in schizophrenia and how this has informed our understanding of the disorder. MRI based research of the impact of treatment on the structure and function of the brain is then discussed, along with the potential confounds of studying pharmacological changes with this method.

1.3 Neuronal communication

The 85 billion-plus neurons present in the healthy human brain are arranged and interconnected to allow for the multitude of complex motor, cognitive and affective functions humans are afforded (Herculano-Houzel, 2012). With an estimated number of connections in excess of 100 trillion (Drachman, 2005), communication between these cells is a critical component of intact functioning of the brain, and chemical transmission is the primary form of communication here. In its simplest form, it allows the electrical signal from a given cell to be transferred across the synaptic cleft (the gap between two neurons) by means of a release of chemical messenger (a neurotransmitter), which on contact with a receptor on the receiving cell can produce a range of desired effects (see Figure 1.3-1).
The different properties of these neurotransmitters and the receptors they bind with allow for a broad and complex range of responses. They can be both excitatory and inhibitory; act in an anterograde or retrograde fashion; or produce diverse signal transduction cascades within the target cell, allowing for a high degree of flexibility and specialisation in their function. As such, dysfunction in this process of communication has been identified as a likely cause for a large range of neurological and psychiatric disorders. A deficiency or excess in the operation of a given neurotransmitter or its associated processes could have profound downstream effects and impact virtually any aspect of brain function. It is this same fact that psychopharmacological agents attempt to exploit to produce some modulatory effect.

1.4 Basic psychopharmacology

Psychotropic drugs can target several different aspects of neurotransmission, and a summary of this relating to a dopamine (DA) neuron is given in Figure 1.4-1. The focus here will be on those drugs which target a class of metabotropic receptors called G-protein-coupled receptors. When activated by their relevant neurotransmitter, or first messenger, these receptors trigger a complex signal transduction cascade. This involves the activation of second, third or fourth messengers within the cell, the final stage being modulation of phosphoproteins which can heavily influence the ability of the cell to communicate with
others, by modifying enzyme activity and promoting or inhibiting the further release of neurotransmitters for example.

Following release, DA stimulation of postsynaptic receptors can result in different signal transduction cascades depending on the receptor subtype stimulated.

Note the presence of presynaptic autoreceptors, stimulation of which typically inhibits further release of DA.

DA may also be broken down by enzymes such as COMT or MAO, or taken up presynaptically by DAT.

**TH:** tyrosine hydroxylase, **DAT:** dopamine transporter, **DDC:** DOPA decarboxylase, **VMAT:** vesicular monoamine transporter 2, **DOPAC:** Dihydroxyphenylacetic acid, **MAO:** Monoamine oxidase, **COMT:** Catechol-O-methyl transferase, **HVA:** Homovanillic acid

![Figure 1.4-1 Dopamine (DA) synthesis and activity in a synapse. Image adapted from Wikimedia Commons](https://commons.wikimedia.org/wiki/File:Dopaminergic_synapse.svg), used under CC BY SA 4.0 license

Drugs with an affinity for a given receptor can bind to the site where a neurotransmitter would usually interact, and produce a variety of effects including imitating (an agonist), blocking (an antagonist) or reducing (an inverse agonist) the effect that neurotransmitter would normally produce (Stahl and Muntner, 2013). Drugs differ in their action by expressing different affinities for different receptor types. Single drugs can express affinities for more than one receptor type over several different neurotransmitter systems, and can be variable in their binding potential, dissociative time or some other pharmacodynamic element, all which means it can have the potential to produce highly variable effects on neuronal communication, and thus brain function.
**1.5 Development of antipsychotic medication**

Initial breakthroughs in the pharmacological treatment of the illness occurred in the 1950s with the development of chlorpromazine. Originally developed for its antihistaminergic properties, it was also found to reduce psychotic agitation with what came to be termed ‘neuroleptic’ effects (Lopez-Munoz et al., 2005). Further breakthroughs were made with the discovery of clozapine and the development of newer ‘atypical’ antipsychotics throughout the 1980s and 1990s, which promised broader therapeutic and reduced side effect profiles (Shen, 1999).

The introduction of the first antipsychotics during the 1950s transformed the treatment and management of people with psychosis, but they were also instrumental in understanding the chemical changes in the brain that underpin the disorder (Matthysse, 1973). Almost all antipsychotics interact with the dopaminergic system to some extent, with pimavanserin, used to treat psychosis in Parkinson’s disease, being one exception (Howland, 2016). Carlsson and Lindqvist (1963) were among the first to make this link when they noted an accumulation of the dopamine metabolite 3-methoxytyramine in mice treated with chlorpromazine and haloperidol, concluding this was due to a blockade of monoaminergic receptors. This first wave of treatments such as chlorpromazine and haloperidol (termed ‘typical’ or ‘first generation’ antipsychotics (FGAs)) are indeed now known to express a high affinity for D2-type dopamine receptors where they act as full antagonists, and subsequent research further highlighted the importance of dopamine in the illness. CNS stimulants such as amphetamine and methylphenidate (which increase synaptic dopamine levels) were observed to induce psychotomimetic effects in both humans (Griffith et al., 1972) and animals (Groves and Rebec, 1976), with these effects found to be attenuated using FGAs (Angrist et al., 1980, Wald et al., 1978).

Further evidence correlating the clinically efficacious dose of FGAs to their D2 receptor affinity (Seeman and Lee, 1975) contributed to the development of the ‘dopamine hypothesis’ of schizophrenia (Matthysse, 1973). Its basic tenet blamed an excess of the neurotransmitter for the disorder, which antipsychotic medication attenuates by blocking its action at D2 receptors (Meltzer and Stahl, 1976). The hypothesis has been reconceptualised several times in the years since (Davis et al., 1991) but the neurotransmitter at its centre is still prevalent within the psychopharmacology research of antipsychotics today (Howes and Kapur, 2009).
1.6 Pharmacology of dopamine

Dopamine is a monoamine neurotransmitter which binds to metabotropic G-protein coupled receptors. There are five known subtypes of dopamine receptor (D1 – D5), which are further divided into two families: D1-like (D1 & D5) and D2-like (D2, D3 & D4). Stimulation of these two families of receptors has opposing effects – D1-like stimulation promotes the production of the second messenger cyclic adenosine monophosphate (cAMP), while D2 receptor stimulation inhibits it. This functional antagonism between D1 and D2-like families has been well characterised within the basal ganglia, with stimulation of D1 receptors exciting medium spiny neurons (MSNs) in the striatum that project to basal ganglia efferents (the direct pathway) while stimulation of D2 receptors has an inhibitory effect on MSNs projecting to the indirect pathway (Lanciego et al., 2012), see Figure 1.6-1. Although a full description of these pathways and interactions is outside the scope of this introduction, it does illustrate dopamine’s significant mediating role in downstream brain function.

Animal histology was instrumental in identifying three major ascending dopaminergic pathways projecting from midbrain dopamine nuclei that are thought to be particularly relevant for the action of antipsychotics (see Figure 1.6-2): i) the nigrostriatal pathway, projecting from the substantia nigra to the caudate and putamen of the dorsal striatum; ii)
the mesolimbic pathway, projecting from the ventral tegmental area to the nucleus accumbens in the ventral striatum; and iii) the mesocortical pathway, projecting from the VTA to the cortex (Moore and Bloom, 1978), with a more widespread distribution than the simplified projection in Figure 1.6-2. The anatomical and functional segregation of these projections has been supported by subsequent studies, although their individual origins may be more mixed within the midbrain than previously thought (Björklund and Dunnett, 2007).

The delineation of these pathways in addition to newer post-mortem and imaging data helped develop the dopamine hypothesis to give a fuller explanation for the disorder, by asserting regional differences in DA dysfunction (Davis et al., 1991). Positive symptoms were proposed to be a result of excessive dopaminergic activity (measured primarily at the time by plasma HVA) in the striatum and mesolimbic system, while negative symptoms were a result of a hypodopaminergic state within the mesocortical pathway. The high proliferation of D2 receptors in the striatum compared to their relative sparsity in the cortex (where D1 receptors predominate) was further evidence for this cortical-subcortical split, as antipsychotic medication expresses a relatively higher affinity for D2.

Regulation of the overactive mesolimbic pathway by antipsychotics was thought to alleviate the positive symptoms of schizophrenia, while the concurrent effects of the medication on the nigrostriatal pathway, involved in movement regulation, was thought to
give rise to the classic extrapyramidal side effects (EPS; such as dystonia, akathisia, and tardive dyskinesia) caused by extended antipsychotic use. Conversely, hypodopaminergic functioning of the mesocortical pathway was linked to negative and cognitive symptoms and therefore relatively unaffected by D2 blockers. Therefore, although D2 antagonism was shown to be particularly effective at treating the positive symptomology of schizophrenia, it remained ineffective at addressing negative or cognitive symptoms.

1.7 Beyond Dopamine

Dopamine dysfunction is unquestionably a large piece of the puzzle and the dysfunction of this system has been convincingly clinically linked to the symptomology of the disorder - a compelling body of neuroimaging studies to date place it in a central role, with a recent meta-analyses of 17 studies indicating considerable effect size increases in presynaptic dopamine synthesis in patients (n=231) compared to healthy controls (n=251) (Howes et al., 2012). However, even these substantial analyses are not straightforward to interpret – by the author’s admission, significant variations between the studies in treatment history, PET tracer, imaging approach and resolution, outcome measures, exclusion criteria, symptomology/illness duration of cohort and additional compounds used prior to scanning may influence the overall effects observed. One major consideration is the effect of previous or current antipsychotic treatment – of the 15 original studies only two of the oldest had completely drug naïve patients (Hietala et al., 1995, Hietala et al., 1999). Although both indicated increases in presynaptic DA function, these studies alone were not sufficient for a separate meta-analysis and therefore the resolution of the precise influence of antipsychotic treatment remains incomplete. These considerations highlight the complications in assessing this population and the issues identified with this meta-analysis are often found when attempting to summarise the wider literature.

Additionally, it has long been accepted that in a disorder as complex and heterogeneous as schizophrenia there is likely more than one neurotransmitter involved in its aetiology. This was strongly evidenced with the development of a second wave of medications, termed second generation antipsychotics (SGAs), or ‘atypicals’, which exhibited a broader neurotransmitter receptor profile and professed to treat a wider spectrum of symptoms with fewer side effects (Leucht et al., 1999).
The major initial breakthrough came with the synthesising of clozapine in the late 1950s. Initially developed as an “antidepressant with neuroleptic qualities”, it was marked out for providing antipsychotic benefits without the EPS normally produced by FGAs (although this was not licensed for use in the US until the 1990s due to the high risk of agranulocytosis (Crilly, 2007)). Clozapine was shown to be a highly efficacious drug, even with those patients that had shown treatment resistance to other antipsychotics (Kane et al., 1988), and there followed an effort to reproduce the benefits of clozapine without its heavy clinical management burden. Critically, clozapine did not display the same level of D2 receptor affinity as FGAs at clinically effective doses suggesting that its affinity for other neurotransmitters systems was contributing to its efficacy. This led to this newer range of atypical drugs which promised more efficacious treatment and fewer side effects (Kerwin, 1994), although there remains some debate over what exactly confers ‘atypicality’.

Initially used as a term to refer to drugs that did not cause catalepsy in animal models (Mackin and Thomas, 2011), atypicals have a broader mechanism of action than their typical counterparts, exhibiting higher affinities for other neurotransmitter systems such as acetylcholine, histamine and serotonin (or 5-hydroxytryptamine (5-HT)). Others ascribe factors such as the speed of dissociation from receptors (Kapur and Seeman, 2001), the ratio of 5-HT2A to D2 antagonism (Meltzer et al., 1989), extent of EPS or regional selective binding as the defining feature of atypicality (Miyamoto et al., 2012). 5-HT2A antagonism (or inverse agonism) is a common feature of atypicals – this feature has been proposed to indirectly increase dopamine transmission in the nigrostriatal pathway by means of blocking the activation by serotonin of glutamatergic pyramidal neurons (Stahl and Muntner, 2013). This then prevents the activation of connecting inhibitory GABAergic interneurons in the midbrain which synapse onto DA neurons, and therefore allows for increased DA release in the nigrostriatal pathway, reducing the incidence of EPS produced by the concurrent blockade of D2 receptors in this area (Stahl and Muntner, 2013). It has also been proposed that this may help address the negative and cognitive symptoms of the disorder by promoting the release of dopamine and/or acetylcholine in the prefrontal cortex (Miyamoto et al., 2012, Horacek et al., 2006). Several atypicals also act with variable potency at other serotonin receptor subtypes, including 5-HT1A agonism and 5-HT6 and 5-HT7 antagonism, which have been proposed to confer pro-cognitive traits potentially due to their interaction with the glutamatergic system (Meltzer and Massey, 2011), although 5-HT2A antagonism is generally seen as the most important factor in addressing both
symptoms and side effects (Amato et al., 2017). An overview of receptor subtypes is given in Figure 1.6-2. This data is based on each drug’s individual affinity for different receptors, and should not be compared across compounds. Here it is used to illustrate the fact that certain antipsychotics such as risperidone are more potent at the 5-HT2 than D2 receptor, while haloperidol is more potent at D2 that 5-HT2A. In addition, this figure highlights that all these drugs bind to multiple receptors.

There are notable inconsistencies within this atypical-atypical distinction however. Amisulpride, a highly selective D2/D3 receptor antagonist with limited affinity for 5-HT2A, is still considered an atypical due to its efficacious clinical profile with reduced EPS, thought to be achieved by its action at presynaptic dopaminergic autoreceptors (Leucht et al., 2002). Clozapine, the prototypical atypical, remains unmatched in its efficacy within treatment resistant schizophrenia (Lewis et al., 2006) but has a low affinity for D2 and a much broader neurotransmitter system affinity than most other atypicals, including adrenergic, histaminergic and muscarinic receptors. There has therefore been debate as to whether this typical-atypical distinction is appropriate - using a broad two level categorisation may be overly simplistic within these groups as there is a marked difference in their receptor profiles. These inconsistencies are further compounded by some studies suggesting that, as a group, they are not as universally promising as first thought in terms of efficacy, with modest improvements over FGAs at best (Leucht et al., 2009), and

Figure 1.7-1 Simplified antipsychotic receptor binding profiles. Adapted with permission from Gareri et al. (2006).
although they do often reduce EPS they produce other side effects such as sedation and weight gain.

These inconsistencies are partly due to problems in attempting to assess drug efficacy and function within such a heterogeneous patient group, and one which exhibits a high degree of variability in individual treatment response and outcome (Tandon et al., 2010). The neurochemistry of schizophrenia remains only partially characterised, as do how the current drugs available produce both desirable and undesirable effects. Dopamine does appear to be extremely important, with the required 60% D2 receptor occupancy threshold for therapeutic benefit a well replicated finding, and receptor occupancy level in humans is a strong predictor of clinical improvement of positive symptoms (Kapur et al., 2000a). It is however clearly not the whole story. Therapeutically effective doses of quetiapine and clozapine do not appear to reach this threshold (Kapur et al., 2000b, Kapur et al., 1999), and it appears highly likely that other neurotransmitter systems have a major part to play. Glutamate for instance, although not directly manipulated by current medications, has long been thought to play a significant part in the disorder, particularly the negative and cognitive aspects (Howes et al., 2015), and emerging research has indicated poor clinical outcome (Egerton et al., 2014) and treatment resistance (Mouchlianitis et al., 2016) are more related to higher cingulate glutamate levels than to striatal dopamine synthesis capacity (Demjaha et al., 2014).

1.8 Dopamine dependant behaviour

If antipsychotics at least partly bring about their therapeutic effects by targeting dopamine, an important question is how this modulation provides its clinical benefit. Dopamine is not the most prevalent neurotransmitter in the brain, but it is possible it has been implicated in more aspects of brain function than any other. Initially held to be most critical in the control of motor function, its importance to several other more complex functions have since been identified, with reward, motivation and reinforcement arguably at the forefront.

Early animal work identified areas of the brain linked with reward processing, with rats producing repetitive behaviours in order to self-stimulate particular areas of the brain that were implanted with electrodes (Olds and Milner, 1954). These areas were subsequently found to predominately contain dopamine neurons (Corbett and Wise, 1980) which would
respond spontaneously to rewarding stimuli (Schultz, 1986) with 75% - 80% of midbrain dopamine neurons responding in a phasic manner to unpredicted rewarding stimuli (Schultz, 2010). Combined with behavioural theories of reinforcement, dopamine was then conceptualised as a mediator of motivation and learning – in its simplest form a rat presses a lever and receives a morsel, which results in ‘rewarding’ dopamine release, and the rat is therefore more likely to perform the same behaviour. Several variations on this theme have been hypothesised (Wise, 2004), but dopamine function has been central to all. Dopaminergic midbrain centres and their ascending striatal pathways have been previously shown to be critical in this behaviour, with animal studies indicating lesioning or dopamine depletion of the striatum (particularly the ventral striatum) reduced approach behaviour for stimuli that had previously been associated with a reward (Parkinson et al., 1999, Parkinson et al., 2002).

In a series of studies using single cell recording (see Figure 1.8-1), Shultz and colleagues indicated that dopamine signalling in response to external stimuli was far more complex than a simple reaction to the presentation of a reward. If a reward was reliably paired with a predicting cue, the midbrain dopamine neurons would no longer respond to the reward, but instead temporally shift to the presentation of the cue, acting as an alerting signal to behaviourally significant stimuli (Schultz et al., 1993). This implies that dopamine is important in not only learning and modifying behaviour in response to rewarding stimuli, but is also used to direct attention to the salient information in the environment to help affect a rewarding or beneficial change to that organism (Berridge and Robinson, 1998).

Dopaminergic activity returns to the ‘presentation’ phase if the reward is larger than the cue indicated (in which case dopaminergic firing increases) or if the reward is less than expected, or absent (in which case dopaminergic firing decreases) (Schultz et al., 1997). The dopamine response was thus conceptualised to code for a reward prediction error signal (RPE) which can be described by the simple difference between obtained and predicted reward, and is utilised to update expectations about the animal’s environment. This RPE has been reliably produced in both humans (D’Ardenne et al., 2008) and animals (Cohen et al., 2012), using a variety of different task paradigms and imaging techniques (Schultz, 2016), with RPE midbrain firing proposed to induce mesolimbic dopamine release in the nucleus accumbens (Nacc) within the ventral striatum (Garrison et al., 2013, Hart et al., 2014).
Prior to any learning, a drop of appetitive fruit juice occurs in the absence of a conditioning stimulus and the unpredicted nature of the stimulus activates the neuron.

After learning a cue is associated with the reward and predicts its occurrence, the dopamine neuron responds to the cue but fails to be activated by the predicted reward itself.

After learning, the conditional cue elicits a response, but the reward fails to occur because the monkey not making the required behavioural response. The activity of the dopamine neuron is depressed exactly at the time when the reward would have occurred. Original sequence of trials is plotted from top to bottom. CS: conditioned, reward-predicting stimulus; R: primary reward.

Figure 1.8-1 Peri-event time histogram and raster of impulses from the same neuron in the midbrain of a monkey. Reprinted from Schultz et al. (1997) with permission.

It is here that the capacity of dopamine function moves beyond something as simple as a neurotransmitter released in response to a pleasant stimulus into something more sophisticated. This signal not only alerts the animal both to behaviourally significant or incentively salient stimuli in its environment (inferring motivation and attentional aspects of cognition) but also allows updating of what is expected from the environment (inferring learning). This has since been extended beyond highlighting just primitive, primary rewards such as food, to include more sophisticated cognitive ‘rewards’. For instance, Bromberg-Martin and Hikosaka (2009) illustrated that macaque monkeys preferentially sought out advance information about upcoming rewards (even if had no effect on the reward itself) and that midbrain dopamine neurons selectively fired in response to receiving this information. They concluded that the same system that drives animals to seek basic reward such as food also teaches the brain to gain information about future events, suggesting dopamine neurons may additionally promote knowledge-seeking.

Dopamine’s contribution to modulating arousal, motivation and action initiation has been well characterised in a recent collection of studies. Syed et al. (2016) employed a task whereby rodents learnt that different auditory cues required them to either move and press a lever or remain still in order to receive a food reward. They reported that dopamine
release in the Nacc (measured using fast-scan cyclic voltammetry) increased in response to the cue indicating movement and a motor response was required, but did not increase in response to the cue indicating inhibition of movement was required for the reward, suggesting that dopamine release may be related to motivating rewarding goal-directed actions. Using microdialysis, voltammetry and optogenetics during an adaptive decision-making task, Hamid et al. (2016) also illustrated that Nacc dopamine release in rodents flexibly alters in response to reward value and availability, again suggesting its role in motivated task engagement. Taken together, these studies indicate that although there may be a role in dopamine release in learning, it also strongly associated with coding for the value of a motivation to promote action (Collins and Frank, 2016).

Dopamine’s precise contribution to learning (via prediction error), ‘wanting’ (via modulation of motivational salience) or ‘liking’ has been a source of much debate (Berridge, 2007, Berridge et al., 2009, Collins and Frank, 2016) and the literature examining these facets of behaviour is vast and outside the scope of this thesis. However, it has been essential in understanding how dopamine dysfunction relates to the symptoms of schizophrenia, and how antipsychotics may provide their benefits. The ability of midbrain neurons to response differentially to a wide array of environmental stimuli and contexts and the highly interconnected nature of dopamine neurons throughout the brain via complex cortico-striatal loops means it is well placed to modulate numerous behaviours including memory, expectation, attention (facets which are all impacted by psychotic illness) in a highly sophisticated manner (Cohen and Carlezon, 2007), and how antipsychotic manipulation of these networks could produce powerful results.

1.9 Aberrant salience hypothesis

Traditionally split into neurodegenerative (Lieberman, 1999) and the now prevalent neurodevelopmental (Fatemi and Folsom, 2009) camps, the various specific theories concerning the aetiology and pathogenesis of schizophrenia are extensive and outside the focus of this discussion (but see Owen et al. (2016) for an overview). However, particularly relevant to this thesis is the aberrant salience hypothesis (Howes and Nour, 2016, Kapur, 2003, Swerdlow and Koob, 1987, Schmajuk et al., 2001), a compelling biobehavioural theory linking the operation of dopamine back to the development and presentation of psychotic symptomology.
In schizophrenia, excessive dopamine levels may cause the dopaminergic reward system described above to malfunction, impairing the individual’s ability to pick out and attend to rewarding or salient information in their environment, or by misattributing irrelevant stimuli as behaviourally significant. Psychotic symptomology is therefore generated as higher order cortical processes attempt to make sense of these signals in the context of that individual’s cultural and environmental experiences, leading to the idiosyncratic hallucinations and delusions that are consistent with a psychotic episode. According to this theory, antipsychotics provide their therapeutic effect by suppressing this aberrant salience attribution (Kapur et al., 2006), primarily by blocking the action of dopamine at D2 receptors.

Directly linking the behavioural clinical improvement of patients treated with antipsychotics directly to this attenuated salience attribution is difficult, although patient self-reports of a sense of being detached from symptoms rather than them being eradicated (Mizrahi et al., 2005) conceivably match up with this underlying process being supressed. A particularly persuasive element of this theory is that in many cases it does appear to mimic the trajectory of the early stages of the disorder. Patients in the prodromal phase of the illness often report a period of having a vague or subtle sensation of being ill at ease - of something being not quite right that they cannot put their finger on - which may be their experience of these misfiring signals ‘from below’ (Fusar-Poli et al., 2008). Once a narrative is formed around these aberrant signals to make sense of them, the delusional thinking is crystallised, leading to the clinical onset of psychosis. Pharmacological treatment essentially breaks this link and stops the ‘support’ for the narrative, allowing circumstances for the aberrant salience to extinguish (Kapur et al., 2005b). For instance, animal studies have shown that antipsychotic treatment reduces the condition avoidance response (CAR) in rats (Li et al. 2004) which has been characterised as the intervention of the medication reducing the salience of the stimulus which would normally produce such a response.

It is prudent to note here that there is risk of conflating a range of potential environmental experiences as all being ‘incentively salient’, all of which are processed with the same dopaminergic response. Clearly, human interaction with the environment is far more nuanced than this, with salience conceivably including hedonistic, appetitive, aversive, and surprising events (Winton-Brown et al., 2014). There is still debate regarding the specificity
of this response (Ungless, 2004), such as whether this population of midbrain neurons primarily react to rewarding events (Fiorillo et al., 2013, Schultz, 2016) or whether they are diverse in their response capability (Roeper, 2013, Collins and Frank, 2016), with some research suggesting there are distinct populations and pathways of neurons encoding for different events (Bromberg-Martin et al., 2010). However, for clarity, and given the reward response is one of the most reliable and well replicated phenomenon produced in experiments of this kind, the focus of this thesis will be on reward related processing elicited by the Monetary Incentive Delay task (MID; described in next section) and antipsychotic modulation thereof.

1.10 MR imaging of antipsychotic modulation of reward

Much of the evidence cited so far has primarily been from the preclinical domain, which is highly informative terms of the basic neuroscience of antipsychotic drugs and the neuronal systems they operate on. However, the more nuanced effects these drugs have on complex aspects of human cognition requires non-invasive inspection in humans, which MRI allows. The utility of using MRI to study these compounds in vivo has obvious benefits. By observing, for instance, localised changes in blood flow or deviations in grey matter volume after treatment with these drugs (and with comparison with an appropriate control group), it improves understanding of the sites of functional modulation of these drugs. MRI can therefore be highly informative in terms of how these brain systems change both functionally and structurally with treatment. In view of the theories of schizophrenia outlined above, task based fMRI allows the utilisation of cognitive systems thought to be disrupted in the disorder, such as reward or salience processing, and how they may be affected by antipsychotics.

Research into antipsychotic modulation of reward function is multifaceted, even when limited to just the fMRI literature (see recent review by Martins et al. (2017) of pharmacological dopamine manipulation of reward related function). There are several considerations if attempting to get a ‘clean’ understanding of a given drug, and there is a high degree of variability within the literature. For instance, studies often use varying task paradigms which are geared towards different stages or elements of reward processing (such as reward anticipation or reward prediction error), or may be more focused on other reward related cognitive processes such as motivation, learning or attention. Varying
analysis methods are often employed, with differences in the contrasts set between task conditions and differing statistical analysis approaches employed.

This thesis will focus primarily on research using the Monetary Incentive Delay (MID) task (Knutson et al., 2000). A full description will be given in the methods chapter, but in brief, this task typically comprises of an anticipatory phase (during which the participant is primed to receive a potential reward), a response phase (during which the participant responds to a stimulus in order to receive the reward) and a consummatory or outcome phase (during which feedback on the reward is delivered). Variations on the task include introducing elements of punishment or varying the complexity of the response phase (from requiring no response from the participant (modelling Pavlovian conditioning), to responding to a complex rule based array (to model varied elements of salience or learning).

The task can be broken down into two major sections of interest: 1) anticipation and 2) consumption of reward, which do appear to rely on different systems (Rademacher et al., 2010b). During anticipation of reward, the task has been shown to reliably activate the ventral striatum/nucleus accumbens, caudate, putamen, thalamus and dopamine midbrain areas (Knutson et al., 2001, Knutson and Greer, 2008, Wittmann et al., 2005). The responses to the outcome or receipt of reward are less consistent, but the ventral striatum does not appear to be as readily involved (Knutson et al., 2001), with medial prefrontal areas instead being activated (Knutson et al., 2001, Knutson et al., 2003) – although many of these studies report activation in both areas across both phases as illustrated in Table 1.10-1, highlighting the issues caused when introducing subtle differences in paradigm or analysis methods between studies.
Table 1.10-1: Selected summary of studies using MID based paradigms in healthy volunteers.

<table>
<thead>
<tr>
<th>Study &amp; Year</th>
<th>Paradigm</th>
<th>Sample</th>
<th>Analysis</th>
<th>Anticipation</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knutson <em>et al</em> (2000)</td>
<td>Standard</td>
<td>12</td>
<td>VOI</td>
<td>↑BL Cau, Put, mPFC (single regressor for both phases)</td>
<td></td>
</tr>
<tr>
<td>Knutson <em>et al</em> (2001)</td>
<td>Standard</td>
<td>9</td>
<td>WB p&lt;0.0001 uncor., VOI</td>
<td>↑BL Nacc, Cau, Thal, mPFC; L Put; R Amy</td>
<td>↑ Thal, vmPFC (VOI only) ↓ R Put, OFC, parietal</td>
</tr>
<tr>
<td>Knutson <em>et al</em> (2003)</td>
<td>Standard</td>
<td>12</td>
<td>WB p&lt;0.0001 uncor., VOI</td>
<td>↑BL Nacc, Cau, Thal, mPFC; R Ins</td>
<td>↑ Pcc, parietal</td>
</tr>
<tr>
<td>Wittmann <em>et al</em> (2005)</td>
<td>Adapted memory task</td>
<td>22</td>
<td>WB p&lt;0.0005 uncorr.</td>
<td>↑ BL Put, Nacc; R Cau, Ins, Acc, SN</td>
<td>↑ R mPFC; Acc, Pcc, Thal</td>
</tr>
<tr>
<td>Abler <em>et al</em> (2006)</td>
<td>Probabilistic cue &amp; rewards</td>
<td>11</td>
<td>WB p&lt;0.001, cluster corr p&lt;0.05</td>
<td>↑BL Nacc, Thal</td>
<td>(reflecting RPE) ↑ BL Nacc, L mPFC, medial temporal cortex</td>
</tr>
<tr>
<td>Preuschoff <em>et al</em> (2006)</td>
<td>Adapted cue uncertainty</td>
<td>19</td>
<td>WB p&lt;0.001, uncorr</td>
<td>↑ BL Nacc, Put; L SN</td>
<td>↑ BL Cau, Thal, superior frontal gyrus, pCC</td>
</tr>
<tr>
<td>Dillon <em>et al</em> (2008)</td>
<td>Standard</td>
<td>8</td>
<td>WB p&lt;0.001 MC corr, various con masks</td>
<td>↑ Acc, R Nacc, L Put</td>
<td>↑ BL OFC, vmPFC</td>
</tr>
<tr>
<td>Knutson &amp; Greer (2008)</td>
<td>Varied</td>
<td>21 studies</td>
<td>ALE Meta-analysis</td>
<td>↑ (over outcome) BL Nacc, Thal, R Ins, L Cau</td>
<td>↑ (over anticip.) L mPFC, Amy, Put, R Cau</td>
</tr>
</tbody>
</table>

Cau: Caudate; Put: Putamen; A/Pcc: Anterior/Posterior cingulate cortex; (v)mPFC: (ventro)medial prefrontal cortex; Ins: Insula cortex; Thal: Thalmus; Amy: Amygdala; Nacc: Nucleus accumbens/ventral striatum; SN: substantia nigra; WB: Whole brain; VOI: Volume of interest. Main reward win anticipation/outcome results reported only.
Several variants of the MID have since been developed to assess various facets of reward function, and reward anticipation again reliably involves the ventral striatum in these tasks, with this response also featuring information about the probability (Abler et al., 2006) and magnitude (Preuschoff et al., 2006) of the expected outcome. Reward outcome phases also often report more inconsistent results, partly because the outcome phase tends to be more variable in terms of paradigm design than the anticipatory phase across studies, including addition of gamble elements introducing outcome uncertainty (Preuschoff et al., 2006); different probabilities of win not dependent on performance (Abler et al., 2006); unexpected and/or surprising events (D’Ardenne et al., 2008, Ramnani et al., 2004); and variability in definition of fMRI contrasts with control conditions (Dillon et al., 2008). The consummatory phase itself is a complex period with multiple processes occurring, including the response to the reward itself (including information about its pleasurable or value), the evaluation against expectation, integration into memory and preparing for the next trial, and activation here can be influenced by other brain systems such as in participants employing cognitive control strategies which may modulate prediction error encoding in the ventral striatum (Staudinger et al., 2009).

Importantly, areas typically activated closely marry up with the anatomical architecture of the dopaminergic reward system (Haber & Knutson, 2010), and these hubs appear further subdivided based on more specific and nuanced aspects of reward functioning. For instance, the striatum itself also appears to be functionally subdivided for certain aspects of reward process, with dorsolateral, dorsomedial and ventral striatal regions coding different elements of classical and operant reward based learning, or tracking prediction of reward (for review see Chen et al. (2015)). The two main phases of the standard MID task (anticipation and outcome) are also thought to rely on functionally different systems, and reflect dissociable components of reward (Berridge et al., 2009) although the extent dopamine differentially mediates these processes is still debated (Kumar et al., 2014). The anticipatory phase is associated with motivational processes that drive achievement of a desired goal (or the feeling of ‘wanting’), whereas the consummatory phase has been proposed to be more reflective of the experience of pleasure (or the feeling of ‘liking’) (Dillon et al., 2008). It has been argued that dopamine is more important for incentive salience or ‘wanting’ rewards, but less so for ‘liking’ them (Berridge and Robinson, 1998), although adding more layered elements to the outcome phase such as punishment, unexpected or surprising events, or probabilistic outcomes (to produce RPEs for instance)
adds complexity to the response. This partly highlights the problem at assessing variations of similar paradigms to gauge overall function in that it may create “can’t see the wood for the trees” situation. It is perhaps partly for this reason the relative impact of antipsychotics on these processes is still poorly characterised, particularly within the outcome phase (see below), and why this thesis will focus on the most common form of the MID task employed to date to allow more straightforward comparison with the existing literature.

There is in fact an extensive fMRI literature exploring the effects of antipsychotics on reward related activation, typically involving patient cohorts in various stages of the disorder. A major issue in interpreting such studies is disentangling the contribution of the disorder from the contribution of the medication. The reward network has been shown to be dysfunctional in individuals with schizophrenia, potentially outside the effect of medication. Several studies employing the MID task or similar variants have reported reduced ventral striatal activity in medication-naïve patients during reward anticipation (Juckel et al., 2006b, Esslinger et al., 2012), in addition to attenuated activity in other reward related areas such as the ACC and midbrain (Nielsen et al., 2012b), and the extent of these deficiencies has been shown to correlate with both positive (Nielsen et al., 2012b) and negative (Juckel et al., 2006b) symptoms. Reduced activation in the ventral striatum during reward anticipation on the MID has also been reported in first-order relatives of patients (Grimm et al., 2014) and unaffected siblings (de Leeuw et al., 2015) – additionally these siblings also exhibited a correlation between decreased ventral striatal signal and sub-clinical negative symptoms suggesting this effect is related to the genetic underpinning of the disorder.

Interestingly, in the latter of these studies (de Leeuw et al., 2015), reward activation was differentially affected over the course of the task. Siblings of patients showed reductions in activity during anticipation, but then exhibited increased activation in the ventral striatum compared to controls during reward outcome. As the responses in this region during anticipation and outcome were correlated, the authors proposed the suppression of the reward signal was instead being expressed during reward outcome. Processing of the outcome phase of the task does appear widely disrupted in unmedicated patients, with deficiencies in ventral striatal signal during avoidance of loss (Schlagenhauf et al., 2009) and exaggerated responses to both non-rewarding events (Nielsen et al., 2012b) and omission of expected reward (Schlagenhauf et al., 2009) in the dorsolateral and medial
prefrontal cortex (DLPFC/mPFC) respectively (although the Schlagenhauf cohort contained a mix of drug naïve and previously medicated patients).

Although there are some inconsistencies in the literature, a recent meta-analysis of 478 patients against 439 healthy controls across 23 studies reported significant hypoactivation in bilateral ventral striatum during reward anticipation, with an association between left ventral striatal reductions and negative symptoms. They also reported hypoactivation in the same region during reward outcome, albeit with a reduced effect size and sample (183 patients vs 175 controls). Although cumulatively these studies included variably medicated patients at different stages of illness using a range of methodological and analysis approaches, no effects of age or antipsychotic medication use were found and the authors concluded the reductions in activation observed are not purely a result of treatment (Radua et al., 2015).

However, antipsychotics do appear to make a unique contribution to some of the functional brain changes seen in schizophrenia patients (Snitz et al., 2005, Fusar-Poli et al., 2007) and some studies have indicated that this is specifically true of reward related processes in patient populations. Higher doses of medication were shown to be associated with reduced prediction error related activation in the mPFC and striatum during a reward reinforcement learning task in a patient cohort (Insel et al., 2014). Smieskova et al. (2015) reported medicated first episode patients exhibited reduced bilateral cingulate activation compared to an at risk mental state (ARMS) group and reduced insula activation compared to healthy controls in response to reward salient cues in medicated first episode patients.

A handful of studies have used a longitudinal design to explore the influence of antipsychotics more directly. Nielsen et al. (2012a) reported the reduced ventral striatal response in 23 antipsychotic naïve patients at baseline was subsequently found to be indistinguishable from controls after 6 weeks of treatment with amisulpride (a moderately specific D2/D3 antagonist), with a similar trend found more recently in a larger cohort of 39 medication naïve patients (Nielsen et al., 2016). However, these studies used different paradigms such as the salience attribution task (SAT; Roiser et al. (2009)) to evaluate putatively more selective elements of reward processing, while the Nielsen studies used a modified version of the MID to introduce extra levels of uncertainty to the anticipatory phase. As previously mentioned, the differences in task paradigms used, which are
sometimes subtle, and variation in the grouping together of different events in contrasts of interest (Nielsen et al., 2012a) can make collating the trend in the literature with regards to the influence of antipsychotics problematic.

In those studies that have used the standard version MID, the subtype of drug appears to be of particular importance in how reward related activation is modulated. Juckel et al. (2006a) examined patients on either typicals (n=10; flupentixol (4), haloperidol (4) or fluphenazine (2)) or atypicals (n=10; risperidone (4), olanzapine (4), aripiprazole (1), or amisulpride (1)) compared to healthy subjects on the MID task and found patients treated with atypicals showed ventral striatal activation during reward anticipation (as did healthy controls) while patients on typicals did not (although direct head-to-head comparisons between the treatment groups were not reported). Kirsch et al. (2007) similarly found reduced activation in 13 patients treated with both typical antipsychotics in the ventral striatum during reward anticipation compared to 17 patients treated with atypicals. Schlagenhauf et al. (2008) found a similar reduction in ventral striatum activation during reward anticipation in 10 patients treated with typical antipsychotics compared to controls, but the same pattern was not observed after switching the patients to an atypical antipsychotic (olanzapine) after 4 weeks. Although some of these studies have relatively small sample sizes, when taken together (and along with the large sample Nielsen group studies) this does highlight the importance of drug subtype, and suggests the receptor profile of the medication may be more influential that the disease state itself on the BOLD response. Nevertheless, these studies are unable to remove the confound of illness altogether. Observing the modulatory effect dopaminergic medication has in healthy human allows for this, in addition to affording a higher level of experimental control.

1.11 Acute dopaminergic modulation in the healthy brain

Studies of this type in healthy humans typically use a single dose of dopaminergic agonists or antagonists. Although these results are not comparable to studies looking at the effects of longer term use, antipsychotics have been shown to produce rapid functional effects (Kapur et al., 2005a), so single dose studies can still be informative in a pharmacological context.
In the healthy human brain, the dopamine precursor L-3,4-dihydroxyphenylalanine (L—DOPA) has been shown to increase connectivity of reward networks at rest, with haloperidol reducing connectivity (Cole et al., 2013). Various task paradigms have also shown pharmacological agonism and antagonism of D2 receptors to have counteractive effects on prediction error signalling (Menon et al., 2007, Diederen et al., 2017, Pessiglione et al., 2006) and learning related activation (Jocham et al., 2011) – again see Martins et al. (2017) for review of these and similar studies. Fewer studies have studied the effect of pharmacologically manipulating dopamine purely in terms of processing reward anticipation or outcome on the standard form of the MID task, although Admon et al. (2017) reported a single dose of amisulpride caused a reduction in caudate to dACC connectivity during reward outcome in a healthy control group, using a dose to putatively increase dopamine transmission by preferentially blocking striatal presynaptic D2/D3 autoreceptors and limiting the likelihood of postsynaptic blockade.

Many of the studies above used selective D2 antagonists, allowing for a more selective manipulation of the dopamine system and it may be experimentally desirable to study drugs with a selective profile in terms of interpreting outcomes. However, most contemporary antipsychotics have a broad receptor profile, and therefore drugs selective for a particular neurotransmitter system do not give a full account of how many of these ‘dirty’ drugs that are used in clinical practice operate in vivo. As seen above, the specific subtype does appear to have a profound effect on the neural signature for reward processing, in patients at least (Juckel, 2016).

In terms of acute dosing studies in humans using the MID specifically, Abler et al. (2007) gave eight healthy volunteers a single clinical dose of olanzapine (the second most commonly prescribed antipsychotic by the NHS, which expresses an affinity for dopamine, 5-HT, alpha-1, histamine & muscarinic receptors) in a repeated measures placebo controlled design. Using a version of the MID that included low and high potential wins, they reported that olanzapine extinguished the difference in the ventral striatal response between magnitude of reward seen in the placebo session (although by the authors own admission this was using a small sample with liberal statistical thresholds). Conversely, Mathews et al. (2012) tested healthy participants before and after 1 week of olanzapine on a version of the MID which incorporated the delivery of different tastes as levels of reward (tomato juice, chocolate milk, or tasteless water). Olanzapine was found to increase the
BOLD response during anticipation of rewarding tastes in the inferior frontal cortex, striatum, and anterior cingulate cortex, while also increasing reward receipt responsivity in the caudate and putamen and decreasing it in the lateral orbital frontal cortex. It should be noted that this study was chiefly examining neural correlates of antipsychotic induced weight gain over an extended period, and the physical delivery of rewarding tastes (a primary reward) is clearly qualitatively different to the indirect reward of money (a secondary reward) in the standard MID (Sescousse et al., 2013). More recently however, Dean et al. (2016) administered 150mg of bupropion (an antidepressant dopaminergic and noradrenergic reuptake inhibitor) to 17 healthy volunteers for 7 days in a placebo controlled trial, and reported increased activity in the mPFC and caudate during anticipation and increased activity during the outcome phase in the orbitofrontal cortex, amygdala and ventral striatum. The similarity in results across both stages of reward processing between these last two studies is interesting, given bupropion’s dopamine reuptake inhibitor qualities would presumably increase synaptic dopamine levels in comparison to olanzapine’s D2 antagonist qualities, and does perhaps point to the other receptor features of olanzapine as being critical in this context – although again the difference in paradigms used in these studies is enough to prevent tight comparison.

These inconsistencies make interpretation of the precise action of different subtypes of antipsychotics on reward processing problematic. A particular paradox is the reduction of reward anticipation activity in the ventral striatum of unmedicated schizophrenia patients (Radua et al., 2015) follows the same pattern as seen in (an albeit small sample of) healthy individuals given a single dose of the drug and performing a similar reward task (Abler et al., 2007)) which raises questions about how this transfers to the drug’s clinical effect. One interpretation of these findings in relation to the excess striatal dopamine present in schizophrenia is that the phasic dopaminergic related activity associated with reward learning is ‘drowned out’ by the high tonic baseline level, resulting in reduced BOLD responses (Heinz and Schlagenhauf, 2010). This notion is lent weight by Knutson et al. (2004), who used the indirect dopamine agonist dextroamphetamine to increase dopaminergic tone in eight healthy individuals, and found a similar reduced reward anticipation BOLD signal in the ventral striatum. A putative action of medication may be to correct this imbalance.
Indeed, alterations in the firing rate of dopaminergic neurons appear particularly relevant in this context, as they mediate the characteristics of dopamine release (see Figure 1.11-1). Baseline dopamine midbrain firing follows a slow and irregular pattern, and is associated with tonic, extra-synaptic dopamine release. Phasic burst firing on the other hand results in high-amplitude, intra-synaptic release (Floresco et al., 2003).

Under normal conditions, midbrain dopamine neurons fire tonically in a slow and irregular pattern, and only around half of the neurons in the VTA/SN fire. This sets the baseline tone of the dopaminergic system, as only active neurons can increase their firing rate. Non-active neurons held silent by GABAergic inputs from the ventral pallidum (VP) can therefore set the tone, or possible level of amplification of the phasic signal. Depending on the context (e.g. threatening v non-threatening), the VP can alter the number of active dopamine neurons, thus allowing an alteration in responsiveness when transferring to a phasic burst pattern in response to a rewarding or salient stimulus (Grace, 2016).

In situations whereby tonic dopamine release occurs independently of the controlled firing pattern of the neurons (due to the potential influence of a disease or drug on the system, for instance) this ‘excess’ dopamine will stimulate autoreceptors and inhibit further dopamine release driven by phasic activity. It is important to point out that this interpretation is necessarily conjecture as it is difficult to actually measure these features in

Figure 1.11-1 Tonic and phasic patterns of dopamine neuron firing and dopamine release. Reprinted from Grace (2016) with permission.
humans in vivo. What this does illustrate however is observing the action of the drug in clinical populations cannot therefore to be as readily transferred to its action in a ‘clean’ system as the baseline firing characteristics may be different. This promotes the case for study in healthy individuals, and specifically the within-subject differences between subtypes to pick apart their mode of action.

1.12 Acute structural changes due to antipsychotic exposure

The effects of antipsychotics are not limited to the functional domain. Although debate continues around the underlying cause and dynamics of the structural brain changes that have been associated with schizophrenia (Nenadic et al., 2015, Filippi et al., 2014, Chan et al., 2011, Zhang et al., 2015, Torres et al., 2013), there is a consistent body of evidence that indicates antipsychotics contribute to these changes in the long term (Ho et al., 2011, Lieberman et al., 2005). Recent meta-analyses have attributed more of the observed change in brain structure over time to antipsychotic exposure than to other contributing factors such as duration of illness, illness severity, age or substance use (Fusar-Poli et al., 2013, Vita et al., 2015, Haijma et al., 2013). Drug subtype and dose appear to be important factors, with typicals, such as haloperidol, associated with greater increases in basal ganglia volumes and more pronounced cortical thinning compared to atypicals such as risperidone and olanzapine (Vita et al., 2015, Lieberman et al., 2005, Corson et al., 1999). Many of the areas most affected are regions of the brain expressing a high density of dopamine receptors such as the striatum.

Again, identification of the unique contribution antipsychotics make to these structural brain changes is inherently problematic to study in patient populations, where placebo control and manipulation of dose and drug type are not fully possible. However, several well controlled animal studies have indicated that chronic exposure to antipsychotics at clinically relevant doses may cause structural changes over time in the absence of disease pathology, mapped both post-mortem (Dorph-Petersen et al., 2005) and through longitudinal in-vivo MRI assessment (Vernon et al., 2011). Notably, these data reflect similar patterns of cortical reductions and subcortical increases in volume that are seen in some clinical studies (Vernon et al., 2012, Vernon et al., 2014).
These observed changes in brain structure due to antipsychotic medication may occur on a far more rapid time scale than previously thought. Recent studies have shown dopaminergic medication causing apparent changes in grey matter (GM) volume or density in healthy humans as soon as 1-2 hours after administration (Salgado-Pineda et al., 2006, Tost et al., 2010), typically in areas heavily innervated by midbrain dopaminergic neurons. These data resonate with other reports of acute administration of baclofen (Franklin, et al., 2013), lithium (Cousins, et al., 2013) and even cigarette smoking (Franklin et al., 2014) causing apparent rapid localised changes in GM volume or density. Similar reversible changes following single doses of either sodium valproate or levetiracetam have been reported in rhesus monkeys (Tang et al., 2015). Changes on this time scale are not limited to pharmacological interventions – other factors such as dehydration (Duning et al., 2005, Kempton et al., 2011), learning (Kwok et al., 2011) and environmental enrichment (Scholz et al., 2015) have been shown to rapidly influence in vivo measures of brain volume when measured using longitudinal MRI. There is also evidence that this apparent MR measured plasticity is correlated with histologically assessed structural changes (Lerch et al., 2011, Blumenfeld-Katzir et al., 2011, Sagi et al., 2012).

Changes on this relatively short time scale after single dose administration given the millimetre resolution that MRI offers are nevertheless surprising given what is already known about brain structural plasticity. This does raise questions as to what the potential biological correlate of these measured changes could be, and/or whether there are other potentially confounding factors contributing to these signal changes. This is true for both the structural and functional applications of imaging drug action.

1.13 Considerations for functional imaging of antipsychotic effects

Implementing a placebo controlled design with healthy volunteers provides considerable benefits when attempting to gain an interpretation of the action of these drugs while minimising potential confounds. However, our measuring tool of choice may also be influenced by the presence of these compounds.
A fuller description of the relevant principles of MR imaging and the BOLD contrast will be given in the methods chapter. Briefly however, the BOLD signal is derived from the magnetic field inhomogeneity created due to changes in the relative concentrations of oxygenated and deoxygenated haemoglobin in blood vessels serving the brain. As a measure of brain activity, it is based on the principle that active neurons carry with them an energy supply requirement, which is satisfied by a local increase in blood flow and oxygen supply (Attwell, 2010). To allow this, active neurons and supporting glial cells signal to the local vasculature that controls cerebral blood flow. This results in a vascular response which allows for an influx of oxygenated blood, subsequently giving a detectable change in the BOLD signal. This complex chain of events is referred to as neurovascular coupling and is an essential assumption of relating the BOLD signal back to its putative underlying neural activity.

In the context of drug studies however, interpreting change in the BOLD signal is more complex as the drug could be influencing one or more of the elements within this cascade. An undetected drug induced increase or decrease in baseline blood flow, vascular signalling or vascular responsiveness could therefore either produce a BOLD response in the absence of underlying neural activity, or ‘mask’ a neuronal response by reducing, for instance, vascular reactivity (see Figure 1.13-1; Iannetti and Wise (2007)). In Figure 1.13-1 the neural response (left) is of most interest, but can be ‘masked’ by drug or disease influences on signalling or vascular response. In rows 2 & 3, these are not affected and the BOLD signal reflects the underlying activity. In rows 4 & 5 the BOLD response is confounded by an influence on these factors.
This is particularly relevant in respect of D2 antagonists, as rapid alterations in blood flow produced by single doses of these drugs have consistently been reported (Mehta et al., 2003, Fernández-Seara et al., 2011, Handley et al., 2013). The picture is complicated further in view of the fact stimulation of D1/D5 receptors has a positive hemodynamic effect in increasing CBF, while stimulation of D2/D3/D4 receptors results in the opposite. Other neurotransmitters have their own effects on vascular receptors - serotonin for instance has a vasoconstrictive effect through receptors on the vasculature as well as via astrocytes (Cohen et al., 1996). Astrocyte signalling is also implicated (Attwell et al., 2010) as pharmacological modulation of these cells may also influence blood flow. For instance, D3 receptors are present on astroglial cells and are positioned to mediate regional blood flow, with D3 agonists having been previously shown to cause vasoconstriction (Choi, et al., 2006). Many antipsychotics exhibit affinity for D3 receptors (Girgis et al., 2015, Stahl and Muntner, 2013) so it follows that antagonism of these receptors may contribute to increases in CBF through vasodilation. Therefore, when exploring a class of drug that includes compounds with varying receptor affinity profiles, the potential for a highly
variable effect on the vasculature is present and it cannot be ruled out that the differing receptor profiles of different drugs could be altering baseline CBF between conditions which ideally needs accounting for.

Similarly, if cerebrovascular responsiveness or reactivity (CVR; a vessel’s ability to respond to a vasoactive stimulus) is altered by the presence of a drug it could potentially interfere with neurovascular coupling and interpretation of a drug effect on the BOLD signal. Carbon dioxide (CO$_2$) is one such substance that can create increases in CBF and cerebral blood volume, but without an increase in the cerebral metabolic rate of oxygen (CMRO$_2$). Therefore, by experimentally increasing the levels of CO$_2$ in the blood, the reactivity of the vascular system can be assessed with minimal increases in neural activity. The breath hold task is one such method that has been employed in fMRI research. A fuller explanation of method will be given in the methods chapter, but this approach has been shown to be a reliable method of assessing CVR (Urback et al., 2017a). Abler et al. (2007) included a breath hold task in their assessment of olanzapine on reward processing in the healthy brain. Although they did not include the response to this task directly in their analysis of the drug effect on reward anticipation, they reported a significant drug effect on vascular reactivity in three ROIs (insula, cingulate and occipital cortex), but no effect in a ventral striatal ROI where the treatment effect on reward anticipation BOLD signal was found. Nevertheless, this does highlight that the vascular reactivity can be altered by the presence of a drug, and should be considered.

Several recommendations have been made in recent years to address these issues since pharmacological MRI (phMRI) became commonplace in the literature (Iannetti and Wise, 2007, Bourke and Wall, 2015, Jenkins, 2012). Factors such accounting for baseline CBF, assessing vascular reactivity, adding control tasks and including a placebo condition are all minimum recommendations for phMRI – however, it remains rare that more than one of these factors are addressed at any one time.
1.14 Considerations for structural imaging of antipsychotic effects

Similarly, in the structural domain, drug induced physiological changes may be interfering with our ability to accurately assess changes in grey or white matter. In terms of antipsychotic induced changes, a critical question is whether they alter the MR signal sufficiently to then influence macro-level measurement MRI analysis techniques (Thomas and Baker, 2013). It is important to note here that MRI does not measure brain structure directly, but relies on the magnetic resonance properties of the surrounding tissue environment. These properties may be influenced by several non-structural factors which are yet to be fully explored (Weinberger and Radulescu, 2016). One such factor is potential pharmacologically induced changes in blood flow, which could exert an influence on the MR signal used to construct standard structural images, leading to an apparent acute remodelling at the macro-level. Indeed, Franklin, et al. (2013) posit that changes in blood flow induced by pharmacological agents may be ‘masquerading as volumetric changes.’

Their study reported changes in CBF, measured using arterial spin labelling (ASL), overlapping with same-direction changes in GM volume from standard T1-weighted images following acute administration of the GABA agonist, baclofen. This notion is particularly relevant in the context of volumetric changes induced by acute antipsychotic administration, as rapid alterations in blood flow produced by single doses of these drugs have consistently been reported (Fernández-Seara, et al., 2011; Handley, et al., 2013; Mehta, et al., 2003). Resolving this issue requires the assessment of the acute effect of antipsychotics on both regional blood flow and structural MR metrics in a placebo controlled setting (Hoflich et al., 2017).

How might blood flow influence the outcome of structural MR techniques? Typical methods used to gauge structural changes in vivo in both human and animal subjects using MRI are morphometry based techniques such as voxel based morphometry (VBM). These analysis techniques make use of gradient recalled, high resolution anatomical images in which the signal intensity in each voxel is primarily governed by the longitudinal relaxation or spin lattice relaxation time, T1 (and to a lesser extent the inhomogeneous transverse relaxation time T2*). T1 is highly sensitive to the physical properties of the tissue surrounding the 1H spins that generate the MR signal. In brief, the 1H longitudinal relaxation is faster in densely packed matter (such as white matter and bone), slower in
grey matter tissue, and slower still in less-restricted fluid environments (such as the ventricles). VBM relies on classification of images into tissue types based on the distribution of these T1-weighted image intensity values, as well as the a priori information from probabilistic tissue prior maps. Theoretically, pharmacological related blood flow changes may alter the relaxation times of \(^1\)H spins in certain regions due to the relative change in the movement of blood in that region. Although the related increase in cerebral blood volume (CBV) itself is unlikely to be sufficient to register a change in GM at this resolution, a biophysical influence of the change in CBF could alter the MR signal to result in such apparent changes. For instance, Franklin et al. (2013) point out the similarity of the T1 relaxation time of blood and grey matter (Stanisz et al., 2005), which may contribute to the apparent probability of a given voxel belonging to a particular tissue class, leading to an artefactual change in volumetric outcomes.

Allowing the accurate assessment of the potential influence of blood flow on the measurement of brain structure requires careful consideration of how exactly this structural information is gathered using MRI. A T1-weighted image, the standard for structural MR acquisition, is a qualitative measure heavily dependent on the TR and TE parameters of the acquisition protocol. However, recent developments in relaxometry imaging have allowed the relatively rapid acquisition of quantitative T1 maps, which provide a precise metric of the T1 relaxation time within each voxel. These absolute measures are more readily comparable across time points and could give a more informative measure of the underlying structure and possible drug-dependant tissue changes than T1-weighted values can provide (Draganski and Kherif, 2013, Draganski et al., 2014, Lorio et al., 2016, Tardif et al., 2016, Weiskopf et al., 2015). Several studies have used quantitative MR techniques to attempt to assess brain microstructure changes on a short-term time scale in both humans and animals (Blumenfeld-Katzir et al., 2011, Ding et al., 2013, Hofstetter et al., 2013, Sagi et al., 2012), although the majority of these examine use-or experience-dependant neuroplasticity. By using quantitative MR methods to assess T1 values following a single dose of an antipsychotic, it would allow the clear assessment of the pharmacological affect an acute dose has on the MRI signal. Only Fujimoto et al. (1987) have assessed the effect of an acutely administered antipsychotic on quantitative T1, reporting increased T1 in the striate body of dogs following a single large 20mg IV dose of haloperidol, albeit at a very low spatial resolution by current standards. Furthermore, by
concurrently assessing cerebral blood flow using ASL (also a quantitative method), the impact of potential changes in blood flow on T1 can also be explored.

1.15 Aims & Hypotheses

This thesis will explore putative acute antipsychotic induced structural and functional changes to the brain using MRI. To increase precision, several methodological steps will be taken. Testing will be conducted in healthy human subjects, allowing a double-blind, placebo controlled repeated measures design, whereby both dose and subtype of drug can be manipulated. Quantitative measures of T1 will be collected alongside quantitative ASL, to allow an assessment of the effect of blood flow on structural measures. Basic reward processing will be elicited using the MID task, and the effect of antipsychotics on both reward anticipation and outcome will be assessed, while considering potential drug effects on elements of neurovascular coupling, such as baseline blood flow and vascular reactivity. Together, these factors will help produce a clearer and more precise picture of the acute dynamics of different antipsychotics, which may help inform our knowledge of how they provide their therapeutic effect.

1.15.1 Acute structural analysis

The first part of the thesis will examine potential acute changes to structural metrics. Following drug administration, the likely contribution of regional CBF changes to the local spin lattice relaxation time, T1, will be determined by quantitatively measuring the T1 of each voxel using ‘Driven equilibrium single pulse observation of T1’ (DESPOT). Regional CBF will also be determined using 3D pseudo-continuous Arterial Spin Labelling, which has been shown to be sensitive to the effects of a single dose of pharmacological agents (Zelaya et al., 2015). For completeness, a range of automated morphometric analyses of standard T1-weighted images will also be carried out (to allow a more direct comparison with the majority of the published literature on acute structural changes following antipsychotic exposure), in addition to a confirmatory reverse-translational preclinical study.

Blood flow is hypothesised to increase in the striatum in response to antipsychotic administration. If quantitative T1 measures change and correlate with the quantitative measures of blood flow, it would suggest that the primary MRI signal used to assess brain volume is influenced by transient drug induced CBF changes. Alternatively, if T1 remains
stable in the face of the expected blood flow changes, this would suggest CBF changes are not sufficient to influence structural metrics alone. This would provide some validation of the structural methods employed in studies examining the chronic effects of brain structure, as it would suggest these measures are not unduly influenced by acute blood flow changes at the point of measurement, over and above more pervasive long term effects.

1.15.2 Acute functional analysis
The second part of this thesis will focus on the functional domain. The contribution of single dose antipsychotics to both anticipatory and consummatory reward functioning will be determined using the MID task during BOLD acquisition. To account for the indirect effects of these drugs on the vasculature and the underlying BOLD parameters, the effects of the drug on cerebrovascular reactivity (assessed using a breath hold task) and CBF will be taken into account when assessing the BOLD data.

Antipsychotic administration is hypothesised to suppress reward related BOLD activation during reward anticipation in reward related areas (the striatal structures of the putamen, caudate and ventral striatum) in line with previous findings (Abler et al, 2007). This design allows for exploration of the differences between these drugs. For risperidone, a dose response effect is expected – a high dose will produce suppression of this region, while low dose will not.

During reward outcome, antipsychotics are again hypothesised to modulate BOLD activation. However, due to the scarcity of research of the effect of these drugs in healthy humans, two possible impacts are considered. First any suppression of the anticipatory phase may influence the impact of the outcome such that the response increases (in line with a more novel or salient reward). Second, if the drug influences the outcome phase directly (not via anticipation) then we would expect a suppression of activity due to a reduction in the reward related signal.

Finally, given one previous study has indicated single doses of olanzapine affects CVR (Abler et al., 2007), the BOLD response to the breath hold task is hypothesised to decrease in response to antipsychotic administration.
Chapter 2 Methods

2.1 Magnetic Resonance Imaging

This thesis involves measures of brain grey and white matter contrast with T1-weighted images, quantification of T1 relaxation times and cerebral blood flow and BOLD imaging. Therefore, a short summary of the physics and physiology of these measurements is provided.

2.1.1 MRI physics

Hydrogen is the most abundant element in the human body (in terms of number of atoms) and is therefore a useful marker for medical imaging. MRI can exploit the characteristics of the physical properties of hydrogen protons and how they respond to magnetic fields and radio frequency excitation to create detailed images based on their distribution and immediate environment in the body.

As is the case with all protons, the single hydrogen proton within a hydrogen nucleus displays an angular spinning motion around its axis which creates a small magnetic field of its own, known as a magnetic dipole moment. The ratio of this magnetic moment to the angular momentum of the proton is known as the gyromagnetic ratio, which varies for different protons (referred to as ‘spins’ in MR terminology). Hydrogen has a relatively high gyromagnetic ratio (42.6 MHz/T), which along with its ubiquity makes it an ideal candidate for use in MRI.

In normal circumstances, the random arrangement of spins in the body (in terms of their north-south orientation) are such that these magnetic forces cancel each other out. However, when placed in an external magnetic field ($B_0$) the spins align themselves either parallel (in what is called a low energy state) or anti-parallel with $B_0$ (in a high energy state). A very small majority of spins will align parallel with $B_0$ as this requires less energy than aligning against the field, with the extent of the difference being dependant on the strength of the external magnetic field.
The sum of the magnetic moments of the spins therefore results in a net magnetisation ($M_0$) along the same vector as $B_0$. In addition to this, the protons precess around $B_0$ at a rate dependant on the gyromagetic ratio (GMR) of the element and the external magnetic field strength, called the *Larmor Frequency*, given here:

$$\omega = \gamma B$$

**Larmor freq.**  
(MHz)  
**Gyromagnetic ratio***  
(MHz/T)  
**Field strength**  
(T)

The two critical points from this overview that are exploited for MR imaging is i) there is a small but observable difference in the longitudinal plane and ii) the spins precess around $B_0$ at the Larmor frequency, albeit out of phase (see Figure 2.1-1).

![Figure 2.1-1 Characteristics of spins in an external magnetic field. Figures adapted from McRobbie et al. (2017)](image)

On the left of Figure 2.1-1, slightly more protons align with $B_0$ than against, resulting in a net magnetisation, $M_0$, in the same vector as $B_0$. Using a 3D rotating frame of reference allows illustration of the magnetisation vectors on the right of the figure – the coordinate system $x', y', z'$ is rotating at the same frequency as $\omega$ (the Larmor frequency) in the same direction as the spins, which therefore appear stationary.
2.1.2 MR excitation

If a radio frequency (RF) pulse is applied at the Larmor frequency, only the protons spinning at that same frequency will response or resonate in a specific manner. MR can therefore use prior knowledge of the GMR and the field strength to selectively manipulate protons of interest – in this case hydrogen protons.

RF pulses at this resonant frequency result in two important changes to the behaviour of the spins. First, a number of those spins in a low energy state are moved into a high energy state by absorbing the energy of the pulse. A RF pulse at the correct frequency, amplitude and length of time will therefore alter the net magnetisation previously in parallel with $B_0$ to the extent that $M_0$ will rotate 90° into the perpendicular or transverse plane (this is often called a 90° pulse). Second, the spins begin to precess in phase or achieve phase coherence. These two principles create a magnetisation vector that rotates in the X-Y plane around the Z axis, and provides the basic signal detected by MRI.

![Magnetisation vectors in a rotating frame illustrating the two components of M.](image)

Figure 2.1-2 illustrates this. The net magnetisation, $M$, consists of the longitudinal component, $M_z$, and the transverse component, $M_{xy}$. After a 90° RF pulse $M$ will rotate into the xy plane, as the more spins will be in high energy state and the spins will have phase coherence. This signal can be measured by a receiver coil which is sensitive only to magnetization perpendicular to $B_0$. Once the RF pulse is turned off the spins rapidly lose phase coherence (causing the signal in $M_{xy}$ to decay) and more spins will return to a low energy state, resulting in the recovery of the signal along $M_z$. 
MRI can determine a large amount of information about the characteristics of the tissue by observing how this signal changes when the RF pulse is turned off. When this happens, the magnetisation starts to recover back to its original state, but the manner in which this occurs reveals information about the characteristics of the environment in which the spins are present. This process is dependent on two independent but simultaneous processes – longitudinal relaxation and transverse relaxation.

2.1.3 Longitudinal relaxation

After the RF pulse is switched off, those spins which were forced into the high energy state will start to return to their low energy state (as this is their preferential position). This results in the original net magnetisation being restored, and the longitudinal relaxation can be said to ‘regrow’ or recover along the z-axis as the small majority of spins in a low energy state is restored.

Importantly, the rate at which this occurs is dependent on the biophysical environments of the spins, as the extra energy from the RF pulse is released into the surrounding lattice. For this reason, longitudinal relaxation is also known as spin-lattice relaxation. As hydrogen protons are bound to their molecules in different ways, the speed at which they can release their energy differs and therefore the relaxation rate will be different depending on the tissue type the spins are present in. Using a somewhat basic example, in fat tissue the protons are tightly bound and can release their energy quickly whereas in water they are more loosely bound and energy transfers back to the lattice occurs more slowly.

In MR, this rate of relaxation is defined as a time constant, T1, and is the time it takes for the longitudinal relaxation to recover to 63% of $M_0$. Because different tissue types will release energy at a different rate and therefore recover the longitudinal signal at different speeds, this results in a contrast in signal between different tissue types. Note the T1 constant (63% of recovery, red line) varies between the two tissue types in Figure 2.1-3.
2.1.4 Transverse relaxation

In addition to spins returning to their previous orientation, the removal of the RF pulse results in the loss of phase coherence. The interaction between the spins cause some to precess at a higher rate than others, resulting in them collectively becoming out-of-phase and losing the net magnetisation vector created by phase coherence. The rate at which this occurs is therefore dependant on the spins in the immediate environment, and for this reason transverse relaxation is also known as spin-spin relaxation. In fat tissue, for instance, dephasing is rapid and T2 is short, whereas in water the spins dephase relatively slowly and T2 is therefore longer. This rate of relaxation is defined as a time constant, T2, and is the time it takes for 37% of the signal created by phase coherence to decay following the removal of the RF pulse. Whereas T1 was the time for the signal to recover along the z-axis, T2 refers to the decay of the signal in the x-y plane, and occurs on a far more rapid time scale than T1. Note that in a similar fashion to the situation with T1, in Figure 2.1-4 the T2 constant (37% of decay, red line) varies between the two tissue types.

Figure 2.1-3 T1 curve for tissues approximating grey (orange line) and white (blue line) matter at 3T (values calculated from Stanisz et al. (2005)).
In reality, dephasing is further accelerated by inhomogeneities in the external magnetic field, causing differences in the precessing frequencies of spins. The combined effect of both spin-spin interactions and $B_0$ field inhomogeneities is defined by the time constant $T2^*$. 

Although $T1$ and $T2$ are independent processes, and react differently to each other and to the tissue type present, both contribute importantly to the MR signal and the creation of different types of image.

2.1.5 Image formation

Although the MR signal is highly informative about the qualities of the tissue it is produced from, extra steps are required to localise its origin in 3D space. This is achieved by manipulating the original external magnetic field of the scanner in a specific manner with the use of additional magnetic fields produced by three gradient coils across the z, x and y planes respectively. This allows for the precise localisation of the signal, as the specific conditions that the spins will respond to are limited to a known area within the scanner.
The slice encoding gradient creates an additional magnetic field in the z-plane ($G_z$), which is superimposed on $B_0$, resulting in $B_z$ differing slightly along the slope of the field. This results in the protons spinning at different frequencies along the field, given the principles of the Larmor equation. When the RF pulse is applied, only the protons in a specific region of the scanner react because only they are at the resonant frequency of the pulse. This effectively produces an axial slice of known position, with protons in this slice spinning at the same frequency and in phase. The frequency encoding gradient (sometimes referred to as the ‘read-out’ gradient) is used to orient the signal in the x-axis ($G_x$). It acts in a similar way to the slice encoding gradient, and creates a range of spin frequencies along the left-right orientation. When the phase encoding gradient is switched on along the y-axis ($G_y$), it results in protons spinning at differing frequencies along the length of this field. When the gradient is turned off, the protons return to spinning at the same frequency but they will now have a different phase depending on their position.

The use of each of these gradient coils and the controlled manipulation of spin frequency and phase therefore narrows down the location of the signal in three dimensions. By rapidly switching these coils on and off in a specific manner, in addition to the delivery of RF pulses (collectively called a pulse sequence), multiple data points can be collected in a rapid fashion, each with their own frequency and phase which code for its position in the brain. This raw data is finally analysed using a Fourier transformation to calculate the exact location and intensity of each data point to create a recognisable MR image, with each data point represented by a 3D volume element, or voxel, analogous to a pixel on a 2D display.

As described earlier, the MR signal decays very rapidly after the first 90° RF pulse is removed and dephasing occurs. This produces problems in sampling the signal, as by the time the different gradients have been applied to localise the signal it will have already decayed. A commonly used pulse sequence used in MR is a spin echo sequence as this ‘rebuilds’ the signal using a second 180° pulse. In short this second pulse causes the spins to rephase and allows sampling of the signal. As illustrated in Figure 2.1-5, the initial radiofrequency (RF) pulse flips the magnetisation 90°, and the gradient coils ($G_z$, $G_y$) localise the signal with slice selection (SS) and phase encoding (PE) pulses respectively. A second RF pulse of 180° causes a rotation of transverse magnetisation within the slice and rebuilds the signal, refocusing it to form a spin echo at time TE (echo time). At this point a
frequency-encoding (FE) pulse is applied by Gx, which isolates the signal in 3 dimensions by making it vary with its location in the x dimension. The signal can then be sampled.

This illustrates two of the most important parameters in MR – the echo time (TE) and the repetition time (TR). As described earlier, the MR signal is dependent on two relaxation processes, T1 and T2, and the type of image acquired is dependent on the extent to which each of these is allowed to occur. TR and TE are the primary parameters used to control this, and can be set to allow the signal to be more dependent on T1 (T1-weighted image) or T2 (T2-weighted) image.

T2 is particularly important for functional imaging. However, most structural imaging relies primarily on T1, and a brief overview of these methods will now be given.
2.1.6 Structural MR imaging & Relaxometry

2.1.6.1 T1-weighted imaging

Altering the TR allows the change in contrast between different tissue types that is typical of MR images, and is particularly sensitive to changes in T1. By allowing longitudinal relaxation to recover to a point at which there is a large difference between tissue types (see T1 graph in fig 2.1-3), another 90° pulse at this point (TR) would mean the subsequent signal will be different throughout the brain, resulting in a large contrast in the resulting image which is primarily due to differences in T1. This is illustrated in Figure 2.1-6: Under a long TR (left), the longitudinal relaxation has time to fully recover in both tissues (albeit at different rates), and therefore there is no appreciable difference in signal once the next pulse is applied. Under the short TR (right), the tissues recover at different rates as before but are not allowed to fully return to $M_0$ before the next RF pulse is applied, resulting in the contrast in the signal produced.

![Figure 2.1-6 Longitudinal relaxation under different TRs in different tissues (blue and orange schematics)](image)

However, there are some caveats to T1-weighted (T1-w) imaging. In practice, they are qualitative images in that they are dependent on changeable scanner parameter variables (TR & TE), as well as less controllable factors (such as scanner hardware, temperature,
participant hydration, and time of day), and therefore produce unitless intensity values that are problematic to compare across sessions (Draganski and Kherif, 2013).

2.1.6.2 Quantitative T1
Relaxometry is a quantitative form of structural imaging, which allows the mapping of absolute, reproducible and directly comparable T1 times per voxel in the brain. Compared to T1-w intensity values, they produce a quantitative value of T1 (in seconds) which is immediately more informative and comparable across individuals and sessions. The basic principle in creating these maps involves acquiring a T1-w image, altering the TR & TE in a precise and measured fashion, acquiring another image, and then measuring the observed difference in outcome to build a pure T1 measure. A standard pulse sequence for this method is phase-sensitive inversion recovery. This uses a 180° pulse to flip magnetisation, resulting in no transverse magnetisation (as the magnetisation vector is not in the horizontal plane). After waiting a given inversion time (TI), a 90° pulse is applied which flips magnetisation into transverse plane so it can now be measured (see Figure 2.1-7).

![Figure 2.1-7 IR sequence. Figures reproduced with kind permission of Tobias Wood.](image)

This can be repeated several times with different inversion times, with the result being a different strength signal on each occasion. As there is no transverse magnetisation, T2 contributes minimally to the signal. The outcome from each differing TI can then be plotted and T1 calculated by the relationship between them, as illustrated in Figure 2.1-8.
An issue with this approach is the extremely long total scanning time, as enough time has been given to allow for full longitudinal relaxation several times at each voxel. Therefore, instead of using a spin echo sequence (as above), the TR can be shortened to just a few milliseconds using a gradient echo sequence. The benefit here is a gradient/RF spoiled sequence destroys transverse magnetisation which means at the end of the TR there is only longitudinal magnetisation present – the same effect as the spin echo sequence but achieved far more rapidly.

$$S_{SPGR} = cM_\infty \frac{(1 - E_1) \sin(B_1 \alpha)}{1 - E_1 \cos(B_1 \alpha)}$$

$$\frac{S_{SPGR}}{\sin(\alpha)} = E_1 \frac{S_{SPGR}}{\tan(\alpha)} + cM_\infty (1 - E_1)$$

**Figure 2.1-8** Phase sensitive IR curve. Figure reproduced with kind permission of Tobias Wood.

**Figure 2.1-9** Linear expression of relationship between T1 signal and flip angle. Figure reproduced with kind permission of Tobias Wood.
Because the relationship can be expressed linearly (see Figure 2.1-9), only two flip angles are required for the calculation of T1 which reduces scanning time as both the TR and number of acquisitions have been reduced. The method used to determine T1 in this thesis was Driven Equilibrium Single Pulse Observation of T1 (DESPOT1; (Deoni et al., 2003). The parameters for this protocol are described in full in the MR Acquisition section but in brief, this involved collecting spoiled gradient recalled echo (SPGR) images over two flip angles while keeping the repetition time constant. Because this method spoils the transverse magnetisation by altering the phase of the RF pulse, this provides a signal intensity curve characterized only by T1.

2.1.7 Functional imaging

2.1.7.1 The Blood Oxygen Level Dependant (BOLD) Signal

Many of the principles described above for imaging structure can also be applied to imaging the putative functional state(s) of the brain. The differing magnetic properties of oxygenated haemoglobin (diamagnetic) and deoxygenated haemoglobin (paramagnetic) can be exploited by MR imaging to reflect the contrast between the two.

When oxygen is released from haemoglobin due to a local energy demand, the haemoglobin gains a magnetic dipole moment due to now having four unpaired electrons per iron atom. The presence of this paramagnetic haemoglobin therefore distorts the magnetic field, causing spins in this environment to experience different field strengths and therefore precess at different frequencies. As described above, T2* relaxation is influenced by spin-spin interactions and this distortion results in more rapid phase dispersal and subsequent decay of the T2* signal. The measurable outcome of this process in terms of T2* sensitive MR sequences is that the T2* signal will be weaker in areas with a high concentration of deoxygenated blood compared to oxygenated blood.

The process producing this blood oxygen level dependant (BOLD) signal has been applied as an indirect measure of brain function since the early 1990s. The basic initial premise was that neuronal activity produced an increased energy demand, which was satisfied by the delivery of oxygen to the cell, and which would also therefore increase the level of deoxygenated blood and reduce the signal intensity of T2*-w images (Ogawa et al., 1990). In fact, due to the complex hemodynamic relationship with active neurons, the first BOLD
experiments in humans found the T2* signal actually increases due to a large increase in cerebral blood flow over that of blood oxygen extraction in response to neuronal activity (Kwong et al., 1992).

### 2.1.7.2 The Hemodynamic Response

The dynamics of blood flow in response to neuronal activity has a significant impact on the BOLD signal. Following neuronal stimulation there is a very large increase in cerebral blood flow (CBF) of oxygenated haemoglobin to the area, in excess of the metabolic requirements of the neurons (Malonek and Grinvald, 1996) and to the extent that it flushes out the deoxygenated blood from the capillaries. This relative reduction of paramagnetic haemoglobin results in the stronger T2* signal and higher intensity T2*-w images.

The temporal dynamics of this response are an essential component of understanding what this change in signal represents. The hemodynamic response (HDR) describes the typical change in the BOLD signal following neural activation, although this will differ slightly depending on the stimulus eliciting the response, the area of the brain stimulated and the individual themselves (Handwerker et al., 2004). Following the onset of a single, short-duration stimulus, the shape of the response is characterised by a sharp increase after around 2 seconds, which peaks at 5 seconds then falls rapidly, undershooting the baseline for an extended period, before returning to the pre-stimulus level. There is also debate about the presence of an initial dip (Hu and Yacoub, 2012), thought to be reflective of the initial increase in deoxyhaemoglobin as the active cells make use of the local immediately available oxygen in the blood.

Although this response has been shown to be somewhat variable depending on the conditions (an extended stimulus may result in the signal plateauing around the peak for a period of time related to the stimulus for instance), the canonical response function has been well characterised and is commonly used to model the data from a typical fMRI experiment. However, it is important to note that the BOLD signal is an indirect measure of neural activity which is dependent on several assumptions, and relating it back to what it represents in terms of brain function requires further consideration.

### 2.1.7.3 Neurovascular Coupling and the Neural Correlates of the BOLD signal
The BOLD signal can be said to depend on three major factors – CBF, CBV and the cerebral metabolic rate of oxygen (CMRO$_2$), in addition to oxygen extraction fraction. BOLD signal is crucially dependant on changes on blood flow; therefore a clear association between blood flow and neuronal activity is required for this signal to be related back to brain function. This relationship is referred to as neurovascular coupling and is an important concept in the interpretation of the BOLD signal (Lecrux and Hamel, 2011, Petzold and Murthy, 2011).

Cerebral blood flow is subject to a range of mediators, from the global stabilising effects of central autoregulation down to the physical characteristics of individual blood vessels, such as their diameter and orientation. In the context of the BOLD signal, functional hyperaemia is the process by which local blood flow is increased in response to neuronal activity. This is achieved through a complex interplay of neurotransmitter signalling, release of vasoactive substances and blood vessel dilation/constriction across different levels of the vascular system. This was initially thought to be controlled through a negative feedback loop: active neurons produced changes in the concentration of CO$_2$ or O$_2$ which promoted the dilation of surrounding blood vessels, reducing resistance and increasing flow - the active neurons are subsequently provided with oxygenated blood and neurovascular coupling is maintained. However, more recent research has suggested that a more active feedforward process may be involved, with both neuron and glial cells (Attwell et al., 2010) directly manipulating the vasculature through neurotransmitter release to alter blood flow to their needs using an highly complex array of substances. In summary, it is likely that a combination of the release of vasoactive substances (such as potassium ions or nitric oxide) and direct neurotransmitter signalling is required, depending on the location of vessels and the time course of the change in flow.
Astrocytes and neurons regulate blood flow by sending messengers (arrows) to influence the smooth muscle around the arterioles that supply oxygen and glucose to the cells. mGluR: metabotropic glutamate receptors; NMDAR: N-methyl-D-aspartate receptors; NO: nitric oxide; nNOS: nitric oxide synthase; AA: arachidonic acid; PLA2: phospholipase A2; PG: prostaglandins; EET: epoxyeicosatrienoic acids; cGMP: Cyclic guanosine monophosphate; 20-HETE: 20-Hydroxyeicosatetraenoic acid.

Figure 2.1-10 Pathways from astrocytes and neurons (left) that regulate blood flow. Reprinted from Attwell et al. (2010) with permission.

The actual neurophysiological activity that elicits these haemodynamic changes is multifaceted, and efforts have been made to define the precise elements contributing to the signal to allow for a more meaningful interpretation. This remains an area of debate, but current evidence suggests the signal is most reflective of local field potentials (the sum of postsynaptic potentials of a given group of cells, as can be recorded from the extracellular medium with a microelectrode and low-pass filtered to remove the spiking signal of actively firing cells), and which are thought to represent the integrative activity of a neural population (Logothetis, 2008). Nevertheless, there are studies indicating that the BOLD signal and LFPs can dissociate under some circumstances (Ekstrom, 2010) highlighting the caution that is required in interpreting this signal.

2.1.7.4 Arterial Spin Labelling (ASL)

BOLD is a relative measure, as it requires comparison between a baseline and experimental state, and is therefore essentially unitless which makes its quantitative interpretation difficult. As highlighted in the previous chapter (and explored in detail within this thesis) this complicates examining populations where this baseline state may be altered outside the effects of the experimental design (D’Esposito et al., 2003). This is in addition to the issues highlighted above in interpreting the precise physiological contributors to the BOLD
signal (i.e. CBF, CBV or CMRO2, and the underlying cellular processes that promote these changes).

Arterial spin labelling (ASL; Alsop et al. (2014), Detre et al. (1992), Williams et al. (1992)) is a method that allows the quantitative determination of cerebral blood flow in a given voxel, in physiologically meaningful units of ml/g/min, reflecting the volume of flow per gram of brain tissue per unit time. It is a non-invasive technique in that it uses an endogenous marker in the form of arterial blood water, the longitudinal magnetisation of which is manipulated as it enters the vascular system of the brain, and which allows it to be traced as it perfuses into tissue.

In its simplest form, an ASL technique typically involves ‘tagging’ a bolus of arterial blood by applying an inversion RF pulse to a slice or slab below the area of interest. A labelled image is then acquired comprising of both the tagged blood water and tissue water. These images can then be compared with a second control image – acquired without the use of the earlier RF pulse and therefore in the absence of any tagged blood – and the difference between the labelled and control images represents the delivery of arterial blood to brain tissue by perfusion. By creating several pairs of these labelled and control images and averaging the resulting subtraction image, the signal-to-noise ratio (SNR) can be improved and a CBF map can be created.

Different ASL protocols have been developed since it’s widespread introduction in the mid-90s. The major difference between methods involves the application of the tagging pulse. Continuous (cASL) methods use long RF pulses (on the order of seconds) to continually invert the flow of blood through a given slice below the imaging plane (as process called flow driven adiabatic inversion), while pulsed (pASL) methods rely on shorter pulses (on the order of milliseconds) to invert the blood water but in larger slabs of tissue, proximal to the imaging region. cASL provides a superior signal-to-noise ratio compared to pASL, but presents certain technological and practical issues, such as an increase in the specific absorption rate (SAR; the amount of energy deposited in tissues by the RF pulses), magnetisation transfer effects (MT; the RF-pulse indirectly lowering signal in the imaged slice), and demands on scanning hardware (due to its requirement for a sustained RF pulse and a separate coil for labelling and imaging). The method employed in this thesis was originally developed to maintain the SNR ratio of cASL with the efficiency of pCASL. Pseudo
continuous ASL (p-CASL; Dai et al. (2008)) achieves flow driven adiabatic inversion by applying many RF pulses in extremely rapid succession over a similar period as a standard cASL pulse. This produces the same labelling effect as the standard cASL RF pulse but with reduced energy deposition and MT effects.

ASL has proved a hugely informative addition to the repertoire of neuroimaging methods. Although it does not offer the same temporal resolution or SNR of BOLD, it does provide a quantitative measure of CBF and has been shown to be sensitive to drug effects (Wang et al., 2011).

2.2 Study Design

The majority of data in this thesis was collected in a double-blind, placebo-controlled, randomized, fully counterbalanced three-period cross-over study, in two parallel groups of healthy male subjects receiving a single dose of pharmacologic agents during each period. A reverse-translational preclinical pilot study was also conducted based on the results of the human trial and will be discussed in the relevant chapter.

2.2.1 Study participants

Healthy right handed male participants were recruited using newspaper/radio advertisements. Screening procedures were conducted between 28 and 2 days before the first imaging session. An initial session assessed general physical suitability for the study, including medical history, a full physical exam, vital signs, electrocardiogram, blood and urine chemistry profiles, serology (HIV1, HIV2, hepatitis B and/or hepatitis C), tests for alcohol and drugs of abuse, and concomitant medication. Inclusion criteria required normal ECG, standard laboratory blood screens and urinalysis, and alcohol consumption within the recommended guidelines at the time of the study (less than 21 units per week). Exclusion criteria included smoking more than 10 cigarettes per day, a history of neurological or psychiatric illness, physical illness and positive drugs of abuse or alcohol breath test on the screening or study days.

A second screening session within one week of the planned first visit was conducted to familiarise the participant with the scanning protocol and fully assess their psychiatric history via an unstructured interview with a study psychiatrist. MRI safety screening was
conducted and a mock scanning session prepared participants for the scanner environment and allowed assessment of their ability to perform the tasks. Standardised instructions were given for each task. Participants who could not tolerate the mock scanner environment or were unable to understand or perform the task adequately were excluded. Participants who passed screening were pseudo-randomly assigned to one of two parallel groups. All subjects were scanned three times, with seven days separating each scan. Participants from each group were scanned at the same time of day per visit. During each visit, one group received either a single oral dose of risperidone 0.5 mg, risperidone 2 mg or placebo prior to their scan (herein referred to as group RIS-H/L); while in the other group subjects received either a single oral dose of olanzapine (7.5 mg), haloperidol (3 mg) or placebo (herein referred to as OLAN/HAL). Within-group treatment order was randomised using a Williams square design.

A target number of 42 subjects (21 in Group A and 21 in Group B) was chosen based on previous studies (Handley et al., 2013, Marquand et al., 2012, Zelaya et al., 2012) indicating 12-24 subjects in a cross-over design are typically sufficient to detect pharmacological effects of clinically efficacious doses of CNS compounds in healthy volunteers. A sample size of 21 evaluable subjects per group provides more than 90% power to detect a statistically significant difference at a 1-sided significance level of 5%, assuming an effect size of 1 in a within-subject comparison performed at the region of interest level.

![Participant recruitment and progress through study](image-url)
Figure 2.2-1 illustrates recruitment and screening throughput. 42 participants successfully completed the study: 21 in RIS-H/L (age range 19-41, mean age 27.56 ± 6.87 years) and 21 in OLAN/HAL (age range 19-42, mean age 28.34 ± 6.34 years). The age difference between the two groups (0.78 years) was not significant (t(40) = 0.383, p = .704). Following QC of the imaging and behavioural data, some further data required excluding from the final analysis within specific modalities. Full details on these exclusions will be given within the appropriate chapter for that data modality.

2.2.2 Data collection

MRI data were acquired at the approximate point after dosing when the agents would be at their predicted peak plasma concentrations. This was 5 hrs post dose for olanzapine and haloperidol, and 2 hrs post dose for risperidone (de Greef et al., 2011, Kodaka et al., 2011, Midha et al., 1989, Nyberg et al., 1993, Tauscher et al., 2002). Scan times were interspersed to allow data acquisition from one participant from each group was acquired per scanning day (see Figure 2.2-2 for scanning day schematic).

Blood plasma samples were taken 90, 230 and 510 minutes post-dose for RIS-H/L, and 90, 270 and 600 minutes post-dose for HAL/OLAN to allow modelling of total drug exposure at the time of MRI acquisition, calculated using the trapezoid method. The half-life for oral risperidone, olanzapine and haloperidol is 22 hours, 33 hours and 37 hours respectively (Kudo and Ishizaki, 1999, Mauri et al., 2014), allowing for full washout of treatment between scans.

![Figure 2.2-2 Study day protocol](image-url)
As a measure of subjective sedation, the Visual Analogue Scale was administered prior to dosing and at regular points throughout the visit (see Figure 2.2-2), including prior to each scan. The VAS is a 16-item self-rated dimensional analogue scale, which can be categorised into two main factors to represent subjective alertness and tranquillity (Herbert et al., 1976). It has been well-validated to quantify sedative drug effects in healthy volunteers including dopaminergic agents and antipsychotics (Liem-Moolenaar et al., 2011). Only the alertness factor was used in the analysis.

2.3 MR Acquisition & Analysis

For the structural arm of this thesis, both T1-weighted images and quantitative measures of T1 were collected. Arterial spin labelling was used to obtain a quantitative of CBF, and an echo-planar imaging sequence (EPI) was employed to obtain BOLD measurements during reward processing and breath hold tasks.

All scans were conducted on a GE MR750 3 Tesla scanner using a 12-channel head coil. A T2-weighted image (FOV = 240mm, TR/TE = 4380/46.992ms, 320x256x156 matrix, slice thickness = 2mm), required for the preprocessing of the ASL images, was acquired during the first visit. A T1-weighted MPRAGE scan, for use in DARTEL normalisation, (FOV = 270mm, TR/TE/TI = 7.312/3.016/400ms, 256x256x156 matrix, slice thickness = 1.2mm) was acquired on the second visit.

All preprocessing and analysis of imaging data was conducted in SPM12 (Functional imaging Laboratory, UCL, London, UK), running on Matlab 8.2.0.701 (MathWorks, Natick, MA) unless stated otherwise.

2.3.1 Image acquisition

2.3.1.1 BOLD

Within a 64 x 64 matrix, 3 mm thick slices were obtained at 38 spatial positions with 3.3 mm slice gap. Functional scans were obtained using T2*-sensitive gradient echo planar imaging optimised for parallel imaging (repetition time [TR] = 2000 ms; echo time [TE] = 28
ms; flip angle = 75 degrees; number of volumes = 414; field of view [FoV] = 214 mm). The initial four volumes were discarded from each time series to minimise non-steady-state effects.

2.3.1.2 Structural

Driven Equilibrium Single Pulse Observation of T1 (DESPOT) was the method used to acquire quantitative T1 maps. Two spoiled gradient recalled T1-weighted images (SPGR; FOV = 220 mm, TR/TE = 8.1/3.7 ms, 220x220x172 matrix, slice thickness = 1 mm) at two flip angles (4° & 18°) and an inversion recovery (IR) SPGR image (FOV = 220 mm, TR/TE/TI = 8.1/3.7/450 ms, 220x110x86 matrix, slice thickness = 2 mm) were acquired at each visit. The 4° flip angle SPGR image from this scanning protocol was also utilised for the automated morphometric analysis. Briefly, this involved resetting the origin of the IR image and the SPGR image at both flip angles to the anterior commissure and reorienting the images to the AC-PC line, and then registering and resampling all images within subject to the SPGR acquired on the second visit. The DESPOT1HIFI protocol (described in detail in Deoni (2007)) was then used to create a T1 map for each visit.

2.3.1.3 ASL

ASL image data was acquired using a pseudo-continuous Arterial Spin Labelling sequence (pCASL) with a multi-shot, segmented 3D stack of axial spirals (8-arms) readout with a resultant spatial resolution of 2x2x3 mm. Three control-label pairs were used to derive a perfusion weighted difference image (Dai, et al., 2008). The labelling RF pulse had duration of 1.5 s and a post-labelling delay of 1.5 s. The sequence included background suppression for optimum reduction of the static tissue signal. A proton density image was acquired in 48 sec using the same acquisition parameters to compute the CBF map in standard physiological units (ml blood/100 gm tissue/min). Two runs were acquired per visit.
2.3.2 BOLD data acquisition

2.3.2.1 Scanner tasks

2.3.2.1.1 MID task

The Monetary Incentive Delay (MID) task (Knutson et al., 2000) has been extensively used to elicit and study reward related activation within fMRI designs (Knutson and Greer, 2008), and has been shown to be reliable over time in healthy volunteers (Plichta et al., 2012). The version used in this study is most closely comparable to that used in Knutson et al. (2001).

The task involves the display of a cue which the participant has previously learnt to associate with a certain level of monetary reward. To win the reward, the participant must press a button as quickly as possible in response to the brief appearance of a white square, which appears a variable amount of time after the cue. An outcome display then reveals if the reward was won or not, depending on if the participant pressed the button within the response window. The reaction time window adapts to the performance of each participant with a pre-set target of a 66% win-rate to ensure an adequate number of win and lose outcome trials for subsequent analysis. The task comprises of four randomised trial types: three active reward-level cues (high (£2), low (£0.20) and control (£0)) to which the participant was asked to respond as quickly as possible to, and a passive trial denoted by the presence of a single X to which the participant was told required no response. If the participant pressed the button during the presentation of the fixation cross or within 100ms of the presentation of the target (an unrealistic reaction time), the trial would set as a no win. Each condition was presented 24 times within a total duration of approximately 13.8 minutes. The 3 active trial types (see Figure 2.3-1) were conducted within a fixed 10 second window, while the passive trial was a simple X displayed for 4.25secs without the fixation, target or feedback screens displayed in the active trials. Four separate ‘playlists’ with the 96 trials randomly arranged in each were created which participants randomly received on each visit according to a Latin square design, to ensure there were no learning effects from completing the same task on each visit.
Breath hold task

The breath hold paradigm has been used extensively in fMRI research as a measure of the ability of cerebral vasculature to modulate blood flow in response to vasoactive stimuli (Urback et al., 2017b), or vascular reactivity (CVR). Hypercapnia induced by breath holding increases the concentration of CO$_2$ in the blood. As CO$_2$ is a vasodilator this results in widespread increases in CBF, increasing the BOLD signal without the assumed increase in CMRO$_2$ that would normally accompany such a signal change (Kastrup et al., 1998). It is therefore a useful putative measure of vascular reactivity in the absence of neural activity, and has been shown to be altered in both disease states and healthy aging (Raut et al., 2016), and in the presence of pharmacological agents (Pattinson et al., 2009, Friedman et al., 2008).

Breath holding has been shown to be comparable to alternate methods of assessing vascular reactivity, such as CO$_2$ inspiration (Tancredi and Hoge, 2013, Kastrup et al., 2001) or resting state physiological fluctuation amplitude (Kannurpatti and Biswal, 2008, Lipp et al., 2015), but has the benefit of being non-invasive and easy to implement, with minimal discomfort or distress to the individual.
Participants were instructed to follow a simple set of instructions on screen alternating between paced breathing (45 seconds) and breath holding (16 seconds), with this cycle repeated four times. The task started and ended with a period of paced breathing. Breath holding was instructed to commence at the end of expiration (or on a ‘out’ breath) which has been shown to produce a quicker CVR peak, in addition to removing some of the confounds produced by an end-inspiration approach, such as a biphasic response within the time course signal and marked inter-subject variability in inspiration depth (Urback et al., 2017a).

![Breath Hold task diagram](image)

**Figure 2.3-2 Breath Hold task**

Standardised verbal reminders of the instructions were given prior to entering the scanner and immediately before the task started. Participants were advised not breath more heavily or deeply than they normally would during the paced breathing segments; rather they should just attempt to breath regularly but in time with the instructions. They were also reminded to hold their breath after exhaling, to ensure end-expiration holding. Participants were supplied with a respiratory bellows sensor during each scan, which allowed for the monitoring of their breathing in the control room to ensure they were following the task correctly, as well as analysis of group adherence to the task.

2.3.3 BOLD data preprocessing and analysis

For both the MID and the BH task, the first 4 volumes (8 seconds) were discarded to allow the tissue to reach a steady state of radiofrequency excitation.

2.3.3.1 Slice timing correction

As each volume of functional data is collected over the duration of the TR, it is necessary to correct for the temporal difference between the acquisition of the slices. In the protocol employed for this thesis, 38 slices were acquired sequentially and in a descending fashion
for every TR of 2 seconds – therefore there is an appreciable time gap between the point of acquisition of, for example, slice 5 to slice 30. This is a particularly important consideration in the context of event related designs when task events (and their related neural activity) are occurring in rapid succession.

Using temporal interpolation, slice timing correction considers the acquired values either side of the required reference time point, and gives a weighted average of these (using weights inversely proportional to the distance of the new point from the original values). The middle slice (slice 19) was chosen as the reference slice to which all others were adjusted, as this slice was relatively close to regions of interest such as the striatum, and therefore was subject to minimal interpolation. Sinc interpolation was employed, which uses more distant data points to calculate the new value compared to linear interpolation, and therefore smooths the data less.

2.3.3.2 Realignment

Head movement presents a particularly troublesome issue for fMRI analysis, as each sequentially acquired volume is assumed to have the same spatial characteristics (Friston et al., 1996). Uncorrected head movement of just a couple of millimetres between two given volumes means that the positioning of the voxel in one volume will be spatially altered in the next. Aside from the anatomical inaccuracy this introduces, it will often lead to erroneous changes in BOLD signal being detected as the observed change in intensity is due to the voxel being replaced by a spatially distant one of a different intensity, rather than any change in the hemodynamics within the voxel itself. This is particularly an issue in boundary areas between tissue types, as intensity levels deviate strongly within a few voxels of each other. Given that natural head movement is almost impossible to avoid, especially during longer scanning sessions, several techniques are applied to reduce its impact on the data.

Realignment of functional volumes applies a rigid body transformation to realign each volume to a reference volume, involving rotations and translations along the x, y and z axis (Jiang et al., 1995). This has the effect of minimising the positional deviations between the reference volume and the acquired volumes while maintaining the integrity of the original data as only linear transformations are applied.
To reduce the effect of session on the data, an initial between session alignment was performed for each participant where the first scan from sessions two and three were aligned to the first scan of the first session. A ‘two-pass’ realignment approach was then applied: first all images within each session were aligned to the first image of that session. Second, the images were realigned to the mean image of all three sessions. Images were then resliced to original voxel sizes, as per the current recommendation in the SPM12 manual (Ashburner et al., 2016) when using DARTEL to normalise data (see ‘Normalisation’, below).

As motion related residual fluctuations may still be present in the data following realignment (Friston et al., 1996), it is also standard practice to include the motion parameters estimated during the realignment process as regressors in the first-level design matrix (see ‘First-level modelling’, below). This approach has been shown to be particularly appropriate for event-related designs (Johnstone et al., 2006). In addition to correcting this displacement from the reference scan, accounting for volume to volume (or framewise) displacement has also been shown to reduce both residual error in GLM estimation and the variance in parameter estimates within- and across-subjects, and increase the magnitude of statistical effects (Siegel et al., 2014). Therefore, the calculated framewise displacement was included as a further regressor of no interest (in addition to the six standard motion parameters). Additionally, any volumes with a frame to frame displacement of 1mm or more were flagged and marked with a 3-TR regressor (to include the volumes either side) in the first level design matrix, a process referred to as ‘scrubbing’ (Power et al., 2012). Any scan that resulted in more than 10% of the volumes of the full run being regressed out in this manner resulted in that individual being removed from the analysis. This is an arbitrary, but pragmatic threshold, and limited the number of suspect volumes being entered into group analysis.

Despite these attempts to account for motion related artefacts, there are occasions where head movement is too excessive or structured in such a way that post acquisition correction cannot be satisfactorily guaranteed. One such issue is stimulus correlated motion, whereby head motion accompanies the presentation of a stimulus and therefore the temporal period of interest, producing erroneous task related activity (Bullmore et al., 1999, Field et al., 2000). During the MID task for instance, the button press required in rapid response to the reward stimulus is associated both with the onset of a regressor of
interest and an inevitable increased likelihood of body and head movement due to the speed of the response required.

Therefore, following the initial realignment procedure but prior to first level modelling of each subject, the realignment parameters produced by the two-pass procedure in SPM were visually inspected (note in Figure 2.3-3 the noticeable head movement was associated with volumes in which a stimulus/response for a principal regressor was made which resulted in this subject being excluded from the analysis). Time-series’ for which the maximum detected translation from the first volume was greater than the dimensions of one voxel (i.e. 3mm)/2 degrees rotation or involved patterns of movement which were temporally related to the principal regressors for the analysis (e.g. response to reward cue during the MID task) were flagged and considered for exclusion. This process resulted in five participants (three from RisH/L, two from Olan/Hal) being excluded based on excessive head motion alone. These participants were identified and excluded during the data

Figure 2.3-3 Stimulus correlated motion during the MID task
collection phase, and were therefore able to be replaced by new recruits to maintain a suitably powered study.

2.3.3.3 Co-registration

Since the spatial resolution of the functional data is relatively poor compared to the structural images, it is necessary to coregister the functional images to that same individual’s structural image to provide more precise spatial localisation of any signal changes. It is also a critical step in the normalisation method employed in this thesis. This procedure is slightly more complicated than the realignment step above, as it concerns registering images from different modalities together (and therefore with different contrast, intensity ranges and voxel sizes). Briefly, the process involves creating a frequency histogram of the intensity values in each image, and replacing the intensity value with the histogram bin it was assigned to, so that each voxel has a bin value from each image. If the two images are in perfect alignment, the underlying voxels within a given area should be in the same bins, as although their intensities and sizes may be different their distribution should be the same. The mutual information cost function is typically used as the similarity measure, as it allows for linear and non-linear measures of association. Coregistration therefore involves applying the rigid body registration that maximizes the mutual information between the histogram bins of the voxels in the two images (Ashby, 2011).

To reduce the likelihood of the coregistration routine settling at a local minima, the origin of both the T1-w images and functional volumes were manually reset to the anterior-posterior commissure line prior to any other preprocessing step. This gave the cost function the best chance of success as the functional and structural modalities already occupied similar spatial orientations. The T1-w image for each subject was then coregistered to the resampled mean functional image from the realignment step, using the normalised mutual information objective function in SPM. Registering the structural image to the functional (as recommended in the SPM manual (Ashburner et al., 2016)) instead of the other way around results in the functional images being manipulated as little as possible.
2.3.3.4 Normalisation

Because of the considerable anatomical variation in the size and shape of brains within the population, the functional data must also be normalised to a standard template to minimise differences in brain morphology and allow for analysis to be conducted at a group level. This template will typically be set to a standardised coordinate system to allow subsequent comparison of results between studies. Various normalisation methods are used throughout the literature, with many studies using non-linear registration methods to warp functional images to the MNI coordinate system (Fonov et al., 2009).

Diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL; Ashburner (2007)) is a normalisation method that uses the segmented grey and white matter tissue images (derived from the high resolution structural images from each participant) to build a cohort specific group template in MNI space, which the functional data is then warped to. In brief, it first simultaneously creates a mean of all segmented grey matter and white matter images together as an initial template. Deformations from this template to each of the individual images then are computed, and the template is regenerated by applying the inverses of the deformations to the images and averaging. This generates an increasingly crisp average template data to which the data are iteratively aligned. A final affine transformation moves the template into MNI space.

![Initial template](image1.png) ![Final template](image2.png)

*Figure 2.3-4 DARTEL template creation, first to sixth iteration*
After 6 repetitions of this process, each individual will have a unique set of flow fields that encode the deformations required to move their data from native space into template space. Because it uses a very large number of parameters (around $6 \times 10^3$ parameters per subject) and does not rely on a pre-existing template, DARTEL allows for improved characterisation of brain shapes, resulting in more precise inter-subject alignment, improved group analysis, more accurate localization and increased sensitivity. Although it was originally developed to improve the normalisation of structural data for morphological analysis (Klein et al., 2009), it has also been shown to be a superior normalisation method compared to standard EPI template techniques for both fMRI (Tahmasebi et al., 2009) and PET (Martino et al., 2013) data.

DARTEL was therefore used for normalisation of all modalities of functional and structural data in this thesis. The segmentation tool in SPM12 (an extension of unified segmentation (Ashburner and Friston, 2005)) was used to create grey, white and CSF class images from each MP-RAGE T1-weighted image. This uses both the intensity value of each voxel in the images along with a priori tissue probability maps (TPMs, from the ICBM tissue probabilistic atlas (Mazziotta et al., 2001)) to assign each voxel as belonging to a particular class. This segmentation routine models intensities using a mixture of Gaussians (MoG) to allow non-Gaussian distributions to be modelled – this is particularly the case for voxels with partial volume effects, where there may be signal from more than one tissue type present (such as at tissue boundaries). Unified segmentation also includes a bias correction step, as the smoothly varying magnetic field inhomogeneity caused by MR imperfections is known to disrupt the intensity values across the brain (and thus impact segmentation). The subsequent grey and white matter images were entered into the DARTEL protocol in SPM12 to create a cohort specific template for the RisH/L and Olan/Hal cohorts, and a set of flow fields for each participant which were used to warp each fMRI session into MNI space.

The cohort specific template for each group was also used as a grey matter mask for the functional analysis in this thesis. Following an affine registration to MNI space, the template was binarised at a threshold of 0.95 and used within the permutation testing protocol described in the analysis section below.
2.3.3.5 Smoothing

The final stage of preprocessing of functional data involves the smoothing of the voxels, which helps improve the signal to noise ratio and increase sensitivity. The process involves convolving the fMRI signal with a Gaussian function of a specific width which replaces a given voxel value with the spatially weighted average of its neighbours. This helps reduce remaining anatomical differences between subjects, as well as limiting high frequency signal and boosting low frequency signal. Smoothing also has important statistical benefits. Because it produces weighted averages at each data point it causes the data overall to become more normally distributed, or spatially smooth. This an assumption of Gaussian random field theory (see ‘First-level statistical modelling’, below) which a cornerstone of fMRI statistical analysis (Poldrack et al., 2012).

The extent of smoothing is dictated by the size of the smoothing kernel (typically between 4-12mm), and will depend on the modality and resolution of the data, and the research question being asked. 8mm has been indicated as appropriate size for fMRI analysis (Mikl et al., 2008) and was applied to the functional data in this thesis (i.e. the MID and breath hold data). The other modalities required different levels of smoothing, which are explained within their relevant section.

2.3.3.6 First-level statistical modelling

Prior to examining any group effects on the BOLD signal, each participant’s functional time series requires modelling according to the experimental task they performed during scanning. The standard approach is to model, within-subject, each individual voxel in isolation using the general linear model (GLM) to create a statistical parametric map for each participant, with the goal of identifying which voxels responded to a given experimental manipulation more than others. This is known as single-subject or first level modelling. Because each voxel is analysed independently in a data-set containing many tens of thousands of voxels, it is a mass-univariate approach.
The GLM relates a continuous dependant variable to one or more continuous or categorical independent variables. In the case of fMRI single-subject analysis, the dependant variable is the time series of our voxel and the independent variables are the parameters of the experimental task. It is described by the following equation:

\[ Y = \beta_0 + \beta_n X_n + \epsilon \]

where the observed time series (Y), is explained by a linear combination of the explanatory variables (X_n) and their associated weights (\(\beta_n\)), including some residual error (\(\epsilon\)) and the constant (the mean voxel signal over time). By defining X as the independent or explanatory variables in terms of the known experimental paradigm used, this model can be fitted to the data and the subsequent predicted beta terms reflect how well these explanatory variables explain the observed data - the unknown parameters (\(\beta + \epsilon\)) are therefore estimated from the data available (X and Y).

The key to building an accurate model involves including regressors that best explain the data without the inclusion of regressors that are unrelated to the signal, and which ‘water down’ the model by reducing the degrees of freedom. In simple linear regression, with one explanatory variable, the beta parameter can be estimated using the method of ordinary least squares (OLS), which involves fitting a line which minimises the squared differences (the error) between Y and the estimates predicted by the line (\(\hat{Y}\)). \(\beta_1\) is then given by the slope of the line and \(\beta_0\) is the intercept on the Y axis (the value of Y if X is 0). Multiple linear regression is normally required to model Y in an fMRI task, as it will typically have several explanatory variables. In this form X becomes the design matrix (see below), with each column specifying an explanatory variable or regressor.

One assumption of the GLM is for all observations to be independent and have equal variance. However, the inherent noise in the time series of a given voxel is highly correlated from one time point to the next, which results in a high degree of temporal autocorrelation. This means each time point cannot be regarded as independent as it is partially explained by the time point before and after it. An ordinary least squares estimation assumes uncorrelated errors, and if this temporal autocorrelation is ignored the standard errors of the model coefficients will be underestimated resulting in an increased false positive rate.
Temporal filtering is included in first level modelling in SPM to address this issue and further improve SNR. Much of the noise associated with fMRI data is low frequency noise, such as physiological signals (e.g., cardiac (~0.15 Hz) and respiratory (~0.3 Hz) effects) and slow scanner drift (~<0.01 Hz). SPM applies a high pass 128 second filter to remove this noise from the data. It additionally incorporates an autocorrelation estimation (a first order autoregressive process or AR(1)) to assess the level of correlation in the noise and remove it from the time series (a method also known as ‘prewhitening’). The restricted maximum likelihood method (ReML; Patterson and Thompson (1971), Harville (1974)) is then used to give an unbiased estimate of the error in the model. Although there is still a great deal of debate about the best way to address the issue of autocorrelation in fMRI (Eklund et al., 2012), in event related designs at a TR of 2 seconds (as for the main data set of interest herein) this approach is shown to limit the false positive rate (Eklund, 2012).

As described in above, due to the haemodynamic characteristics of the response there is a delay in the BOLD signal following stimulus onset. The onsets of the explanatory variables in the model are therefore convolved with a canonical haemodynamic response function to provide a more accurate model fit (modelled in SPM12 as a double gamma function). This, along with the six motions parameters, framewise displacement and any scrubbing regressors that were created as part of the realignment stage of preprocessing, are added to the design matrix and the model estimated at each voxel.

Figure 2.3-5 Design matrix – typical event-related model for the MID task. The first 11 columns are task regressors, the next 7 are movement regressors and the final column is the constant.
Examining the difference in BOLD response between regressors of interest can be explored using linear combinations of the estimated parameters. A t-contrast tests if the parameter weights between two different conditions is significantly different from zero, given the error in the model, and creates a t-statistic at every voxel. T-contrasts therefore allow comparison between an experimental condition and a control condition which can then be tested at the group level.

For task activated designs, a typical contrast will be made between an experimental condition and a control task which includes as much of the experimental task without the element of interest. For instance, in the MID task employed in this study, the control task requires attending to a cue and making a fast motor response, but as there is no chance to win money it does not include the element of reward present in the experimental task – therefore the difference between the two conditions more plausibly reflects reward processing alone (compared to comparison to an implicit resting/task-free baseline for instance).

### 2.3.3.7 Second Level Statistics/Permutation testing

A standard approach to analysing neuroimaging data at the group level would be to enter these individual contrast images into a second level analysis, whereby a statistical test (such as two-sample t-test) which accounts for between subject variability can be performed to test for differences in activation between groups of participants. Setting an appropriate statistical threshold for these tests has been a source of debate in the neuroimaging literature for some time (Eklund et al., 2016)

Because a statistical test has been performed at every voxel in a data set containing many thousands of voxels this produces a multiple comparison problem. A single statistical test will typically set the type I error rate at $\alpha = 5\%$, meaning the likelihood of obtaining a ‘significant’ result and incorrectly rejecting the null hypothesis would occur at a rate of 1 in 20, a reasonable threshold for a single test. In a single functional brain image of ~100000 voxels however, this threshold would produce 5000 false positives. A standard correction for this family wise error (FWE) is the Bonferroni correction, but requires all tests be independent from each other which is not the case in a spatially dependant fMRI image. Additionally, applied to neuroimaging data this would set $\alpha$ for a given voxel at around the
0.00005% mark – a far too conservative level for BOLD data with its relatively modest range in signal change – and drastically increase the type II error rate.

As the data has been smoothed during preprocessing, the principles of random field theory (RFT) can be applied to provide a more appropriate corrected statistical threshold by taking into account the spatial correlations in the data. For a full review of this procedure see Ashby (2011) - but in principle the approach works by estimating the smoothness of the data in each statistic image and calculating the number of resolution elements (or resels), a virtual voxel based on the size of the smoothing kernel applied during preprocessing. By then calculating an estimate of the Euler characteristic (an assessment of the topology of the data) an appropriate threshold for the likelihood that voxels (or clusters) with a particular statistic level would appear by chance (given the number of resels) can be found, which is then employed as the FWE-corrected α-level.

Examining each voxel individually to pick out those that exceed this threshold is known as voxel-level inference. Random field theory uses the Euler characteristic to account for the fact that all voxels are part of a ‘smooth’ field, but does not account for contiguous voxels displaying similar effects. Cluster-level inference first applies a cluster-forming threshold, with contiguous voxels above this threshold then being assessed on a second threshold based on the cluster’s size. RFT allows for the calculation of the expected number and size of clusters in a data set, and neuroimaging analysis packages will therefore report both voxel and cluster level statistics. Due to its superior sensitivity over voxel-based statistics, cluster-based thresholding has dominated the recent neuroimaging literature, accounting for the correction method used in 75% of studies published in a sample of seven leading neuroimaging journals between 2010 and 2011 (Woo et al., 2014).

Cluster correction usually relies on the assumptions that spatial smoothness in fMRI data is constant over the brain and that the spatial autocorrelation is normally distributed. However, recent research has cast some doubt on these assumptions which may lead to an inflated false positive rate. In an assessment of group level analysis using SPM, FSL and AFNI, Eklund et al. (2016) reported that cluster-based thresholding in SPM produces a false positive rate of up to 25% in an event-related design with 8mm smoothing. This increased to almost 90% for a cluster defining threshold of \( P = 0.001 \) (uncorrected) combined with an arbitrary cluster extent threshold of 10 (an ad hoc approach commonly reported in the
literature). The authors attributed this inflated rate to the distribution of the spatial autocorrelation not meeting the assumptions of normality, specifically having larger tails than a Gaussian distribution (meaning distant voxels are more correlated than assumed).

Permutation testing did not produce any significant increase in false positives, presumably due to being a non-parametric method which does not rely on any assumptions of normality. Instead, non-parametric methods use the data itself to create the distribution under the null hypothesis. Permutation testing achieves this using the premise that under the null hypothesis there is no experimental effect and therefore any group labels are arbitrary. By switching the labels into many thousands of possible arrangements and calculating a t-statistic for each of these permutations, a distribution is created from which a 5% threshold can be calculated.

In terms of the data and design within this thesis, this would involve taking a given set of contrast images from the first level for one treatment session and comparing them with the contrast images for a second treatment session, creating a statistics map of the differences between them. The within-subject data labels are then shuffled and another statistic map is calculated, but only the largest statistic over the whole brain from this test is saved. The labels are shuffled again, and the largest statistic is again saved. This process is repeated 5000 times, with a different arrangement of data for each permutation, allowing the creation of a null distribution of test statistics against which the original test (from the ‘real’ arrangement of group labels) can be tested. Because the null distribution is built from the data itself, this type of permutation testing routinely accounts for the smoothness in the data. Cluster-based permutation testing simply involves the same process as above, but the largest cluster is saved after each permutation (after application of a cluster forming threshold) instead of the largest test statistic. Switching the labels in this fashion is straightforward if these observations are independent but with a repeated measures design as in this thesis, the correlation that exists cannot be broken by moving one individual’s first scan and grouping it with a second individual’s second scan. However, permutation testing in a design involving more than one measurement per subject is permitted with the definition of exchangeability blocks, which stipulate that permutations of observations will only occur within a block, i.e., only observations with the same group label will be exchanged.
The second level analysis throughout this thesis was therefore conducted using non-parametric permutation testing, using the *randomise* feature in FSL and threshold free cluster enhancement (TFCE; Smith and Nichols (2009)). TFCE was developed to address the arbitrarily defined initial cluster forming threshold in cluster correction, and integrates both magnitude and spatial extent in determining a test statistic. A voxel’s ‘score’ is derived from the sum of the supporting section underneath it, given by its height multiplied by its extent, with the extent and height constants optimised for fMRI data. The resulting TFCE score is therefore a weighted sum of the entire clustered signal, without requiring the arbitrary initial cluster-forming threshold described above, and allows the detection of both spread out clusters with a relatively low signal maxima as well as smaller, more focal signals with a larger magnitude (which standard cluster-thresholding may fail to detect).

2.3.3.8 Modelling MID task data

The anticipation, target and feedback periods are the main modelled components of the MID task and were defined as in Figure 2.3-6.

![Figure 2.3-6 Regressors defined for each manifestation of a MID task](image)

Three cue regressors (high win (HCue), low win (LCue) and neutral (NCue)) were defined for the anticipatory period depending on the cue presented, with the regressor set to include both the cue and the fixation cross (a variable total amount of time between 4050ms and 4400ms, depending on the trial). The target was defined by a single regressor of variable time, dependant on participant performance (150ms – 350ms). Five feedback regressors (high win (H+Win), low win (L+Win), high no win (H-Win), low no win (L-Win), neutral win (NWin)) were defined for the feedback period depending on the cue type and outcome (win or no win). These regressors were set for a fixed period (1450ms). The entire duration
of passive trails were defined as a single regressor of 4250ms. This resulted in the entire run being modelled by the ten regressors above (and seven movement parameters).

In addition, some performance related regressors were also set. When participants failed to make a response to an active task (ie no button press was attempted on non-passive trials), the entire trial of 10 seconds was defined as a missed response regressor of no interest. Any trials with an RT of +/-3SDs of that individual’s mean were also classified as a missed response. This was to ensure only trials the individual was attending to were included in analysis. If more than 10% of a single active trial type was regressed out after applying this threshold, that subject was removed from the analysis. Further performance related exclusions are described in the behavioural analysis section of Chapter 4.

Contrasts of interest were set to explore main effect of anticipation of reward (HCue & LCue > NCue), and main effect of receipt of reward (both for win situations (H+Win & L+Win > NWin) and no win situations (H-Win & L-Win > NWin)). Drug influence on the main effect of reward anticipation and outcome was of primary interest, but exploratory analyses of the level of reward for both anticipation and outcome was also examined using three contrasts (HCue > LCue for anticipation; H+Win > L+Win and H-Win > L-Win for outcome).

To examine the effect of drug on reward processing, contrast images from the first level were taken forward into second level permutation testing using RANDOMISE in FSL, with TFCE. 5,000 permutations were conducted for each treatment-placebo comparison in order to create a non-parametric null distribution and calculate a 5% significance threshold, familywise error corrected. Exchangeability blocks were specified to ensure permutations would only occur within subject, to take account of the repeated measures nature of the data. Paired sample t-tests were conducted between each drug condition and placebo scan. A second round of analyses were conducted which included both the ASL and BH maps (see below) as voxelwise covariates of no interest to explore the influence of the drug induced changes to baseline CBF and CVR on the BOLD signal.

2.3.3.9 Modelling Breath Hold task

The breath hold task is often modelled with a simple box car function, although the hemodynamic response to the task does not follow the same temporal characteristics as a typical block design task convolved with the HRF as it is reliant on arterial accumulation of
CO₂ over time (Sasse et al., 1996). Murphy et al. (2011) examined the most accurate manner to model the breath hold task for fMRI data, and reported that delaying the onset of the hold regressor by 9 seconds and including its temporal derivatives (allowing for a shift in time between the regressor and the BOLD signal) explained more of the whole brain variance in the signal than the block design alone. The data in this thesis was therefore modelled as such. Contrasts between the paced and held breathing portions of the task were then employed to examine the effect of task on BOLD signal, which was used as a metric of CVR.

The task required individuals to hold their breath after expiration. Although this is perhaps a slightly less ‘natural’ fashion in which to hold one’s breath, end expiration holding has been shown to produce a faster peak signal than end inspiration (Kastrup et al., 1999), which itself produces a bi-phasic response (Li et al., 1999) and is problematic to model. During the regular breathing portions of the task, participants were given instructions to breath at a controlled rate (breath in for 3 seconds, out for 3 seconds) as this approach produces a larger peak BOLD signal and improved SNR than self-paced breathing (Scouten and Schwarzbauer, 2008). Finally, as the breath hold signal has been shown to plateau around 20 seconds (Liu et al., 2002, Magon et al., 2009), a 16 second hold was chosen as to be long enough to produce a peak response whilst not being uncomfortable for the participant (and reduce the likelihood of head movement).

To examine the effect of drug on CVR alone, contrast images from the first level were taken forward into second level permutation testing. Paired sample t-tests were conducted between each drug condition and placebo scan, and thresholded at 5% FWE corrected.

2.3.3.10 ROI choice

In addition to the exploratory whole brain analysis described above, a priori regions of interest were also examined based on areas of the brain involved in the processing of reward anticipation or receipt.

2.3.3.10.1 Striatum

The striatum is a large subcortical structure and forms part of the limbic system, acting as the main input module to the basal ganglia. It is involved in number of processes, such as elements of motor control, and has been shown to be a major hub of the reward system.
It has a high density of D2 receptors, the common target of all antipsychotic drugs.

Anatomically the striatum can be divided into three main sections. The putamen, the caudate (which are separated by the internal capsule and together form the dorsal striatum), and the ventral striatum (which contain the nucleus accumbens). It is a heavily interconnected structure, receiving afferents from the midbrain, thalamus and cortex, and sending efferents to widespread subcortical and cortical areas via the substantia nigra pars reticulate and globus pallidus.

The ventral striatum has been most strongly associated with reward processing, although dorsal areas have also been implicated. Some research suggests the dorsal striatum is more involved in learning about the actions that produce reward (O'Doherty, 2004, Tricomi et al., 2004), compared to the passive prediction of reward overseen by the ventral striatum. Therefore, to elucidate the relative involvement of this complex structure in reward processing, probabilistic bilateral putamen and caudate ROIs were defined from the FSL Harvard-Oxford subcortical atlas, thresholded at 0.20 and binarised using the fslmaths tool as implemented in fslutils (Jenkinson et al., 2012). A bilateral ventral striatum ROI was also defined as described in Montgomery et al. (2006), based on previous work by Mawlawi Mawlawi et al. (2001). These ROIs were also combined with the SPM probabilistic grey matter mask (thresholded at 0.20) to ensure any areas extending into non-grey matter areas such as CSF were removed.

In addition to its functional involvement in reward and its modulation by antipsychotic drugs, there are also well replicated reports of acute and chronic structural changes due to antipsychotic administration in patients, healthy volunteers and preclinical subjects in this region, in addition to changes in striatal blood flow in these regions following antipsychotic administration. Therefore, these striatal regions were the main region of interest for all modalities within this thesis, and were also employed in the structural analysis described in Chapter 3.
2.3.3.10.2 VTA

The ventral tegmental area (VTA) in the midbrain is the major source of DA neurons projecting to the ventral striatum and has previously been shown to be involved in reward processing. The substantia nigra is also an important origin of DA projections to the dorsal striatum - however, due to the limited resolution of fMRI, the small nature of these structure and their close spatial proximity within the midbrain, only the VTA was selected from this region. The VTA ROI was created based on Murty et al. (2014), which created ROI templates suitable for fMRI.

In addition to these areas heavily involved in reward anticipation, a further 3 ROIs were defined to explore reward outcome.

2.3.3.10.3 Amygdala

A critical part of the limbic system sharing direct connections with the ventral striatum and VTA, the amygdala has been implicated in the non-receipt of expected reward, as well as ‘positive’ reward processing, and has been shown to be sensitive to DA modulation during reward processing in healthy volunteers (Murray, 2007a, O’Daly et al., 2014, Russo and Nestler, 2013, Tye et al., 2010a). A bilateral amygdala ROIs was defined from the FSL Harvard-Oxford subcortical atlas, thresholded at 0.20 and binarised.

2.3.3.10.4 Frontal regions: Ventro-medical prefrontal (vmPFC) and anterior cingulate (ACC) cortex

The vmPFC shows strong connections to the ventral striatum (Pujara et al., 2016), with the anterior cingulate being more connected to dorsal striatal regions (Knutson & Haber, 2010). Both regions have been shown to be involved in the processing of outcome phase of reward (Vassena et al., 2014). The ACC ROI was defined from the AAL atlas (Tzourio-Mazoyer et al., 2002). The vmPFC ROI was defined from coordinates in Vassena et al. (2014) based on (Liu et al., 2011) and centred a 10mm sphere on MNI coordinates x=0 y=51 z=-10. Frontal areas are thought to be more involved in the processing of the outcome of reward, and therefore these regions will only be applied in the feedback analysis section of this thesis.

After first level modelling as described above, these ROIs were used to extract beta estimates using the MarsBar plugin in SPM12. The extracted values were analysed in SPSS.
using generalised estimating equations, which allow the inclusion of covariate ROI measures of CBF and CVR, derived from their respective scans.

2.3.4 ASL data preprocessing and analysis

The T2-weighted images were co-registered to the MP-RAGE T1-w image used in the creation of the DARTEL template (described above). Each raw Proton Density image (acquired at the end of the pCASL sequence) was then co-registered to the T2 image, as this provided superior contrast for the mutual information cost function. The parameters for this transformation were then applied to the CBF maps (as they were already in alignment with the Proton Density image) prior to their normalisation using the earlier generated DARTEL flow fields. Finally, the normalised CBF maps were smoothed using a 6mm full width at half maximum (FWHM) kernel. As increasing the number of pCASL scans per session has been previously shown to increase sensitivity to drug effects (Marquand, et al., 2012), image calculator in SPM was then used to create an average image of the two CBF maps produced per visit for each volunteer.

Global CBF values were extracted using the MarsBar toolbox (Brett et al., 2002) and the default whole brain mask provided in SPM8, and analysed using a one-way repeated measures ANOVA for each group in SPSS. Mean CBF values for each of the a priori striatal ROIs were also extracted from unsmoothed images and analysed by the same means. Absolute CBF values were used as the main metric of interest in this analysis as they provide the most meaningful comparison with T1 values from the same region. However, CBF calculated relative to whole brain CBF has been shown to be more sensitive in detecting regional differences (Aslan and Lu, 2010, Stewart et al., 2015) - therefore for the whole brain analysis the average global CBF values were included as a covariate. For completeness, whole brain relative ROI CBF was also examined, calculated simply as whole brain CBF minus ROI CBF, and analysed in the same manner as the absolute values in a one-way repeated measures ANOVA.

Non-parametric whole brain analysis was conducted using Threshold Free Cluster Enhancement (TFCE; (Smith and Nichols, 2009) within FSL’s RAMDOMISE (Winkler et al., 2014). 5,000 permutations were conducted for each treatment-placebo comparison to create a non-parametric null distribution and calculate a 5% significance threshold, familywise error corrected. Exchangeability blocks were specified to ensure permutations
would only occur within subject, to take account of the repeated measures nature of the data. Data were modelled using a General Linear Model (GLM) with global CBF values added as a covariate, to account for inter-individual differences in global perfusion (Handley, et al., 2013). Paired t-tests were conducted between each drug and placebo condition to assess treatment effects on perfusion.

2.3.5 Structural imaging processing and analysis

2.3.5.1 Quantitative analysis

Driven Equilibrium Single-Pulse Observation of T1 (DESPOT1; Deoni (2007)) was used to create voxel-wise quantitative T1 maps for each subject, in all scanning sessions. Briefly, this involved resetting the origin of the IR image and the SPGR image at both flip angles to the anterior commissure and reorienting the images to the AC-PC line, and then registering and resampling all images within subject to the SPGR acquired on the second visit. The DESPOT1HIFI protocol (described in detail in Deoni (2007)) was then used to create a T1 map for each visit.

A ROI approach was primarily conducted to assess T1 times in the a priori regions in the striatum, in addition to an exploratory voxel-wise whole brain approach. For whole brain analyses a DARTEL template was created for each group using the 4-degree flip angle SPRG images from each participant’s second visit. The subsequent flow fields were applied to the native space T1 maps to warp them into standard space before smoothing with a 6mm FWHM kernel for the voxel-wise analysis, allowing for assessment of T1 values throughout the whole brain. For the ROI analysis, the same flow fields were utilised to inverse-warp standard space ROIs into each individual’s native space using nearest neighbour interpolation, producing a set of ROIs for each individual T1 map. These native space ROIs were eroded with a 3x3x1 kernel in FSL to endure the warping procedure did not extend any of the ROIs into CSF. This allowed more precise assessment of a priori regions while controlling for any errors related to the normalisation or smoothing steps of the T1 maps (Aribisala et al., 2011). After extraction, mean T1 from each ROI was entered into a two way repeated measures ANOVA in SPSS (within subject factors: drug and ROI) for each group. For completeness the same analysis was conducted on ROI values extracted from the normalised T1 maps using the standard space ROIs described above to rule out any potential issues related to the ROI warping procedure.
2.3.5.2 T1-weighted analysis methods

To fully elucidate any apparent structural changes due to drug exposure and account for any differences in analysis methodology, four standardised methods were employed to assess the T1-weighted images: 1) Voxel-Based Morphometry (VBM) in SPM; 2) Longitudinal Registration in SPM; 3) Structural Image Evaluation, using Normalization, of Atrophy (SIENA) within FSL; 4) Longitudinal stream analysis for cortical thickness and subcortical volume within Freesurfer. The processing steps for each of these methods, and their relative strengths and weaknesses for the addressing the hypotheses are discussed below.

Standard T1-weighted analysis was also undertaken to fully elucidate any apparent structural changes due to drug exposure and account for any differences in analysis methodology. Similar to the ASL analysis, non-parametric inference was used for the voxel-wise VBM analysis, and standard parametric approaches were used for the other methods. For completeness, the VBM analysis was repeated with parametric inference as results have been shown to differ significantly depending on which approach is employed (Rajagopalan et al., 2014).

To allow clearer comparison between the quantitative T1 maps and the T1-weighted images, the same SPGR images used in the creation of the T1 maps were employed in these analyses.

2.3.5.3 VBM

Images were segmented into GM, white matter (WM), and CSF class images in SPM8 (Ashburner and Friston, 2005). Employing the same DARTEL template and flow fields used in the normalisation of the T1 maps (see above), each GM class image was normalised, and smoothed with a 6mm FWHM kernel using the DARTEL toolbox. A 6mm smoothing kernel was chosen based on recommendations in Shen and Sterr (2013). This DARTEL framework includes a modulation step whereby the GM segments are multiplied by their Jacobian normalisation determinates in order to preserve the original local volumes in the final images.

Total brain GM volume was derived using SPMs get_totals function and drug effects assessed with a repeated measures ANOVA in SPSS. Non-parametric whole brain analysis
was conducted in FSL as described above, with paired t-tests run between each drug and placebo. Standard parametric whole brain analysis was also conducted with a series of paired t-tests in SPM between each drug/dose group and placebo, voxelwise thresholded at p<0.05 FDR corrected. Mean values were also extracted from each of the a priori ROIs which were included in a repeated measures ANOVA with drug and ROI as within-subjects factors.

2.3.5.4 Longitudinal registration

VBM has been used extensively in cross sectional analysis between two or more groups but presents some challenges when applied to repeated-measures or longitudinal studies, primarily with regards to the definition of an unbiased template (Thomas et al., 2009, Yushkevich et al., 2010). The longitudinal registration toolbox (Ashburner and Ridgway, 2012), introduced in SPM12, attempts to avoid this bias from asymmetry. The framework interleaves a rigid body registration, an intensity inhomogeneity correction and a non-linear diffeomorphic registration of temporally separated within-subject pairs of anatomical MRI scans.

It produces an average midpoint template and a ‘difference’ image which encodes the differences between the deformations from the midpoint template to time-point one (placebo) and the deformations from midpoint to time-point two (drug). These midpoint images were segmented in SPM and the GM class images multiplied by the Jacobian differences to create images reflecting the GM volume change between the pairs of scans. Group templates were created in DARTEL from the midpoint segmentation, and the GM volume change images normalised and smoothed with a 6mm FWHM kernel, producing an unbiased normalisation. One sample t-tests were conducted to assess voxelwise change between each drug and placebo, thresholded at p<0.05 FDR corrected. Small volume corrections were also conducted within the a priori ROIs. This deformation based approach to assessing GM volume is also particularly suited to longitudinal based analysis, as the direct deformations between pairs of scans encode all the salient morphometric information (Tardif et al., 2016).

2.3.5.5 SIENA

The precision of VBM is highly dependent on the accuracy of the initial segmentation procedure, which itself is vulnerable to partial volume (PV) effects throughout tissue type
boundaries in the brain (Rueda et al., 2010). Further, the use of different segmentation algorithms has been shown to produce significantly differing outcomes in the assessment of GM changes (Callaert et al., 2014). In assessing the effect of lithium on brain volume, Cousins et al. (2013) used both VBM and SIENA; (Smith et al., 2002), part of FSL (Smith et al., 2004)) to examine within-subject longitudinal change. SIENA detects the brain-non brain edge displacement over pairs of scans, and only uses segmentation to detect the brain-CSF border. It may be less vulnerable to the PV effects that may occur in deep sulci or mixed tissue types (Cousins et al., 2013) such as the pallidum, and therefore this method was also employed here.

SIENA starts by extracting brain and skull images from the two-timepoint whole-head input data (Smith, 2002). The two brain images are then aligned to each other (Jenkinson and Smith, 2001, Jenkinson et al., 2002) using the skull images to constrain the registration scaling; both brain images are resampled into the space halfway between the two. Next, tissue-type segmentation is carried out (Zhang et al., 2001) in order to find brain/non-brain edge points, and then perpendicular edge displacement (between the two timepoints) is estimated at these edge points. Finally, the mean edge displacement is converted into a (global) estimate of percentage brain volume change between the two timepoints, which were assessed for each drug versus placebo using a one-sample t-test.

**2.3.5.6 Freesurfer**

VBM cannot readily distinguish between GM changes that are due to cortical thickness and those due to cortical folding (Jbabdi, 2009). Approaches which attempt to measure cortical thickness directly have been shown in some case to be more sensitive to GM changes (Hutton et al., 2009), and it is a measure which has been reliably shown to be affected by long term antipsychotic use in both humans (Thompson et al., 2009) and rodents exposed to clinically comparable doses of antipsychotics (Vernon et al., 2014). FreeSurfer (v5.3.0) is a commonly used package to assess cortical thickness and subcortical volumes. As it able to more accurately delineate the folds of the cortex to a sub voxel accuracy, it may be less vulnerable to PV effects in deep sulci that could be influenced by blood flow. It uses a surface based approach to image registration, which may also be superior to volume based methods in some instances (Hinds et al., 2008, Ghosh et al., 2010), although direct comparison between the two techniques is problematic (Klein et al., 2010). It also avoids the modulation step inherent to VBM, the suitability of which has recently come under
scrutiny (Radua et al., 2014). Taken together, these advantages may allow a clearer interpretation of the biological basis of the outcome measure (Lovden et al., 2013).

Cortical reconstruction and volumetric segmentation was performed with the Freesurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). To extract reliable thickness estimates, images were automatically processed with the recently developed longitudinal stream (Reuter et al., 2012). Specifically, an unbiased within-subject template space and image (Reuter and Fischl, 2011) was created using robust, inverse consistent registration (Reuter et al., 2010). Several processing steps, such as skull stripping, Talairach transforms, atlas registration as well as spherical surface maps and parcellations were then initialized with common information from the within-subject template, significantly increasing reliability and statistical power (Reuter et al., 2012). Measures of total cortical thickness and subcortical volume measures of the caudate, putamen and nucleus accumbens were extracted for each hemisphere and quality control checked using the freely available ENIGMA protocols (http://enigma.ini.usc.edu/). Measures for each time point were entered into a repeated measures ANOVA in SPSS, with drug and hemisphere as factors.
Chapter 3  Antipsychotic modulation of blood flow and structural metrics

3.1 Introduction

Some recent studies have indicated acute antipsychotic exposure in healthy individuals produces structural remodelling as assessed with MRI, although the contribution of alterations in blood flow (concurrently elicited by the drug) has not been experimentally assessed. This chapter will first examine the changes in CBF induced by a single dose of three different antipsychotics using both a whole brain and *a priori* ROI approach. Results of a comprehensive structural analysis will then be presented allowing any influence of blood flow changes on structural metrics to be evaluated.

Data are mean +/- standard deviation unless otherwise stated.

3.1.1 Plasma levels

Total drug exposure calculated using the trapezoid method as area under the curve (AUC) from administration to 4.5 hours (30 minutes before start of the scan) for haloperidol and olanzapine, and from administration to 3.8 hours (end of the scan) for risperidone were as follows: Haloperidol 1530 +/- 720 (pg*hr)/ml; Olanzapine 16.8 +/- 6.83 (hr*ng)/ml; Risperidone 0.5mg 13.8 +/- 4.53 (ng*hr)/ml; Risperidone 2mg 54.0 +/- 17.6 (ng*hr)/ml.

3.1.2 Cerebral Blood Flow

3.1.2.1 Global blood flow

Group average global mean CBF values (ml/100g/min) for the RIS-H/L group were: Risperidone 2mg 47.98 +/- 10.37, Risperidone 0.5mg 49.52 +/- 8.71, placebo 49.21 +/- 8.90; and for the HAL/OLAN group: Olanzapine 7.5mg 43.10 +/- 7.76, Haloperidol 45.3 +/- 7.93, placebo 46.29 +/- 7.3.

A one-way repeated measure ANOVA revealed there were no global differences in CBF in the RIS-H/L group (*F*(2,40) = 1.380 *p* < .263), but a borderline significant difference was observed in the OLAN/HAL group (*F*(2,34) = 3.395 *p* < .045). Pairwise comparisons with
Bonferroni adjustment revealed the largest reduction in CBF was after olanzapine compared to placebo, a non-significant reduction of 3.1 (95% CI, 0.36 to -6.43) ml/100g/min, $p = .056$.

3.1.2.2 Whole brain analysis

Both haloperidol and risperidone produced significant increases in striatal blood flow (Figure 3.1-3 & Figure 3.1-2) Haloperidol produced greater perfusion than placebo in two large bilateral clusters encompassing the right and left putamen, while 2mg risperidone produced a very large continuous cluster with a peak centred around the left caudate but extending into bilateral caudate, putamen and anterior cingulate. 0.5mg risperidone produced a similar but less pronounced pattern to 2mg, and was limited to left and right caudate and putamen. 2mg risperidone also produced large reductions in blood flow within the cerebellum Figure 3.1-1 – these were not found after the 0.5mg dose.

Non-parametric whole brain analysis did not reveal any significant changes in blood flow with Olanzapine compared to placebo.

![Figure 3.1-2 Whole brain blood flow 2mg Risperidone>Placebo: 5,000 permutations, peak -11.3, 5.6, 9; 9486 voxels, p < 0.05 (FWE corrected)](image)

![Figure 3.1-1 Whole brain blood flow Placebo>2mg Risperidone: 5,000 permutations, peak at 5.64, -73.3, -21; 7800 voxels, p < 0.05 (FWE corrected)](image)
3.1.2.3 ROI analysis

Absolute CBF values (figures 2 & 3, left) sampled from the a priori ROIs were entered into a two-way repeated measures ANOVA (within subject factors: drug and ROI) which revealed a significant main effect of drug on CBF in both the RIS-H/L group ($F(2,40) = 6.476, p = 0.004$) and in the OLAN/HAL group ($F(2,34) = 6.838, p = 0.003$). Pairwise comparisons revealed a significant increase in CBF compared to placebo after 2mg risperidone (4.545 ml/100g/min (95% CI 1.117 to 7.973) $p = 0.07$), 0.5mg risperidone (3.243 ml/100g/min (95% CI 0.553 to 5.933) $p = 0.015$), and haloperidol (4.125 ml/100g/min (95% CI 0.926 to 7.324) $p = 0.01$). There was no significant pairwise difference between olanzapine and placebo. However, when ROI values for olanzapine were analysed relative to whole brain values, a significant increase did emerge compared to placebo (2.755ml/100g/min (95% CI 0.788 to 4.722) $p = 0.005$). Whole brain relative ROI blood flow after risperidone and haloperidol also produced similar significant increases in the ROIs to that of the absolute values.

3.1.3 Quantitative T1 analysis

There was no significant effect of drug on T1 in either group, for ROIs extracted both from native space T1 maps (RIS-H/L: $F(2,40) = 0.383, p=.684$; OLAN-HAL: $F(2,34) = 0.253, p=.778$) and normalised maps (see figures 2 & 3, right; RIS-H/L: $F(2,40) = 0.407, p=.668$; OLAN-HAL: $F(2,34) = 0.253, p=.779$).

Voxel-wise analysis of normalised T1 maps also failed to reveal any significant changes, which remained the case with an exploratory height threshold of 0.001 uncorrected (cluster threshold 50).
Figure 3.1-4 Comparison of CBF and T1 changes due to acute exposure to 2mg risperidone in striatal ROIs (*p<0.05 Bonferroni corrected)

Figure 3.1-5 Comparison of CBF and T1 changes due to acute exposure to 3mg haloperidol in striatal ROIs (*p<0.05 Bonferroni corrected)
3.1.4 T1-weighted image analysis

None of the automated morphometric techniques applied to the T1-weighted images detected an effect of drug on brain structure.

3.1.4.1 VBM

Analysis of whole brain volumes extracted from GM segmented images revealed no change between either drug dose and placebo in RIS-H/L (Placebo 795.39 cm³ +/- 71.49: 2mg Risperidone 792.09 cm³ +/- 71.48 (t = -1.143, p=0.267); 0.5mg Risperidone 790.4 cm³ +/- 69.41 (t = -1.634, p=0.118)) and no change between either drug type and placebo in HAL/OLAN (Placebo 794.04 cm³ +/- 69.34: Olanzapine 793.36 cm³ +/- 70.91 (t = -.153, p=0.880); Haloperidol 793.08 cm³ +/- 68.81 (t = -.266, p = 0.824)).

Non-parametric whole brain analysis did not reveal any significant voxels between drug and placebo. Similarly, parametric voxelwise paired t-tests failed to reveal any volume change between drug and placebo at the FWE correction threshold of p < .05.

ROI analysis also showed no significant change in grey matter metrics on any of the drugs compared to placebo. For completeness, and to rule out any effect of the normalisation procedure, paired t-tests between the mean extracted tissue probability values from unmodulated native space grey matter images segmented from the T1-weighted scans were conducted, and also revealed no significant effect of any treatment level vs placebo.

3.1.4.2 Longitudinal registration

One sample t-tests of the jacobian difference images between placebo and drug GM maps revealed no significant differences in either group. Small volume corrections in each of the ROIs did not reveal any difference between time points.

3.1.4.3 SIENA

One-sample t-tests of percentage brain volume change between placebo and each drug revealed no significant change (0.5mg risperidone 0.02% +/- 0.39 (t = .784, p=.784); 2mg risperidone -0.23% +/- 0.72 (t=-1.48, p=.154); Olanzapine -0.07% +/- 0.28 (t = -1.09. p=.292); Haloperidol -0.008% +/- 0.41 (t = -0.085, p=.933)).
3.1.4.4 Freesurfer

A two-way repeated measures ANOVA (factors: drug and hemisphere) revealed no significant effect of drug on whole brain cortical thickness in either group (RIS-H/L: $F(2,40) = 0.301, p = 0.742$; OLAN-HAL: $F(2,34) = 0.047, p = 0.955$). A second repeated measures ANOVA analysis on the subcortical ROIs automatically defined by Freesurfer that closest matched our pre-defined ROIs (caudate and putamen) was conducted with factors drug, hemisphere and ROI, and revealed no significant main effect of drug in either group (RIS-H/L: $F(2,40) = 0.373, p = 0.691$; OLAN-HAL: $F(2,34) = 0.519, p = 0.600$).

3.1.5 Preclinical confirmation

Given the negative nature of the structural findings and to exclude the possibility that drug dose was a limiting factor, we explored the effects of a high dose of risperidone using a reverse-translational design. As many of the structural changes linked to chronic antipsychotic exposure have been replicated in animal studies, a preclinical investigation of the acute effects of exposure would supplement the human investigations appropriately. As a pilot study, this was initially limited to risperidone as this drug produced the most robust increases in blood flow in the human cohort, and a focus was again placed on quantitative T1 metrics for precision.

Male Sprague-Dawley rats (n=16; Charles River UK, Kent, United Kingdom), strain CD, initial body weight 269g to 289g were housed 4 per cage under a 12-hour light–dark cycle (7 AM lights on) with food and water available ad libitum. Room temperature was maintained at 21° ±2°C and relative humidity at 55% ±10%. Animals were habituated for 7 days before experimental procedures. Animal experiments were carried out with local ethical approval and in accordance with the Home Office Animals (Scientific Procedures) Act 1986, United Kingdom.

In a repeated measures, within-subject design, each rat was administered a 0.1ml per 100g body weight subcutaneous dose of saline 90 mins prior to the first scanning session. After recovery, each animal was returned to housing for 48hrs before receiving a 2mg per 1kg body weight subcutaneous dose of risperidone (Sigma-Aldrich) 90 mins prior to the second scanning session at the theoretical cmax. Animals were then euthanised by terminal anaesthesia.
Within each scanning session, a fast spin echo multi-slice T2-weighted image (matrix: 256x128x55; TR/TE: 4/0.06 secs), a 3D multi gradient echo image SPGR at multiple flip angles (matrix: 160x160x64; TR/TE: 0.02/0.005 secs; flip angles 3, 13, 21 degrees) and an actual flip angle (AFI) sequence (matrix: 64x32x32; TE: 0.003388; TR: 0.02) were acquired in vivo under isoflurane anaesthesia (5% induction, 1.5% maintenance delivered in 70% medical air/30% oxygen mix), using a 7.0T horizontal small bore magnet (Varian, Palo Alto, California) with custom-built head radiofrequency coil (David Herlihy, Imperial College London, United Kingdom) connected to a console running VnmrJ acquisition software (v2.3; Varian).

A B1 map was created from the AFI sequence as previously described (Yarnykh, 2007), which was used along with the SPGRs to create the T1 maps. Due to their superior contrast, the T2-weighted images were used for the initial preprocessing of the data, and these parameters then applied to the T1 maps (which are in the same orientation and dimensions of the T2-weighted scans). After an initial within subject rigid body registration of the T2-weighted images to the T1 maps, the T2-weighted images were then affine registered to a group template created by the Advanced Normalisation Tools suite (Avants et al., 2011) from the 16 baseline placebo images.

Figure 3.1-6 T1 map of rodent brain

Structural analysis was conducted using an in-house unbiased automated analysis pipeline (Pre-clinical Image Processing Pipeline In Neuroimaging (PIPPIN); (Crum et al., 2013a, Crum et al., 2013b)). A group mean of the placebo T2-weighted scans was created as a reference image and each individual image was non-rigidly warped to this reference, with a map of the deformations required for this transformation also derived. These processing parameters were then applied to the T1 maps to move them into alignment, and a paired t-test conducted between the placebo and drug images to assess the effect of the drug on T1
values throughout the brain, with a statistical threshold set at p<0.05 FDR corrected for multiple comparisons.

3.1.5.1 Results
The voxelwise analysis between the placebo T1 images and drug T1 images did not reveal any significant differences in values of T1 due to exposure to drug.

3.2 Discussion
3.2.1 Introduction
In agreement with the hypothesis and the results of previous studies (Handley et al., 2013) acute antipsychotic administration induced pronounced dose and drug dependent changes in cerebral blood flow across the three antipsychotics tested herein. However, following a thorough examination of structural metrics, it was found that in healthy individuals, no significant changes were observed in quantitative T1 relaxometry in the face of these significant blood flow changes relative to placebo. Additionally, extensive exploration of T1-weighted images using several volumetric analysis techniques also showed no apparent volumetric changes in response to the doses administered, nor were any risperidone related changes to T1 detected in a supplementary rodent study.

3.2.2 CBF
The rapid increases in blood flow observed following acute antipsychotic administration replicate earlier findings in healthy humans (Handley, et al., 2013 (Michels et al., 2016)). Increased postsynaptic metabolism in striatal areas due to the large density of D2 receptors is a possible interpretation (Goozée et al., 2014) with blockade of D2 receptors in the striatum potentially resulting in disinhibition of D2 receptor containing medium spiny neurons (Fernández-Seara et al., 2011). However, CBF may not be solely influenced by neuronal activity. Astrocyte signalling is heavily implicated (Attwell, et al., 2010) and pharmacological manipulation of these cells may also influence blood flow. For instance, D3 receptors – of the same family of receptors as D2 – are present on astroglial cells and are positioned to mediate regional blood flow, with D3 agonists having been previously shown to cause vasoconstriction (Choi, et al., 2006). The antipsychotics used in this study also exhibit affinity for D3 receptors (Girgis, et al., 2015; Stahl, 2013), so it follows that
antagonism of these receptors may contribute to the observed increase in CBF through vasodilation.

In all, it appears likely that both neuronal and glial receptor expression and the differing receptor profiles of the antipsychotics in question can give rise to the CBF increases observed. The comparatively less pronounced CBF change in striatal areas produced by olanzapine could be due to its receptor profile, as it displays less affinity for D2 receptors than haloperidol and risperidone, while exhibiting a higher affinity for histaminergic, cholinergic and 5-HT2A receptors. Nevertheless, the distinctive blood flow profile elicited by these three different drugs highlights the fact that broad categorisation of these drugs into either typical/first generation or atypical/second generation classes does not take into account the precise differences in receptor profiles within these groups. These profiles should prove to be more informative in understanding their physiological and therapeutic impact than the typical/atypical nomenclature.

The decreases in CBF observed in the cerebellum following risperidone are in line with previous observations (Shcherbinin et al., 2015). These effects of risperidone differ from the effects of aripiprazole, a partial D2 agonist that increased CBF in the cerebellum (Handley, et al., 2013). It is noteworthy that the basal ganglia and cerebellum are more heavily integrated than previously thought, both anatomically (Bostan et al., 2010, Hoshi et al., 2005) and functionally (Neychev et al., 2008) with Dasgupta et al. (2014) proposing their interactions are modulated by striatal dopamine release. However, such an account does not easily accommodate that cerebellar changes were only observed after risperidone, and even consideration of the involvement of other systems such as serotonin (Schweighofer et al., 2004) does not predict that cerebellar effects would be limited to this drug. Understanding the precise mechanism behind this change and its associated impact on brain function, therapeutic or otherwise, would require concurrent measures of function, or confirmation in a patient group.

3.2.3 T1

In addition to the pronounced relative CBF changes observed in the unbiased whole brain analysis, risperidone and haloperidol both produced significant absolute increases in CBF in all a priori striatal regions, the largest being a 9.5% increase in absolute CBF for the ventral striatum following 2mg of risperidone. Olanzapine also produced significant CBF changes in
these ROIs, relative to whole brain CBF. Within these same regions we acquired quantitative measures of T1, an absolute metric of the MR signal that standard volumetric analyses are based upon, and were unable to find a significant change following drug exposure. This suggests that the blood flow changes produced by clinical doses of antipsychotics do not measurably alter T1 at this resolution.

Furthermore, none of the automated volumetric T1-weighted analyses employed returned any significant changes; either using (when possible) whole brain or ROI approaches, in either standard or native space. A reverse-translational preclinical study examining a larger systemic dose of risperidone also failed to produce any voxelwise changes to T1. Given a variety of automated techniques were employed, each with their own application of registration, segmentation, modulation and statistical analysis methods, it is also unlikely the absence of any detectable changes is due to an idiosyncrasy of a methodological approach.

What are the likely causes of our apparent discrepancy with the results of other investigations? The two studies that previously reported acute changes in T1 values or GM volume in response to antipsychotic exposure (Fujimoto, et al. (1987) and Tost, et al. (2010) respectively) both used large doses of haloperidol administered intravenously, as compared to the clinically relevant oral doses used in the current study. An intravenously administered dose produces large and almost immediate increases in drug plasma levels compared to that achieved by oral dosing, which takes several hours to reach peak concentration in the blood and is dependent on factors such as absorption rate and first pass metabolism. Consequently, oral exposure is considerably more gradual, making direct comparison between the two methodologies problematic – for instance, the physiological impact of sudden and extreme exposure to an antipsychotic compound could include factors such as highly pronounced off-target effects. High occupancy levels of serotonergic, histaminergic or adrenergic systems could potentially produce changes, transient or otherwise, to the biophysical environment which could be sufficient to influence the MR parameters underlying structural measurement, with or without a ‘real’ structural change. Indeed, Fujimoto, et al. (1987) concluded that the increase in the T1 values in the striate body of dogs 30 minutes after IV administration of haloperidol was due to the functional effects of haloperidol rather than any morphological change. However, without a clearer understanding of the physiological processes occurring after a dose of this extremity, the
underlying cause of the related T1 change remains unspecified - although the clinical relevance of the impact of such doses in respect of understanding the contribution of antipsychotic doses to longer term structural changes is unclear. Additionally, the larger systemic dose of risperidone used in the preclinical investigation here did not produce any changes to T1, although this involved a different drug (from a different subclass), was not given intravenously and the analysis methods were different to Tost et al. (2010), thus a direct comparison is difficult.

It has been argued (Franklin, et al., 2013) that blood flow changes could influence accurate MR assessment of brain structure. The similarity of the T1 relaxation times of blood and grey matter (T1 of grey matter at 1820 +/-114, and blood at 1932 +/-85 at 3T; Stanisz, et al. (2005)) suggests the potential to influence structural metrics. However, it should be noted that to a simple approximation, the apparent T1 of each tissue voxel may be viewed as a weighted sum of the individual contributions of the T1 of tissue and the T1 of the capillary blood compartments. Thus, the longitudinal magnetization recovery \( M_z(t) \) of the signal in each voxel, in an SPGR scan such as the one used herein, will be given by:

\[
M_z(t) = M_0 \sum_{i,j} (1 - 2A_{ij} e^{-t/T_{1,ij}})
\]

where \( M_0 \) is the equilibrium magnetisation, \( A_{ij} \) are the relative contributions of each domain (tissue and capillary respectively) to the spin density; and \( T_{1,ij} \) represents the individual T1 relaxation times of \(^1\)H spins in each compartment. Therefore, the most likely explanation for the absence of significant changes in T1 (in our study), is that the blood capillary domain contributes to the whole T1 weighted signal, with a maximum of its overall \(^1\)H density. Since this is known to be \(~1\%\) in human grey matter (Alsop, et al., 2014) any changes in the T1 of blood have a low likelihood of making a measurable change in the overall signal.

This of course does not rule out that structural remodelling can occur on an acute timescale, but rather that blood flow is unlikely to be driving its putative detection using MRI within the current context. Indeed, one recent study reported macro level MR assessed structural changes in response to balance training in the absence of ASL measured blood flow changes (Taubert et al., 2016). Several other transient processes could be responsible for the apparent acute structural change following antipsychotics observed in previous studies aside from blood flow, such as the influence of drug on cell microstructure, cell hydration, concentration of iron content or microglial activation...
(Cousins, et al., 2013; Salgado-Pineda, et al., 2006; Tost, et al., 2010), while numerous other biophysical factors that are known to influence T1 could be involved, such as myelination and axonal growth (Deoni, 2011). Further, while T1 is the primary contributor to contrast in T1-weighted images, the other parameters that determine MR contrast (T2, PD) were not quantitively assessed in this study. Lorio et al. (2016) recently reported that altering the relative contribution of R1(1/T1), R2* (1/T2*) and proton density (PD) in the creation of synthetic T1-weighted images resulted in changes in GM volume and thickness as assessed by VBM and Freesurfer, suggesting changes in these other parameters (in addition to T1) could significantly impact the structural information derived from T1-weighted images using automated methods.

Nevertheless, in the current study neither current T1 mapping techniques or T1-weighted morphometric analyses detected a change in response to single clinical doses of antipsychotics. This does provide some validation for studies examining the chronic impact of clinical dosing regimens of these drugs on brain structure, as it appears acute effects of clinical doses are not capable of confounding the long-term effects assessed by current structural analysis techniques (Lovden et al., 2013). A full understanding of the phenomena outlined above will be best achieved by future, careful studies of the effects of clinically relevant oral doses; whilst relevant interpretations from studies using large, intravenous doses are likely to remain limited.

3.2.4 Limitations

The counterbalanced approach to treatment levels meant not all treatments followed placebo in a longitudinal fashion, and it could be argued that if there were persistent structural changes following a single dose these would remain during later scanning/treatment sessions. However, the purpose of this study was to primarily explore acute effects (which had previously shown to be reversible). As a precaution, subjects were split into groups depending on which visit they undertook their placebo scan, and separate one-way ANOVAs for each placebo ROI was conducted – the findings were not altered based on this analysis.

The ASL protocol employed in this study deviates slightly from that recommended in Alsop et al. (2014) as data collection was already in progress at the time of publication, although the parameters used remain appropriate for a healthy sample as employed herein. It
should also be noted that the failure to detect a change in structure using the various methods explored above does not rule out that acute structural changes are occurring to a more finite extent than that detectable by the resolution clinical MRI currently offers. However, also worth noting is that the current sample size is superior to other studies that have reported such acute changes in structure, and includes a placebo control. Further research, potentially in larger preclinical samples with the option for histological confirmation, will allow a more sensitive analysis of the microstructural changes that may occur after clinically-relevant doses of antipsychotic drugs.

3.3 Conclusions

By means of a careful and direct determination of voxel-wise values of T1 and CBF, in a within-subject, placebo controlled experiment design with healthy volunteers, it has been demonstrated that changes in regional blood flow as a result of acute antipsychotic administration are not likely to be the cause of the volumetric changes observed in some previous investigations. Other physiological and bio-chemical factors must be evaluated in order to gain a deeper understanding of the factors that underpin the influence of this family of compounds on brain structure.
Chapter 4  Antipsychotic modulation of reward anticipation

4.1 Introduction

As discussed in Chapters 1 and 2, the Monetary Incentive Delay (MID) task has been extensively used to explore brain activation during reward processing. This chapter will focus on the effects of a single dose of different antipsychotics on both the behavioural performance and BOLD signal during the anticipatory phase of the task, which has been found to be altered in both patients with schizophrenia and in response to antipsychotic administration.

Only one study has examined single dose antipsychotics influence on reward anticipation on the MID in placebo controlled healthy humans (Abler et al., 2007) using a single drug (olanzapine) with a small sample size (n=8), and few studies examining the influence of these drugs on the BOLD signal in patient populations also measure their indirect vascular effects. The current approach will add clarity to the function of these drugs by examining a range of subtypes and doses, as well as taking into account the influence of these drugs on cerebral blood flow and cerebrovascular reactivity.

Antipsychotics were hypothesised to reduce activity in the ventral striatum during reward anticipation on the MID. In addition to the CBF results that have been discussed in Chapter 3, these drugs were also hypothesised to reduce CVR (measured here with a breath hold task). The influence of any indirect vascular effect will be accounted for in the analysis of the reward response, along with the CBF results from the previous chapter.

First, the behavioural results of the task will be presented, examining both the effect of reward within the task, and the effect of drug on task performance. Analysis to confirm participant’s engagement in the task will also be conducted.

The ROI and whole brain results of drug effect on reward anticipation processing will then be presented both before and after correction for cerebrovascular reactivity (CVR) and cerebral blood flow (CBF). The effects of drug on CBF has already been presented in Chapter 3, so the breath hold results will also be presented in isolation here.
4.2 Behavioural results

4.2.1 Performance issues
To ensure only data from participants who were actively and appropriately engaged in the task was included in the final analysis, performance from each treatment session was explored and any subject performing more than 2 SDs of the session average either in percentage of hits on win trials or average reaction time (RT) response across all response trials were removed. Participants performing below this threshold typically represented a win rate of less than 15% or an average RT of >0.35ms. To further ensure participants were actively engaged throughout the task, an attempted response rate (i.e. a button press within the entire 500ms response window, regardless of win or lose) of >66% was also required for a participant to be included in the final analysis. Although this is a somewhat conservative cut off, it ensures only those individuals that were actively involved in the task would be included in the final analysis.

On this basis, four participants were removed from each group following data screening due to falling below threshold in one or more of these criteria, leaving 17 participants in each group.

4.2.2 Adherence to task
Across all sessions participants maintained engagement throughout the duration of the task, responding to at least 90% of trials that required a button press (see Figure 4.2-1 & Figure 4.2-2) – importantly this includes both win and neutral trials indicating attention was held even on trials when no monetary reward was available.
Figure 4.2-1 Cumulative response rate (with SD bars) over duration of task on trials requiring a response (high win, low win, neutral in RisH/L).

In the Ris-H/L group, a one-way repeated measures ANOVA revealed a significant effect of treatment on overall response rate ($F(2,32) = 4.480, p = 0.043$), although this difference was not significantly significant after Bonferroni correction for the number of pairwise
comparisons (the largest change was between high risperidone (95.51 ± 1.31%) and placebo (98.2 ± 0.35%), a non-significant change of 2.96% (p = 0.176)).

In the Olan/Hal group, a one-way repeated measures ANOVA revealed a significant effect of treatment on response rate ($F(2,32) = 4.978, p = 0.013$). Bonferroni corrected pairwise comparisons revealed the only significant difference was a 5.64% decrease in response rate under olanzapine compared to placebo ($91.91 ± 2.65$ vs $97.55 ± 0.78$, $p = 0.013$).

In terms of actual money won per session (Figure 4.2-3), a one-way repeated measures ANOVA revealed no significant effect of treatment on total winnings in the Ris-H/L group ($F(2,32) = 2.680, p = 0.084$). However, in the Olan/Hal group, the same analysis revealed a significant effect of treatment on total winnings ($F(2,32) = 4.382, p = 0.021$), with Bonferroni corrected pairwise comparisons revealing the largest change was between olanzapine (£38 ± 2.25) and placebo (£44.62 ± 1.76), a non-significant change of £6.62 ($p = 0.07$).
Figure 4.2-3 indicates attention to the task was well maintained across subjects, with response rate dropping below 70% during only one session. Despite the effect of drug on response rate, the adaptive nature of the task resulted in consistent winnings across sessions in the RisH/L group. Interestingly for the Olan/Hal group one subject (participant 3 in fig 4.2-3) performed worse than the others on Olan. While they did not meet our formal criterion for an outlier it is noteworthy that the differences between Olan and placebo were all non-significant after their exclusion.

4.2.3 Effect of task

4.2.3.1 Placebo

Both placebo groups were collapsed into one to illustrate the effect of the levels of the task alone. As expected from the existing literature, participants responded both quicker to higher levels of reward, and were more successful at winning in high reward trials (see Figure 4.2-4).

A one-way repeated measures ANOVA revealed significant effect of trial reward level on reaction time ($F(2,66) = 58.427, p < 0.001$), with Bonferroni corrected pairwise comparisons revealing significant differences between no reward (0.287 secs ± 0.005) and low reward (0.270 secs ± 0.004), a difference of 0.017 secs ($p < 0.001$); no reward and high reward
(0.263 secs ± 0.004), a difference of 0.025 secs (p < 0.001); and low reward and high reward, a difference of 0.007 seconds (p = 0.001).

The same was true of percentage of trials won, the same analysis revealing a significant effect of reward level on hit rate ($F(2,66) = 43.176, p < 0.001$), with subsequent Bonferroni corrected pairwise comparisons revealing significant differences between no reward (41.01% ± 2.99) and low reward (53.32% ± 2.16), a difference of 12.26% (p = 0.001); no reward and high reward (69.86% ± 2.8), a difference of 28.8% (p < 0.001); and low reward and high reward, a difference of 16.54% (p < 0.001).

4.2.4 Effect of drug on task performance
4.2.4.1 RisH/L

A two-way repeated measures ANOVA (factors: treatment and reward level) revealed no interaction between treatment and reward level on reaction time ($F(2,64) = 1.035, p = 0.372$), but significant main effects of treatment ($F(2,32) = 7.286, p = 0.002$) and reward level ($F(2,32) = 43.173, p < 0.001$).

Similarly, there was no interaction between treatment and reward level on percentage of trials won ($F(2,64) = 1.131, p = 0.350$), but significant main effects of treatment ($F(2,32) = 4.241, p = 0.023$) and reward level ($F(2,32) = 80.151, p < 0.001$).
4.2.4.2 Olan/Hal

A two-way repeated measures ANOVA (factors: treatment and reward level) revealed no interaction between treatment and reward level on reaction time \( F(2,64) = 0.201, p = 0.873 \), but significant main effects of treatment \( F(2,32) = 19.465, p < 0.001 \) and reward level \( F(2,32) = 31.605, p < 0.001 \).

There was also no interaction between treatment and reward level on percentage of trails won \( F(2,64) = 0.264, p = 0.852 \), but significant main effects of treatment \( F(2,32) = 16.944, p < 0.001 \) and reward level \( F(2,32) = 33.520, p < 0.001 \). Pairwise comparisons again indicated olanzapine was driving these reductions with a 12.19\% (±2.39, \( p < 0.001 \)) reduction in hit rate and a 0.025sec (±0.005, \( p < 0.001 \)) increase in reaction time – haloperidol was not significant for either.
4.2.5 Effect of drug on Alertness

The alertness subscale of the Visual Analogue Scale was used as a metric of subjective sedation. Within visit changes in alertness scores from arrival at testing centre to the closest measure to the T-max of the drug was used as a sedation metric per visit, and entered into a repeated measures ANOVA (factor: treatment) for each cohort. RIS-H/L revealed no significant effect of treatment ($F(2,32) = 2.458, p = 0.102$). Olan/Hal on the other hand revealed a significant effect of treatment ($F(2,32) = 9.975, p < 0.001$). Bonferroni corrected pairwise comparisons revealed a significant decrease in alertness rating due to both olanzapine ($21.41$ (95% CI 8.71 to 34.11) $p < 0.001$) and, to a lesser extent, haloperidol ($12.03$ (95% CI 1.18 to 22.89) $p = 0.03$).

Figure 4.2.6 Estimated marginal means of percent win rate (left) and reaction time in seconds (right) across task conditions and treatment levels for the Olan/Hal group
4.3 Neuroimaging results

4.3.1 Reward Anticipation – Placebo session

To explore whether the MID task was recruiting relevant brain regions outside the effect of drug, the placebo scans from both groups were analysed during reward anticipation. Widespread activation was observed in the striatum, thalamus, cingulate, and insula, areas all purported to be involved in reward processing. There were additional significant clusters in visual, motor and supplementary motor cortex, and the cerebellum.

Reporting cluster information in relatively large n data sets with widespread activation can be problematic using Threshold Free Cluster Enhancement as clusters can often merge into one or two very large masses. For this reason, and under recommendation from FSL collaborators (Mumford Brainstats, 2017), for cluster reporting in the combined group analysis the threshold was reduced to 0.01 to ‘break up’ very large clusters (see Chapter 2). This is not a case of statistical analysis being ‘rerun’, but is rather for illustrative purposes and allows the identification of localised peaks which can be informative when interpreting drug influence within the individual groups.

![Image](image-url)
Similar patterns of activation were observed when the placebo scans were analysed separately for the two cohorts of participants.

4.3.2 Risperidone (Ris-H/L) and reward anticipation

4.3.2.1 Whole brain results

Non-parametric whole brain permutation testing revealed a 2mg dose of risperidone reduced activation in reward related areas during reward anticipation, including the caudate, putamen, ventral striatum, cingulate and thalamus, in addition to visual and supplementary motor cortex.
0.5mg risperidone did not reveal any significant activation (although lowering the threshold to an exploratory 0.8 revealed a similar pattern of reductions to that in the 2mg comparison).

The same analysis between the low and high risperidone conditions did not reveal any significant change in activation, even with the exploratory threshold applied.

4.3.2.2 ROI results

The a priori ROIs were entered into a generalised estimating equation with drug and ROI as factors, and revealed an effect of drug on the extracted parameter estimates (Wald chi-sq = 19.619, df=2, p < 0.001), with Bonferroni corrected pairwise comparisons revealing a significant reduction after 2mg risperidone compared to placebo across all ROIs (0.212 (95% CI 0.11 to 0.31) p < 0.001), but not after 0.5mg risperidone (reduction of 0.97 (95% CI -0.06 to 0.25) p = 0.23).

Bonferroni corrected post-hoc tests of each ROI revealed these changes were limited to the striatal regions, with reductions in the caudate (0.2176 (95% CI 0.07 to 0.42) p = 0.003), putamen (0.42 (95% CI 0.22 to 0.62) p<0.0001) and ventral striatum (0.24 (95% CI 0.08 to 0.39) after 2mg risperidone alone.

4.3.2.3 Level of reward

The same analyses were conducted to explore the effect of drug on reward valence (ie High reward anticipation vs Low reward anticipation), but no significant results were returned, either on the whole brain or ROI analysis.
4.3.3 Haloperidol/Olanzapine and reward anticipation

4.3.3.1 Whole brain results
Permutation testing revealed neither olanzapine or haloperidol caused any detectable change during reward anticipation.

4.3.3.2 ROI results
The *a priori* ROIs were entered into a generalised estimating equation with drug and ROI as factors, and revealed an effect of drug on the extracted parameter estimates (Wald chi-sq = 8.927, df=2, \( p < 0.012 \)), with Bonferroni corrected pairwise comparisons revealing a significant reduction after olanzapine compared to placebo across all ROIs (1.17 (95% CI 0.04 to 0.28) \( p < 0.011 \)), but not after haloperidol (reduction of 0.048 (95% CI -0.14 to 0.22) \( p = 0.611 \)).

Post-hoc tests of each ROI revealed the largest difference was between placebo and olanzapine in the caudate, a non-significant reduction of 0.20 (95% CI 0.05 to 0.36).

4.3.3.3 Level of reward
The same analyses were conducted to explore the effect of drug on reward valence (ie High reward anticipation vs Low reward anticipation), but no significant results were returned, either on the whole brain or ROI analysis.

4.3.4 Behavioural correlations
Reaction time was taken as the most informative metric of behaviour, and has been used in MID tasks previously as an indication of motivation. Correlations were conducted between reaction time and extracted beta values for each ROI to assess any link between BOLD signal and behaviour. No significant correlations were observed between reaction time and beta value, even before correction for multiple comparisons, in either group.
4.3.5 Breath Hold (Vascular reactivity) analysis

4.3.5.1 Adherence to task

Physiological data from the respiratory bellows was analysed to ensure participants were attending to task, and to confirm that there was not a systematic effect of the drug on breathing dynamics during this phase (which would potentially confound the BOLD results).

Figure 4.3-4 indicates that the timing of breath holding was highly similar across drug conditions, and that participants adhered to the task well. This was confirmed by conducting paired t-tests between placebo-HRis and placebo-LRis for the bellows reading at the commencement of the task (ie the extent to which the participant had breathed in at the display of the command instructing them to breath out and hold). Both HRis ($t(20) = -0.23, p = 0.82$) and LRis ($t(20) = -0.42, p = 0.68$) were not significantly different from placebo at this time point.
Figure 4.3-5: Average respiratory bellows readings (and SD) of the 4 breath hold blocks per condition for the Olan/Hal group. Vertical red lines denote start and end of task as displayed to participant.

T-tests at breath hold commencement revealed both Hal ($t(20) = 1.04, p = 0.30$) and Olan ($t(20) = 1.79, p = 0.08$) were not significantly different from placebo.
4.3.5.2 Effect of task on BOLD signal

To examine the effect of the breath hold task on BOLD signal under normal conditions, the placebo scans from each group were analysed as described in Chapter 2. The task elicited widespread increases in BOLD signal throughout the grey matter during held breath compared to paced breathing.

Figure 4.3-6 Whole brain permutation testing RisH/L Placebo scans during breath hold task (Hold>Paced breathing), 5000 permutations, FWE corrected p<0.05 (n=17)

Figure 4.3-7 Whole brain permutation testing Olan/HaL Placebo scans during breath hold task (Hold>Paced breathing), 5000 permutations, FWE corrected p<0.05 (n=17)
4.3.6 Effect of Drug on vascular reactivity

4.3.6.1 RIS-H/L

Neither whole brain or ROI analysis revealed an effect of high or low dose risperidone on vascular reactivity.

4.3.6.2 OLAN/HAL

4.3.6.2.1 Whole Brain

Whole brain analysis revealed no effect of haloperidol on the breath hold task. However, olanzapine produced widespread reductions in vascular reactivity compared to placebo.

![Whole brain permutation testing Plac>Olan vascular reactivity (Hold>paced breathing), 5000 permutations, FWE corrected p<0.05 (n=17)](image)

4.3.6.2.2 ROI analysis

A one-way repeated measures ANOVA of extracted ROI values revealed a significant main effect of drug (F(2,32) = 6.769, p = 0.004) and ROI (F(2,32) = 19.659, p < 0.001). Pairwise comparisons revealed a significant reduction after olanzapine compared to placebo (0.531 (95% CI 1.36 to 9.26) p = 0.007), but not for haloperidol compared to placebo (0.013 (95% CI -0.434 to 0.459) p = 0.98), adjusted for multiple comparisons.
4.4 Reward anticipation after controlling for baseline blood flow and vascular reactivity

4.4.1 Ris-H/L

4.4.1.1 Whole brain

Whole brain permutation testing with vascular reactivity and CBF maps as covariates revealed a reduction in BOLD response in the thalamus and visual and supplementary motor cortex. However, the reductions previously observed in the striatal areas were no longer present.

![Whole brain permutation testing, Placebo>Risperidone 2mg during Reward anticipation (n=17), 5000 permutations with voxelwise CBF and CVR maps included as covariates. FWE corrected p<0.05](image)

4.4.1.2 ROI Analysis

The a priori ROIs were entered into a generalised estimating equation, including CBF and CVR values as covariates, and revealed a main effect of drug (Wald Chi-sq 19.357, p<0.001). Bonferroni corrected pairwise comparisons revealed significant reductions after 2mg risperidone in the putamen (0.2171 (95% CI 0.0768 to 0.35759) p = 0.002) and caudate (0.4164 (95% CI 0.2209 to 0.6119) p<0.0001).
4.4.2 Olan/Hal

4.4.2.1 Whole Brain

Whole brain permutation testing with voxelwise vascular reactivity and CBF maps as covariates did not reveal any significant activation differences following haloperidol. After olanzapine, one small cluster of reduced activation was revealed in the right cerebellum (MNI: 10, -72, -26; t=6.3, voxels=17).

![Brain Image]

**Figure 4.4-2** Whole brain permutation testing, Placebo>Olanzapine 3mg during Reward anticipation (n=17), 5000 permutations with voxelwise CBF and CVR maps included as covariates. FWE corrected p<0.05

4.4.2.2 ROI analysis

The *a priori* ROIs were entered into a generalised estimating equation, including CBF and CVR values as covariates, and revealed a main effect of drug (Wald Chi-sq 6.630, *p*<0.036) within this group. Bonferroni corrected pairwise comparisons revealed a significant reduction after olanzapine compared to placebo across all ROIs (0.1522 (95% CI 0.0201 to 0.2843) *p* < 0.024), but not after haloperidol (reduction of 0.0282 (95% CI -0.1331 to 0.1895) *p* = 0.732).

Post-hoc tests of each ROI revealed a significant difference between placebo and olanzapine in the putamen, a reduction of 0.1980 (95% CI 0.04 to 0.36) *p*=0.048.
4.5 Discussion

4.5.1 Summary

Both whole brain and ROI analysis revealed acute exposure to different antipsychotic medications caused dose and drug dependant effects on the BOLD signal during reward anticipation, both before and after correction for CBF and CVR. As hypothesised, increasing doses of risperidone resulted in dose-related reductions in activation in multiple reward-relevant brain regions, including the striatum (although corrections for CBF and CVR altered the results in this area). Olanzapine also produced reductions in some striatal areas as hypothesised, with whole brain analysis corrected for CBF and CVR revealing additional reductions in the cerebellum. However, haloperidol failed to produce any changes to reward anticipation processing, either in the ROI or whole brain analysis, before or after correction for CVR and CBF.

Discussion on the effect of the drug on CBF and CVR itself will be conducted in more detail in the final chapter. Throughout the rest of this chapter, the neuroimaging results discussed regarding anticipation will be those accounting for CBF and CVR unless otherwise stated.

4.5.2 Drug effect on behavioural performance

Participants maintained good engagement with the task across sessions, and the expected behavioural changes in response to levels of reward were observed, indicating active acknowledgement of the reward salience and valence of the cues. However, exposure to different antipsychotics, specifically olanzapine and risperidone, resulted in significant increases in reaction time (although the adaptive nature of the task attenuated this effect in terms of amount won on the task across sessions). Response rate and win rate were also significantly reduced in these treatment sessions.

This is to be expected with compounds of this type, which have frequently been associated with sedative side effects. Olanzapine in particular has been found to be acutely sedating, followed by risperidone and haloperidol (Gao et al., 2013). Particularly relevant here is the histaminergic system, with higher affinity for the H1 receptor linked to increased sedation or somnolence (Miller, 2004). Olanzapine expresses strong affinity for the H1 receptor, as does risperidone to a lesser extent (Stahl and Muntner, 2013). Haloperidol on the other
hand has a negligible affinity for H1, and may be expected to produce relatively little
disruption to task performance. However as seen in these results, while both olanzapine
and haloperidol (to a lesser extent) reduced self-reports of alertness, risperidone did not
produce a significant change in alertness ratings. Therefore, subjective sedation may only
partially explain these differences, and some behavioural changes may be due to the direct
effects of the compounds on the reward system, which will now be discussed.

4.5.3 ROI analysis of drug effect on reward anticipation

The suppression of reward anticipatory signals in striatal areas by risperidone and
olanzapine replicates earlier findings in eight healthy humans (Abler et al., 2007), but with a
larger sample size and more stringent statistical threshold. This finding is arguably
consistent with the general concept of aberrant salience (due to dysfunctional
dopaminergic activity in the striatum) being suppressed by antipsychotic action (Kapur et
al., 2005b), although the context here must be acknowledged - these findings are specific
to healthy individuals and caution should be taken in extrapolating the observed action of
these drugs in healthy humans to their action in patients suffering from schizophrenia, who
have been shown to exhibit altered striatal function on this task prior to pharmacological
treatment.

Indeed, one paradox in this comparison is that unmedicated patients with schizophrenia
have consistently been shown to already show reduced activity in the striatum during
reward anticipation on the MID compared to healthy controls (Juckel et al., 2006b,
Esslinger et al., 2012, Nielsen et al., 2012b), so the notion that some antipsychotics may
further suppress this activity appears counterintuitive. Additionally, studies that have
examined the change in striatal activity after onset of treatment have indicated that ‘first
generation antipsychotics’ (FGAs; primarily D2 antagonists) produce no change to this
attenuated striatal activity, while second generation antipsychotics (SGAs), such as
risperidone and haloperidol, increased reward anticipation activity in the striatum (Juckel et
al., 2006a, Schlagenhauf et al., 2008, Nielsen et al., 2012a). Juckel and colleagues
characterised this ‘reactivation’ of the ventral striatum on SGAs resulting from DA activity
being maintained in the ventral striatum, due to the SGAs reduced blockade of D2
receptors, their faster rate of dissociation from D2 receptors and their increased 5HT
affinity (as antagonism of 5HT2A receptors is believed to increase DA release in the
striatum (Stahl, 2013)). They explained these changes in terms of negative symptomology –
the lack of reward related VS activity in unmedicated patients being responsible for symptoms such as apathy and anhedonia, with FGAs potentially worsening these symptoms due to their additional suppression of DA activity – supported by the observation that the reduction in VS activation during reward anticipation was inversely correlated with the extent of negative symptoms (Juckel et al., 2006a). SGAs were therefore proposed to restore ventral striatal activity and improve negative symptoms by virtue of their “antagonism of the 5-HT2 receptor...resulting in a sufficient and permanent flow of dopamine within the ventral striatum, thus maintaining affectivity and drive” (Juckel, 2016). It is worth noting this explanation is a slight departure from the view that, in the main, dopaminergic dysfunction in the striatum has been related to the positive symptomology of schizophrenia (Howes et al., 2007), explaining the efficacy of D2 antagonism in the treatment of positive symptoms and its limited effect on negative and cognitive symptoms. Indeed, other studies exploring reward anticipation in treated patients found inverse correlation with reward related activation and positive symptomology rather than negative symptoms in both striatal (Nielsen et al., 2012a) and cortical areas (Walter et al., 2009). Although a recent meta-analysis of fMRI reward anticipation in patients linked left ventral striatum reductions with negative symptomology, it should also be noted that negative symptoms are difficult to define and measure and there is an ongoing debate as to whether they are uniquely disease related or secondary to other environmental factors including medication (Bobes et al., 2010).

Regardless of this side debate, the data in this thesis runs somewhat counter to the narrative that SGAs ‘boost’ striatal activity, in that it was the two SGAs or atypical class medications that resulted in suppression of signal (albeit in more dorsal areas of the striatum). Haloperidol, a FGA, did not produce any significant effect, promoting quite the opposite picture to many of the studies discussed above. There are several possible reasons for this, in addition to the issues around comparing drug action between healthy participants and patients: (i) the studies above were based on multi-dose treatment regimes, conducted over a period of weeks to months, and scanning was not timed to coincide with the peak level in the blood as with the current study; (ii) dose and duration of exposure differences between the studies may have influenced the results; (iii) the sample sizes were generally small; and (iv) when comparing drug subtypes, a varied mix of medications was used within each ‘class’ – for instance the 10 atypical treated patients from Juckel et al. (2006a) were treated with risperidone (four), olanzapine (four),
ariprazole (a partial D2 agonist, and often referred to as a third generation antipsychotic; one) and amisulpride (which has a novel receptor profile; one). As the data in this thesis illustrates, the atypical/typical distinction has limited utility in a pharmacological sense and there are differences between the specific effects of these drugs within classes, meaning merging them into one group may obfuscate findings.

One notable exception to the last three of these points was Nielsen et al. (2012a), with a sample of 23 treatment naïve patients, scanned before and after 6 weeks treatment with amisulpride. They found a reduced VS signal at baseline compared to untreated healthy controls, with this difference no longer being significant after treatment, a finding consistent with that of Juckel and colleagues. However, amisulpride has a unique receptor profile, markedly more selective to D2/D3 receptors to that of olanzapine or risperidone, and has a poor affinity for 5-HT2A receptors. Furthermore, the main anticipation contrast included both reward and loss indicating cues (a ‘salience’ contrast), while the reduction in the healthy control signal between baseline and follow-up may have driven the loss of difference between the two groups (the patient group producing only a limited increase in ventral striatal activation in comparison).

The fact there are no placebo controlled studies in patients is relevant here. These are clearly difficult to implement, but this does limit the confidence of determining drug effects without the same controls affordable to healthy volunteer studies such as the design used here. Nonetheless if we take the patient studies at face value, what could explain the apparent opposing effects of these drugs between patients and healthy participants? An important consideration here is how changes in dopaminergic function are reflected in the related BOLD signal. One proposed mechanism for the reduced striatal reward activation in schizophrenia is the increased baseline dopamine tone in the striatum (due to the disorder’s well characterised hyperdopaminergic state (Howes et al., 2012, Fusar-Poli and Meyer-Lindenberg, 2013)) means the phasic signals that mark rewarding stimuli are drowned out and do not appreciably change the BOLD signal (Heinz and Schlagenhauf, 2010). Knutson et al. (2004) modelled this in healthy volunteers by administering amphetamine, which causes large releases of striatal dopamine, and subsequently found a reduced BOLD response to reward anticipating cues. Similarly, Francois et al. (2016) showed a reduction in ventral striatal activity during reward anticipation in both fMRI assessed human and oxygen amperometry assessed rats after a dose of ketamine (a MDNA
antagonist which has been proposed to indirectly increase striatal DA levels (Smith et al., 1998, Breier et al., 1998), although more recent work has cast doubt on this mechanism (Can et al. (2016), Rabiner (2007).

Extensive work by Anthony Grace (1991, 2016) provides an expanded explanation into the regulation of midbrain DA neurons. Under normal circumstances, around half of the DA neurons in the midbrain are inhibited by the ventral pallidum, and only the uninhibited neurons will provide spontaneous tonic firing. This effectively determines the responsiveness of these midbrain neurons, as only the uninhibited tonically firing population are free to switch to phasic firing in response to a behaviourally rewarding or salient stimulus. If tonic firing is increased due to disinhibition of further midbrain DA neurons, any phasic firing in this context should also increase in amplitude. However, if dopamine release occurs independently of the uninhibited midbrain neurons (as potentially in the case of increased presynaptic DA synthesis and release in schizophrenia, or due to pharmacological blockade of dopamine stimulation or reuptake), this results in attenuation of phasic dopamine release through DA autoreceptor inhibition (Floresco et al., 2003, Grace, 1991). This provides a context for the ‘drowning out’ of the phasic signal described above. Alternatively, the time course of treatment could be an influencing factor. Although acute doses of antipsychotics have been shown to increase midbrain DA firing (Chiodo and Bunney, 1983, Di Giovanni et al., 1998), animal models have shown that repeated exposure results in DA neuron inactivity (Moore et al., 1998) and this depolarisation block has been linked to treatment response (Grace et al., 1997). This complicates comparison between acute studies in treatment native patients and those treated for a relatively short period of time, or in acute single dose studies in healthy subjects as here.

Antipsychotics may therefore act in a context dependant manner, and differences in baseline tonic firing, DA sensitisation or extracellular levels of dopamine could dictate the changes that are reflected in the BOLD response to their actions. In the previously unmedicated and healthy brain (as per the placebo scans herein) with assumed normal baseline tonic DA firing, a clear striatal signal has been observed in response to anticipatory cues, presumably reflective of phasic DA firing in response to the rewarding cue. The increase in BOLD signal observed in the striatum associated with reward anticipation has previously been linked with responsive DA release (Schott et al., 2008a), with the signal most likely reflective of DA stimulation of the postsynaptic membrane (Knutson and Gibbs,
2007). If acute antipsychotic administration does modulate the release of DA in the striatum, either directly or via manipulation of midbrain firing dynamics, the associated BOLD signal could be expected to change.

A parsimonious explanation for the reduction in striatal signal seen here following risperidone or olanzapine administration would be that D2 blockade on striatal postsynaptic membranes results in suppression of the postsynaptic potential and the associated BOLD signal. Why then does haloperidol, primarily a D2 blocker, not produce the same effect as olanzapine or haloperidol, which have a relatively lower affinity for D2 receptors? A likely explanation is the extended receptor profile that the two ‘atypical’ compounds exhibit over haloperidol. Both risperidone and olanzapine express an affinity for serotonin receptors, particularly 5-HT2A, with olanzapine additionally exhibiting an affinity for histamine and muscarinic receptors. Haloperidol on the other hand is reasonably selective for D2-like receptors, with limited affinity for other neurotransmitter types. This suggests that not only direct dopaminergic modulation is required to alter the BOLD signal in reward related areas during anticipatory processing. A clear candidate to further explore this notion experimentally would be amisulpride, which has negligible affinity for 5-HT2A, but clinical affinity for D2/D3 receptors while exhibiting clinical atypicality (although as is becoming clear, these terms should be regarded with caution). In line with this principle Admon et al. (2017) reported no change in the caudate, putamen or ventral striatal signal of a healthy control group (n=43) given either a single 50mg dose of amisulpride or placebo during reward anticipation on the MID (although this is a subclinical dose thought to increase dopamine signalling via presynaptic autoreceptor blockade). Further investigation in healthy cohorts with clinical doses (400mg - 800mg) would help extend these findings.

Of the serotonin receptors, the group that has been most consistently implicated in the treatment of schizophrenia is 5-HT2A which is found in large numbers in both the cortex and subcortical areas such as the striatum and midbrain (although 5-HT2C is also thought to be important). The serotonergic system innervates multiple brain regions, including the striatum, and is therefore well placed to modulate the action of other neurotransmitter systems. The antagonism of 5-HT receptors by antipsychotics have been proposed to be responsible for the reduced incidence of EPS by increasing DA release in the nigrostriatal pathway, via inhibition of GABAergic interneurons. Modulation of serotonergic
neurotransmission was therefore not seen as directly influencing the processing of reward information, but rather by modulating the levels of DA release and action to a level that both reduced symptoms and alleviated side effects within the relevant pathways (Alex and Pehek, 2007, Di Giovanni et al., 2008). If 5-HT2A antagonism has the downstream effect of increasing DA release in the striatum, it may result in the suppression of the phasic DA signal related to reward anticipation, explaining the reduction in signal seen on risperidone and olanzapine, but not haloperidol.

However, recent research has suggested serotonin may be more directly involved in modulating the reward system (Kranz et al., 2010, Li et al., 2016). The literature is somewhat disputed on this point with some proposing 5-HT opposes the function of dopamine (Daw et al., 2002) and encodes punishment rather than reward (Schweimer and Ungless, 2010), or is more responsible for coding the timescale of reward prediction (Tanaka et al., 2007) rather than the reward itself. However, serotonergic neurons have been shown to respond to reward predicting cues (Cohen et al., 2015) with preclinical work indicating neurons in the dorsal raphe nucleus, a major serotonin centre in the midbrain, increase firing during reward tasks and drive learning (Liu et al., 2014). Much of this work is preclinical in nature and involves direct stimulation or depletion of 5-HT. How acute pharmacological antagonism of 5-HT receptors in the human brain may directly alter reward signals in the context of these results is less clear, although it could be speculated direct antagonism of 5-HT mediated signals may be contributing to the reductions in BOLD seen here.

The putative role of serotonin in the sustainment of the effectiveness of reward predicting cues (Doya, 2002) may also be relevant here, given the MID task requires a delayed response to achieve reward. 5-HT2A function has been implicated in performance in a delayed reward choice task in studies which used dietary control of tryptophan (the precursor to serotonin), with Schweighofer et al. (2008) reporting lower serotonin levels resulted in more impulsive responses. Using the same task and procedure, Tanaka et al. (2007) reported a stronger correlation between ventral striatal activity and short term reward prediction in low serotonin conditions, while dorsal striatal activity activation was correlated with reward prediction at longer time scales in high serotonin conditions. Although the MID task is not as well suited to explore the effect of delay and choice (because delay and choice do not vary systematically but instead vary in order to maintain
performance accuracy), it does require inhibition of response and a delayed reaction to acquire the reward, which would implicate serotonergic involvement during the anticipatory phase. Disruption of this system by 5-HT2A antagonists could partially explain the reduction in activation in striatal areas, particularly in dorsal areas of the striatum as seen here (see below for further discussion on differential activation within striatal subdivisions).

5-HT2A receptors are also found directly on the cell bodies of dopamine neurons in the VTA (Doherty and Pickel, 2000), which may suggest antagonism of these receptors would modulate the firing rate (and potentially the subsequent signal in the striatum). As already noted, preclinical electrophysiological and microdialysis studies have indicated acute administration of antipsychotics have been shown to increase the firing rate of midbrain cells, possibly due to blockade of dopamine midbrain autoreceptors and/or by antagonist effects at postsynaptic sites promoting excitatory feedback systems (Grace et al., 1997). More recent research has indicated the latter of these two is more likely, with antipsychotic action in the ventral striatum diminishing dopamine midbrain activity via a ventral striatum-ventral pallidum-ventral tegmental area feedback pathway, rather than direct action on the autoreceptors of the neurons themselves (Valenti and Grace, 2010).

This brings us back to the activity of midbrain brain neurons being an important factor in the differences between these drugs. Some research has suggested that certain antipsychotics may differentially alter specific sub regions of the midbrain, the effects of which would presumably be seen in their respective projection sites. For instance, Hand et al. (1987) reported acute IV haloperidol and clozapine in rats had opposing effects on the single unit activity of VTA and substantia nigra pars compacta (SNC) neurons, the origins of the mesolimbic/cortical and nigrostriatal pathways respectively. Clozapine increased firing of VTA cells but not SNC cells, while haloperidol increased the firing rate of both populations. Similarly, Di Giovanni et al. (1998) reported IV doses of amisulpride increased firing throughout the midbrain, but VTA firing was increased significantly more than that of the SNC. This gives a mechanism for different antipsychotics altering dopamine release in different brain regions, depending on the extent and projection site of the midbrain area manipulated. As Grace (2016) illustrates (and as discussed in Chapter 1), changes in the basal activity of dopamine midbrain neurons alters the context in which further signals are processed, such as the reward related signals elicited by the MID. The changes produced by
olanzapine and risperidone over that of haloperidol may be at least in part to their differential effects on midbrain firing. However, electrophysiological studies of large acute IV doses of these drug in rats should of course be treated in caution when comparing to single clinically relevant oral doses in humans with fMRI.

The difference observed between risperidone/olanzapine and haloperidol may well be down to some other aspect of the characteristics of the drugs. A competing hypothesis for ‘atypicality’ to 5-HT2A/D2 affinity ratio is the “D2 fast-off theory” (Kapur and Seeman, 2001), which postulates the reductions of EPS in atypicals are produced by their increased dissociation rate from the D2 receptor which allows endogenous DA to bind to receptors and reduces the EPS that are produced from high D2 blockade. However, although olanzapine does have a reasonably rapid dissociation rate, risperidone in fact dissociates at slower rate than dopamine, and at a comparable rate to that of haloperidol (Seeman, 2002). Although there may be utility within the fast-off theory in explaining part of the mechanism of action of antipsychotics, it does not appear to explain the differences in reward anticipation suppression observed between the drugs here.

Alternatively, the idiosyncratic action the drugs have at other receptor subtypes may be involved. Both olanzapine and risperidone are antagonists at the 5-HT2C receptor (which has been implicated in therapeutic action via its reciprocal role with 5-HT2A (Kuroki et al., 2008)) and antagonism of this receptor has been shown to increase both tonic striatal dopamine release and midbrain dopamine firing (Alex et al., 2005, Di Matteo et al., 1999). Additionally, risperidone acts as an antagonist at the 5-HT7 receptor while olanzapine has the same action at 5-HT6, both implicated in the treatment of cognitive impairments (Kusumi et al., 2015) and antagonism of both has inconsistently been implicated in altering DA transmission (Di Matteo et al., 2008). Olanzapine meanwhile acts as an antagonist at the muscarinic M1 receptor and histamine-1 (H1) receptor, antagonism of which has been linked to cognitive functioning, such as impairment of attention (Van Ruitenbeek et al., 2010), and its affinity here has been more related to undesirable side effects such as sedation and weight gain (He et al., 2013).

Ascribing the changes seen to one or more of these receptor systems is highly speculative however, and the precise mechanism by which the signal is modulated cannot be determined with fMRI alone. It may be mediated by modification of tonic or phasic DA
firing, by modulation directly within the striatum, and/or of descending signals from the
cortex, and the interaction may be direct (by receptors directly on the cell body) or via
interneurons or feedback loops involving other neurotransmitter systems. However, if the
difference in receptor profile does explain the difference in BOLD signal change, and given
risperidone’s higher affinity for the 5-HT2A receptor than olanzapine, this would suggest 5-
HT2A as a plausible candidate for the differences seen.

4.5.4 Changes in activation across the striatum

Many of studies examining pharmacological modulation of reward have focused on the
ventral striatum, the area most reliably activated by reward anticipation. However, in the
current study the dorsal striatum was most influenced by drug exposure, with the putamen
modulated by both risperidone and olanzapine, while caudate activity was also supressed
by risperidone. Ventral striatum activity on the other hand was only modified at trend level
after risperidone (although there was a significant reduction in this area prior to correction
for CBF and CVR effects). Although ventral striatum activity has been shown to have been
modulated by antipsychotic exposure, dorsal areas of the striatum have been implicated
both in reward related activation and possible disruption in psychosis (Balleine et al., 2007,
Kegeles et al., 2010).

The caudate and putamen, although anatomically separated by the internal capsule, are
thought to be functionally closely linked and the two structures are often grouped together
as the dorsal striatum, to be distinguished from the ventral striatum containing the nucleus
accumbens. These dorsal areas can be further subdivided into associative striatum (rostral
to the anterior commissure) and sensorimotor striatum (post-commissural putamen). The
associative striatum receives dopaminergic input from the substantia nigra, with efferents
to areas such as the thalamus and dorsolateral prefrontal cortex (DPFC), and has been
linked to aspects of cognitive function including reward processing. However, in
comparison to the response signal to reward anticipation seen in the ventral stratum,
associative striatal activation (particularly in the caudate) is thought to relate to action
preparation for reward and has therefore been conceptualised as being involved in effort
or motivational processes related to reward (Balleine et al., 2007, Kurniawan et al., 2013).
Miller et al. (2014) used a modified MID task that effectively allowed the delineation of
motivation and reward expectation (by including and manipulating reward magnitude and
difficulty information during the cue phase). They reported responses in the caudate and
putamen increased with motivation (measured by reaction time), while nucleus accumbens activation increased with reward magnitude, indicating a functional division of processing within the striatum for the anticipatory stage of the task. A variation of the “actor-critic” model could encapsulate this view (Montague et al., 1996), whereby midbrain DA projections to the ventral striatum signal reward prediction, while afferents to the dorsal striatum are involved in stimulus-response associations. It terms of the task employed here, the dorsal striatum may have been recruited as motivational preparation for the motor action required to win the reward.

What relationship does modulation of this process by antipsychotics (specifically risperidone and olanzapine, but not haloperidol), have with their potential therapeutic action? The dorsal striatum has shown aberrant activity in other psychiatric disorders that have had symptomology linked to reward processing dysfunction such as bipolar disorder (Yip et al., 2015) and ADHD (Furukawa et al., 2014) and therefore modulation of this region by antipsychotics could conceivably address some of the symptomology of these disorders in a similar fashion to how its action in the ventral striatum has been proposed (Kapur, 2004) – indeed APDs are increasingly prescribed for ADHD, bipolar disorder, autistic spectrum disorder and even major depressive disorder suggesting there may be transdiagnostic features that these drugs work on. Although the hyperdopaminergic state in the striatum as a whole has been well replicated (Howes et al., 2012, Fusar-Poli and Meyer-Lindenberg, 2013), some PET and fMRI research has in fact suggested some of the most elevated levels of DA in the striatum and disruption to connectivity with cortex are in associative areas (Howes et al., 2009, Kegeles et al., 2010, Dandash et al., 2014). Reductions in caudate activity have been reported in medicated, chronic schizophrenia patients during reward anticipation, with hypoactivation of this region correlated with symptoms of avolition (Mucci et al., 2015), linking back to the potential motivational role of the dorsal striatum. The action of the olanzapine and risperidone in this region over that of haloperidol may reflect the potential efficacy of atypicals in treating negative symptoms (Leucht et al., 2009), although these effect sizes are small. Alternatively, it may be reflective of secondary medication related side effects to motor function, although there was no correlation with activation in dorsal striatum and reaction time here.

How can we understand the non-replication of reductions in ventral striatal activation seen in previous studies? Given the superior sample size (17 per group vs 8 in Abler et al (2007))
it is unlikely to be a power issue. There was a significant ROI result prior to correction for
CVR and CBF in the ventral striatum, and the whole brain results were also similarly
affected by the voxelwise covariates in this region. As previous investigations did not
include such measures in their analysis this is likely a primary reason for the difference (a
fuller discussion of the CBF/CVR influences is given in Chapter 6). One possible additional
caveat in assessing the ventral striatum using BOLD is the higher likelihood of signal
dropout and susceptibility artefacts in this area. Although steps were taken to address this,
such as visual inspection of each scan for loss of signal from regions of interest, there is still
the possibility of loss of sensitivity or other confounds in these areas (Sacchet and Knutson,
2013, Bischoff-Grethe et al., 2015, Miller et al., 2014). Worth noting is that the
measurements of CVR would also be similarly affected in this area, which could complicate
the attempts to control for vascular effects.

A related issue is the definition of these regions throughout studies. The term ‘ventral
striatum’ can often relate to anatomically varying areas across studies, and is often used
interchangeably with ‘nucleus accumbens’ and may or may not include varying portions of
the ventral caudate and putamen. Here, the ventral striatum was defined based on detailed
work by Mawlawi et al (2001), while a structural atlas approach was used to define the
caudate and putamen. Importantly there is no distinct anatomical landmark marking the
dorso-lateral boundary of the VS (Haber, 2011) and thus the definitions in other studies
may differ, potentially including more dorsal parts of the striatum relative to this study
making direct comparison problematic.

### 4.5.5 Whole brain results

Risperidone produced further changes within the whole brain analysis, reducing activity
during reward anticipation in the thalamus, supplementary motor area (SMA) and visual
cortex. The thalamus is a critical relay centre for the brain and is a central component of
the basal ganglia-cortical loops. It has also been shown to be an important part of the
reward network (Haber and Knutson, 2010), responding to reward anticipating cues
(Komura et al., 2001) and has long been implicated in the pathophysiology of schizophrenia
(Oke and Adams, 1987, Yasuno et al., 2004, Talvik et al., 2003). One theory proposes NMDA
dysfunction resulting in excess dopaminergic activity in the thalamus within a thalamus-
hippocampus-VTA circuit as responsible for the onset of psychotic symptoms (Lisman et al.,
2010). Here, increased dopamine stress-related firing from the VTA results in increased
dopamine release and bursting in the thalamus and produces psychotic symptomology. This is sustained by the increases in thalamic bursting resulting in stimulation of the hippocampus, which subsequently promotes further dopamine release and thus stronger thalamic bursting. Although in this context the excessive dopamine release is stress-related, this feedback loop could also presumably be activated by possible aberrant dopamine responses to rewarding or salient stimuli (Moghaddam, 2010). D2 antagonism has been shown to suppress thalamic bursting previously (Zhang et al., 2009) and suppression of this signal in the thalamus by risperidone during reward anticipation may therefore reflect an element of its antipsychotic action.

SMA activation during the anticipatory phase of the MID task is expected as the individual is preparing to make a motor response to win the reward. Antipsychotics have previously been shown to influence BOLD activation in this area, with Braus et al. (1999) reporting both typical (including haloperidol) and atypicals (clozapine and risperidone) reduced SMA activation during a sequential finger opposition task, a reduction not seen in healthy controls or neuroleptic naïve patients (Braus et al., 2000). This would suggest the reductions seen here could be more related to elements of the motor disturbance related side-effects of treatment, and may even contribute to the reduction in reaction time observed on the drug (although the correlation analysis between the difference in beta estimates and reaction time between the placebo and drug scan was not significant). In terms of the reductions seen in the visual cortex, some studies have indicated antipsychotics influence visual processing (Kelemen et al., 2013) although it seems unlikely to be reflective of any direct action of the drug on reward processing itself.

However, together with the striatum, the SMA and visual cortex have been indicated to be 3 of the 4 primary nodes of the network recruited during reward anticipation on the MID in a very large cohort of 1,544 adolescents (Jia et al., 2016). The results from the current study therefore lend support to the notion that antipsychotics have the potential to modulate the network of areas responsive to reward predicting cues without any one region capturing the effect of these systemically administered drugs. Why risperidone had a particularly profound effect on these areas over and above olanzapine and haloperidol is difficult to ascertain with fMRI alone, although it again highlights the lack of utility in a ‘typical-atypical’ nomenclature.
Finally, the whole brain analysis revealed olanzapine caused a reduction in a localised area within lobule VI of the right hemisphere of the cerebellum, close to the vermis. The cerebellum, including this area, was significantly recruited during reward anticipation in the placebo session. This is could be reflective of the increased load required for predictive motor timing, as the participant readies themselves for a response within an implicitly learnt set window, which the cerebellum has been shown to be involved in along with the basal ganglia (although the cerebellum has been more linked with sub-second timing (Lungu et al., 2016, Coull et al., 2011)). While this has been linked to stable dopamine function (Rammsayer, 1990), it is unclear why olanzapine alone would modulate this region. However, the cerebellum has also been implicated in several aspects of cognitive function (Stoodley, 2012, Stoodley et al., 2012) and has been consistently linked to cognitive dysfunction in schizophrenia (Phillips et al., 2015, Yeganeh-Doost et al., 2011).

Critically for this discussion, recent research has indicated it may be directly involved in the expectation of reward. Using two-photon Ca\(^{2+}\) imaging, Wagner et al. (2017) reported specific granule cells within lobules VIa and VIb of the cerebellum of mice respond to the anticipation of delivery of a sucrose-water reward (with other granule cells also uniquely responding to reward receipt and reward omission) and postulated these cells encoded a cognitive state of expectant waiting. In terms of human fMRI, Jia et al. (2016) cited the cerebellum as part of one of the four main hubs of the network serving reward anticipation (see above).

Research to date does then implicate cerebellar regions (including lobe VI) in reward anticipation in line with our findings on placebo. The modulation of this area with olanzapine was unexpected and may reflect the broad receptor profile it has relative to the other compounds studied. For example, serotonergic fibres innervate the cerebellum Saitow et al. (2013) and muscarinic receptors in the cerebellum have an excitatory influence on granular cells (Takayasu et al., 2003). However, how these influences come together to modulate reward related activations cannot be determined from this study alone. Nonetheless our findings do show the advantage of whole brain imaging in revealing the functional consequences of the complex and interacting effects of a compound acting on multiple receptor systems. This would not be possible with those drugs that target single receptor systems.
4.5.6 Conclusion

Both olanzapine and risperidone produced suppression of reward anticipation as hypothesised, even when covarying for CBF and CVR effects. The relative lack of action haloperidol appears to have on reward functioning of this kind would seem to suggest simple D2 blockade is not sufficient to meaningfully alter activation of this network, with compounds with a broader receptor profile causing location specific alterations to anticipation of reward. This highlights the potential involvement of other neurotransmitter systems, primarily for additional modulatory effects on dopamine, but also their potentially direct effects on systems that may be disrupted in psychosis.
Chapter 5  Antipsychotic modulation of reward outcome

5.1 Introduction

In Chapter 4 we observed varying effects of antipsychotics on the brain's response to reward predicting cues. It was also expected that there would be an impact on the response to reward outcomes given the putative involvement of neurotransmitter systems that antipsychotics target. This could be due to dopaminergic modulation during the outcome phase or a propagation of the effects during the anticipation phase to the outcome phase. As already discussed in detail, a view of antipsychotics as dopaminergic modulators alone does not neatly account for our findings. An appreciation of the broader profile of these drugs may be important and only through empirical testing can we address the impact on these reward processes, which are thought to reflect the complex inter-play between the drugs' binding to multiple receptor targets.

Here the whole brain results of the MID task during the placebo sessions will be presented to illustrate the effect of receipt or non-receipt of reward under normal conditions. The ROI and whole brain results of drug effects on reward consummation processing will then be presented both before and after correction for CVR and CBF, and the vascular corrected results discussed.
5.2 Reward Feedback – Placebo session

The first aim of this chapter is to explore whether the MID task was recruiting relevant brain regions outside the effect of drug. The placebo scans from both groups were analysed during reward outcome, and win and no win outcomes were compared against neutral outcome phases.

5.2.1 Outcome win vs Neutral

In the contrast between neutral outcome and winning any reward, increased activation was unexpectedly found during neutral reward outcome in several brain areas including the putamen, SMA, insula and orbitofrontal cortex.

![Figure 5.2-1 Control feedback>win feedback N=34 (placebo), 5000 permutations, FWE corrected p<0.05](image)

5.2.2 Outcome no win vs Neutral

A similar pattern was observed in the contrast between neutral outcome and failing to win any reward, with increased activation again found during neutral outcome, particularly in the putamen, with smaller clusters spread throughout frontal and parietal areas.

![Figure 5.2-2 Control feedback>No Win feedback N=34 (placebo), 5000 permutations, FWE corrected p<0.05](image)
5.2.3 Outcome Win vs No Win

To explore this unexpected result further, comparisons were conducted between win and no win outcomes during the feedback phase, a more common analysis in the MID literature. This revealed a bilateral cluster in the parietal lobe.

Whole brain analysis was also conducted for the contrast between the level of win (i.e. high win vs low win) for those trials when a win was achieved, but no cluster passed the threshold for significance for this comparison. Contrasts used in the drug analysis therefore involved both levels of reward collapsed into one (as for the reward anticipation analysis in chapter 4).

*Figure 5.2-3 Win>No Win feedback N=34 (placebo), 5000 permutations, FWE corrected p<0.05*
5.3 Risperidone (Ris-H/L) and reward outcome

5.3.1 Outcome Win vs Neutral
5.3.1.1 Whole Brain Results
Non-parametric whole brain permutation testing revealed a 2mg dose of risperidone compared to placebo resulted in widespread increases in BOLD response during receipt of high/low reward vs neutral reward.

The same analysis comparing 0.5mg risperidone with placebo also revealed less widespread, but more delineated increases in BOLD signal during reward feedback in bilateral insula, superior temporal gyrus, putamen and hippocampal areas.

Figure 5.3-1 Whole brain permutation testing, 2mg Risperidone>Placebo during Win Feedback (All Win>Neutral feedback) N=17), 5000 permutations, FWE corrected p<0.05

Figure 5.3-2 Whole brain permutation testing, 0.5mg Risperidone>Placebo during Win Feedback (All Win>Neutral feedback) N=17), 5000 permutations, FWE corrected p<0.05
When comparing low and high dose risperidone scans during win outcome there was one small cluster of increased signal on 2mg risperidone in the visual cortex.

![Figure 5.3-3 Whole brain permutation testing, 2mg>0.5mg Risperidone during Win Feedback (All Win>Neutral feedback) N=17), 5000 permutations, FWE corrected p<0.05](image)

### 5.3.1.2 ROI results

The *a priori* ROIs were entered into a generalised estimating equation with drug and ROI as factors, and revealed an effect of drug on the extracted parameter estimates (Wald chi-sq =30.912, df=2, *p* < 0.001), with Bonferroni corrected pairwise comparisons revealing a significant increase after 2mg risperidone compared to placebo across all ROIs (0.95 (95% CI 0.61 to 1.3) *p* < 0.0001), but not after 0.5mg risperidone (increase of 0.45 (95% CI -0.02 to 0.92) *p* = 0.12).

Bonferroni corrected post-hoc tests of each ROI revealed these increased occurred in the putamen (1.31 (95% CI 0.94 to 1.7) *p* < 0.0001), caudate (1.36 (95% CI 0.74 to 1.98) *p* = 0.0002), ventral striatum (0.92 (95% CI 0.57 to 1.26) *p* = 0.0002) and amygdala (0.85 (95% CI 0.48 to 1.21) *p* < 0.0001) after 2mg risperidone alone.
5.3.2 Outcome No Win vs Neutral

5.3.2.1 Whole brain results
2mg risperidone also produced large but more localised increased in BOLD signal during failure to win reward contrasted with neutral outcome. Increases were observed in the caudate, putamen and thalamus, as well as frontal and parietal regions.

![Figure 5.3-4 Whole brain permutation testing, 2mg>Placebo during No win Feedback (No Win>Neutral feedback) N=17), 5000 permutations, FWE corrected p<0.05](image)

The same analysis comparing 0.5mg and placebo did not reveal any significant changes during no win outcome vs neutral outcome. Comparing 0.5mg with 2mg revealed one cluster centred around the supra marginal gyrus.

![Figure 5.3-5 Whole brain permutation testing, 2mg>0.5mg during No win Feedback (No Win>Neutral feedback) N=17), 5000 permutations, FWE corrected p<0.05](image)
5.3.2.2 ROI results

A generalised estimating equation with drug and ROI as factors revealed an effect of drug on the extracted parameter estimates (Wald chi-sq = 9.799, df=2, p = 0.007), with Bonferroni corrected pairwise comparisons revealing a significant increase after 2mg risperidone compared to placebo across all ROIs (0.734 (95% CI 0.22 to 1.25) p = 0.01), but not after 0.5mg risperidone (increase of 0.22 (95% CI -0.35 to 0.8) p = 0.89). Bonferroni corrected post-hoc tests of each ROI revealed significant increases were limited to the striatum, occurring in the putamen (1.15 (95% CI 0.68 to 1.62) p < 0.0001) and caudate (1.61 (95% CI 0.87 to 2.36) p < 0.0001).

5.3.3 Outcome Win vs No Win

5.3.3.1 Whole brain results

Whole brain permutation testing of the Win vs No win contrast revealed a focused increase in BOLD centred around the anterior hippocampus and amygdala on 2mg risperidone compared to placebo.

Figure 5.3-6 Whole brain permutation testing, 2mg risperidone>placebo during Win vs No win Feedback (N=17), 5000 permutations, FWE corrected p<0.05

0.5mg risperidone compared with placebo did not produce any significant changes.

5.3.3.2 ROI results

A generalised estimating equation with drug and ROI as factors revealed no effect of drug on the extracted parameter estimates (Wald chi-sq = 2.35, df=2, p = 0.308) for outcome win vs no win.
5.4 Olanzapine & Haloperidol (Olan-Hal) and reward outcome

5.4.1 Outcome Win

5.4.1.1 Whole brain results

Olanzapine produced increases in BOLD signal in the bilateral putamen during win outcome. Haloperidol did not produce in any significant changes.

![Whole brain permutation testing](image)

*Figure 5.4-1 Whole brain permutation testing, Olanzapine>Placebo during Win Feedback (Win>Neutral feedback) N=17), 5000 permutations, FWE corrected p<0.05*

5.4.1.2 ROI results

The *a priori* ROIs were entered into a generalised estimating equation with drug and ROI as factors, and revealed a non-significant effect of drug on the extracted parameter estimates (Wald chi-sq =5.23, df=2, p = 0.073).
5.4.2 Outcome No Win

5.4.2.1 Whole brain results
Olanzapine produced increases in one cluster in the pars triangularis of the inferior frontal gyrus during no win outcome. Haloperidol did not produce any significant changes.

5.4.2.2 ROI results
Running the generalised estimating equation with drug and ROI as factors revealed a non-significant effect of drug on the extracted parameter estimates (Wald chi-sq = 2.89, df=2, p = 0.236).

5.4.3 Outcome Win Vs No Win

5.4.3.1 Whole brain results
Neither olanzapine or haloperidol produced any significant changes for the win vs no win contrast.

5.4.3.2 ROI results
ROI analysis revealed no significant effect of drug on the extracted parameter estimates (Wald chi-sq = 0.50, df=2, p = 0.746).

5.4.4 Level of reward
No significant results were found for the high reward vs low reward win/no win contrasts at the whole brain or ROI level, for either RisH/L or Olan/Hal.
5.5 Reward outcome controlling for baseline blood flow and vascular reactivity

The same analyses were conducted with the addition of the CBF and CVR voxelwise maps as covariates.

5.5.1 Risperidone (Ris-H/L) and reward outcome controlling for CVR and CBF

5.5.1.1 Outcome Win vs Neutral

5.5.1.1.1 Whole brain results

The widespread changes on 2mg risperidone during reward receipt remained after controlling for CVR and CBF, with significant increases compared to placebo throughout cortical grey matter, striatum, thalamus and other subcortical areas.

Increasing the threshold to an exploratory 0.01 to break up the cluster and isolate local peaks revealed localised increases in the bilateral putamen, amygdala, thalamus and frontal middle gyrus, and SMA and related motor cortex.
The same analysis with the low dose risperidone scans revealed smaller clusters of increases in bilateral hippocampus, insula, and posterior cingulate cortex.

5.5.1.1.2 ROI results

The a priori ROIs were entered into a generalised estimating equation with CVR and CBF included as covariate and drug and ROI as factors, and revealed an effect of drug on the extracted parameter estimates (Wald chi-sq =33.776, df=2, p < 0.0001), with Bonferroni corrected pairwise comparisons revealing a significant increase after 2mg risperidone.
compared to placebo across all ROIs (0.98 (95% CI 0.65 to 1.32) p < 0.0001), but not after
0.5mg risperidone (increase of 0.46 (95% CI 0.01 to 0.92) p = 0.09).
Bonferroni corrected post-hoc tests of each ROI revealed the same 2mg risperidone
induced increases prior to vascular correction in the putamen (1.34 (95% CI 0.97 to 1.7) p <
0.0001), caudate (1.43 (95% CI 0.83 to 2.01) p = 0.0001), ventral striatum (0.97 (95% CI 0.55
to 1.40) p < 0.0001) and amygdala (0.84 (95% CI 0.50 to 1.19) p < 0.0001). Notably, changes
were additionally seen in the vmPFC (1.13 (95% CI 0.46 to 1.79) p = 0.012).

5.5.1.2 Outcome No Win

2mg risperidone produced increases in the caudate, putamen and thalamus, as well as
frontal and parietal regions changes, during failure to win reward contrasted with neutral
outcome. The 0.5mg placebo comparison produced no results.

5.5.1.2.1 ROI results

The a priori ROIs were entered into a generalised estimating equation with CVR and CBF
included as covariate and drug and ROI as factors, and revealed an effect of drug on the
extracted parameter estimates (Wald chi-sq = 11.512, df=2, p = 0.003), with Bonferroni
corrected pairwise comparisons revealing a significant increase after 2mg risperidone
compared to placebo across all ROIs (0.756 (95% CI 0.27 to 1.25) p = 0.006), but not after 0.5mg risperidone (increase of 0.22 (95% CI -0.34 to 0.77) p = 0.89).

Bonferroni corrected post-hoc tests of each ROI revealed significant increases were again limited to the striatum, occurring in the putamen (1.16 (95% CI 0.70 to 1.61) p < 0.0001) and caudate (1.65 (95% CI 0.94 to 2.37) p < 0.0001). However, borderline changes were also additionally seen in the vmPFC (1.04 (95% CI 0.33 to 1.75) p = 0.045).

5.5.1.3 Outcome Win vs No Win

5.5.1.3.1 Whole brain results

Covarying for CVR/CBF reduced the hippocampal cluster previously seen after 2mg risperidone during the Win vs No win contrast to just three voxels centred around the amygdala, and there were no results for 0.5mg risperidone

![Figure 5.5-5 Whole brain permutation testing with voxelwise CBF and CVR map covariates, 2mg Risperidone>Placebo during Win vs No Win Feedback N=17), 5000 permutations, FWE corrected p<0.05](image)

5.5.1.3.2 ROI results

There was no a significant main effect of treatment on ROI values (Wald chi-sq = 3.01, df=2, p = 0.222).
5.5.2 Haloperidol and Olanzapine (Hal/Olan) and reward outcome controlling for CVR and CBF

5.5.2.1 Outcome Win vs Neutral

5.5.2.1.1 Whole brain results

Haloperidol failed to produce any significant changes. However, covarying for CVR/CBF reduced the bilateral putamen cluster previously seen after olanzapine in the Win>Neutral contrast to just 16 voxels centred around the right ventral putamen:

![Whole brain results](image)

*Figure 5.5-6 Whole brain permutation testing with voxelwise CBF and CVR map covariates, 3mg Olanzapine>Placebo during Win vs No Win Feedback N=17), 5000 permutations, FWE corrected p<0.05*

5.5.2.1.2 ROI results

Inclusion of the CVR and CBF covariates in the model revealed that there was now a significant main effect of treatment on ROI values (Wald chi-sq = 6.86, df=2, p = 0.032). Bonferroni corrected pairwise comparisons between placebo and each drug condition across all ROIs revealed a significant increase after olanzapine (0.636 (95% CI 0.16 to 1.10) p = 0.018), but not after haloperidol (increase of 0.27 (95% CI 0.14 to 0.67) p = 0.392). Bonferroni corrected post-hoc tests of each ROI revealed these increases occurred in the putamen (1.08 (95% CI 0.63 to 1.52) p < 0.0001), amygdala (1.36 (95% CI 0.29 to 1.06) p = 0.008) and VTA (0.74 (95% CI 0.40 to 1.08) p = 0.0003) after olanzapine.
5.5.2.2 Outcome No Win

5.5.2.2.1 Whole brain results

Controlling for vascular effects removed the frontal cluster seen earlier, and neither olanzapine or haloperidol produced significant changes.

5.5.2.2.2 ROI results

No significant effect of treatment was revealed by adding the vascular covariates to the model.

5.5.2.3 Outcome Win vs No win

5.5.2.3.1 Whole brain results

Neither olanzapine or haloperidol produced any significant changes for the win vs no win contrast.

5.5.2.3.2 ROI results

No significant effect of treatment was revealed by adding the vascular covariates to the model (Wald chi-sq = 1.228, df=2, p < 0.541).

5.5.3 Level of reward

No significant results were found for the high reward vs low reward win/no win contrasts at the whole brain or ROI level, for either RisH/L or Olan/Hal.
5.6 Discussion

Due to the lack of previous studies examining antipsychotic effect on the outcome phase of the MID, two possible impacts of these drugs on consummatory processing were considered in the initial hypotheses. If any suppression of the anticipatory phase was shown, this was proposed to influence the impact of the outcome such that the response increases (in line with a more novel or salient reward). Alternatively, if the drug influences the outcome phase directly (not via anticipation) then a suppression of activity due to a reduction in the reward related signal was expected.

In a similar trend to that seen in the previous chapter, the newer, ‘atypical’ antipsychotics with broad receptor profiles produced dose and subtype dependant effects during reward receipt, both before and after correction for CBF and CVR, while haloperidol did not. Importantly, while during anticipation these drugs suppressed reward related signals, during consummation of a reward exposure to the drugs produced relative increases in activation, both when compared to a neutral phase or when compared to failure to win reward. A dose response was additionally seen on risperidone, as hypothesised, with low dose risperidone producing limited changes compared to high dose, which were widespread. These findings would fall into line with the first proposal hypothesised – however, the results will be discussed in a broad context here and the relationship with reward anticipatory processing will be additionally discussed in Chapter 6.

As in the previous chapter, the neuroimaging results discussed regarding receipt of reward will be those accounting for CBF and CVR unless otherwise stated, and discussion on the effect of the drug on CBF and CVR itself will be conducted in more detail in the final chapter.

5.6.1 Reward feedback during placebo

An important issue to first note are the results seen on placebo alone. In line with the Schultzian model (and assuming the signal in this area is primarily reflective of dopaminergic activity), winning an expected reward would be expected to produce little change in the response observed in striatal areas, with reductions in activity being produced in response to the failure to win a reward. In fact, compared to the neutral trial outcome phase (where participants were aware no reward was on offer but were still required to make a prior motor response to the cue), both winning and failing to win a
reward reliably produced less activation in the putamen and other cortical areas. The results from this particular contrast are in fact somewhat underreported in the literature, and therefore the results observed here warrant a short discussion before moving on to examining drug effects on the outcome phase of the task.

Early studies using the MID highlighted medial prefrontal cortex (mPFC) activation in response to receipt of anticipated reward, in addition to striatal activation (Knutson et al., 2001, Knutson et al., 2003). However, these studies focused on win vs no win, which contrasts activation associated with consummation of an anticipated reward with activation associated with failing to win an anticipated reward (i.e. only within incentivised trials). These are arguably two distinct processes and therefore a departure from the typical ‘neutral’ task element used for simple contrasts of interest in investigations of this type, such as the neutral cue used in the anticipatory regressors both herein and consistently throughout the MID related literature. A failure to win a reward towards which a motivated effort has been made could recruit additional brain regions. Several studies have additionally included a loss condition in the MID (Knutson and Cooper, 2005, Knutson et al., 2003), which involves requiring to make a motor response to a cue to prevent a loss of money (rather than just failing to win a reward on offer), and report different patterns of activation for reward and loss outcome. However, the aversive nature of a financial penalty (being worse off) compared to a failure to win a reward (no change) may still be considered qualitatively different.

Knutson et al. (2001) did in fact conduct a control contrast of the outcome of all incentivised trials (e.g. both win and non-win) against a neutral outcome. They reported strikingly similar results to those found here with peak reductions in the putamen, parietal, and orbitofrontal cortex, although these were only briefly mentioned and not discussed in detail. A ROI analysis within the ventral mPFC also indicated increased activation during the outcome of neutral trials compared to trials when rewards were won or not won. Conversely, more recent studies have examined the outcome phase using a neutral control element and reported increases in activation. Dillon et al. (2008) reported increased activation in the inferior and middle frontal gyrus, temporal lobes, fusiform gyrus, calcarine sulcus, and cerebellum during a win outcome>neutral outcome akin to the one here. However, the MID task used in this study was a significantly extended version of that developed by Knutson and included an additional delayed inter-stimulus interval (ISI)
between the target and visual feedback of between 4.4 and 8.9s, in addition to using a longer cue – target interval. This resulted in trails being twice the length of the trails used here with feedback being presented after a considerably longer delay, and temporal differences in feedback have been shown to influence activation (Foerde and Shohamy, 2011). Rademacher et al. (2010a) used a MID more temporally consistent with the task used here and reported a large increase in the occipital lobe, along with far smaller clusters in the ACC, thalamus and hippocampal area, but like Dillon et al. (2008) used a very liberal statistical threshold (p<0.001, uncorrected). In both cases, the reverse outcome contrast (neutral>win), where extensive activation is seen here, is not reported, although Dillon et al. (2008) did replicate the reduction in striatal activation seen during failure to win compared to neural outcome. In many other instances of the MID being used in healthy humans, only a win>failure to win contrast or win>loss contrast is reported or discussed.

The reduction in response in the no win-neutral condition has been related to decreased dopaminergic firing observed following the non-presentation of an expected reward, which is a compelling interpretation (Dillon et al., 2008). However, viewed in the context of the similar results observed in the win-neutral comparison here it loses some plausibility, as the ‘expected’ reward produces a similar outcome. These examples are indicative of an issue highlighted in Chapter 1: there is a great deal of inconsistency in the fMRI reward literature even within those studies using the MID, not only with the specific details of the task (e.g. stimuli used, variations in ITIs and ISIs etc.) but with the precise contrasts employed. This is particularly an issue for the outcome phase of the task, and part of the rationale for displaying the various contrasts in full in the results section above.

Why would winning or failing to win a reward produce reduced activation in comparison to a neutral phase? One possible explanation could be due to the type of feedback displayed in the version of the MID used here. Because a running total of the amount currently won was displayed during the neutral feedback phase, this stimulus may be inherently rewarding thus diminishing or confounding the contrast with activity when rewards are won. In both the studies referenced above which did report increases in win-neutral contrasts, the neutral outcome phase included no information about current/total winnings. Lutz et al. (2012) illustrated that the quality of the feedback given to motivated tasks such as the MID does appear to impact striatal activation, reporting dorsal striatal activation in response to rewarding feedback with a monetary element but less so to
performance feedback or monetary gain. However, given that all conditions (win, no win and neutral) also fed back a running total, it is not clear that feedback during the neutral phase should be more rewarding that the outcome win phase. Timing of the feedback phase may be important, with Dillion et al. (2008) reporting increases on a more temporally extended version of the MID employed here.

Another important difference between the win and neutral trials is the longer reaction times for the latter. This is thought to indicate the motivational difference between the conditions. Participants in the neutral trials may have a tendency to look ahead to the next trial, as they have learnt that their responses don’t ‘matter’ (as evidenced by the significant increase in reaction time on these trials). The increased signal in the putamen may therefore be reflective of increased preparatory and/or anticipatory activation for the next trial (in the expectation they may to their total) compared to potential win trials where attention will be focused on the task at hand and the outcome of their efforts. Much of this is highly speculative – we cannot assess the level preparation of the next trial, and participants would not know what was coming without the cue being known – however, the difficulty in interpreting this activation in the context of reward processing and any related modulation by antipsychotics remains.

Comparison with a neutral outcome phase may still be informative as is most comparable to the anticipation stage, also contrasted with a neutral cue. However, only a short discussion will be given over to drug effect on the contrasts with the neutral phase here. This is primarily due to 1) the interpretability of this contrast by itself obscures understanding of the potential mechanism of drug effect 2) the win>no win contrast is used more frequently in the literature and therefore the drug effect will be more relatable to current research.

5.6.2 Antipsychotic modulation of neutral phase contrasts
Risperidone produced robust and widespread increases throughout cortical and subcortical regions on both the win>neutral outcome and no win> neutral outcome contrasts, again highlighting its involvement in the modulation of reward related processing. Increasing the threshold to isolate peaks of signal change revealed that 2mg risperidone particularly increased relative activation in the bilateral putamen, thalamus and frontoparietal regions, while ROI analysis showed further increases in the amygdala and mPFC. Olanzapine also
produced increases in activation in the win>neutral contrast alone, albeit far less widespread than risperidone, being focused to a small cluster in the right ventral putamen (although ROI analysis also revealed increases in amygdala). Again, following the trend seen during the anticipatory phase in Chapter 4, haloperidol failed to produce any changes to activation in either of the contrasts involving the neutral phase suggesting a non-DA antagonist feature of risperidone and/or olanzapine may be responsible for the changes not seen on haloperidol.

Given the possible issues with these contrasts highlighted above, perhaps the most parsimonious explanation for this finding is that risperidone and olanzapine may have suppressed regions that were more active during the neutral phase of the task. Risperidone in particular produced changes in strikingly similar areas to those were more heavily recruited during the neutral phase contrasts. Antipsychotics have previously been shown to modulate attentional systems during a motor task (Ikuta et al., 2014) which may have contributed to the changes seen if attention systematically varied between neutral and reward outcome phases, although again it must be stressed this interpretation of these changes is speculative.

Alternatively, the presence of the drug could be enhancing the response to winning or failing to win the reward. Some research has indicated that antipsychotic use increases sensitivity to reward, and has been linked to increased vulnerability to substance abuse and addiction in medicated individuals (Samaha, 2014). However, this ‘dopamine supersensitivity’ has been more readily linked to chronic, continuous use rather than acute exposure (Bedard et al., 2011), as well as being more associated with typical rather than atypical medication (Bedard et al., 2013) and it is unlikely the same process is occurring here on an acute dose. Nevertheless, the results with olanzapine here do partially replicate those in Mathews et al. (2012), who reported an increase in activation of the caudate and putamen of healthy individuals in response to receiving an anticipated taste reward following one week of dosing with olanzapine. Comparing results on the MID with those paradigms that deliver primary rewards such as pleasant taste should be made with caution however (Sescousse et al., 2013).

Dorsal striatum activity, modulated here by both olanzapine and risperidone, has been shown to be important in assessing the magnitude or valence of received rewards (Delgado
et al., 2003), and has been associated with the ‘actor’ element of the actor-critic model, modulating stimulus-response-reward associations (Doherty et al., 2004). Within the dorsal striatum, olanzapine only increased activity within the putamen, while risperidone’s action included both caudate and putamen (in addition to widespread cortical increases) suggesting the drugs may have a differential effect on subsections of this region. Brovelli et al. (2011) indicated there is an apparent division of action in terms of reward processing within the dorsal striatum, suggesting the putamen tracks the reward received in relation to the initial stimulus, while the caudate is involved in performance monitoring. In the context of the MID this could be analogous to the caudate providing feedback as to whether the individual’s reaction time was fast enough to receive a reward, while the putamen maintains the connection between the level of reward and the initial stimulus. Once more, caution needs to be taken in consolidating some of this research with the current results as many of these studies use learning paradigms which arguably recruit additional neural system. Nevertheless, it does strongly implicate the action of these drugs at sites involved across multiple stages of reward processing. Dorsal striatum dysfunction during reward receipt has been linked to a range of psychiatric disorders, with exaggerated responses seen in ADHD (Furukawa et al., 2014) and reductions in activation observed in unmedicated patients with major depression (Pizzagalli et al., 2009). In schizophrenia, reduced dorsal activation has been reported during the consummatory phase of the MID (Mucci et al., 2015), with this attenuated response to reward linked to negative and depressive symptoms of the disorder (Simon et al., 2010, Mucci et al., 2015).

Risperidone additionally produced increases in several cortical areas, including the ventral medial prefrontal cortex (vmPFC) and amygdala (which was also increased on olanzapine). The vmPFC has been particularly implicated in processing of the receipt of reward, identified in the very earliest studies using the MID (Knutson et al., 2001), with subsequent studies and meta analyses showing it to play an important role in consummatory processes (Haber and Knutson, 2010, Diekhof et al., 2012, Vassena et al., 2014). Patient studies have also shown aberrant responses in this area during the outcome phase of the MID (Waltz et al., 2010, Schlagenhauf et al., 2009), with Waltz et al. (2010) also reporting abnormal responses in the amygdala, an area which both olanzapine and risperidone also increased activity within during the win > neutral outcome here. The involvement of this region will be discussed in more detail in the next section.
Taken together, these findings do suggest a selective action of risperidone and, to a lesser extent, olanzapine on the processes associated with receiving or failing to receive a cued reward, in areas frequently affected in the disorder these drugs treat. As with modulation of the reward anticipatory phase, manipulation of DA transmission may be the most likely candidate for driving the changes in activation seen here, either directly or indirectly via other neurotransmitter systems. Studies conducted in healthy volunteers in which DA is manipulated pharmacologically have shown similar disruptions to reward receipt in the context of learning or RPE paradigms. Jocham et al. (2011) reported a low 200mg dose of amisulpride increased a vmPFC reward tracking signal during reinforcement learning task while van der Schaaf et al. (2014) report a single 400mg dose of the selective D2 antagonist sulpiride increased the striatal response to rewards and punishments in a reversal learning task (although the learning aspects of these paradigms prevent direct comparison with the MID). Conversely, in a more simple design with no learning component, McCabe et al. (2011) reported that 400mg sulpiride reduced BOLD in the ventral striatum in response to the taste of chocolate (although this study used primary rewards which were not cued). Generally, the results of studies examining reward processing under pharmacological dopamine manipulation are inconsistent, which are likely due to marked differences in paradigms used, in addition to variations in dosage resulting in changes in the relative action of the compound pre- or post-synaptically (Martins et al., 2017). Interestingly, one study that putatively (but non-pharmacologically) modulated DA levels by increasing stress levels (thought to acutely increase DA release) during performance of the MID showed the inverse relationship to that seen here, with high stress levels increasing BOLD signal in the caudate and amygdala during reward anticipation, and reducing activation in the putamen and caudate during consumption (Kumar et al., 2014).

The changes produced by these drugs to the tonic-phasic status of midbrain neurons is again most likely relevant here. In Chapter 4, one potential explanation was the increase of VTA/SNC firing produced by exposure to the antipsychotics (Chiodo and Bunney, 1983, Di Giovanni et al., 1998) altered the fining of midbrain neurons or the basal level of extracellular dopamine, meaning the context in which reward relating signalling was altered. Applying optogenetic stimulation to the VTA, Decot et al. (2017) reported increased VTA activity resulted in widespread changes in CBF when assessed using brain-wide voxel-based principal component analysis. Given that VTA stimulation can produce distal detectable changes in CBF, even in areas not typically associated with dopamine function, the manipulation of midbrain firing mechanics in some unique manner by risperidone is a plausible mechanism
for the cortical increases in BOLD seen here. However, given the inability of the D2 antagonist haloperidol to produce changes specifically to MID related activation in the current results, it is again likely that manipulation of other neurotransmitter systems are involved in producing the changes to consummatory activation seen here.

5.6.3 Antipsychotic modulation of Win Vs No Win contrast

In the context of understanding the mechanism of the drugs employed in this thesis, applying the win vs no win contrast may offer more insight. This contrast does assuage some of the possible issues with the neutral phase, in that the participant will have been equally engaged in the task up to the point of reward delivery and aware they had some agency in the outcome (compared to the neutral trial, which from the time of the presentation of the cue they would be aware the outcome was a foregone conclusion). Given responses to predictable reward have been shown to be relatively small in comparison to, say, the response to the predictor of that reward (O'Doherty et al., 2002, Berns et al., 2001), a win vs no win contrast may actually be more sensitive to processes during this phase of reward processing and the drug interactions thereof. The parietal activation produced this contrast on placebo replicates the highest peak value reported for the same contrast in Knutson et al. (2001) and activation in this region during reward processing has been linked to task-reward value associations (Wisniewski et al., 2015, Kahnt et al., 2014).

The modulatory effect of the antipsychotics used were found to be far more localised using this contrast. Only 2mg risperidone produced increased activation in the amygdala which suggests, along with the same direction changes seen in this area after both high and low dose risperidone during the neutral contrast analysis, that this region is selectively modulated by this drug during feedback regarding a rewarding outcome. The amygdala has long been associated with the processing of emotion, particularly those related to negative or aversive stimuli (Zald, 2003). However, its scope of operation has been considerably extended in recent years and the amygdala has been shown to display remarkable heterogeneity in its functional repertoire than merely the ‘fear centre’ (Paré and Quirk, 2017, Murray, 2007b). Reward processing is one area in which the amygdala has been consistently implicated, (Baxter and Murray, 2002), such as involvement in encoding expected outcome (Kosson et al., 2006).
Anatomically the amygdala is well placed to modulate information processing within the reward network, and is thought to play an important role in adjusting motivational levels in response to emotional assessment of environmental stimuli (Haber, 2011). It projects directly to the nucleus accumbens in the striatum as well as to frontal areas including the vmPFC, and is directly innervated by midbrain dopamine and 5-HT neurons (Russo and Nestler, 2013, Haber and Knutson, 2010). These connections have been shown to be important in establishing expected reward representations (Hampton et al., 2007), facilitating motivated responses to reward (Stuber et al., 2011) and assessing the valence of a reward (Correia and Goosens, 2016).

Animal work has also strongly implicated the amygdala’s role in the valence of reward. Neurophysiological studies have shown amygdala activity tracking the expected outcome of a salient olfactory cue for both pleasant and aversive tastes (Schoenbaum et al., 1998, Schoenbaum et al., 1999), suggesting a role in the reinforcement of stimulus-valence associations. An early fMRI meta-analysis of human MID studies indicated amygdala activation is more involved with reward consummation than anticipation in this context (Knutson and Greer, 2008), responding in particular to differences in outcome (Ernst et al., 2005). Other animal studies have further implicated the amygdala in reward receipt: (Belova et al., 2007) reported subsets of neurons in the rat amygdala responding selectively to either reward or aversive stimulus (or both), pharmacological inactivation of the amygdala in rats results in a failure to update behaviour in response to changes in rewards received (Salinas et al., 1993), and electrophysiological recording from the primate amygdala reveals different populations of neurons encode for elements of both the extent and valence of a conditioned outcome (Belova et al., 2008, Bermudez and Schultz, 2010, Tye et al., 2010b).

An important point to note here is that amygdala activation was not observed on placebo during the win vs no win contrast in the ROI analysis. However, the ROI covered the entirety of the structure, and as seen above, the amygdala exhibits selective activity to different stimuli within the structure itself, while much of the animal work is centred around the basolateral sub portion of the amygdala. Therefore the ROI employed here may not allow for the required sensitivity to detect a change on placebo, whereas acute pharmacological manipulation on the other hand may be profound enough to inhibit or promote a significant relative change that would be observed.
Given a clinically relevant dose of risperidone increases signal in the amygdala here, with olanzapine also increasing activation in this area in the neutral contrast analysis, what could be the mechanism of action for this effect? Animal studies have suggested reward related signalling in the amygdala is mediated by dopamine, with lesioning of rat VTA resulting in the loss of amygdala activity in response to unexpected changes in the timing of extent of the delivery of a reward, compared to sham lesioned rats (Esber et al., 2012) with Darvas et al. (2011) indicating intact DA functioning is required for other aspects of amygdala mediated learning.

The basolateral amygdala (the sub region most consistently implicated in reward signalling within the preclinical research) receives direct input from the VTA, and its activity has been shown to be directly modulated by dopamine (Kroner et al., 2005). D1-type receptors appear to be most numerous in this region, although D2-type receptors are also present (de la Mora et al., 2010). Amphetamine sensitization has been used to mimic the mesolimbic DA dysfunction thought to underlie schizophrenia in healthy humans (Featherstone et al., 2007), and in applying this model to healthy humans O’Daly et al. (2014) reported reductions in amygdala signal in response to reward outcomes on a gambling task. They posited the increased DA levels reduced sensitivity to differences in outcome on the task, driven by either reduced responses to winning or increased responses to losses. Taken together, DA blockade by antipsychotics could therefore modulate amygdala activity during reward outcome – for instance, one interpretation in the context of the MID results here could be suppression of DA transmission may mediate amygdala activity in response to a reward not being received (as it is the marginally less likely of the two outcomes), resulting in a relative increase in activity during the receipt of reward. However, as seen with the anticipatory phase, haloperidol did not produce any significant changes, so D2 antagonism alone would not appear to be sufficient to produce the effect seen, and other neurotransmitter systems are therefore likely involved.

Serotonin is a likely candidate in this respect. The DRN shares both afferent and efferent connections with the amygdala (Nakamura, 2013) and as outlined in chapter 4, 5HT function has been linked to reward processing (although its potential modulating effects have not been studied to the extent of dopamine). Having said that, a more straightforward role for the DRN has been shown outside the effects of learning or prediction error in the context of processing the consummation of an expected reward.
Nakamura et al. (2008) reported that the DRN neurons of primates responded to both the expectation and subsequent receipt of rewards during a learnt saccade task, while midbrain DA neurons only responded if the outcome was different than expected. Similar results were reported in active mice by Li et al. (2016), with a range of rewards (including food, social interaction and sex) producing responses from 5HT neurons in the DRN, while aversive stimuli failed to change firing patterns. Serotonin function may therefore be particularly salient in the context the MID task used here, where RPEs are low given a certain amount of unpredictability of the task. Specific to the serotonin action in the amygdala, Rygula et al. (2015) reported that surgical 5-HT depletion in this structure in monkeys resulted in impairments in responding appropriately to reward or punishment during a probabilistic discrimination task, suggesting 5HT function in the amygdala is involved in processing feedback to an anticipated reward.

Pharmacological manipulation of serotonergic systems is a main mechanism of action of antidepressants, with obvious links between changes in the ability to accurately process reward when it is received and symptoms of depression, such as loss of motivation and pleasure. For instance, a single 30 mg dose of mirtazapine (an adrenergic and serotonin antagonist) enhanced activations in the bilateral parietal cortex of healthy volunteers during receipt of a monetary reward, the same region activated in the win>no win contrast here (Völlm et al., 2006). Conversely, healthy volunteers given citalopram (a selective serotonin reuptake inhibitor) reduced activity in striatal areas in response to a passive chocolate taste reward (McCabe et al., 2010). Interestingly, the same group found that 7 days treatment with bupropion (a dopamine and noradrenaline reuptake inhibitor) boosted both the signal in frontal regions in response to a pleasant taste reward, and the signal in frontal, amygdala and striatum in response to unpleasant reward (Dean et al., 2016). The same drug increased the reward related response to viewing erotic images in healthy males treated for 7 days, compared to paroxetine (an SSRI) which reduced it (Abler et al., 2011).

Of course, these are all quite different compounds with different mechanisms of action, and comparison with results here should be made with caution, but they support the general principle of 5-HT modulation in vivo altering reward-related processing. Additionally, much of the research above (including the majority of the preclinical work) is based on food reward (or, in one case, eroticism), in comparison to monetary reward used in the current study, and primary and secondary rewards most likely involve different or
additionally processes or pathways (Sescousse et al., 2013). Nevertheless, it demonstrates the complex interplay of different neurotransmitter systems involved in mediating the neural activity of the consummation of reward, and does help explain the marked difference between the three drugs examined here as well as highlighting the notion these systems do not work in isolation.

On this point, risperidone exhibits the highest affinity for 5-HT2A of the drugs examined here, with additional unique affinity for 5-HT7, which may suggest a role for these receptors in increasing the amygdala signal on the win>no win contrast, and the more widespread increases seen on the neutral contrasts over and above those seen on olanzapine. For instance, selective 5-HT7 antagonists have been shown to decrease DA and 5-HT turnover in the amygdala (Takeda et al., 2005), while 5-HT2A receptors in the mPFC are known to modulate amygdala reactivity (Fisher et al., 2009). However, and as in the previous chapter, it is impossible to tell with this method whether the effects on the amygdala are from direct modulation of the DA of 5HT systems, or by indirect modulation – but it does give an indication that some feature of the drug is required to produce this effect.

5.6.4 Summary

As with modulation of reward anticipatory processes, it appears direct DA antagonism is not sufficient to produce changes to the BOLD signal elicited during reward outcome and further highlights the importance of other neurotransmitter systems in modifying this system.

As seen in Chapter 4, a primary finding from these data are the differential effects these drugs have on processing of reward outcome, regardless of the precise contrast employed. Once again, the relatively selective D2/D3 antagonist haloperidol did not produce any observable changes to reward processing during the outcome phase of the MID task. Instead, risperidone (and to a lesser extent, olanzapine) produced increases to several brain areas further highlighting the importance of other neurotransmitter systems in modifying this system.

A compelling feature of these results when viewed alongside those in chapter 4 is the change in direction of the action of these drugs across the stages of reward processing,
from supressing activity during reward anticipation, to increasing it during reward consumption. In the context of the aberrant salience hypothesis, one interpretation could be that risperidone and olanzapine, most likely through indirect DA modulation, are ‘boosting’ the signal in these critical reward areas in response to salient information, hence the increased amygdala and striatal activity during reward wins, which may be reflective of their therapeutic action. This is admittedly a highly speculative conclusion however, and further research with carefully designed paradigms is required to clearly delineate the multifaceted aspects of reward consummation and their drug effects. The relationship between these two phases of processing in the context of the drug effects will be discussed in the following chapter.
Chapter 6  General Discussion

6.1 Introduction

This thesis set out to investigate the acute effects of single doses of commonly prescribed antipsychotics on the structure and function of the brain using MRI, while considering potential influences of the drugs on the cerebrovasculature that may have an additional impact on these MRI metrics. It additionally offered increased precision over previous investigations of the acute effects of these drugs, in terms of sample size, a repeated measures design and placebo control. As such, this is the first investigation examining the acute effects of these three commonly prescribed drugs on a commonly used and well validated neuroimaging task, together with a concerted attempt at dealing with the potential confounds of measuring drug related BOLD signal changes.

It was found that the three drugs investigated produced significant dose and subtype dependant alterations to both the blood flow and vascular reactivity of the brain. A comprehensive analysis of MR structural data revealed that single doses do not alter structural metrics, even in areas of large increases in blood flow. However, single doses of olanzapine and risperidone do alter functional imaging in a differential manner during anticipation and consummation of reward, even after accounting for drug effects on cerebral blood flow and vascular reactivity.

These findings offer clarity to both the MR based imaging of antipsychotics and the potential mechanism of action of these drugs, and highlights important considerations for future imaging studies of drug effects.

6.1.1 Structural

The lack of evidence for structural changes produced by an acute dose of all three antipsychotic drugs tested indicates that, counter to recent investigations (Tost et al., 2010), a single, clinically relevant oral dose of risperidone, haloperidol or olanzapine is not sufficient to alter the standard metrics employed to assess structure of the brain in a typical sample size. This conclusion was replicated across all three drugs and also in a pilot rodent study using a larger dose of risperidone. Additionally, and importantly for structural
MR studies investigating drug related structural alterations over longer time scales, the significant blood flow changes that are induced by these drugs do not appear to be sufficient to alter the parameters that govern these structural metrics using MRI suggesting the literature indicating long term use of these medications alters MRI metrics of structure are not confounded by these acute physiological effects.

Again, it should be noted that the inability to detect a change using the methods employed here does not permit confirmation that there are not structural changes occurring on this time scale, and there may be microstructural changes occurring at a more finite resolution than currently offered by MRI, and detectable by the structural analyses used here. Nevertheless, the large MRI sensitive macro level changes in response to single doses previously reported would have considerable ramifications for the interpretation of studies looking at both longer term effects of these drugs on structure, and for those studies examining the functionally correlates of these drugs, with structure and function being of course closely linked (Lesh et al., 2015).

In the context of the précis of this thesis, the structural dynamics of the brain are assumed to be unchanged by single dose antipsychotics and the discussion moves to assessment of the functional changes observed.

6.1.2 Functional

In comparison to the structural analysis, the investigation into the acute functional effects revealed significant and varied drug specific changes across different stages of reward processing, and similarly, for the blood flow and cerebrovascular reactivity of the brain. Some of the overarching issues and questions raised by these findings will now be discussed in more detail.
6.2 Differential effects of antipsychotics across stages of reward processing

One novel finding from this study is the bi-directional effect olanzapine and risperidone had across reward anticipation and reward outcome during the monetary incentive delay task (MID), supressing activity during anticipation and increasing activity during outcome in drug specific localised cortical and subcortical areas.

Some studies have shown suppression of the anticipatory BOLD signal in healthy individuals after a single dose of an antipsychotic on the MID (Abler et al., 2007), and in anticipation of a taste reward (McCabe et al., 2011), but to the author’s knowledge none have examined the effect of a single dose in healthy humans on the outcome phase of this type of task. One study has investigated the effect of olanzapine after 7 days of treatment across both anticipatory and consummatory phases in healthy participants, and found increases in the striatum during both phases, and decreases in lateral orbital frontal cortex during consummation, during a taste reward paradigm (Mathews et al., 2012) – but the differences in dose duration and reward quality make direct comparison problematic.

Understanding the relevance of the opposing effects of risperidone and olanzapine to these two stages of reward processing requires an understanding of the relationship between anticipatory and consummatory phases of reward processing itself. How neural circuits separately control and track these processes aside from other aspects of reward related functions (such as Pavlovian learning or motivation) has been much debated (Berridge and Robinson, 2003, Rademacher et al., 2010b), and a solid appreciation of their level of independence or integration is paramount in understanding subsequent drug effects.

For instance, within the nucleus accumbens-ventral pallidum reward circuity, Smith et al. (2011) identified separate systems for predictive, incentive and consummative signals of the same reward in rats, with the same separable elements also selectively enhanced with amphetamine (increasing incentive salience) and/or μ-opioid stimulation (increasing both incentive salience and consummative signals), while predictive signals were unchanged. This suggests separable processes with potentially their own underlying neuroanatomical and neurochemical mechanisms, which pharmacological manipulation could conceivably selectively modulate as seen with the results in this thesis.
In terms of the MID in humans, fMRI studies showing the different patterns of activation between the anticipatory and consummatory phases also indicate separate processes (Dillon et al., 2008), although there are likely additional separable processes within each of these phases (such as between motivation and expected reward (Miller et al., 2014)). FMRI will be limited in parsing these processes due to its relatively low temporal resolution, so it is of note that EEG studies have also identified specific and separable ERPs related to anticipatory and consummatory processes during the MID (Novak and Foti, 2015, Angus et al., 2017).

Since anticipatory and consummatory phases are mediated - at least in part - by different processes, it allows for a situation by which experimental manipulation may differentially influence them. As such, Kumar et al. (2014) used acute stress to report the inverse relationship produced by risperidone and olanzapine reported in Chapters 4 & 5; stress increasing activation in the amygdala and caudate during the anticipatory phase of the MID, but decreasing activation in the caudate and putamen during the consummatory phase. Given the putative increase of dopamine release in response to stress (Vaessen et al., 2015, Suridjan et al., 2012) and the known modulation of dopamine activity by the compounds studied here, the results here support the findings of Kumar et al. (2014) and suggest dopamine may have a primary function in mediating these two processes. Interestingly, similar dissociations have been observed in clinical populations where dopamine transmission is thought to be dysfunctional. Using the MID, Yan et al. (2016) reported localised increases in prefrontal cortex activity during reward anticipation in patients with positive schizotypy compared with controls, but reductions in reward consummation activation in the amygdala and putamen activation in patients with negative schizotypy. Siblings of schizophrenia patients on the other hand show a similar pattern and direction of change in activation to the results presented here, with reduced activation in the ventral striatum, insula, and SMA during reward anticipation and increased activation in the ventral striatum and orbitofrontal cortex during reward consummation compared to controls (de Leeuw et al., 2015). Although the consummatory phase may be more associated with the feeling of pleasure associated with the hedonic experience of a rewarding stimulus (Berridge and Kringelbach, 2015) and is potentially primarily mediated by the opioid system (Castro and Berridge, 2014), DA does still appear to be involved in mediating the more qualitative response to the relative importance of reward.
How do the changes in each phase affected by the drugs studied here relate to each other? The changes seen in the consummatory phase may not be fully independent of the effects during the anticipatory phase. That is, they may be a downstream effect of the pharmacological alteration of the anticipatory phase, which may possible if they are indeed mediated by common neurotransmitters. Under normal circumstances, the dopamine related response to the anticipatory cue in the MID has been ‘shifted’ there from the receipt of the reward phase when the participant familiarised themselves with the task prior to testing and learnt that the cue predicted the potential reward. In circumstances where the drug has suppressed the normal dopamine mediated signal during that anticipatory phase, it may result in a relative increase in signal during outcome as the delayed dopamine related signal is then provided at the actual presentation of the reward. In agreement with this premise, de Leeuw et al. (2015) reported that the reduced response to reward anticipation in siblings of schizophrenia patients was correlated with the increased response during reward outcome suggesting the dysfunctional outcome was related to the dysfunctional cue processing. In this thesis, exploratory correlations were conducted between the ROI values during anticipation and reward receipt. For both the neutral contrast and win vs no win contrasts the correlations were found not to be significant. Although this is still a plausible mechanism of action, it does mean that these drugs may modify the processing of these two phases independently (albeit via modulation of common neurotransmitters).

The processes by which this may occur are unclear and difficult to parse with fMRI. This is partly due to a varied etymology of reward related terms, with wanting, liking, prediction, prediction error, anticipation, consumption, consumption, hedonism and motivation all frequently being used in the literature in differing contexts. Some of these terms are used interchangeably, some are overlapping constructs, some independent; with different paradigms eliciting them under different levels of motivation and with different qualities of reward (Rademacher et al., 2010b, Sescousse et al., 2013, Tibboel et al., 2015, Winton-Brown et al., 2014). In the context of this, response to the reward predicting cue is referred to as anticipation which is expected to involve elements of prediction of the outcome. A win during the outcome phase is referred to as reward receipt, but may also capture consumption. While the task may be useful in assessing motivation and hedonia as well, these are interpretations that depend on associations with these measures. That is, this task can be used to probe brain systems involved in motivation and (an)hedonia, but the
relevance to these processes will need to be demonstrated using tasks and questionnaires that have been separately validated. The results here do however significantly extend our knowledge in the context of the MID task at least, and shows for the first time that antipsychotics have a dose, subtype and ‘reward phase’ dependant effects in healthy humans.

6.3 Level of selectivity in mode of action across drugs
Another novel finding is the high level of specificity exhibited by these drugs across the functional outcome measures employed. Differential drug specific patterns of alterations were observed across CBF, CVR, and different levels of reward functioning, as illustrated in Figure 6.3-1.

<table>
<thead>
<tr>
<th></th>
<th>2mg Risperidone</th>
<th>7.5mg Olanzapine</th>
<th>3mg Haloperidol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral Blood Flow</td>
<td>Increase</td>
<td>Bilateral putamen, caudate, ACC</td>
<td>Striatum**</td>
</tr>
<tr>
<td>Decrease</td>
<td>Cerebellum</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Vascular reactivity</td>
<td>Increase</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Decrease</td>
<td>None</td>
<td>Widespread cortex and subcortical regions</td>
<td>None</td>
</tr>
<tr>
<td>Reward Anticipation</td>
<td>Increase</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Decrease</td>
<td>Caudate, putamen, ventral striatum, cingulate, thalamus, SMA, visual cortex</td>
<td>Caudate*</td>
<td>None</td>
</tr>
<tr>
<td>Reward feedback</td>
<td>Increase</td>
<td>Widespread cortex and subcortical regions</td>
<td>Bilateral putamen and visual cortex</td>
</tr>
<tr>
<td>Decrease</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

Figure 6.3-1 Divergent antipsychotic effect on imaging metrics. Results are from whole brain permutation testing unless otherwise marked. Reward results prior to correction for CVR/CBF. Reward feedback is WinVsNeutral *ROI only **ROI after accounting WB CBF
First, the marked lack of influence haloperidol appeared to assert on reward functioning is of interest, as it highlights D2 antagonism for these drugs to mediate these networks as not sufficient in isolation. This could arguably be expected in a broad sense, given its status as an older drug with a limited mechanism of action, which may be expected to ‘under perform’ against the newer atypical medications with their wider range of action. Although this may raise questions around the importance placed on D2 antagonism for the therapeutic impact of these compounds it must be stated that single dose impact on reward related BOLD signal in healthy volunteers is clearly a considerable distance from D2 occupancy studies and treatment response in patients. Furthermore, some evidence even suggests the actual therapeutic benefit of atypicals is limited, with a reduced EPS profile being the primary defining feature (Crossley et al., 2010). Nevertheless, these patient studies invariably include a large mix of medications within each of those two categories, and as highlighted within this thesis there is significant heterogeneity in their mechanism of action which may be lost in the noise of the group. If reward system modulation is indeed how these drugs achieve at least part of their therapeutic benefit, these findings highlight the critical importance of the additional neurotransmitter systems in providing clinical benefit over and above that of just alleviating EPS.

The marked differences in changes produced by the drugs within the atypical subtype were less expected. The widespread changes produced by risperidone – particularly the BOLD signal during reward feedback and the changes in CBF - were not replicated by olanzapine. Olanzapine produced considerable changes to CVR, which risperidone did not. This divergence of functional impact across both task-elicited signal and vascular activity is extremely striking, given these are two drugs that have resided for many years under the same ‘atypical’ banner as well as exhibiting overlapping molecular binding profiles. This does raise the question of how much of the difference in changes between compounds seen here and in previous studies is due to actual modulation of neuronal functioning, and how much is due to one drug influencing parameters underlying the BOLD signal more than another.
6.4 Influence of drug induced CBF and CVR changes

6.4.1 Drug specific effects

Including the CBF and CVR variables as covariates in the reward related analysis had the effect of revealing some results that were not present prior to correction (a main effect of olanzapine on reward outcome in extracted ROI values) and removing others (reduction in striatal activation during reward anticipation due to risperidone) both in the whole brain and ROI analysis. This highlights that direct and indirect effects of these drugs on the vasculature may account for some of the BOLD contrast effects observed during the task, and that these influences vary systematically depending on the drugs used. This finding has particularly strong implications for patient studies using fMRI to assess cognitive or drug function as they will typically include cohorts taking a mix of subtypes of antipsychotics. If these drugs systematically vary in how they potentially confound the BOLD signal, it may increase the noise of the sample and reduce sensitivity or even produce erroneous results. In a situation where two treatments are being compared (e.g. risperidone vs olanzapine) these potential problems would be compounded further.

The mechanism of the CBF changes observed were discussed in Chapter 3 in the context of the structural analysis, and although they did not appear to influence structural metrics they are very important in the context of BOLD. Two out of three of the drugs (haloperidol and risperidone) produced significant increases of CBF to striatal areas. Increases in baseline CBF have been shown to reduce the BOLD response (Vazquez et al., 2006), which would have modified the BOLD results on haloperidol and risperidone in a manner that would not have been reproduced on olanzapine. This creates clear issues when comparing the drugs on BOLD related tasks.

The drugs also differentially altered the BOLD response to the breath hold task, which is yet to be discussed in detail. The breath hold task increases blood levels of CO₂, a potent vasodilator, which produces elevated CBF and therefore an increase in BOLD signal (Hsu et al., 2010). The apparent reduction of this effect by olanzapine could be a result of drug mediated interference with the signalling process between neurons and/or glial cells and the vasculature, the concern being this may also occur with other vasoactive signallers that are released in response to changes in neuronal activity and which medicate neurovascular coupling.
The substances and mechanisms that mediate this signalling to the vasculature are highly complex and not yet fully characterised – however, in brief there is some agreement that synaptic activity results in an increase of intracellular calcium (CA++) in both neurons and astrocytes, which results in the release of vasoactive substances such as adenosine, nitric oxide (NO), potassium (K+) and the metabolites of arachidonic acid such as prostaglandins and epoxyeicosatrienoic acids (EETs). These mediate the neuron-astrocyte-endothelial signalling pathway, resulting in vasoconstriction or dilation (Jakovcevic and Harder, 2007, Attwell et al., 2010, Carmignoto and Gómez-Gonzalo, 2010, Howarth et al., 2017b). In terms of response to increased levels of CO2, these processes are thought to be primarily mediated by increased K+ and NO resulting in decreased CA++ concentration in the endothelial cells of vessels and subsequent relaxation of the vascular smooth muscles (Kuznetsova and Kulikov, 2014, Battisti-Charbonney et al., 2011), although again the specifics of these processes remain a source of debate (Glodzik et al., 2013). It should be noted that the responses of vasoactive substances such as CO2 or NO vary in different regions of the brain, complicating the picture further (Sato et al., 2012, Duchemin et al., 2012). The presence of a drug which modulates different neurotransmitter systems could have diverse effects on this cascade, thereby influencing neurovascular coupling and altering BOLD in the absence of change of neuronal signal. For instance, astrocytic glutamate reuptake has been shown to increase the release of arachidonic acid (Stella et al., 1994), while dopamine has well characterised influences both on astrocyte control of CBF and directly on the microvasculature itself (Choi et al., 2006, Jenkins, 2012). Olanzapine had profound effects on CVR here over that produced by risperidone and haloperidol, which suggests some unique feature of its pharmacology may be responsible. Only olanzapine exhibits meaningful affinity for the histaminergic (H1 receptor) and muscarinic (M1 receptor) systems in addition to its affinity for several of the other receptors shared with the other drugs examined here. H1 receptors are found directly on cerebral blood vessels (Stanimirovic et al., 1994) with some evidence suggesting histamine neurons may interact with the vasculature directly (Takagi et al., 1986). Histamine itself has been shown to produce specific and localised increases (Suzuki et al., 1999) and reductions (Yang et al., 2010) in the CBF of rats, mostly likely via interaction with the H1 receptor. Similarly, stimulation of cholinergic neurons projecting from basal forebrain areas produces increases in cortical blood flow (Biesold et al., 1989, Hotta et al., 2011), with stimulation of M1 receptors found on smooth muscle cells producing vessel dilation (Hamel, 2004). Interestingly, this response is thought to be dependent on NO production (Sato et al.,
2001), a major component of neurovascular signalling described above, which could be telling in explaining the changes produced by olanzapine in the vascular response to CO2, also thought to be reliant in part on preserved NO signalling.

However, attempting to isolate the cause of the effects seen on olanzapine using fMRI alone is highly limited, especially given its very broad receptor profile, with the widespread changes seen also making it difficult to narrow down potential driving causes based on localised alterations in signal. This again could be due to a number of factors, most likely centred around the complex interplay in the neurotransmitters it modulates. These could cause potential direct and downstream effects, while direct drug interactions with vasoactive signallers may also be occurring. Other considerations include, but are not limited to, the influence of olanzapine on prostaglandin E2 (Bakare et al., 2009, Cheon et al., 2011), a vasoactive metabolite which causes vasodilation (Howarth et al., 2017a); and olanzapine’s inhibitory effects on noradrenaline transporter (Yoshimura et al., 2005), with noradrenaline linked to cerebral vasoconstriction (Busija and Leffler, 1987). In sum, it appears that theoretically there are a broad range of potential influences, but in practice the relative importance of these is unknown. In general, olanzapine’s pharmacological profile does make it problematic identifying which feature would produce it changes on CVR seen here, and a full examination is outside the scope of this discussion. It does however highlight the importance of accounting for these effects when interpreting drug effects on cognitive neuronal function using BOLD.

A slight paradox worthy of mention here is the fact olanzapine had a considerable influence on CVR but little influence on resting CBF, the opposite pattern to risperidone and haloperidol. If direct action of olanzapine on histamine and/or muscarinic receptors are responsible for the changes seen to CVR, why are these not producing changes to resting brain perfusion as well? CBF assesses changes in the baseline blood flow while CVR changes are maybe more reflective of the drug effect on the signalling mechanisms that are in play in response to changes in vasoactive substances, rather than direct effects on the vasculature (although these would also presumably contribute). It seems more likely therefore that haloperidol and risperidone were acting more directly on the vasculature (Choi et al., 2006) while olanzapine was interacting with the signalling systems in response to a change in blood CO2. These are separate processes but both contribute to the BOLD signal. This highlights the complex interaction pharmacological agents exert on the
parameters of the BOLD signal, and that both resting perfusion and cerebrovascular reactivity need to be assessed to fully appreciate drug influences on task activated BOLD. Just one of the potential further explanations for the limited changes in CBF produced by olanzapine is that its affinity for several receptor subtypes may produce a counteractive mechanism which ‘cancels out’ widespread changes (see Figure 6.4-1) - although localised changes may still be expected in this event. Again, the actual interaction for these receptors is likely more complex than a simple CBF up/down relationship, as single dose quetiapine and pramipexole (a D2 antagonist and agonist respectively) both produced increases in striatal CBF in healthy humans (in addition to producing opposite effects in other brain areas; (Michels et al., 2016)).

One final note on the assessment of CVR is that CO2 is one of several vasoactive substances, and different vasoactive cascades may occur in response to neural activity which subsequently may react in a different fashion to the presence of a drug. Other methods have been used, such as injection of an exogenous chemical such as acetazolamide (Siero et al., 2015), although the manipulation of CO2 levels has obvious practical advantages (Fierstra et al., 2013).
6.4.2 Accounting for CBF and CVR alterations

An attempt was made to account for the considerations outlined above in the BOLD analysis in this thesis by including the assessments of CBF and CVR as covariates. This method is relatively conservative and basic, assuming a linear relationship between the parameters which may not be the case (Lin et al., 2010). It is recognised the actual relationship is likely to be more complicated with a more sophisticated approach required, given the assumed close relationship between the parameters making up the BOLD signal (although an exploratory analysis of the ROI metrics of CVR and CBF here revealed them not to be correlated, which adds some utility to their use of separate covariates in this analysis).

Indeed, such sophisticated approaches are already in use. Calibrated BOLD methods (Blockley et al., 2013, Davis et al., 1998, Hoge et al., 1999) use acquisition of BOLD, ASL and a calibration task (normally administering a hypercapnia or hyperoxia inducing gas) to assess the change in cerebral metabolic rate of oxygen (CMRO2), a more direct measure of underlying tissue metabolism. These methods have been continually developed, using a combination of both hypercapnia and hyperoxia to estimate oxygen extraction fraction (OEF) and provide a quantitative measure of absolute CMRO2 (Merola et al., 2016, Wise et al., 2013), and have been applied to observe drug effects on the brain with increased clarity. For instance Merola et al. (2017) used dual calibrated fMRI in a placebo controlled pre-post treatment design to explore the effects of 250mg caffeine, which has previously been shown to both increase neuronal activity (Fredholm et al., 1999) while concurrently decreasing CBF (via its antagonist action at different adenosine receptors (Pelligrino et al., 2010)). They reported an increase in OEF alongside larger decreases in CBF, resulting in a net decrease in CMRO2. This highlights an apparent paradox in a drug which is known to increase neuronal firing actually reducing O2 metabolism, which the authors propose may be due to caffeine’s disinhibitory effects changing the balance between active excitatory and inhibitory neurons - this then produces a net decrease in excitatory activity and subsequent reduction in energy demand. Once more this highlights the extremely complex interplay of pharmacological agents on the physiological parameters of the BOLD signal and the nuanced interpretations that are required.

This is still a rapidly developing field with the assumptions and fixed parameters within the mathematical models used being regularly updated, such as challenges to the assumption
that hypercapnia does not induce CMRO2 itself being addressed (Driver et al., 2017), the precision of inducing hypoxia (Lajoie et al., 2017) or issues around the accurate estimation of OEF contribution to the signal (Blockley et al., 2015). Additionally, there are some extra practical issues in collecting this type of data in terms of the extra scanning time and the more precise inducement of hypercapnia and/or hyperoxia, which may be problematic in patient groups. Nevertheless, given the importance of the divergent effects of antipsychotics on the underlying vascular dynamics revealed here, methods such as calibrated BOLD likely offer the most precise assessment of drug impact on fMRI metrics.

6.5 Interpretation of BOLD changes

A strong theme running through this thesis is that the interpretation of BOLD is not straightforward, and is muddied further when accounting for pharmacological influences. Care must be taken in the conclusions drawn from the action of these drugs, even when accounting for the vascular issues described above, and a change in BOLD doesn’t not necessarily mean a similar change in direction in the integrative activity of neurons, nor does it guarantee the site of action.

The main putative change affected by antipsychotics is via modulation of neurotransmitter action, with DA being of a major focus in reward processing, psychosis and its treatment. Therefore, BOLD imaging of changes in DA mediated transmission would hope to give an indication of the site and extent of any drug induced change (Schultz, 2016). Although there is evidence of an increase DA release being spatially and temporally correlated to an increase in BOLD signal in the context of reward processing (Schott et al., 2008b, Knutson and Gibbs, 2007), there have also been reports in primates of similar DA mediated related rewarding stimuli resulting in decreases in BOLD in visual areas (Arsenault et al., 2013). Additionally, using direct injections of L-DOPA into V1 of macaques, Zaldívar et al. (2014) reported a reduction in visually induced BOLD signal while CBF and neural activity in the same region increased. In humans, Lohrenz et al. (2016) compared the RPE related BOLD signal produced by a stock market investment paradigm against the direct DA signal measured by fast-scan cyclic voltammetry in a separate group of patients with Parkinson’s disease performing the same task and found a dissociation between BOLD and DA release in the striatum (although these comparisons were made between clinical and healthy cohorts). Furthermore, D1 and D2 receptors in the striatum are known to have opposing
excitatory and inhibitory influences respectively and DA modulation can therefore produce differing neuronal and BOLD related effects upstream depending on the context (Planert et al., 2013, Takahashi et al., 2010, Surmeier et al., 2007), while signals and neuromodulators from other cortical sites can alter the BOLD signal in a different direction to DA release (Morita et al., 2012, Ferenczi et al., 2016).

Drug modulation of these systems can complicate the picture further, by altering the tonic or phasic firing rate of DA neurons, changing the extracellular DA tone and altering the context in which signals are processed (Grace, 1991, Grace et al., 1997, Knutson et al., 2004). The action of autoreceptor blockade appears particularly relevant here, which can both increase and decrease midbrain firing of DA release depending on the basal level of DA, influence of feedback mechanisms or length of stimulation (Jenkins, 2012), while it requires keeping in mind that blockade of one neurotransmitter system may lead to modulation of one or more other systems, and a subsequent change in BOLD signal. In the context of reward processing, these principles have relevance for the divergent direction in changes seen between the anticipatory and consummatory phase. As they do seem to be separate systems, this allows for the situation whereby the same changes in basal levels of dopamine result in the divergent action of different neural systems when recruited. It should be said however that much of this is conjecture at this point – however, the clear message is that none of these neurotransmitters work in isolation, and it should not necessarily be surprising that the presence of drugs that modulate so many different systems would have such divergent effects.

This is not to say these results are uninformative! The dissociations discussed above are most likely due in part to the influence of other neurotransmitter systems, resulting in competing inputs which have complex effects on synaptic activity and energy use (Hall et al., 2016). It is impossible to quantitively ascertain the relative contribution of the modulation of each receptor type using fMRI, with one work around being to study highly selective drugs to pick apart relative contributions. However, as informative as this is, many of these more selective drugs are rarely used in clinical practice for the very reason that they are not clinically efficacious, and the interaction of the different systems may be a critical feature of antipsychotics (indeed the most selective one used here, haloperidol, produced the fewest observable changes).
For instance, one finding that can be asserted here is that DA2 antagonism alone is not sufficient to produce a change to reward related functioning, but coupled with other neuromodulator manipulation (most likely 5HT2A) it does, which would have not been ascertained if each system was studied in isolation. Nevertheless, the lack of findings on haloperidol may be a dose related effect, which under higher doses may reveal itself. The estimated occupancy for this dose is in the lower end of the clinical range and the changes in blood flow clearly demonstrate the central activity of the compound. Continuing to investigate clinically relevant doses and compounds in combination with other measuring techniques such as PET, MRS, EEG and electrophysiology will most likely produce the most useful insights.

6.6 Implications for treatment

There is a paucity of placebo-controlled studies in patient populations. Here, a full cross over design was used show that single doses of antipsychotics do indeed influence reward processing. However, without similar studies in patients, extrapolating to the clinical domain needs to be done with caution as patient and control groups will most likely be starting from very different baselines (both in terms of aberrant disease processes and the fact longer term treatment changes the dynamics of the neurotransmitter systems (Goto and Grace, 2007)) which would likely influence a measure such as BOLD. However, these findings do provide some support for the aberrant salience hypothesis, in that it postulates antipsychotics confer their therapeutic qualities during the reward prediction stage by suppressing the putative dysfunctional DA signal to salient stimuli in schizophrenia - although the results here provide evidence for the first time that these effects will differ by drug.

Much less background work has been conducted on the possible mechanism of these drugs during the consummative phase of reward processing, and the interpretation here is more challenging. A simple reading could be that the boosting of the signal is highlighting salient outcomes, representing a more complete corrective action over both stages of reward processing in that it both suppresses an overactive salience system and highlights the salient information when it is presented – although it is impossible to infer from these results if the changes observed to reward consummation are therapeutic or are detrimental to functioning.
Disruption to processing receipt of reward has been observed in schizophrenia (Waltz et al., 2009, Gradin et al., 2011), and has been linked to anhedonia (the inability to experience pleasure from previously pleasurable activities, one of the negative symptoms of the disorder) although dysfunction of feedback processes has also been related to aberrant associations associated with delusional symptomology (Schlagenhauf et al., 2009). Dopamine has again been proposed to play an important part in the symptoms of anhedonia (Wise, 2008) although antipsychotics have been traditionally seen as relatively ineffective at treating this symptom dimension, with some research indicating D2 antagonists to exacerbate or even introduce negative symptomology (Danna and Elmer, 2010, Mizrahi et al., 2007, Wise, 2008).

fMRI studies have reported reductions in BOLD activation during the consummation of pleasurable stimuli in schizophrenia patients, with a recent meta-analysis of 19 fMRI and PET studies (Yan et al., 2015) summarising reductions in areas including rostral MPFC, amygdala, and putamen (although these studies include a range of paradigms and reward types, with a sample including mixed symptomology and medication status which were unable to be controlled for). Nevertheless, the areas exhibiting most suppressed activation were some of the same areas that were seen to be selectively increased during the outcome phase by risperidone and olanzapine here. Medication status may be particularly relevant here, as several individual studies with medicated patients specifically using the MID have not shown differences to controls during the consummatory phase (Kirsch et al., 2007, Simon et al., 2010, Abler et al., 2008) which has been interpreted as the normalising effect of antipsychotic treatment (de Leeuw et al., 2015).

How this relates to the behavioural domain is less clear, as research with self-report scales suggest the consummatory phase of processing is actually preserved in schizophrenia, while anticipatory pleasure is most affected (Chan et al., 2010, Gard et al., 2007, Mote et al., 2014). Some imaging studies have indicated that anticipatory striatal dysfunction is a better predictive of anhedonia (Arrondo et al., 2015, Subramaniam et al., 2015) suggesting the relationship between the functional changes seen during the consummatory phases may be downstream of anticipatory dysfunction. A recent study by Segarra et al. (2016) provides support for this view, finding the reduced fMRI signal in the mPFC to an unexpected reward in schizophrenic patients was associated with self-report measures of
motivation and severity of anhedonia but not pleasure, suggesting the motivational drive (or lack of) applied by the anticipatory phase is related to anhedonic symptomology. It may be that self-rated anhedonia is more linked to disruption of motivational salience processing - associated with dysfunction during the anticipatory phase - resulting in apathy and loss of motivational drive, while disruption to consummation of reward may be more related to depressive symptoms (Simon et al., 2010, Kring and Barch, 2014, Lee et al., 2015). Again, the parsing of these closely linked processes is difficult, more so in a disorder with a highly heterogenous symptomology and often with different drug interactions at work.

Nevertheless, it does seem likely that the results produced by risperidone and olanzapine here are more related to the motivational incentive facets of reward medicated by dopamine, rather than hedonic pleasure, giving them a more plausible context for their mechanism of action. This is actually a limitation of the current study, in that preference or ‘likability’ of the stimuli after each session was not assessed. As the deficit in patients appears primarily motivational in terms of behavioural and imaging metrics, and the drugs appear to be modulating these same systems, it would suggest drugs are acting on motivational aspect of the reward. Whether they are corrective in nature, by supressing a potentially overactive signal during anticipation and boosting the presence of salient feedback, or detrimental by decreasing the reinforcing value of normally positive rewards and reducing motivation cannot be ascertained and requires additional single dose studies using more nuanced paradigms.

### 6.7 Methodological Considerations

The striatum was a major focus in this thesis, given its central role in the reward network and as a major site for the action of antipsychotics. Changes in activation in dorsal areas of the striatum was a common feature in the results here, although in the ROI analysis this was only divided into two relatively large structures (the caudate and the putamen) as is common in neuroimaging investigations when examining this area. Many of the cognitive processes that are engaged by the MID and that have been under examination here are believed to be mediated by striatal sub divisions (Liljeholm and O’Doherty, 2012). Connectivity analysis may offer further insights into this division of function (for example the caudate itself exhibits different patterns of functional connectivity between the head
and the body (Robinson et al., 2012)) and how antipsychotic medication may mediate the connections with distal brain regions. Sarpal et al. (2015) illustrated that parcellation of the striatum into six bilateral regions in a cohort of patients commencing antipsychotic treatment revealed a correlation between treatment response and increase in resting-state connectivity with specific striatal seeds and frontal and limbic areas (with an associated decrease in connectivity with more posterior brain regions). This would suggest splitting the striatum in three regions and examining them in isolation probably may have reduced sensitivity to more subtle changes. However, one issue with this approach is that correcting for vascular effects of the drugs becomes more methodologically problematic in connectivity analysis, and a robust method to account for these issues would be essential given some of the findings here. Additionally, and as with all resting-state connectivity studies, this type of analysis does not say anything mechanistically about the impact of the drugs on reward or motivational processing as it is not task based. Approaches such as generalized psychophysiological interactions (gPPI; McLaren et al. (2012)) may be an interesting avenue of further study in assessing this, but again presents challenges in accounting for indirect vascular effects of the drugs.

The MID was selected for the functional analysis of this study due to it ubiquitous nature in the literature and good repeat reliability over time (Wu et al., 2014). The contrasts chosen reflected similar contrasts used in previous studies looking at processing in schizophrenia or studies examining drug effects, and were therefore appropriate for comparison. No changes were found in the level of reward analysis either during anticipation or receipt of reward, on placebo or on drug. This was unexpected, and may be reflective of the fact that although participants were paid for taking part in the study, their performance on the task did not impact the amount they were paid (which they were aware of). For this reason, the reward component within both the anticipation and outcome phase was collapsed across both high and low wins. Although different processes may be at play, the increased power from including both types of trial in the analysis was felt to be a worthwhile trade off.

It should be noted that the MID was primarily developed to assess reward anticipation. This is reflected in the literature with a large number of studies employing it to specifically examine and report on anticipatory processes in both healthy humans and clinical populations (for instance see Balodis and Potenza (2015), Plichta and Scheres (2014) for recent meta-analyses/reviews of MID anticipatory function in addicted and ADHD
populations respectively). Far fewer studies report on the outcome phase, and although it has it benefits (such as removal of elements of learning from the task), there are some potential issues. For instance, because this phase is variably split into win and no win outcomes for each participant depending on their performance, it may suffer from an associated loss of power in terms of the number of trials that are available for analysis.

A more general note here regarding the outcome phase of the MID is that strong dopamine related activation would not generally be expected as the parameters of the task are pre-learnt and reward prediction error (RPE; i.e. the presentation of an unexpected reward or omission of an expected reward) would not occur in its simplest sense. Both winning or failing to win a reward could arguably not be sufficient to produce an RPE in this context as the participant is aware that both are likely outcomes. Computational models have attempted to encapsulate this signal, by calculating the strength of the signal based on the difference between the predicted outcome and the actual outcome (Anselme, 2015, Morita et al., 2012). However, it may be other paradigms may be more suited to eliciting and manipulating RPEs while the MID used here is more adept at examining processes at play during consumption of reward within comparatively stable parameters, allowing for the observation of anticipation (or ‘wanting’) of reward and the subsequent receipt (or ‘liking’) of reward, separate from reward related learning (Smith et al., 2011). More recently, modified versions of the MID have been developed which relate the amount won directly to the individual response time (Kirschner et al., 2016) rather than a binary win or no win outcome, allowing exploration of the responsiveness or adaptive coding (Tobler et al., 2005) of reward regions to anticipated but unpredictable reward, which could easily be applied to pharmacological investigations.

In relating these findings back to the literature, an attempt has been made to limit comparisons to studies using as similar tasks as possible to ensure broadly the same brain networks are being compared - a recent meta-analysis revealed both overlapping and distinct brain areas for activation produced by different task types (Liu et al., 2011) indicating different tasks may recruit different brain systems, complicating comparisons between them. However, single dose, placebo-controlled studies are relatively rare and therefore studies using other paradigms and reward types were included in the discussion, particularly regarding the reward feedback results. For instance, several studies examining response to taste reward were considered in light of the results in Chapter 5 (as
considerable research has been conducted into antipsychotic propensity to cause significant weight gain). Caution should be applied in these interpretations, as although primary and secondary reward do appear to recruit mutual brain regions and networks, they also rely on areas specific to the nature of the reward (Sescousse et al., 2013). As discussed, picking apart the precise processes involved during the consummatory phase of the MID is generally problematic and alternative paradigms may be of use looking forward. The influence of antipsychotics on learning and reward prediction error would supplement these findings well, and well-designed tasks such as the Salience Attribution Task have been useful in exploring elements of salience in more detail by splitting task stimuli into relevant and irrelevant dimensions to parse adaptive and aberrant salience (Roiser et al., 2009, Roiser et al., 2010). Additionally, computational modelling approaches have been applied to pick apart neural signals during reward processing in healthy humans and patient cohorts and could prove useful in further determining antipsychotic effects (Deserno et al., 2016).

There are limited comparisons that can be made from single dose studies and clinical cohorts. Nevertheless, even without linking them back to patient populations these findings are highly informative from a psychopharmacology viewpoint. It is telling that these three drugs, that clinically have little to separate them, needed to be tested on their own in this controlled fashion to illustrate in fact how different their physiological effects can be. The benefits of testing in untreated healthy individuals extends beyond the removal of the confound of potential disease processes, as current and even prior drug use can produce considerable confounds to measuring drug action. This has been shown to be the case in the development of novel antipsychotics (Gill et al., 2014), while antipsychotic treatment has been linked to the production of dopamine supersensitivity in schizophrenia patients (Seeman, 2011). Using single dose studies allows for a high degree of experimental control that cannot be achieved in patient studies, and the ability to add placebo control is a major strength in allowing a clearer appreciation of the neuropsychopharmacology of these drugs.
6.8 Summary

This thesis explored the effect of single dose antipsychotics on the healthy human brain. It can be summarised in three main points:

1) Structural MR metrics are not acutely influenced by single dose antipsychotics, offering validation to a large body of longer term structural research. This provides clarity in this area for future research as well as a methodology to test the acute effects of other pharmacological compounds on structure.

2) Antipsychotic compounds (including two within the same subtype class) produce markedly disparate effects on elements of cerebrovascular coupling that are known to indirectly influence BOLD signal, the primary method for studying brain function in vivo in humans. Although these confounds had already been well characterised, the extent and disparity in effect they have on the independent parameters underpinning BOLD has large implications for how pharmacological MRI is conducted.

3) These same compounds have similarly disparate effects on reward function. These otherwise clinically similar drugs produce divergent effects on different phases on reward function activation, highlighting the importance of the precise neurotransmitter profile of these drugs over and above their broad class assignment.

The thesis brings increased knowledge, clarity and precision to our understanding of the effect of these drugs on imaging parameters in both the functional and structural domain, and their effect across different phases of reward processing. Further single dose studies accounting for vascular effects and exploring other more precise facets of reward processing and cognitive functions with more sophisticated modelling techniques could provide understanding of the systems-level operations of these drugs, and afford knowledge required develop effective new compounds and improve clinical outcomes.


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