Use of methamphetamine phMRI in humans to investigate the role of dopamine in reward and other functions related to mental illness

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Abstract

This is a thesis submitted by Patrick Horgan on 31/03/2010 for the degree of Master of Philosophy in The University of Manchester for the study “Use of methamphetamine phMRI in humans to investigate the role of dopamine in reward and other functions related to mental illness”.

Introduction

Dopamine is a neurochemical that has an important role in brain processes such as memory, learning and movement. In this study, manipulation of dopamine receptors by methamphetamine and amisulpride was completed in healthy volunteers undergoing functional magnetic resonance imaging (fMRI). Amisulpride is a selective dopamine receptor antagonist whereas methamphetamine increases neuronal dopamine release. The main aim of the study was to examine whether effects of methamphetamine on blood oxygen level dependent (BOLD) signals in the brain could be attenuated by amisulpride.

Methods

The effects of the drugs were examined using three groups. One group was given oral amisulpride and intravenous (i.v.) methamphetamine (AM). Another group was given oral placebo and i.v. methamphetamine (PM). A third group was given oral placebo and i.v. placebo (PP). The effects of methamphetamine were measured by comparing the PM group with the PP group. The effects of amisulpride on methamphetamine responses were shown by the AM group. A challenge pharmacological-fMRI (phMRI) imaging technique was used to examine for the direct pharmacological effects of the drugs. Another imaging technique (modulation phMRI) was used to examine for drug effects on participants performing an N-back task, a finger tapping task and a reward learning task. Performance measures and reaction times for the tasks were examined for effects due to drug group where possible.

Results

An effect on performance due to methamphetamine for some of the tasks was detected but the effect of amisulpride pretreatment was variable. There were different effects of methamphetamine for the reaction time data depending on the task. Amisulpride pretreatment did not clearly alter these effects. There were some demonstrable effects of methamphetamine on BOLD signal changes for the tasks used. Increased BOLD signal was detected in expected areas related to activating effects of methamphetamine for the challenge phMRI, the finger tapping task and (to a lesser degree) for the N-back task. There was some attenuation of the BOLD signal resulting from methamphetamine with amisulpride pretreatment for the challenge phMRI, the finger tapping task and the N-back task. The effects of drug treatment on the reward learning task were weaker; however, this task showed activations somewhat consistent with the role of dopamine in reward prediction error.

Conclusions

There were some demonstrable effects on BOLD signal changes related to methamphetamine and attenuation by amisulpride for the challenge phMRI, N-back task and finger tapping task. There was some evidence of another type of dopamine related effect for the reward learning task.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Preface

The author went to University College Dublin gaining a MB, BCh in Medicine in 1997. The author completed basic psychiatry training in Manchester gaining membership of the Royal College of Psychiatrists in 2003. A taught MSc was completed by the author in University College Dublin in Cognitive Science in 2005.

Abbreviations

5-HT	Serotonin
AC	Anterior cingulate
AM	Amisulpride – methamphetamine
ANOVA	Analysis of variance
BA	Brodmann area
BOLD	Blood oxygen level dependent
BP	Binding Potential
BPRS	Brief psychiatric rating scale
Ca^{2+}	Calcium
cAMP	Cyclic-adenosine-3',5'-monophosphate
Cl^-	Chloride
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CS	Conditioned stimulus
CSF	Cerebrospinal fluid
DA	Dopamine
DARPP-32	Dopamine and cyclic AMP-regulated phosphoprotein, 32
DAT	Dopamine transporter
DLPFC	Dorsolateral prefrontal cortex
df	Degrees of freedom
ECB	Endogenous cannabinoid
FDR	False discovery rate
FC	Frontal cortex
fMRI	Functional magnetic resonance imaging
FWE	Family wise error
GABA	γ-aminobutyric acid
Girk	G protein-regulated inwardly rectifying potassium
GPe	Globus pallidus externa
GPi	Globus pallidus interna
HPC	Hippocampus
i.v.	Intravenous
L-dopa	Levodopa
LFP	local field potentials
Log	Logarithm (to base e)
LTD	Long term depression
LTP	Long term potentiation
Min	Minimum
MINI	Mini International Neuropsychiatric Interview

MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MSN	Medium spiny neurons
Na^+	Sodium
NAc	Nucleus accumbens
NO	Nitric oxide
OC	Occipital cortex
OF	Orbitofrontal cortex
PC	Parietal cortex
PD	Parkinson's disease
PET	Positron emission tomography
PFC	Prefrontal cortex
phMRI	Pharmacological functional magnetic resonance imaging
PKA	Protein kinase A
PM	Placebo – methamphetamine
POMS	Profile of mood states
PP	Placebo - placebo
PP1	Protein phosphatase 1
Sd	Standard deviation
SMA	Supplementary motor area
SPM	Statistical Parametric Mapping
STN	Subthalamic nucleus
SNr	Substantia nigra pars reticulata
TC	Temporal cortex
unc	Uncorrected
US	Unconditioned stimulus
VMAT	Vesicular monoamine transporter
VS	Ventral striatum
VP	Ventral pallidum
VTA	Ventral tegmental area
WHO	World Health Organisation

1. Introduction

1.1. General introduction

Dopamine is a neurochemical that affects a wide range of cognitive processes in the brain. These include reward related processes, learning processes, working memory, and movement (Montague, Dayan et al. 1996; Arnsten 1997; Crossman 2000; Berridge 2007). The importance of its role is readily apparent when diseases related to dopamine dysfunction are considered. Schizophrenia and addiction disorders are amongst the mental disorders where dopamine dysfunction is thought to form an important part of the pathophysiology (Nutt and Lingford-Hughes 2008; van Os and Kapur 2009). One of the motivations for this study was to get a better understanding of the function of dopamine in healthy people in order to better understand the nature of dopamine dysfunction in people with mental illnesses. Another motivation for this study was to examine the use of neuroimaging techniques in healthy people so that these techniques could be used in further research in people with mental illnesses.

In this study, two drugs were used to investigate the role of dopamine in humans. Amisulpride acts mainly as an antagonist of certain dopamine receptors (Sanofi-Aventis Accessed Oct-2009) whereas methamphetamine causes neuronal dopamine release (Sulzer, Sonders et al. 2005).These drugs were used in healthy participants who did various cognitive tasks whilst brain imaging was completed. The terminology is not standardized for this kind of study but pharmacological functional magnetic resonance imaging (phMRI) has been used to refer to the experimental paradigm of completing fMRI together whilst giving a drug challenge (Anderson, McKie et al. 2008). This has been subcategorised into modulation phMRI where a neuropsychological test is undertaken at the same time as a drug challenge and challenge phMRI where the effects on blood oxygen level dependent (BOLD) signal are caused by the acute effects of a drug. This study can be loosely divided into two parts: in the first part, the challenge phMRI technique was used and in the second part, three modulation phMRI tasks were completed.

1.2. *Description of drugs used in the study*

1.2.1. Methamphetamine

Methamphetamine is a drug that is used illicitly worldwide (Cruickshank and Dyer 2009) but is also prescribed for certain medical conditions. In the United States of America, it can be prescribed for attention deficit disorder with hyperactivity as part of a total treatment plan for children over 6 years old. It can also be prescribed for exogenous obesity as a short-term adjunct in a regimen of weight reduction (Lundbeck Inc Accessed Feb-2010). There has been a recent review of studies examining the pharmacokinetics of methamphetamine (Cruickshank and Dyer 2009). Data are reproduced from this review in Table 1.2-1 which were derived from studies of methamphetamine dependent subjects (Newton, Roache et al. 2005; Newton, De la Garza et al. 2005a; Mahoney, Kalechstein et al. 2008).

Dose	Bioavailability	C_{max} (µg/l)	T_{max} (minutes)	$T_{1/2}$ (hour)	Time to peak effect (minutes)
30 mgs	100%	108 ± 22 (64–164)	6 ± 11	9.1 ± 0.8 (8–16)	<15

Table 1.2-1
In this table the key pharmacokinetic properties of intravenous methamphetamine are presented. Data are presented as mean ± standard deviation and the range when this was available. C_{max} is peak plasma methamphetamine concentration. T_{max} refers to the time to reach peak plasma concentration of methamphetamine. $T_{1/2}$ refers to methamphetamine plasma half life.

Methamphetamine operates as an indirect agonist for dopamine, noradrenaline and serotonin receptors (Cruickshank and Dyer 2009). A description of its pharmacological properties which follows is based on recent reviews (Sulzer, Sonders et al. 2005; Fleckenstein, Volz et al. 2007). In common with amphetamine it has the following structural properties (shown in Figure 1.3-1):

(1) an unsubstituted phenyl ring

(2) a two-carbon side chain between the phenyl ring and nitrogen

(3) an α-methyl group.

Although methamphetamine differs from amphetamine in certain ways, the similarity of their biological effects means that they can be considered in the same family of compounds (Sulzer, Sonders et al. 2005). This is important as much of the work on the mechanisms of neurotransmitter release has been done with amphetamine whereas studies examining neurodegeneration has been completed mainly with methamphetamine.

Amphetamine does not facilitate the exocytosis of secretory vesicles and it does not have strong affinity for neuronal receptors. Instead its pharmacological mechanisms comprise the transfer of catecholamines from synaptic vesicles to the cytosol and reverse transfer of catecholamine across the plasma membrane. Two hypotheses have been proposed for the transfer of catecholamine into the cytosol from the synaptic vesicles. The first hypothesis is that amphetamine is able to diffuse into the synaptic vesicle where its action as a weak base alters the pH within the vesicle. The accumulation of intravesicular catecholamines occurs via a carrier mediated mechanism dependent upon an electrochemical protein gradient (Johnson 1988; Sulzer, Sonders et al. 2005). The reduction of the electrochemical gradient interferes with the uptake of catecholamine so increasing its concentration in the cytosol. The other proposed mechanism is that amphetamine acts in competition to the catecholamines (principally dopamine since noradrenaline is mainly synthesized within vesicles) for the vesicular monoamine transporter (VMAT), thus reducing the uptake of dopamine to the vesicle.

The other mechanism of catecholamine release is by reverse transport at the site of the plasma membrane transporter. This transporter differs from the VMAT as it requires co-transport of co-substrate ions whereas VMAT uses counter transport with H^+ ions. In the case of the dopamine transporter (DAT) there is co-transport of one Cl^- and 2 Na^+ ions. The main proposed mechanism for reverse transport is by a facilitated exchange diffusion model. According to this idea, amphetamine acts as a substrate instead of dopamine at the site of the DAT on the external site of the membrane. Since dopamine is at a higher concentration in the cytosol, this means that dopamine preferentially binds on the internal aspect of the transporter resulting in the reverse transport of dopamine out of the cell. In recent years, it has been demonstrated that amphetamine may also cause the release of dopamine in a burst manner, explicable by a channel like mode of DAT, in addition to the exchange model (Kahlig, Binda et al. 2005). This discussion has shown how there are several aspects to dopamine release by amphetamine.

1.2.1.1. Effects of amphetamine on animals

Amphetamine and methamphetamine have been used in numerous imaging studies in animal and humans often as a means of assessing dopamine function. An early fMRI animal study examined the feasibility of using BOLD signals changes to detect the pharmacological effects of amphetamine (Chen, Galpern et al. 1997). This study showed that the main effects of amphetamine occurred in expected dopaminergic areas such as frontal, striatal and cingulate regions. Parietal regions were also activated but the authors suggested that this may have been due to effects in the adjacent striatum. Destruction of the dopaminergic input to the frontal cortex and striatum reduced the BOLD signal yet did not change the regional cerebral blood flow at resting state suggesting that the BOLD effects were due to dopamine. It was also possible to compare the time course of the BOLD signal changes with previous reports on dopamine efflux using microdialysis. This showed highly similar patterns over time. The relationship of BOLD signal changes to particular dopamine receptors has been examined in anaesthetised rats (Dixon, Prior et al. 2005). By comparison with a baseline pretreatment time period, a widespread detectable increase in BOLD signal due to amphetamine was detected in the orbital cortex, striatum, globus pallidus, thalamus and hippocampus. There were also areas that had decreased BOLD signal including frontal cortex, amygdala, substantia nigra and entorhinal cortex. It was shown that the increase in BOLD signal due to amphetamine was markedly attenuated by dopamine D1 receptor antagonist pretreatment. In contrast, dopamine D2 receptor antagonists seemed to attenuate decreases in BOLD signal due to amphetamine. There were some attenuating effects on increases in BOLD signal but this was much less than during pretreatment with the D1 receptor antagonist.

Using neuroimaging based on relative cerebral blood flow changes, another animal study showed an increase in dopamine release (mainly in the first 60 minutes) detected with microdialysis which correlated with cyclic-adenosine-3',5'-monophosphate (cAMP), indicating postsynaptic signal transduction, for a range of amphetamine doses (Ren, Xu et al. 2009). However, the relative cerebral blood flow decreased in the caudate and putamen region with a low dose of amphetamine despite the release of dopamine measured by microdialysis and the increase in neuronal activation as detected by an increase

in cAMP. At higher doses there was an increase in relative cerebral blood flow corresponding to the dopamine release and neuronal activation. The authors suggested that these effects might be due to different dopamine receptor effects at different doses (dopamine D2/D3 receptors at low doses and dopamine D1/D5 receptors at high doses).

1.2.1.2. fMRI effects on healthy volunteers

Studies examining the direct effects of methamphetamine indicate the occurrence of increased BOLD signal changes. These kinds of studies are similar to the concept of challenge phMRI as used in this thesis. A study using fMRI to detect BOLD signal changes in healthy volunteers with intravenous methamphetamine (0.15 mgs/kg) showed activation effects in the medial orbitofrontal cortex, anterior cingulate and ventral striatum (Völlm, De Araujo et al. 2004). A “mind racing” measure was used to examine the subjective effects of methamphetamine. This correlated with activations in the anterior cingulate region and ventral striatum. Another study in healthy volunteers (using 15 mgs of intravenous methamphetamine) showed detectable effects on BOLD signal more pronounced in the subcortical and cerebellum than in the frontotemporal region (Kleinschmidt, Bruhn et al. 1999).

The effects of amphetamine on BOLD signal in participants completing cognitive tasks are fairly mixed and depend on the task used. These kinds of studies would be classified as modulation phMRI studies according to the terminology of this thesis. They have been completed much more frequently than challenge phMRI studies. In an open label study (Willson, Wilman et al. 2004), participants completed cognitive tasks whilst undergoing fMRI and then took amphetamine followed by further fMRI. The sessions before and after amphetamine administration were compared. This showed a decrease in BOLD signal and a decreased number of voxels activated in three different cognitive tasks. These tasks were: a word generation paradigm (subjects required to generate words given a letter prompt); a working memory task (memorization of 5 digits and subsequent testing with single digits) and spatial attention task (subjects required to respond as quickly as possible to the co-occurrence of two shapes presented on a screen). In contrast, a finger tapping task (subjects required to tap their index finger as fast as they could) showed an increase in BOLD signal during right-hand motor activity. The same tasks (except for the

finger tapping task) were completed in a follow up study (using the same dose of amphetamine) which showed similar results (Bell, Willson et al. 2005). In contrast, other studies have shown increases in BOLD signal in participants completing various tasks including a working memory task (Mattay, Callicott et al. 2000), a tone discrimination task (Uftring, Wachtel et al. 2001), reaction to fearful faces (Hariri, Mattay et al. 2002), an aversive conditioning task (Menon, Jensen et al. 2007) and a finger tapping task (Uftring, Wachtel et al. 2001). Amphetamine had mixed effects on a group completing an incentive processing task (Knutson, Bjork et al. 2004).

1.2.1.3. Subjective effects of amphetamine and methamphetamine

In participants given an oral dose (30 mgs) of amphetamine, significant increases in ratings of arousal and significant decreases in sedative effects relative to placebo were demonstrated in a double blind within subject crossover study (Mintzer and Griffiths 2007). There were also significant effects of liking of drug and good effects of drugs. Further details of the subjective effects of amphetamine in healthy volunteers are outlined in the section discussing the blockade of amphetamine effects by various drugs. Research groups have examined the effects of intravenous methamphetamine in methamphetamine dependent subjects (Cook, Jeffcoat et al. 1993; Newton, Roache et al. 2005; Newton, De la Garza et al. 2005a; Newton, De La Garza et al. 2005b; Newton, Roache et al. 2006; Newton, Reid et al. 2008). For example, one study (Newton, De la Garza et al. 2005a) showed that mean ratings for “good effect”, “liking”, “stimulating” were higher for those given 15 - 30 mgs methamphetamine compared to those given 0 mgs.

Amphetamine has clear effects on reaction times. A decrease in reaction time was detected for a spatial attention task and a working memory task in healthy volunteers given amphetamine (Willson, Wilman et al. 2004). In 14 children aged 6 to 12, amphetamine at a dose of 0.5 mgs/kg resulted in a faster reaction time compared to placebo (Rapoport, Buchsbaum et al. 1978). A similar result was also detected in healthy volunteers given amphetamine using an auditory reaction time task (Hamilton, Smith et al. 1983).

1.2.1.4. Blockade of the effects of amphetamine using dopamine receptor blocking drugs

There is mixed evidence for attenuation by dopamine receptor blocking drugs on subjective effects induced by amphetamine. An early study (Angrist, Lee et al. 1974) suggested that haloperidol (5mgs) may attenuate subjective effects of amphetamine in people who were amphetamine users. The effects mainly occurred within 60 minutes. In this study, half of the subjects were given a prescribed dose of amphetamine; the other half experienced symptoms in the context of illicitly used amphetamine. In both cases the antipsychotic was given after the amphetamine effects were present. Another study used a variety of antipsychotics to examine the attenuation of effects of amphetamine in amphetamine dependent subjects (Jonsson 1972). The effects of the pretreatment with antipsychotic drugs were tested in subjects given an intravenous dose of 200 mgs amphetamine. There was a reduction of peak euphoric effects of amphetamine by 50% with a single dose (5 mgs) of pimozide. The same effect occurred with single higher doses (10 - 20mgs) and repeated administration for 7 and 13 days. Single and repeated doses of chlorpromazine also had reducing effects on euphoria but to a lesser degree than pimozide.

Another study showed that 10 mgs amphetamine produced increased arousal rating and decreased hunger but no increased euphoria in a group of healthy females. In this group, 2 mgs of pimozide reduced significantly the arousal rating but had no effect on the decreased hunger rating (Silverstone, Fincham et al. 1980). A study with healthy volunteers examined the effect of 4 mgs pimozide pretreatment on the arousal effect of a single dose of 20 mgs amphetamine (Jacobs and Silverstone 1986). This showed some attenuation of the arousal effects of amphetamine but not to a statistically significant degree. However, in two later studies (Brauer and de Wit 1996; Brauer and de Wit 1997) the effects of pimozide on euphoria were examined using a range of doses of pimozide. Both studies used amphetamine at doses of 10 to 20 mgs. Amphetamine produced detectable effects on mood but pimozide at doses of 1 and 2 mgs in the earlier study and 8 mgs in the later study (Brauer and de Wit 1997) failed to antagonize consistently the effects of amphetamine. A similar finding occurred in a study examining whether subjective effects of methamphetamine could be attenuated by two different antipsychotics

(Wachtel, Ortengren et al. 2002). A number of expected effects (arousal, euphoria, elation, vigour) occurred with methamphetamine (20 mgs given orally). Neither haloperidol (dose of 3 mgs) nor risperidone (dose of 0.75 mgs) consistently reversed the subjective effects of methamphetamine. The inconsistency of these studies could be related to the higher doses of amphetamine (ranging from 70 -190 mgs) used in older studies compared to recent studies (often 25 mgs) (Wise 2008). However, the evidence of the studies in recent years indicates that the subjective effects of amphetamine or methamphetamine are not readily reversed with agents blocking dopamine D2 receptors.

1.2.2. Amisulpride

Amisulpride is a substituted benzamide used in the treatment of schizophrenia (Sanofi-Aventis Accessed Oct-2009).There is also evidence that it has antidepressant properties (Montgomery 2002). The pharmacokinetics of amisulpride is outlined in Table 1.2-2. Amisulpride binds to dopamine D2/D3 receptors rather than dopamine D1, D4 and D5 receptors. It has very low affinity for adrenergic, histamine, cholinergic and most serotonin (5-HT) receptors. Recently amisulpride has been shown to have high affinity (acting as an antagonist) for the serotonin receptors $5\text{-}HT_{2B}$ and $5\text{-}HT_{7a}$. The effects on the latter ($5\text{-}HT_{7a}$) receptor may explain its antidepressant effects (Abbas, Hedlund et al. 2009). It has been demonstrated in animal studies that at low doses, amisulpride preferentially acts on presynaptic dopamine D2/D3 receptors whereas at high doses amisulpride blocks dopamine receptors located in the limbic structures rather than in the striatum (Schoemaker, Claustre et al. 1997).

Bioavailability	C_{max} (µg/l)	T_{max}	$T_{1/2}$ (hour)
48%	39 54	Two peaks 1 hour post dose 3-4 hours post dose	12

Table 1.2-2
Some of the key pharmacokinetic properties of amisulpride (Sanofi-Aventis Accessed Oct-2009). C_{max} is peak plasma amisulpride concentration for 50 mgs for each of the two concentration peaks. T_{max} refers to the time to reach peak plasma concentration of amisulpride. $T_{1/2}$ refers to amisulpride plasma half life.

A recent review indicated that amisulpride reaches 65% dopamine occupancy in both the striatal and extrastriatal areas at an approximate level of 200 ng/ml, or dose of 400 mgs/day (Sparshatt, Taylor et al. 2009). Amisulpride is unable to permeate the blood brain barrier (Hartter, Huwel et al. 2003) so it may have

delayed onset of effects compared to equivalent drugs for single doses. It may need require repeated administration to overcome this effect (Natesan, Reckless et al. 2008). The absorption of amisulpride into the brain may occur via the efflux transport protein P-glycoprotein found on plexus epithelial cells (Schmitt, Abou El-Ela et al. 2006). It may be that amisulpride reaches its targets within the central nervous system (CNS) via ventricular cerebrospinal fluid (CSF) possibly explaining its selective effects on limbic regions (Schmitt, Abou El-Ela et al. 2006).

Several studies have been completed on the effects of amisulpride for cognitive tasks. The effect of amisulpride on memory has been frequently examined. Episodic memory is an aspect of memory concerning the ability to encode and retrieve information relating to one's past (Gibbs, Naudts et al. 2008). Examining recognition memory is a way of testing this aspect of memory and itself can be viewed to consist of two different processes: recollection and familiarity (Gibbs, Naudts et al. 2008). These processes can be seen in the experience of recognition of a person (familiarity) but being unable to remember specific information about the person (recollection failure) (Yonelinas 2002). Amisulpride at a dose of 400 mgs has been used in healthy male volunteers to examine these aspects of memory (Gibbs, Naudts et al. 2008). Plasma levels of amisulpride at encoding were inversely correlated with recollection estimates for emotional stimuli. This effect did not occur for recollection estimates for neutral stimuli. Amisulpride levels were not correlated with familiarity for neutral or emotional stimuli. The authors speculated that amisulpride might interfere with limbic dopaminergic transmission. This could affect the modulation of the hippocampus by the amygdala - possibly interfering with the binding of affective related detail. This is broadly consistent with another study which showed poorer recognition of emotional aspects of a story in an emotional memory task in healthy participants given sulpiride (Mehta, Hinton et al. 2005).

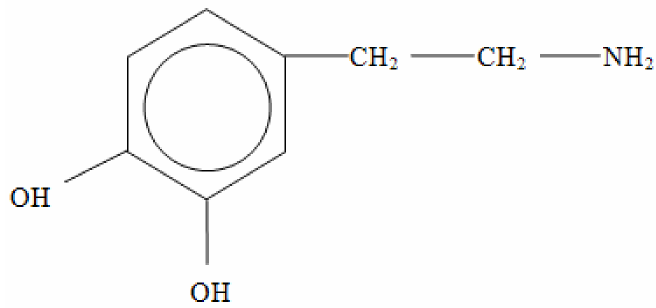
Amisulpride does not have clear effects on a number of other memory tests (working memory, immediate or delayed free recall, picture or word recognition) with single doses (Rosenzweig, Canal et al. 2002). However, after administration of amisulpride for five days there has been some detrimental effects on working memory detected (Ramaekers, Louwerens et al. 1999).

There does not appear to be significant effects of amisulpride on tests examining attention, vigilance, information processing or sensory motor coordination with single doses (up to 400 mgs) of amisulpride (Rosenzweig, Canal et al. 2002). After 5 days of amisulpride administration, a decreased performance in an attention task has been noted on day 5 (Ramaekers, Louwerens et al. 1999). Speed and tracking were also impaired in a motor and perceptual based task after 5 days treatment. Amisulpride appeared to have a minimal effect on affective function in this study - only drowsiness from the present state examination (Kendell, Everett et al. 1968) was significantly different from placebo. No decreases in a reaction time task were detected even with the prolonged administration of amisulpride (Ramaekers, Louwerens et al. 1999).

1.3. Overview of dopamine neurons and receptors

1.3.1. Dopamine structure and dopamine neuron neuroanatomy

Dopamine can be classified as one of the main monoamine neurotransmitters (Nestler, Hyman et al. 2009) in the brain. Monoamines all contain a single amine group and in addition to dopamine, include adrenaline, noradrenaline, serotonin and histamine. The structure of dopamine is presented in Figure 1.3-1. For comparison, the structure of methamphetamine is also shown.



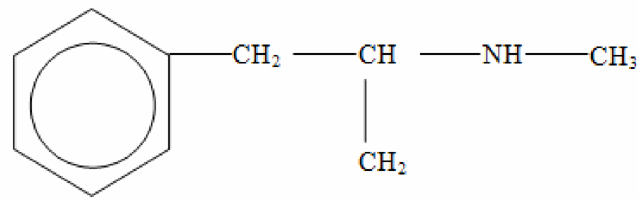


Figure 1.3-1
Structure of dopamine (top) and methamphetamine (bottom).

The monoamine neurons are characterized by having wide projections throughout the brain (Nestler, Hyman et al. 2009). In the human brain the midbrain is the site for a number of dopamine neurons. Three groups of dopamine neurons of particular importance (Schultz 1998) are located in the midbrain as follows:

- A8 Group - dorsal to the lateral substantia nigra (SN): this group has cells that project to the striatum and to the limbic / cortical areas.
- A9 Group - present in the substantia nigra pars compacta (SNc): this group mainly projects to the striatum along the mesostriatal pathway. This group also has cells that project to the cortical and limbic areas.
- A10 - ventral tegmental area (VTA) medial to the substantia nigra: this group mainly projects to the limbic and cortical areas along the mesolimbic and mesocortical pathways. This group also projects to the ventral striatum and to the ventro-medial part of the head of the caudate.

These groups and projections are outlined schematically in Figure 1.3-2.

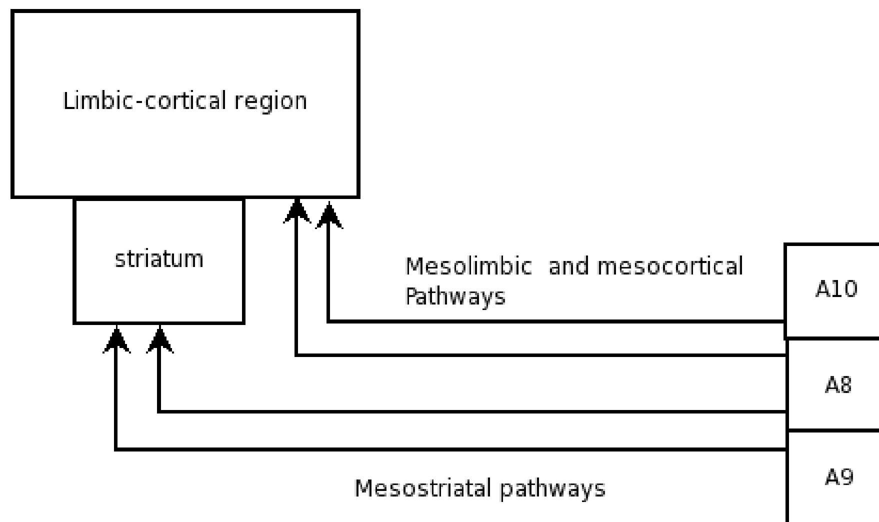


Figure 1.3-2
Main pathways from the three dopamine groups in the midbrain to cortico-limbic regions and striatum.

As presented above there is a number of projections pathways from the midbrain to the striatum. However, there are also projections from the striatum. Using markers that stain for acetylcholinesterase activity, two areas in the striatum can be identified (Graybiel 1990): a larger area called the matrix and smaller groups of areas called striosomes (patches). The former area project to the substantia nigra pars reticulata (SNr) whereas the latter areas projects to the SNc (Graybiel 1990). These areas may have functional distinctions. It has been suggested that these projections may allow both inhibitory and excitatory input to the dopamine neurons in the SNc (Schultz 1998). The inhibitory pathway is via the direct actions of GABA neurons projecting to the SNc from the striosomes whereas the excitatory input would occur via double inhibition (GABA neurons from the matrix area projecting to the SNr and collaterals from SNr neurons projecting to the SNc). Studies on primates have showed the further complexity of the interconnections in this regions by looking at the striatum and the midbrain in a broader perspective (Haber, Fudge et al. 2000). In this work, a description was given of a series of interconnections forming a type of ascending spiral between the ventral striatum, midbrain regions and dorsal striatum. These findings indicate the complexity of the networks that involve dopamine neurons. The identification of these detailed pathways enables complex models of dopamine function to be tested.

1.3.2. Pharmacology of dopamine

Dopamine receptor activation has effects on a number of different channels. The molecular mechanisms underlying these have been reviewed in detail elsewhere (Neve, Seamans et al. 2004). In this section some of these features are briefly discussed. Dopamine receptors are grouped into two main categories:

- D1 like: these include D1 and D5 type receptors
- D2 like: these include D2, D3 and D4 type receptors.

Activation of these receptors affects a number of channels as shown in Table 1.3-1. D1 receptor activation generally increases channel activity whereas D2 receptor activation generally decreases channel activity. There are different effects following D1 or D2 receptor activation on NMDA channels as summarised in Figure 1.3-3. The relationship between dopamine receptor activation and glutamate receptor function is important because it provides the assumption that dopamine receptor activation can be related to BOLD signal changes.

Effect	Mediated by	Channel
D1 receptor effects		
Enhancement	Protein kinase A	NMDA, AMPA, GABA
Enhancement	Protein kinase A Protein kinase C	Sodium P (persistent) channels, L type Calcium channels
Inhibition	Protein kinase A	Voltage gated potassium channels
Inhibition	Unknown	GIRK channels
D2 receptor effects		
Inhibition	Protein kinase A Gβγ protein	NMDA
Inhibition	Gβγ protein	GABA receptors, Sodium channels L,N,P,Q type calcium channels
Inhibition	Uncertain	AMPA
Enhancement	Gβγ protein Protein kinase A	Voltage gated potassium channels
Inhibition	Unknown	GIRK channels

Table 1.3-1
Effects following Dopamine D1 (upper) and D2 (lower) receptor stimulation (Neve, Seamans et al. 2004; Surmeier, Ding et al. 2007). The enhancing effects by D1 receptor activation occur largely via the Gα subunit (and subsequent effects on protein kinase A and protein kinase C) of the G protein whereas the inhibitory effects of the D2 receptor activation largely occur via the Gβγ subunit of the G protein. Abbreviations: Girk is G protein-regulated inwardly rectifying potassium channels.

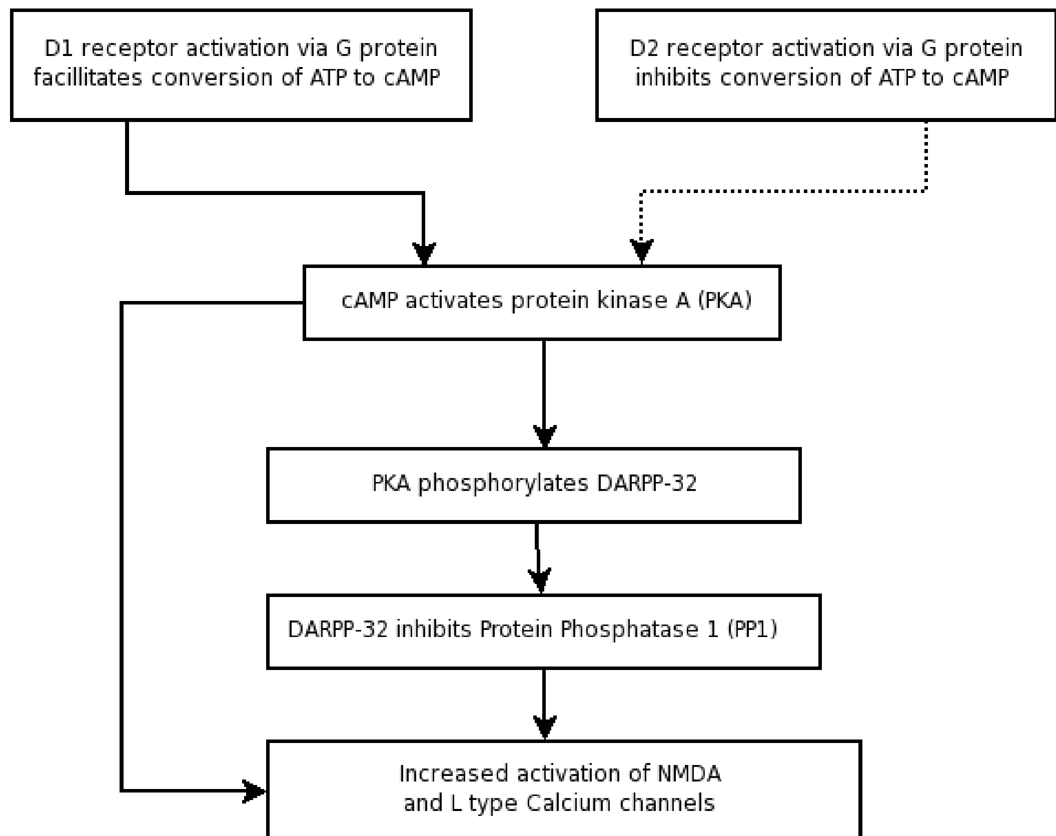


Figure 1.3-3
Different signalling pathways following D1 and D2 receptor stimulation (Greengard, Allen et al. 1999; Neve, Seamans et al. 2004). Continuous lines are activating effects and broken lines indicate inhibitory effects. D1 receptor activation via Gα facilitates the conversion of ATP to cAMP which disinhibits cAMP dependent PKA. This results in the phosphorylation of a number of proteins such as DARPP-32. When phosphorylated in a specific region (Thr34), DARPP-32 inhibits PP1. The inhibition of PP1 increases the state of phosphorylation and activity of NMDA receptors and L type Ca^{2+} channels. PKA also directly phosphorylates NMDA receptors and L type Ca^{2+} channels increasing their activation. D2 receptor activation via Gβγ subunits inhibits the conversion of ATP to cAMP thereby inhibiting the rest of cascade. Abbreviations: PKA is protein kinase A; DARPP-32 is dopamine and cyclic AMP-regulated phosphoprotein, 32 kDa and PP1 is protein phosphatase 1.

Although there are different effects following D1 and D2 receptor activation, there are similarities in the distribution of these receptors in the brain as shown in Table 1.3-2. These data are based on studies using positron emission tomography (PET) to demonstrate the binding potential (BP) of these receptors in different areas in the brain. BP in Table 1.3-2 is taken to be the number of binding sites of a neurotransmitter multiplied by the affinity of the neurotransmitter for the receptor (Mintun, Raichle et al. 1984). This gives a sense of how well an area interacts with a neurotransmitter of interest (in this case dopamine). BP detected by the different groups are broadly in line with each another (Hirvonen, Nagren et al. 2001; Abi-Dargham, Mawlawi et al.

2002). These values are also compatible with in vitro findings (Abi-Dargham, Mawlawi et al. 2002).

	Caudate	Putamen	Amydala	Neocortex	Thalamus	Other
D1 receptors (Abi-Dargham, Mawlawi et al. 2002)	100%	112%	26%	26-30% AC >TC = PC = OC; OC= OF > DLPFC	19%	(HPC) 26%
D2/D3 receptors (Mukherjee, Christian et al. 2002)	16-32%	19-37%	3-2%	1-0% TC >PC > OC >FC	4-3%	(VS) 24-10%

Table 1.3-2
Summary of BP using D1 and D2/D3 radiotracers. The BP values for the D1 receptors (Abi-Dargham, Mawlawi et al. 2002) are normalised whereas those for the D2/D3 receptors (Mukherjee, Christian et al. 2002) are not. Abbreviations: TC is temporal cortex; PC is parietal cortex; FC is frontal cortex; OC is occipital cortex; AC is anterior cingulate; DLPFC is dorsolateral prefrontal cortex; OF orbitofrontal cortex; VS is ventral striatum and HPC is hippocampus.

Anatomical localization has also been judged using methods detecting messenger ribonucleic acid (mRNA) for D1 and D2 receptors. (Hurd, Suzuki et al. 2001). The results of this study on the whole brain were in line with a previous study on selected areas of the brain (Meador-Woodruff, Damask et al. 1996). The highest expression of both D1 and D2 mRNAs occurred in the striatum. In the cortex, there was moderate D1 mRNA in all regions but the highest levels were in medial orbital frontal cortex (Brodmann area (BA) 11 and BA 16), subcallosal / paraterminal gyrus (BA 25, BA 32) and insular cortex (BA 13-16). Moderate to high levels occurred in the striate cortex (BA 17). The lowest levels were detected in the inferior frontal cortex (BA 44, BA 45, and BA 47) and middle frontal cortex (BA 8, BA 9, BA 10, and BA 46). There was minimal D1 mRNA detected in hippocampal formation, thalamus and cerebellum and low amounts in the amygdala. There was very little mRNA for D2 receptors detected in the cortex (although some relatively higher signals occurred in rostral temporal lobe, parietal and occipital cortex). There were weak to moderate levels detected in the hippocampus, high levels in the dentate and moderate levels detected in the uncal gyrus. There were weak levels of mRNA detected in the basal and lateral amygdala nuclear group. There were varying levels of mRNA detected in the thalamus: highest in the paratenial and paracentral nuclei; moderate in the geniculate bodies but many other regions (dorsal thalamus and subthalamic nucleus) showed low levels.

There were fairly high levels of D2 mRNA detected in the hypothalamus. There were also high levels of mRNA expression detected in midbrain regions such as substantia nigra compacta. Low to moderate levels were detected in the red nucleus, pontine nuclei, inferior colliculus and medial lemniscus. The investigators were unable to comment on levels in the ventral tegmental areas or cerebellar cortex with confidence. Overall these findings are similar to the receptor profile above apart from the low D1 mRNA detected in the thalamus, hippocampus and amgydala.

A discrepancy can be seen between the distribution of dopamine receptors as described above and the description of dopamine neuron projections given earlier. There was a wide distribution of dopamine receptors throughout the cortex yet dopamine neurons only project to a few cortical regions – mainly in the frontal and limbic cortical regions. An explanation for this could be that dopamine is released from noradrenaline neurons and as these neurons project throughout the cerebral cortex, this would provide another mechanism for its release in cortical regions (Devoto and Flore 2006). It is possible that dopamine could diffuse from the frontal regions to other cortical regions but in view of the distance involved, this would seem unlikely. It may be the case that dopamine effects are clearer in the target sites of dopamine neurons such as the frontal regions (e.g., rectal gyrus, anterior cingulate) however, other cortical regions are still likely to be modulated in an important manner by dopamine. The review of the neuroanatomy of dopamine receptors presented here from animal and PET studies was important for the study in this thesis as it provided the basis for the expectations of dopamine receptor activation and attenuation using the challenge phMRI technique.

1.3.3. Dopamine physiology

Three main patterns of activity in dopamine neurons in vivo have been described (Goto and Grace 2007):

1. Inactive hyperpolarized state
2. Slow single-spike state - "tonic" firing pattern
3. Burst activity - "phasic" mode.

These different patterns of dopamine activity are controlled via different pathways in the brain.

1.3.3.1. Tonic dopamine neuron activity

Various studies (Floresco, Todd et al. 2001; Floresco, West et al. 2003) indicate the existence of a circuit involving the hippocampus (HPC), nucleus accumbens (NAc), and the ventral pallidum (VP) that influences tonic dopamine neuron activity in the VTA. This is shown in Figure 1.3-4.

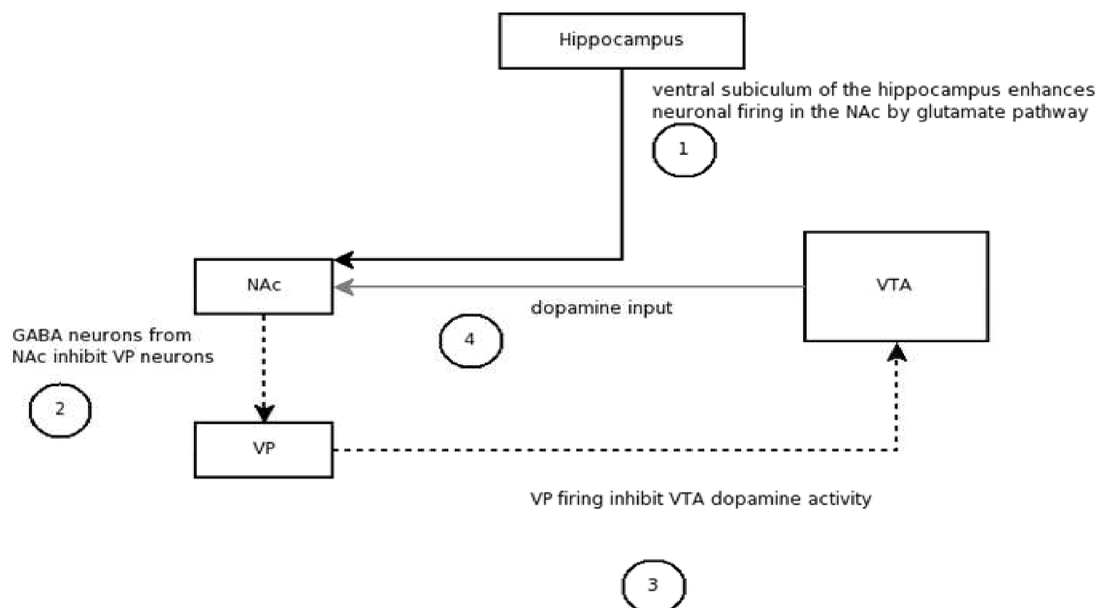


Figure 1.3-4
Interrelationships between HPC, NAc, VP and dopamine neurons in the VTA. Here solid lines represent excitatory input and dashed lines represent inhibitory input. The light coloured continuous line indicates dopamine neurons. (1) HPC input activates NAc neurons. (2) NAc neurons inhibit VP. (3) VP neurons inhibit VTA dopamine neurons (4) Actions of (1), (2), (3) act to disinhibit VTA dopamine neurons. This increases the number of dopamine neurons with tonic activity. It also increase the number of dopamine neurons that could display burst firing. Abbreviations: HPC is Hippocampus; NAc is Nucleus Accumbens; VP is Ventral Pallidum and VTA is Ventral Tegmental Area.

1.3.3.2. Phasic (Burst) dopamine neuron activity

A number of conditions are considered necessary for the occurrence of burst activity in VTA dopamine neurons projecting to the NAc (Grace, Floresco et al. 2007).

- Tonic mode activity: in the VTA, glutamate driven burst firing occurs only in dopamine neurons that are already firing spontaneously. The regulation of this is shown in the circuit in Figure 1.3-4.
- Laterodorsal tegmentum (LDTg) afferent input: without afferent input from LDTg, dopamine neurons revert to activity only seen in vitro (Lodge and Grace 2006).

- Glutamate input: microiontophoretic applications of glutamate induces burst firing in dopamine neurons in vivo (Grace and Bunney 1984). A structure providing the glutamate afferent could be the pedunculopontine tegmentum as this is highly interconnected with the basal ganglia and projects to the VTA (Mena-Segovia, Bolam et al. 2004).

As shown in Figure 1.3-4, when the VP is inactivated, via effects on the NAc by the HPC, there is an increase in tonic activity in VTA dopamine neuron activity. This reduces prefrontal cortex (PFC) input to NAc via activation of D2 receptors but does not affect the input from the HPC (Goto and Grace 2005a). When the VTA dopamine neurons display tonic activity this fulfils a condition for burst activity. With burst activity of dopamine neurons, activation of D1 receptors occurs with an increase in responsiveness of NAc to inputs from the HPC. This reduces the activation of the VP followed by the same sequence of events as described above. These effects are summarised in Figure 1.3-5. Thus, it can be seen that the activation of the circuit as shown in Figure 1.3-4 with the combination of effects outlined in Figure 1.3-5 generates a form of information bias towards HPC input and away from PFC input into the NAc. These effects occur by a combination of effects following phasic and tonic dopamine neuron activity and different actions on dopamine receptors. This idea of dopamine neurons being able to bias input from different neural structures into an area such as the NAc is a useful concept in learning theories involving dopamine. This idea of an information bias towards the HPC following HPC activation mediated by dopamine receptors is also supported by other types of experiments involving stimulation and tetany of the HPC and PFC (Goto and Grace 2005b).

This model can be translated to behavioural responses in situations of expected rewards or situations where there are omissions of rewards. In the case where an animal encounters a rewarding event, phasic dopamine release (burst activity) occurs, facilitating hippocampal input and attenuating PFC input. This results in only highly salient information from the PFC influencing actions to be taken. This is useful for learning appropriate responses for the rewarding events. However, when there is omission of expected rewards, there is reduction of dopamine neuron activity (Schultz 1998) switching the balance from the HPC to the PFC. The greater influence of the PFC enables greater

behavioural flexibility as required by the situation. Features of this model can also be used (presented later) as a way of understanding certain symptoms of schizophrenia.

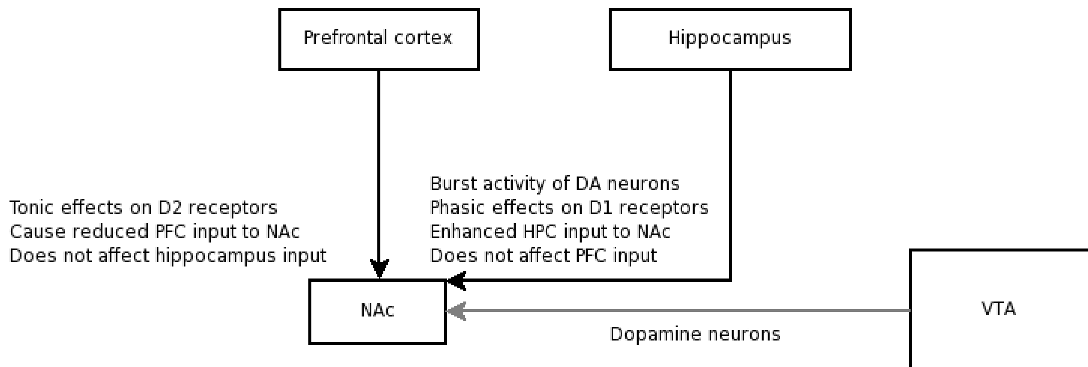


Figure 1.3-5
Overview of some of the different inputs into the nucleus accumbens (NAc) that are modulated by dopamine. The effect of burst activity directly enhances the HPC input to the NAc. This also increases tonic dopamine activity by actions on VP and VTA (Figure 1.3-4) inhibiting the PFC input to the NAc.

Dopamine has an important role for long term potentiation (LTP) and long term depression (LTD). LTP refers to the sustained increase in the postsynaptic neuronal responses following tetanic stimulation of an afferent neuron to a synapse (Nestler, Malenka et al. 2001). LTD is similar but here tetanic stimulation results in a sustained decrease in postsynaptic neuronal responses. The focus here is on the occurrence of LTD and LTP in medium spiny neurons (MSN) with cortical neurons providing afferent input. The features of LTP and LTD in these cells are summarised in Table 1.3-3 (Calabresi, Picconi et al. 2007). D1 and D2 receptor activation are both required for LTD but in the case of LTP, whilst activation of D1 receptors is required, D2 receptor stimulation is inhibitory. D2 receptor activation facilitates LTD by antagonizing the inhibitory influence of cholinergic interneurons. In contrast, D1/D5 receptor activation may be important in the release of nitric oxide (NO) from NO interneurons (Centonze, Grande et al. 2003) - important in the induction phase of LTD (Calabresi, Gubellini et al. 1999). The release of endogenous cannabinoids (ECBs) is also important for LTD (Gerdeman, Ronesi et al. 2002). LTD induced by ECB can be increased by D2 receptor stimulation (Kreitzer and Malenka 2005). The different roles of NMDA receptor activation in these processes (not required for LTD (Calabresi, Maj et al. 1992) but required for LTP (Calabresi,

Pisani et al. 1992)) is interesting in view of how it is affected by dopamine receptor activation. The findings presented here highlight the various roles of dopamine receptor activation in LTD and LTP both of which are important in theories of how learning processes in animals may be implemented (Dayan and Abbott 2001).

Component	LTD	LTP
Dopamine	D1 and D2 receptor activation required	D1 receptor activation required for LTP D2 receptor activation antagonizes LTP
NMDA	Independent of NMDA receptor activation Reduced by glutamate metabotropic receptor antagonism	NMDA receptor activation required
Interneurons	Cholinergic neurons inhibit NO interneurons role in induction phase	Not clearly defined
ECBs	ECB important for LTD Enhanced ECB release by D2 receptor stimulation	Not clearly defined

Table 1.3-3
Summary of the features of LTP and LTD in MSN in the striatum (Calabresi, Picconi et al. 2007). Abbreviations: ECB is endogenous cannabinoid; LTP is long term potentiation; LTD is long term depression; NO is nitric oxide and MSN is medium spiny neurons.

1.4. Aspects of fMRI relevant to the study

1.4.1. Biological basis of fMRI

An account of the basic physics of fMRI can be found in various standard textbooks (Jezzard, Matthews et al. 2001; Westbrook, Roth et al. 2005; McRobbie 2007). In humans the basis of fMRI relates to oxygen changes in haemoglobin. When haemoglobin in red blood cells loses oxygen forming deoxygenated haemoglobin, its iron becomes paramagnetic, which generates local magnetic field distortions especially when compartmentalized as blood capillaries (Ogawa, Menon et al. 1993; Drake and Iadecola 2007). This results in the deoxygenated blood having a lower magnetic resonance signal than fully oxygenated blood. During neuronal activity, an increase of oxygen usage is followed within a few seconds by an increase in blood flow and blood volume. It may be expected that there would be an increase in deoxygenated haemoglobin in this circumstance. However, there is a mismatch between the amount of oxygen delivered and the amount of oxygen taken up by neuronal activity. Thus, the veins and capillaries draining blood from the region become arterialized (Leslie and James 2000) with a reduction in the amount of deoxygenated haemoglobin. The reduction in the deoxyhaemoglobin results in

a relative increase in the magnetic resonance signal. This forms the basis of fMRI BOLD signals and is detected in psychological experiments as differences between tested conditions.

Alteration of blood flow in the brain has been viewed to correspond to its energy consumption (Attwell and Iadecola 2002). As the fMRI BOLD signal reflects changes in blood flow, this suggests a relationship between BOLD signal and energy usage (Magistretti, Pellerin et al. 1999b). The majority (74%) of energy used in signalling in grey matter in primates may be involved in reversing ion fluxes underlying postsynaptic currents; whereas, action potentials has been estimated to consume only 10% of the total signalling energy (Attwell and Iadecola 2002). As 80–90% of cortical synapses are glutamatergic (Attwell and Iadecola 2002), this suggests that the majority of energy used in the brain is related to post synaptic glutamatergic activity. These features could be consistent with a relationship between blood flow and post synaptic glutamatergic activity. However, in certain situations cerebral blood flow and metabolism can be uncoupled (Magistretti and Pellerin 1999a). This suggests that biological processes involving glutamate do not directly control alterations in blood flow (Drake and Iadecola 2007). This implies that the post synaptic glutamatergic activity does not directly affect BOLD signal changes. It may be that parallel regulation of cerebral blood flow (Magistretti and Pellerin 1999a) occurs whereby when glutamate is released from active synapses other mechanisms (e.g., via nitric oxide) could effect the alteration in blood flow and the consequent BOLD signal changes.

Another way to examine the biological basis of BOLD signal is to relate it to other types of neurophysiological data. A signal called the mean extracellular field potential can be detected when a microelectrode is placed in the extracellular space somewhat distant from a spiking neuron (Logothetis and Wandell 2004). By using high and low pass filters this signal can be divided into local field potentials (LFP; cutoff < 200 Hz) and multiple-unit spiking activity (MUA; cut-off 300–400 Hz).These signals represent different types of neuronal activity: the MUA represents local neuronal spiking; the LFP reflects the various types of electrical activity at synapses between dendrites and soma within a particular neuronal region (Logothetis and Wandell 2004). An experiment

completed using the visual cortex of primates examined how well the BOLD signal could be predicted from LFP and MAU (Logothetis, Pauls et al. 2001). The LFP signal predicted statistically significant greater amount of variance than the MUA, suggesting that the BOLD signal reflects local somatic and dendritic activity rather than the long range signals transmitted by action potentials (Logothetis and Wandell 2004; Drake and Iadecola 2007). This is consistent with a study using a perceptual suppression task where BOLD responses were reduced but the spiking response of the population of neurons was not reduced (Maier, Wilke et al. 2008). These studies (which relate the post synaptic effects to BOLD signal changes) are consistent with the previously discussed work relating changes in cerebral flow to post synaptic glutamatergic activity. There are also direct effects of various monoamines (dopamine , noradrenaline and serotonin) on cerebral cortex microvessels (Raichle, Hartman et al. 1975; Krimer, Muly et al. 1998). Therefore it may be that the effects of monoamines such as dopamine and serotonin affect the BOLD signal in a manner unrelated to glutamate related processes. This complicates the interpretation of BOLD signal changes in pharmacological studies that use agents that affect monoamine systems.

1.4.2. Challenge and modulation phMRI

A challenge phMRI technique was used for one part of the study in this thesis whereas three modulation phMRI tasks were used in the other part. Challenge phMRI is a technique used to examine brain activation due to the direct pharmacological effects of drugs (Anderson, McKie et al. 2008). Drugs are often given intravenously in these studies to ensure the effects in the brain regions are rapid enough to be detected in the scanner. As challenge phMRI in humans is a fairly new methodology, different approaches have been taken for the analysis (Anderson, McKie et al. 2008). One method is by using the pharmacokinetics of the drug to generate a model of the expected time course of the drug. A problem with this is that it cannot be certain that the pharmacokinetics in the circulation will match those in the brain. Another approach is to use the psychological response to the drug as a regressor to model the effects of the brain (Anderson, Clark et al. 2002). However, this is based on the assumption that the psychological effects are related to the pharmacokinetics of the drug. A third method is a data driven approach. One way of doing this is by the use of independent component analysis (Beckmann

and Smith 2004) to detect underlying patterns in the data. An alternate data driven approach is the use of time series. This method analyses the BOLD signal changes over time (McKie, Del-Ben et al. 2005). This makes the assumption that there is a change in BOLD signal over time but does not make particular assumptions about the shape of the curve. This was the method that was used for the study in this thesis.

Challenge phMRI was used in the study in this thesis as a way of investigating the direct effects of the psychotropic drug methamphetamine and how its effects could be attenuated by amisulpride. Earlier, an account was about how post synaptic glutamatergic activity affects BOLD signal changes. It was also shown previously how D1 and D2 dopamine receptor activation affects glutamate receptors. As a result, it was assumed that the direct effects of D1 and D2 receptor dopamine activation would affect post synaptic glutamatergic activity resulting in BOLD signal changes. The main difficulties with the assumption is that the effect of post synaptic glutamatergic activity likely affects BOLD signal changes indirectly and dopamine may affect the BOLD signal in a manner unrelated to glutamate related processes.

Modulation phMRI tasks are essentially the same as typical fMRI tasks (Jezzard, Matthews et al. 2001) except in these cases, drug is the condition of interest. The use of a drug condition requires particular consideration in the design (Anderson, McKie et al. 2008). There is a need for a placebo control and vigilance for carry over effects if a within subject design is used. Despite these considerations, the analysis of modulation phMRI tasks is broadly the same as for standard fMRI tasks.

1.4.3. Comparisons with PET

A brief review of PET methods pertaining to dopamine function in humans would seem useful due to the considerable number of studies completed in healthy and clinical populations using these methods (Mishina 2008; Egerton, Mehta et al. 2009; Volkow, Fowler et al. 2009; Patel, Vyas et al. 2010). The fundamental aim of PET when used in humans is the measurement of the distribution of positron emission radioisotopes in the body. When a positron is released from a radioisotope in human tissue, it will only go a few millimetres, at

which point, it is captured by an electron and 2 photons are emitted. These can be sensed by radiation detectors in a scanner and by the use of mathematical models, the position of the radioisotope can be determined (Frackowiak and Jones 2003).

A number of radiotracers can be used to assess for dopamine function. There are radiotracers that have high affinity for D1 receptors and D2 receptors which can be used to examine the dopamine receptor profile of brain regions of interest (Laruelle 2000). This can be usefully employed in comparison studies between control and clinical populations (Okubo, Suhara et al. 1997). There are also a number of functional PET methods that can be used to assess dopamine related activity (Patel, Vyas et al. 2010). As glucose is the main source of energy for the brain, the radiotracer 18F-2-fluoro-2-deoxy-d-glucose (FDG) can be used as an index of neuronal activity as this is metabolised in the same pathway as glucose (Herholz, Carter et al. 2007). This can be used to examine the metabolic rates in brains of people given drugs that affect dopamine receptors (Buchsbaum, Haznedar et al. 2009). Increased blood flow in the brain can be measured using the water related radiotracer 15O-H_2O. Changes in blood flow are taken to represent neuronal activity in an analogous way to BOLD signal changes. Another approach is based on the idea of displacement of D2 receptor radiotracers during episodes of increased dopamine release (as a result of pharmacological challenge or cognitive task) (Laruelle 2000; Egerton, Mehta et al. 2009). This can be used to examine for correlations between the completion of tasks and changes in extracellular dopamine levels. There are also radiotracers (e.g., 18F-FDOPA) that are metabolised in the same pathway as dopamine enabling presynaptic striatal dopamine function to be assessed (Patel, Vyas et al. 2010).

This overview shows how PET can be used to examine many facets of dopamine neuron activity in vivo. However, there are some advantages with the use of fMRI techniques. There is greater temporal resolution (but similar spatial resolution) for BOLD fMRI compared to functional PET (Moser, Stadlbauer et al. 2009). In addition, fMRI does not require radioisotopes: this makes it cheaper, safer and easy to repeat compared to PET. Although a wide variety of PET techniques have been used to investigate dopamine neuron function, the

advantages of fMRI provide a motivation to increase the scope of its use through the challenge phMRI and modulation phMRI techniques that are described in this thesis.

1.5. Cognitive processes related to the study involving dopamine

In the study described in this thesis, there were a number of tasks used to investigate the role of dopamine in man. In the following sections, some of the animal studies and human neuroimaging studies related to these tasks are reviewed to provide relevant background information.

1.5.1. Finger tapping tasks

A number of different brain regions involved in voluntary movement has been described (Penfield and Boldrey 1937; Penfield and Welch 1951; Kuypers 1987; He, Dum et al. 1993; He, Dum et al. 1995; Fink, Frackowiak et al. 1997; Nieuwenhuys, Voogd et al. 2007). In recent decades, the importance of premotor areas (areas that project to the motor cortex) has also been identified. One such region is caudal area 6 (which includes the supplementary motor area (SMA)) but other regions include a number of other spatially separate areas such as the arcuate sulcus, regions adjacent to the superior precentral gyrus and regions in the cingulate gyrus (Dum and Strick 1991). In addition to projecting to the motor cortex, these areas also project directly to the spinal cord (Dum and Strick 1991). A wide number of functions has been attributed to the premotor areas including visual guidance of movement, bimanual coordination, preparation for movement, sequencing of movements and postural aspects to support movements and aspects of trajectory control (Dum and Strick 1991).

These regions receive input from the basal ganglia and cerebellum in different ways. This has been examined in primates using tracer studies (Sakai, Inase et al. 2002). This work indicates that the SMA receives input more from the globus pallidus whereas the primary motor cortex receives input more so from the cerebellum. These different input pathways form part of well described circuits involving the cortex, thalamus and basal ganglia (Alexander, Delong et al. 1986; Alexander and Crutcher 1990). Distinct functional and structural properties have been attributed to these circuits. One of the circuits involving motor activation is

considered to be composed of two different pathways: a "direct" and "indirect" pathway. These are shown in Figure 1.5-1. Functionally, the effects of these pathways are different: the direct pathway is considered to facilitate intended motor behaviour while the indirect pathway may inhibit unwanted motor behaviour (Crossman 2000). Furthermore these pathways are affected by dopamine in different ways at the site of the striatum. The indirect pathway may be inhibited by D2 receptor stimulation whereas the direct pathway may be activated by D1 receptor stimulation (Albin, Young et al. 1989; Crossman 2000; Surmeier, Ding et al. 2007). Functional interpretation of these circuits has been applied to certain diseases with reduction of dopamine in the striatum such as Parkinson's disease (PD) (Samii, Nutt et al. 2004). One of the core symptoms of Parkinson's disease is bradykinesia which could be explained in terms of overactivity of the indirect inhibitory pathway (Albin, Young et al. 1989; Crossman 2000). This may result in hypoactivation of the somatosensory and supplementary motor area (Tost, Meyer-Lindenberg et al. 2006). Furthermore this model can be used as a basis for the prediction of dopamine D2 receptor blockade (Tost, Meyer-Lindenberg et al. 2006).

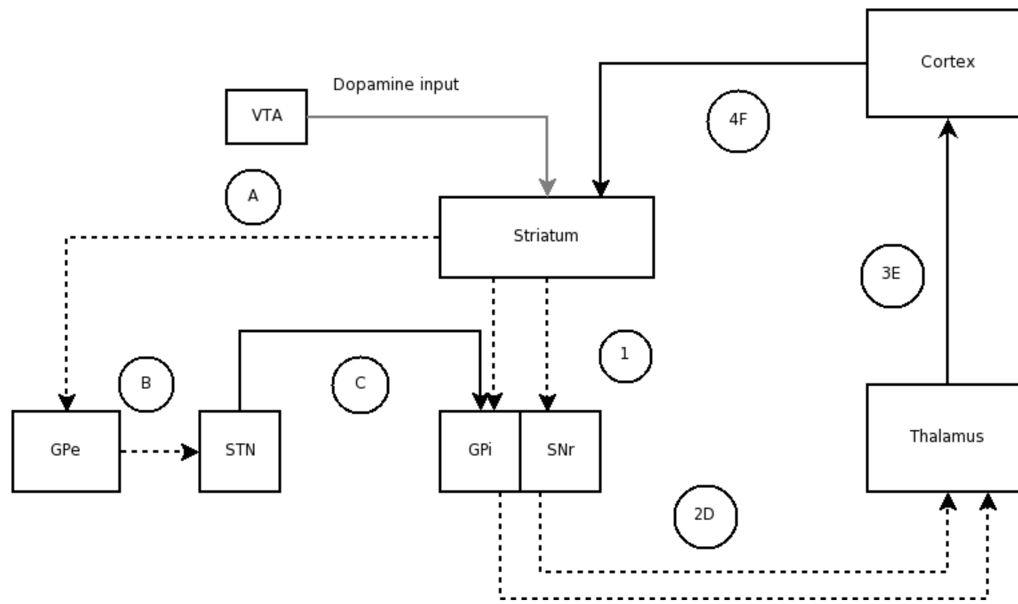


Figure 1.5-1
Diagram of the indirect and direct pathways in the basal ganglia related to motor control (Alexander and Crutcher 1990). Broken lines indicate GABA inhibitory neurons. Continuous lines indicate excitatory glutamate neurons. The light coloured continuous line indicates dopamine neurons. The direct pathway consists of interconnections between the striatum and SNr/GPi. This is indicated by the number 1 in the diagram. The indirect pathway consists of interconnections between the striatum, GPe, STN, and GPi/SNr. This is indicated by the series A, B, C in the diagram. It can be seen that the overall effect of 1,2D is excitatory resulting in the direct pathway having excitatory input to the thalamus and cortex. The effects of A, B, C are excitatory, thus A, B, C, 2D is inhibitory and so the indirect pathway is inhibitory to the thalamus and cortex. The overall circuits can be seen to consist of the series 1, 2D, 3E, 4F (direct input) or A, B, C, 2D, 3E, 4F (indirect input). According to the model, D1 receptor stimulation activates the direct pathway and D2 receptor stimulation inhibits the indirect pathway. Abbreviations: GPe is globus pallidus externa; GPi is globus pallidus interna; STN is subthalamic nucleus and SNr is substantia nigra pars reticulata.

A recent study examined healthy volunteers undergoing fMRI when given a single dose (5 mgs) of haloperidol whilst they completed a left handed finger tapping task (Tost, Meyer-Lindenberg et al. 2006). Using the above model, the expectation had been that haloperidol would cause the following effects:

- increased activation of the basal ganglia
- decreased cortical activation.

fMRI scanning was completed at three times in the study: before administration of haloperidol; 1 hour after its infusion intravenously and 1 day (corresponding to be one half life) after its administration. The comparison between baseline activation and activations one hour after haloperidol indicated a decrease in bilateral pre-supplementary motor area, bilateral SMA (caudal BA6) proper and bilateral dorsal anterior cingulate gyrus. There were reductions of activation in the ipsilateral somatomotor cortex, ipsilateral dorsal premotor cortex,

contralateral cerebellum, ipsilateral putamen, ipsilateral ventral premotor cortex and the contralateral posterior parietal cortex. The recruitment of ipsilateral cortical areas and contralateral cerebellar areas was thought to reflect the complexity of the task. This is consistent with an earlier report (Solodkin, Hlustik et al. 2001) where increased bilateral cortical activations of motor related areas were detected in the comparison between the completion of sequential versus non sequential motor tasks. The reduction in cortical activation following haloperidol fits with the motor architecture described above but the reduction in activity in the basal ganglia is inconsistent with it. The reduction in activation in the SMA is consistent with a study completed on people with PD (Playford, Jenkins et al. 1992). In this study, those with PD failed to show significant increases in blood flow in the SMA during a motor task when compared to healthy controls. This study was repeated using fMRI instead of PET (Haslinger, Erhard et al. 2001) and the results showed underactivity in the SMA and motor cortex.

The effects of amphetamine on BOLD signal in participants completing finger tapping tasks have been examined in a number of studies (Uftring, Wachtel et al. 2001; Willson, Wilman et al. 2004). The former study detected an increase in number of active voxels in the ipsilateral primary motor cortex and right middle frontal cortex with amphetamine. The latter study showed increased activation in the left inferior frontal gyrus with the left hand completing the motor task in the amphetamine condition; however, there was no increased activation when the right hand was used. These studies indicate that amphetamine increases activation in cortical areas consistent with increased activation of the direct and decreased activation of the indirect pathways as in the model in Figure 1.5-1.

From the data described in this section, it seems that functional circuits as described by (Alexander and Crutcher 1990) do not correlate fully with experimental findings (Tost, Meyer-Lindenberg et al. 2006). However, there are at least some consistent findings involving the SMA region between studies using dopamine antagonists and studies in people with Parkinson's disease. In addition there is some evidence of increased BOLD signal changes following amphetamine.

1.5.2. Working memory

In this section both human and animal paradigms relating to working memory will be briefly examined.

1.5.2.1. Animal experiments

A large number of studies emphasize the importance of PFC neurons for particular aspects of working memory (Seamans and Yang 2004). Lesions of the PFC do not affect short term memory (Manes, Sahakian et al. 2002); instead they relate to manipulation of information for thinking or planning. Dopamine plays an important role in the modulation of PFC in working memory with D1 receptor activation probably being more important than D2 receptor activation. A common paradigm used to test working memory is the variable delayed response task. In this task (Arnsten, Cai et al. 1995), bait is put in one of two pots which can be seen by a monkey. The pots are covered with cardboard and an opaque screen is placed between the monkey and the pots for a time interval. The screen is removed after the interval, and the animal responds to obtain the bait. This can be made more difficult by increasing the length of time the opaque screen is kept in place.

Using a task with rodents similar to the variable delayed response task, dopamine levels in the prefrontal cortex have been examined in relation to the phase of the task (Phillips, Ahn et al. 2004). In the training phase of this task, rats were allowed to retrieve food from 4 open arms out of an 8 arm radial maze (the other arms were blocked). This was followed by a delay period where animals were excluded from the maze. In the test phase, animals had to identify the location of the food now placed in the arms that were blocked in the training phase. It was shown that dopamine levels in the PFC were elevated in the training phase and remained elevated for several minutes into the delay phase before falling to baseline. In the test phase, dopamine levels increased again and remained elevated for a few minutes after ending of the test phase. This result indicates that dopamine is involved in encoding and the use of information rather than the storage of the information.

A number of studies by Sawaguchi has examined the role of dopamine in modulating PFC neurons (Seamans and Yang 2004). In a typical study, the activity of prefrontal neurons in living monkeys in a delayed response task

similar to that described above was examined (Sawaguchi and Goldman-Rakic 1991). The activity of the prefrontal neurons was recorded at cue presentation, delay interval and response after the delay period. There were increased neuronal responses at these three time periods. Fluphenazine, haloperidol and sulpiride were applied using iontophoresis on the prefrontal neurons to examine the role of dopamine receptors in the task. Haloperidol and fluphenazine reduced neuronal activity to the cue, delay period and go period. Sulpiride did not have any clear effects. As haloperidol and fluphenazine blocks both D1 and D2 dopamine receptors whereas sulpiride blocks D2 but not D1 receptors this supports the theory that D1 receptors are important in modulating the activity of prefrontal neurons. This theory received further support from subsequent studies using selective D1 receptor antagonists (Seamans and Yang 2004). However, excessive D1 receptor stimulation (as may occur during stress) may inhibit neurons in the prefrontal cortex (Arnsten 1997). As a result, best performance relates to stimulation of an optimal amount of D1 receptors (Williams and Goldman-Rakic 1995; Arnsten 1997).

As suggested by the experiments above, D2 receptors seem to have a less important role in working memory compared to D1 receptors. In order to identify the specific role of D2 receptors, one study examined these receptors in young monkeys using the D2 receptor agonist quinpirole in a delayed response task similar to that described above (Arnsten, Cai et al. 1995). Different effects were found depending on the dose. Low dose quinpirole seemed to cause an impairment in performance but a moderate dose produced an improvement. At high doses of quinpirole, a number of behavioural side effects were evident including increased agitation and enhanced reactivity to stimuli thus impacting upon the performance of the task. In contrast, when animals pretreated with reserpine were tested with quinpirole, improved performances at low, moderate and high doses were noted.

Quinpirole is a compound that affects both presynaptic and postsynaptic D2 receptors. D2 autoreceptors which are presynaptic can inhibit dopamine release whereas antagonism of postsynaptic D2 receptors may inhibit PFC cell firing (Sesack and Bunney 1989). In the study described above, the effect of reserpine was to deplete dopamine in the projection neuron to the PFC. In this

case there is decreased availability of dopamine for release. As a result, quinpirole in the case pretreated by reserpine is unable to affect dopamine release via the presynaptic receptors. Hence the performance enhancing effect of quinpirole in the reserpine pretreated case is likely to be mediated by postsynaptic D2 receptors. The impairment induced by low dose quinpirole was reversed by raclopride a D2 receptor antagonist but was not affected by a D1 receptor antagonist SCH23390. This supported the notion that the effects of quinpirole were due to D2 receptor effects. Raclopride also reversed the improvement induced by moderate dose of quinpirole. However, the improvement by moderate doses of quinpirole was also altered by treatment with the D1 antagonist SCH23390. This indicates that the enhancing effects of moderate doses of quinpirole may involve interactions between dopamine D1 and D2 receptors. Quinpirole has very low affinity for D1 receptors so endogenous dopamine is likely to be mediating the effects on D1 receptors. This experiment is consistent with previous studies showing a role for D1 receptor activation in the PFC in working memory. However, it suggests that D2 receptor activation may have some role in this process as well.

1.5.2.2. Human studies

Working memory in fMRI studies in human subjects can be assessed indirectly by using the N-back task (Drobyshevsky, Baumann et al. 2006). In the 2-back version of this, a series of letters is presented one at a time to subjects who are required to figure out if the current letter presented was the same as that presented 2 previously. A control condition consists of asking the subjects to identify a particular letter (e.g., "X") in a sequence of letters. This task has the advantage that certain areas are activated very reliably when subjects complete it. These areas include the following: bilateral premotor cortex (BA6), dorsal cingulate/ medial prefrontal cortex (BA32, BA6), dorsolateral prefrontal cortex (BA 46, BA9), bilateral ventrolateral prefrontal cortex (BA 47, BA 45), left frontal pole (BA10), right medial posterior parietal cortex (BA 7), bilateral inferior parietal lobule (BA 40) and medial cerebellum (Owen, McMillan et al. 2005). However, the exact relationship between the BOLD changes detected in humans using this task and the experimental work in animals is not certain (Rowe and Passingham 2001; Ragland, Turetsky et al. 2002). Despite this uncertainty, BOLD signal in these areas in human studies may be altered by changes in dopamine activation in the brain. Two factors that influence

dopamine in the PFC include catechol-O-methyltransferase (COMT) and DAT (Bertolino, Blasi et al. 2006). COMT is an enzyme that degrades dopamine whereas DAT (as metioned previously) is responsible for the reuptake of dopamine. It was shown that the genetic polymorphisms of COMT and DAT hypothesised to result in increased activation of dopamine in the PFC was associated with less widespread BOLD signal changes during an N-back task (Bertolino, Blasi et al. 2006). The authors suggested that the increased activation of dopamine in this area allowed a more focused response for the working memory task. This suggests that increased dopamine activation facilitates the efficient performance of the task. In support of this finding, another study looked at siblings of people with schizophrenia and showed a relationship between the val / val genotype (resulting in deceased dopamine activation) and increased neuronal activation in the dorsolateral and anterior cingulate (Egan, Goldberg et al. 2001).

The effect of amphetamine in the performance of the N-back task has been examined in a number of different studies. Using measures of N-back performance such as sensitivity, false alarm rate and hit rate in healthy volunteers, one study (Mintzer and Griffiths 2007) was unable to demonstrate an effect of amphetamine. In addition, no significant effects due to amphetamine on reaction times were detected. Another group (Willson, Wilman et al. 2004) used a variation of a working memory task where participants were given a 5 digit number to remember and were subsequently tested with single digits. After amphetamine there was a reduced number of activated voxels in the left insula with trends towards reduction in the other areas. There was also a reduced BOLD signal in the left dorsolateral and the cingulate cortex. These results (same dose of amphetamine using healthy volunteers) were also found in a follow up study (Bell, Willson et al. 2005). In a double-bind crossover designed study (Mattay, Callicott et al. 2000) amphetamine seemed to have beneficial effects on those with weak baseline performance but caused deterioration in performance in those with strong baseline performance. There were no differences in performances across drug conditions. A three way interaction (load (task difficulty), time, drug condition) was detected in right prefrontal cortex (BA 9). On further examination of this region amphetamine seemed to result in a greater increase in BOLD signal for both the 2-back and

3-back levels relative to the no-back (0-back equivalent) level. Despite the findings of this study, on balance, from the studies using amphetamine and the dopamine genetic studies, it seems decreased activation of BOLD signal would likely occur with methamphetamine in an N-back task.

Another way of examining the role of dopamine in working memory in humans is to examine the effects of D2 receptor modulation. Some of these studies have used memory tests other than the N-back; however, they may still give an indication of the role of D2 receptor activation in these kinds of memory tasks. One group (Mehta, Swainson et al. 2001) examined the performances of healthy human volunteers in a cognitive task who were given the D2 receptor agonist bromocriptine**.** The investigators used the spatial span test taken from the neuropsychological battery CANTAB. This consists of nine white boxes on a screen which change colour in a particular sequence which must be reproduced by the participant. The sequences can vary from the easy condition of a 2 box sequence to the difficult 9 box sequence. The length of sequence produced in the group given bromocriptine was increased compared to the non bromocriptine group. In another study, the effects of sulpiride (a D2/D3 receptor antagonist) was examined (Mehta, Hinton et al. 2005). The task required people to search a number of boxes displayed on a screen for coloured tokens; they also had to remember whether previous searches had been successful. There was no impairment in a group given sulpiride compared to a control group. Dopamine D2 receptors may have a role in the manipulation of information for working memory as distinct from retrieval. This was examined in an experiment where healthy subjects were asked to complete a memory task with separate simple retrieval and manipulation conditions, before and after administration of 400 mgs sulpiride (Dodds, Clark et al. 2009). Lower sulpiride plasma levels were associated with greater activations in the putamen in the manipulation condition rather than the simple retrieval condition. It was suggested that some of the effects of D2 receptor activation in working memory could occur via effects in the striatum. Taking the results of the animal and human studies together, the role of optimal D1 receptor activation looks to be important in working memory however, the role of D2 receptor activation is more uncertain.

It can be useful to look at general theories that have been proposed of the role of dopamine in the prefrontal cortex. One theory describes the role of dopamine in signal to noise mechanisms in the prefrontal cortex. This theory emphasizes how the function of dopamine receptors in the prefrontal cortex can be understood based on their position on both GABA interneurons and on pyramidal cells (Abi-Dargham and Moore 2003). Dopamine receptor activation on GABA interneurons provides inhibition to pyramidal cells and D1 receptor activation decreases glutamate input to the cortical neurons (Gao, Krimer et al. 2001). Stimulation of post synaptic D1 receptors stabilizes inactivation during irregular glutamate input but spike firing and plasticity is enhanced during high levels of glutamatergic stimulation. The overall effect is that dopamine generally provides inhibition to the cortex thereby suppressing spurious spike activity. With sufficient glutamatergic input, D1 receptor activation facilitates spike firing so amplifying the output signal of the cortex. In this way, dopamine facilitates a signal to noise mechanism in the cortex (Abi-Dargham and Moore 2003). Imbalances in this process could lead to problems in cognitive tasks such as working memory. Excessive inhibition may result in too little information being retained whereas insufficient inhibition may result in too much information being retained causing confusion. This may help to explain how performance in working memory studies relates to stimulation of an optimal amount of D1 receptors.

The specific role of dopamine D2 receptors is more difficult to understand. However, Seamans and Yang (2004) propose a two state model based on dopamine's effect on both sets of dopamine receptors in the prefrontal cortex. It incorporates a computational model (Durstewitz, Seamans et al. 2000) where key effects of dopamine on cortical neurons were implemented and assembled into neural networks allowing non linear effects of dopamine to be incorporated within the model. In state 1, the effects of D2 receptors predominate, which causes a reduction of inhibition, thereby allowing access to the prefrontal cortex of multiple inputs. Hence, multiple representations are held within the prefrontal cortex in state 1. However, with D1 modulation, the network switches to state 2. Here inhibition increases, so inputs have less effect on prefrontal cortex but strong inputs that can overcome the increased inhibition have stable representation. This means that in the first case multiple representations can be

held but in the second state, a few strong representations dominate. This may translate to behavioural situations where in the first case an animal may need consider a number of options and decide flexibly; in contrast the second case allows the animals to hold a few representations in order to strongly guide behaviour. These states can be detrimental to optimal performance. Using D1 antagonists, a bias towards state 1 could be generated preventing the animal from reaching a decision. Bias to state 2 (by a D1 agonist) may result in perseveration errors and lack of flexibility.

This model can be applied to a working memory task by considering moderate activation of the mesocortical pathways. This would activate D1 receptors (extrasynaptic) switching the network towards state 2. During phasic bursts of dopamine, D2 receptors (intrasynaptic) may be activated switching the network to state 1. Thus, state 1 may be a transitional state in order to allow new information to reach working memory; with new goal state representation subsequently maintained using state 2 by D1 receptor activation. This model emphasizes the modulatory role of dopamine as the information is carried by glutamate receptors and dopamine determines the way the information is allocated within the prefrontal cortex. In addition, phasic bursts by dopamine neurons do not contain information but determines the representation of information provided by glutamate input. Later in the thesis, an account will be given of how this model can also be used to explain symptoms of schizophrenia.

1.5.3. Learning paradigms

1.5.3.1. Modelling of dopamine neurons using the TD algorithm

Although temporal difference computational models are not used explicitly used in this study. Predictions based on these models are used in the analysis so this is the main learning model presented. To highlight the concepts for these models one type (TD Q learning) will be described in detail. Some definitions of certain terms from the broader field of reinforcement learning (Sutton and Barto 1998; Russell and Norvig 2003) are presented to facilitate the understanding of the concepts in the TD Q learning model as follows:

- An **agent** is something which figures out how to interact with an environment for some goal - a rat in a maze trying to find food could be regarded as an (highly complex) agent.
- The **state** is the representation of the environment available to an agent. This could be the information related to the agent via its sensory processes.
- A **policy** describes how an agent chooses a particular action in a given state.
- A **reward function** defines what are good or bad events for an agent.
- A **value function** gives an agent an overall view of how useful a state is to it. It can be seen as an assessment of the total overall rewards an agent may expect to receive starting from that state.

One type of value function is called the Q function. It relates to an agent the value of taking a particular action in a particular state. In a particular state one action may be advantageous for the agent over the longer term (high Q value) but taking a different action would be disadvantageous for an agent (low Q value). The Q value for a particular state and action is written as Q (s, a). TD Q learning (Watkins 1989; Sutton and Barto 1998; Russell and Norvig 2003) is one way of figuring out a Q values for a particular state and action. In this approach, an agent chooses a particular action in a given state according to some policy. It uses the size of the reward consequent to this action and its estimate of subsequent Q values in order to adjust the Q value that it had (given the state and action it took). This can be summarised in the following equation.

$$Q(s_t, a_t) \leftarrow Q(s_t, a_t) + \alpha \left[r_{t+1} + \gamma \max_a Q(s_{t+1}, a) - Q(s_t, a_t) \right] \qquad (1)$$

Here $Q(s_t, a_t)$ is the estimate by an agent of taking action a at time t. The term $\max_a Q(s_{t+1}, a)$ refers to a process where an agent checks all the actions at time t+1 and identifies which action would give the highest Q value. If this is a high value, it may indicate that an agent is on the "right track" to achieve its goal. Furthermore, if this is much larger than the $Q(s_t, a_t)$ then it may be that it underestimated how useful $Q(s_t, a_t)$ had been. r_{t+1} indicates the reward that follows an action taken by the agent when in state s: this is a form of immediate feedback to the agent. It can use this immediate form of feedback as well as the

"longer term view" from the term $\max_a Q(s_{t+1})$ to improve its value of $Q(s_t, a_t)$. γ is a temporal discounting factor which is a form of bias towards Q values that are likely to occur in the near rather than the distant future.

The term $[r_{t+1} + \gamma \max_a Q(s_{t+1}, a) - Q(s_t, a_t)]$ is a form of error term that is sometimes written as δ_t. This allows the equation to be rewritten as below.

$$Q(s_t, a_t) \leftarrow Q(s_t, a_t) + \alpha [\delta_t] \qquad (2)$$

So it can be seen how the error term is used to update the value of $Q(s_t, a_t)$. The term α is a learning rate parameter which determines how large the adjustments should be when updating the value of $Q(s_t, a_t)$. This allows the effects of updating, using the error term δ_t, to occur on a gradual basis. By applying the above equation repeatedly to all the states an accurate table of Q (s, a) values can be generated. Once these values are generated, it effectively means that the agent, in a given set of circumstances, is better able to choose actions for a desired outcome. It can be seen how this process could be applied to action outcome learning (the completion of an action for the intention of obtaining a goal) as described for animal experiments (Everitt and Robbins 2005).

The TD model has been used in modelling the activation pattern of dopamine neuron activation as can be seen in Figure 1.5-2. This figure (adapted from (Kakade and Dayan 2002) has been derived from experiments where dopamine neuron activity has been directly recorded in animals while learning the association between a stimulus and reward. At the early stage of learning there is increased dopamine neuron activity at the time of the reward but not at the time of the stimulus. After the animal has learnt the association, there is increased dopamine neuron activity at the time of the stimulus but not at the time of reward. The increase in dopamine neuron activity can be related to the concept of a reward prediction error term. At the outset of learning the occurrence of the reward is unexpected as the animal has not yet learnt that the stimulus predicts it (Schultz 2000; Waelti, Dickinson et al. 2001). Hence there is a discrepancy between what the animal predicts and what occurs at the time of the reward: so a reward prediction error occurs which is reflected by the phasic

dopamine neuron activity. After learning, the animal predicts the reward given the occurrence of a stimulus so when the reward occurs there is not a reward prediction error and this is reflected by the absence of phasic dopamine neuron activity at the time of reward. At this stage, the stimulus predicts the reward so now the stimulus acts like a reward in an early stage of learning explaining the phasic dopamine neuron activity at the time of the stimulus. This reward prediction error can be related to the δ_t error term that occur in TD models (as in the Q learning model above) allowing these algorithms to model dopamine neuron activity (Montague, Dayan et al. 1996). Although this model was initially related to impulse frequency of dopamine neurons it has also been shown to be consistent with neuronal dopamine release (Day, Roitman et al. 2007).

One benefit of this computational model is that it enables predictions to be made about the learning of a task to relate to dopamine neuron function. In the study in this thesis, it was assumed that an increase in the size of the error signal (δ_t) would be related to an increase in dopamine release following methamphetamine. From equation (2) above, the final value of Q (s_t, a_t) could be reached with less iterations in those with methamphetamine than those without methamphetamine. In the learning task used in the study in this thesis it was assumed this would mean that participants would select an optimal action more often with methamphetamine.

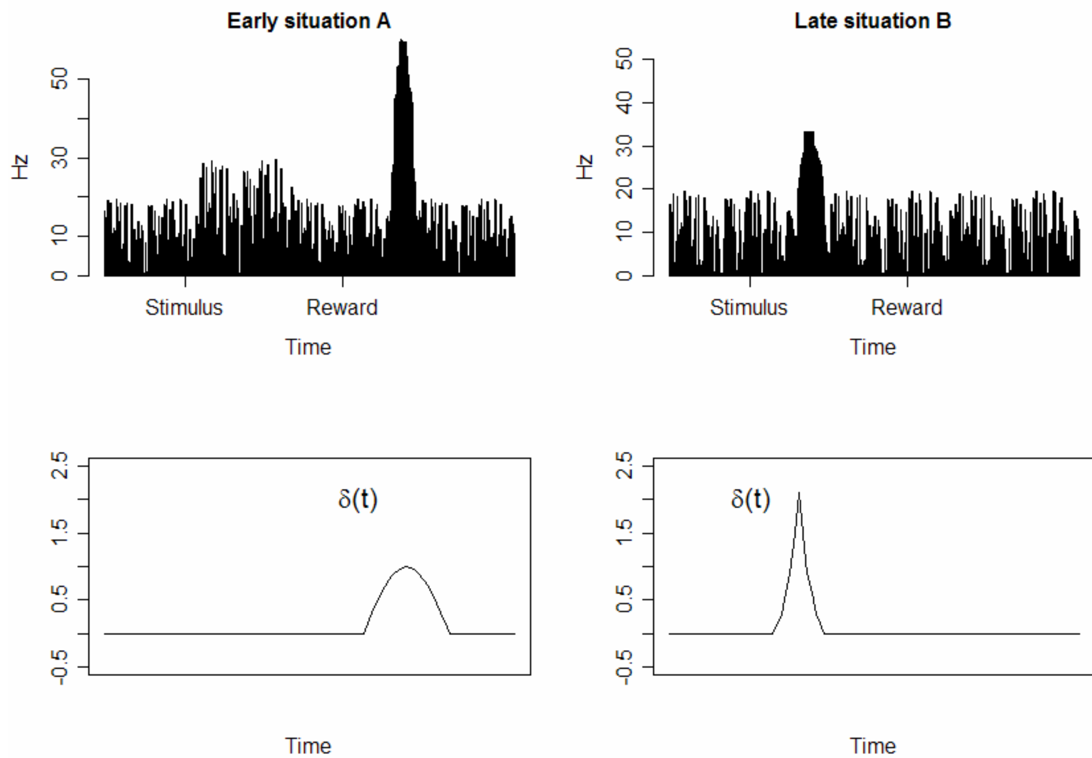


Figure 1.5-2
Histogram of the activity of a dopamine neuron above and representation of Temporal Difference (TD) error signal below. Modification of diagram from the original paper (Kakade and Dayan 2002) for illustration purposes. The TD error term is generated using TD algorithm methods. The TD error term also broadly corresponds to the reward prediction error term described in the text. In situation A (early learning) a dopamine neuron responds to the delivery of reward but not to the presentation of the stimulus that predicts reward. This is similar to the TD error signal (δ (t)). After an animal learns to pair a stimulus with a reward, (situation B), a dopamine cell responds to the delivery of stimulus but not to the reward. Again, this matches the TD error signal (δ(t)).

Another appealing aspect of this model is how it incorporates characteristics of dopamine neurons that have been previously discussed. First, the model needs to have some mechanism for increasing and decreasing dopamine burst activity. The model of Goto and Grace provide plausible ways that this could be done. Another way this could occur is through the inhibition and excitation of the SNc from the striosomes and matrix areas in the striatum (Schultz 1998). As this is a learning algorithm, some form of memory process is also required. As described before, dopamine also has a role in the memory related processes (Nestler, Hyman et al. 2009) of LTD and LTP. This shows how dopamine could influence the biological components required for the implementation of the model. The biological plausibility of the model is also one of its strengths.

1.5.3.2. Modelling learning paradigms in humans

The ability to relate dopamine neuronal activity to the TD error signal in animal studies has led to experiments trying to identify a similar phenomenon in humans. One approach has been to use fMRI to detect alterations in BOLD signal related to predicted changes according to the TD model. A wide variety of such studies has now been completed (Niv 2009; Balleine and O'Doherty 2010). An early study (O'Doherty, Dayan et al. 2003) using this framework examined subjects who learnt the association between an unconditioned stimulus (US) (abstract picture) and a conditioned stimulus (CS) (liquid solution injected into the subject's mouth). Three types of solution were used: fruit juice, solution isotonic with saliva and a tasteless solution. These corresponded to a positive CS, neutral CS and a negative CS. The TD error signal which occurs initially at the time of reward and subsequently at the time of stimulus presentation was used in a regression with the fMRI data. This showed significant BOLD signal changes at the ventral putamen, ventral globus pallidus (GP), left orbitofrontal cortex and dorsal prefrontal cortex. Groups have since used TD models to model BOLD responses in fMRI studies in subjects completing second order conditioning tasks (Seymour, O'Doherty et al. 2004) and subjects indicating their preference within a conditioning experiment (O'Doherty, Buchanan et al. 2006). A similar form of algorithm to the TD model has been used to model the BOLD responses in action selection paradigms involving abstract rewards (Haruno and Kawato 2006). Another algorithm related to the TD model has been used to model BOLD responses in the learning of an instrumental task. The study showed the particular importance of the dorsal striatum in that process (O'Doherty, Dayan et al. 2004).

In view of the animal work suggesting a role of dopamine in the TD error signal, these studies led to further work looking at how the BOLD responses could be altered by drugs which act on dopamine receptors. In one such study (Pessiglione, Seymour et al. 2006), subjects were randomly given either haloperidol, levodopa (L-dopa) (a metabolic precursor of dopamine used as a dopamine agonist) or placebo. In the study, a pair of stimuli was presented simultaneously on a computer screen and participants could select one by a button press or the other by omission. Depending on the stimuli presented, different feedback occurred following the type of action chosen. Thus, there

were three pairs of stimuli and two types of outcome for each pair of stimuli depending on the action taken as shown in Table 1.5-1.

Type of stimuli	Selection of stimulus by taking an action	Default selection of stimulus following omission of action
Gain	Picture of coin and word "GAIN"	Word "NOTHING"
Loss	Picture of coin and word "LOSS"	Word "NOTHING"
Neutral	Word "NOTHING"	Word "NOTHING"

Table 1.5-1
Stimuli and responses of a learning task in humans where dopamine manipulation was used (Pessiglione, Seymour et al. 2006).

Learning which action to press was modelled using the Q learning algorithm similar to the TD Q learning model outlined previously. The error term generated in the model was used in a linear regression analysis with BOLD related brain activity across all trials in all three groups. This revealed positive correlation between the reward prediction error and activation in the bilateral ventral striatum and the left posterior putamen in both loss and gain conditions. In addition there was a negative correlation between the error term and cluster activation in the right anterior insula in the loss trials. The authors suggest that in the loss condition there may be mechanisms both for appetitive (positive correlation) and aversive (negative correlation) processes. In the analysis, a contrast between the gain and neutral condition showed increased activation in the ventral striatum bilaterally; a similar finding was seen in the contrast between the loss and neutral conditions. In the latter contrast, the bilateral anterior insula was also activated. To assess the effect of drugs, the clusters reflecting prediction errors were examined and averages taken of the BOLD signal changes for the different outcomes and drug conditions. There was an increase in both the negative and positive BOLD signal for the L-dopa group compared with the haloperidol group for the gain trials. (This is seen in the top two plots in Figure 3 in their paper). There was no significant effect for the loss trials.

A related study examined the effect of pharmacological manipulation in an aversive learning task (Menon, Jensen et al. 2007). The effects of amphetamine, haloperidol or placebo were examined in participants completing this task whilst undergoing fMRI. In this study two stimuli were presented to

subjects. One of the stimuli was followed by an electric shock one third of the time whereas presentation of the other stimulus was never followed by an electric shock. The former stimulus was referred to as condition stimulus positive (CS+); the later as condition stimulus negative (CS-) and the electric shock was regarded as an unconditioned stimulus (US). The learning model used in the study was a TD model (akin to the TD Q learning model presented earlier). Similar to previous studies (O'Doherty, Dayan et al. 2003), the TD error signal was used in a regression with the fMRI data to obtain the changes in the BOLD response. In the placebo group, changes in the BOLD response in the left ventral striatum could be related to the TD error signal. However, in the amphetamine group the TD error signal could be related to changes in the BOLD response in the bilateral ventral striatum, right GP and putamen, bilateral cingulate, bilateral SN and bilateral insula. It was not possible to relate the TD error signal to changes in the BOLD response in the striatum in the group given haloperidol. In the comparison between the amphetamine group and placebo group there was greater TD error signal related activity bilaterally in the dorsal caudate and in the left SN. This was similar to the comparison between the amphetamine group and haloperidol group. In the comparison between the placebo group and amphetamine group there was greater TD error signal related activity in the right medial orbitofrontal cortex which was similar to the comparison between the haloperidol groups and the amphetamine group. The authors acknowledged that the BOLD response related to the TD error signal might not directly correspond to the phasic increase of dopamine neuron activity as in animal experimental models. Instead the authors suggest that the increased activity may result in increased dopamine release enhancing postsynaptic long term potentiation or long term depression.

An alternate study examined the effects of amphetamine on an incentive processing task (Knutson, Bjork et al. 2004). This was not a learning task but aspects of it can be usefully compared to the other studies above. In the study, participants were given a cue followed by a target presentation during which participants had to press a button followed by feedback. Different cues indicated different degrees of gain and loss that were given in the feedback. A hit occurred when button press was followed by the gain outcome and likewise a miss occurred following a button press without the gain. Compared to placebo,

it was demonstrated that amphetamine decreased BOLD signal changes in the ventral striatum in the comparison between anticipation of gain with non gain. For loss versus non loss anticipation, amphetamine had similar regions of deactivation (decreased BOLD signal changes) compared to placebo (medial prefrontal cortex and bilateral prefrontal cingulate) but also deactivation of medial caudate nucleus accumbens and anterior cingulate. For hit versus miss outcomes, amphetamine caused BOLD signal changes in similar regions as placebo (left medial prefrontal cortex, left nucleus accumbens and posterior cingulate). Amphetamine did not result in activation for loss avoidance versus miss outcome whereas placebo showed activation in the bilateral superior temporal gyri and right putamen. Amphetamine was shown to increase peak activation in the nucleus accumbens for loss anticipation and decreased activation for gain anticipation– the authors interpreted this as amphetamine equalizing the effects of large positive and negative incentives in the nucleus accumbens. In addition, amphetamine seemed to blunt the peak activation but prolong its duration during anticipation of gains. The authors suggested that amphetamine may have more an effect on tonic rather than phasic aspects of the modulation of the ventral striatum.

A different approach to examine the role of dopamine in learning paradigm is to compare the prediction error between people with PD and healthy controls (Schott, Niehaus et al. 2007). It was found that in the PD group the prediction error signal was preserved in the ventral striatum but impaired in the dorsolateral striatum. This reflects the dopamine neuron degeneration pattern in patients with Parkinson's disease. This study offers more indirect evidence that the prediction error signal in humans relates to dopamine neuron activity. In another study examining the relationship of dopamine on reward processes (van Eimeren, Ballanger et al. 2009), a game was used where, in one set of trials there was a high probability of picking the reward but in the other set of trials there was a low probability of picking the reward. The sample consisted of people with Parkinson's disease treated with levadopa and pramipexole (a dopamine D1, D2, D3 receptor agonist (Kvernmo, Hartter et al. 2006)). The participants were shown a stake that was to be used in the trial. Aspects of the paradigm modelled as regressors included: presentation of the stake; button press; the stimulus indicating when outcome was due and, the outcome. In this

study participants were either off medication, given L-dopa or given pramipexole. Irrespective of drug condition, at the presentation of outcome, there was an increase in BOLD signal in cerebellum, visual cortex, putamen, cingulate motor area and ventral premotor cortex. Both L-dopa and pramipexole diminished reward processing compared to being off medication in the ventral striatum. Although this sample consisted of people with PD, this part of the study is inconsistent with some of the studies mentioned above. In the orbitofrontal cortex, only pramipexole diminished local reward processing. Pramipexole seemed to increase the orbital frontal activation in trials with negative RPE values. This was taken to represent pramipexole preventing negative reinforcement which the authors view as a tonic rather than a phasic dopamine effect. The authors suggested that pramipexole may prevent pauses in D2 signalling and in this way, impair negative feedback learning.

From the overview of the above studies it can be seen that modelling BOLD responses using TD related models is a useful approach in learning paradigms. In addition, some other studies not employing the TD algorithm have also detected activations in similar areas to the studies using the algorithm. These effects may be modifiable using dopamine agonist and antagonists although the results are not fully consistent between studies. The studies using algorithms related to the TD Q learning model (Pessiglione, Seymour et al. 2006; Menon, Jensen et al. 2007) were those that provided the main basis of the reward learning task that was used in the study in this thesis.

1.6. General theories of dopamine function

There are a wide number of theories about the function of dopamine in the brain. For finger tapping, the differences between the indirect (D2 receptors) and direct pathways (D1 receptors) constitute a way of understanding the effects of dopamine on the motor system. In the PFC, dopamine has been viewed as part of a signal to noise mechanism (Abi-Dargham and Moore 2003) and also as a way of modulating a 2 state system (Seamans and Yang 2004). The model given for reward based learning functions of dopamine is based mainly on ideas of reward prediction (Montague, Dayan et al. 1996). What follows is a brief review of the other theories of dopamine function particularly in relation to reward and learning.

One influential theory is based on the idea of incentive salience (Berridge and Robinson 1998; Berridge 2007). This framework views reward as a composite construct comprising the components wanting, learning, and liking. Dopamine is viewed as having a role in modulating the 'wanting' component. This is achieved by dynamic attribution of incentive salience to reward-related stimuli. This process consists of three stages. In the first stage, conditioned stimuli are regarded as having no motivational value beyond novelty. They are merely perceptual stimuli. Due to curiosity or chance, an animal may encounter a stimulus and liking may occur as a result of a property of the unconditioned stimulus. For example, it may comprise a food substance with a pleasant taste. The pleasure experienced by the animal is a triggered affective state. In the next stage, associative learning takes place characterized by a development of a correlation between the hedonic activation by the unconditioned stimulus and a preceding external event. The third stage of the process consists of attribution of incentive salience. This stage is needed to transform the perception of a conditioned stimulus at a distance into an incentive which elicits appetitive or instrumental behaviour towards it. After this stage, the stimulus is now both liked and wanted. Furthermore, each time the wanted stimulus is followed by activation of hedonic liking, then the wanting component is reboosted. Without the reboosting, reward extinction would follow. Wanting and liking are regarded as being implemented in separate neural structures. Dopamine neuron input to the striatum subserves wanting whereas liking may be implemented by the GABA systems in the brain stem or ventral pallidum, or by the opioid system in nucleus accumbens shell.

It can be hard to tease apart the processes of wanting and liking components, as even at a first point of contact with an unconditioned stimulus, liking often activates wanting. However, wanting can be selectively identified when two successive stimuli are used to predict a reward (Tindell, Berridge et al. 2005). The first stimulus maximally predicts the reward. However, the second stimulus does not add any further predictive value but being closer to the reward has greater incentive salience. Amphetamine effects seem to amplify the stimulus nearest the reward but not the other stimulus. This is used as evidence that the effects of dopamine specifically relate to incentive salience. There are some reservations about this model (Wise 2006). For example, one of reservations is

that the liking concept in the model may not be able to explain how humans learn to like bitter tastants like broccoli (Wise 2006). Despite reservations expressed about the model, it has been influential and a concept similar to incentive salience has been used in a model of schizophrenia discussed later in the thesis (Kapur, Mizrahi et al. 2005).

Another way to explain the function of the central dopamine system, is to consider it in terms of an “energetic” construct (Robbins and Everitt 2007). This is based on the effect of dopamine on vigour and frequency of animal behaviours in various experimental paradigms. An increase in the mesolimbic dopamine activity to the ventral striatum (e.g., through the use of amphetamine) causes increased responsiveness to cues paired with reinforcement and so enhances appetitive approaches to a goal. For mesostriatal projections to the dorsal striatum, the role of dopamine may be to help preparation for performing a particular response thereby increasing stimulus-response coupling. In this model, the ventral striatum is an area that processes affective and goal-related information generated in the limbic cortical structures. The dorsal striatum is involved in processing of information to and from the motor and premotor regions of the cortex related to the preparation and generation of programmes of well-learnt behaviour. The model also accepts a likely role for striatal dopamine operating in its phasic mode in new learning, for CS-US associations in the ventral striatum and stimulus-response associations (“habits”) mediated by dorsal striatum.

Although reinforcement learning forms part of computational models outlined previously, the concept of reinforcement learning also relates to animal experiments whereby stimulus and response associations are generated (Wise 2006). According to this model, brain dopamine has an important but not necessary role in the “stamping in” of the associations between stimulus and responses. It can be seen that there is some similarity between aspects of this theory and that of the “energetic” construct (Robbins and Everitt 2007) above.

There are some timing issues that are inconsistent with current ideas about phasic dopamine being used as a reward prediction error (Redgrave, Gurney et al. 2008). The time for an animal to bring into focus an unexpected sensory

event is longer that the period between the onset of a stimulus and appearance of the phasic dopamine signal. It could be that this signal instead reflects subcortical processing in the superior colliculus. This pathway would not allow detailed stimulus processing required to enable dopamine neurons to usefully code for reward related events. Instead it could be that the phasic dopamine signal is used to reinforce the selection of the action and (thereby the environment) that preceded the occurrence of the biological event (Redgrave, Gurney et al. 2008). In this way, when a behaviour of an agent has resulted in a sensory event such as a reward the agent can gradually be identified as the cause. In addition, the critical causative behaviour of the agent can be identified.

A variation-selection model using the dopamine system has also been described (Ikemoto 2007). It is suggested that the meso-ventromedial striatal dopamine system allows the generation of unconditioned responses (variation). Selection of particular behaviours subsequently by the animal is achieved by modulating associative learning using the meso-ventrolateral striatal dopamine and the meso-dorsal striatal dopamine systems.

These descriptions give an overview of some of the theories regarding dopamine in animal behaviour. Although disparate, the role of dopamine for increasing drive, and the development of associations is fairly consistent in the models. For this thesis, the main perspective used in the reward learning task is broadly consistent with the TD algorithm.

1.7. Role of dopamine in psychiatric illnesses

Schizophrenia and addiction disorders are two psychiatric illnesses where understanding dopamine function can provide insights into the pathophysiology of the condition. Schizophrenia can be considered as a syndrome that is characterized by long duration, bizarre delusions, few affective symptoms and the presence of negative symptoms (lack of motivation, reduction in spontaneous speech and social withdrawal) (van Os and Kapur 2009). The aetiology of schizophrenia is complex but currently it is viewed as a disorder developing in individuals due to the interaction of a range of factors (genetic, environmental, drug exposure) that occurs throughout a person's life (Murray, Lappin et al. 2008). One of the key feature of schizophrenia is that during acute

episodes of illness, there is increased release and synthesis of dopamine (Laruelle, Kegeles et al. 2003; van Os and Kapur 2009).

Treatment of schizophrenia is by antipsychotic drugs (Lieberman 2006). These drugs are varied in pharmacology, but in each case, their therapeutic action is by acting on dopamine D2 receptors. The majority work as antagonists of the dopamine D2 receptor (Kapur and Mamo 2003) but the relatively new compound aripiprazole acts instead as a partial agonist (Kane, Carson et al. 2002). A complicating factor in trying to understand the role of dopamine dysfunction in schizophrenia is that dopamine dysfunction is often discussed in relation to psychosis (delusions and hallucinations) which is usually present in schizophrenia but can also occur in other disorders. For the purposes of this discussion, the findings of dopamine dysfunction for psychosis are taken to apply to schizophrenia.

The concept of reward prediction and “motivational salience” has been used to explain psychotic symptoms (Kapur, Mizrahi et al. 2005). Motivational salience here is similar to the concept of incentive salience outlined above (Berridge 2007). In this context, it relates to stimuli that become the focus of goal directed behaviour. The authors of the model propose that various genetic and environmental factors combine to result in dopamine dysfunction in schizophrenia. This results in the release of dopamine independently of cue and context leading to abnormal assignment of salience by the affected person to external stimuli and internal representations. Delusions can be viewed as “top-down” attempt by the person to make sense of these abnormal experiences. It is suggested that antipsychotic drugs attenuate the abnormal salience which prevents formation of further symptoms. The previously developed delusions are then gradually worked through psychologically by the patient.

Another way of explaining the strange experiences suffered by people with schizophrenia due to abnormal dopamine function is by considering the 2 state model of the prefrontal cortex (Seamans and Yang 2004) as outlined previously. Using this model, it is suggested that stable persistent activity states are required to maintain information in working memory until an appropriate response is executed. Low dopamine tone on D1 receptors could cause

premature termination of information in working memory prior to the completion of a thought or action. As a result, the networks encoding information could get contaminated by weak stimuli normally ignored. This could result in people being susceptible to distractibility and so experience intrusive or tangential thought patterns as can occur in people with schizophrenia. This would be predicted by a strong state 1 and could be countered by a forcing the system to transition to state 2 by blocking D2 receptors by the use of antipsychotic drugs.

Goto and Grace outline a way of viewing the cognitive problems of schizophrenia (Goto and Grace 2008) in terms of abnormal dopamine function within their model comprising the nucleus accumbens, prefrontal cortex and limbic regions (described earlier). According to this view, the key problem arises from abnormal nucleus accumbens information processing secondary to dysfunction of the prefrontal cortex and the hippocampus. As basal hippocampal activity can be higher in patients with schizophrenia than healthy controls (Heckers, Rauch et al. 1998), this may result in increased release of dopamine at the nucleus accumbens. As a result of the increased dopamine there may be excessive facilitation of the limbic-nucleus accumbens circuit and reduction of the prefrontal – nucleus accumbens circuit. This may then lead to disruption of behavioural flexibility. This could be translated into human behaviour as a reduced ability to switch response strategies - a feature commonly found in people with schizophrenia (O'Grada and Dinan 2007). By blocking D2 receptors, antipsychotic drugs could facilitate LTP at the prefrontal cortex and LTD at HPC (Goto and Grace 2005b) thus generating a more favourable balance between the two circuits. This could explain some of the improvements in executive function due to antipsychotics seen in people with schizophrenia (O'Grada and Dinan 2007).

Addiction is a chronic disorder defined by a number of features such as a compulsion to seek and take drugs, loss of control to limit intake and a negative emotional state reflecting a motivational withdrawal syndrome when access to drugs are denied (Koob and Volkow 2010). The World Health Organisation lists a wide number of substances that can be used for addiction including alcohol, opioids, cannabis, sedatives, tobacco, stimulants, hallucinogens and volatile substances (WHO 1992). The understanding of

addiction to drugs should take consideration of its complex cultural and psychosocial contexts (Lingford-Hughes, Welch et al. 2004). However, it can be useful to consider it in simpler terms using animal models. In this section, the animal model as outlined by Everitt and Robbins shall be described as this model emphasizes the importance of dopamine in the understanding of addiction (Everitt and Robbins 2005).

Everitt and Robbins note that it is important to draw a distinction between the reinforcing aspects of drugs of addiction and their rewarding aspects. The reinforcing aspects of drugs of addiction results in the increased likelihood of responses that result in their administration. The rewarding aspects of drugs relate to the subjective effects associated with these drugs such as the sensing of autonomic activity or distortions in sensory processing. The differences between the reinforcing aspects and the rewarding aspects of these drugs suggest that they are implemented in separate neural structures. The shell of the nucleus accumbens is a likely site for the primary reinforcing effects of drugs as it is an area that is targeted by motor and autonomic centres and it is a necessary structure for the occurrence of the direct psychomotor stimulant effects of amphetamine (Parkinson, Olmstead et al. 1999). The complexity of identifying reward processes distinctly in animals makes it a difficult task to identify their neural implementation with confidence.

An important component in the animal model of Everitt and Robbins is the concept of a conditioned reinforcer. This is a stimulus that initially is motivationally neutral but become reinforcing through association with primary reinforcers such as food or drugs. Animals exposed to conditioned responders will continue to respond for a time period even without the direct reinforcement of drugs of abuse. Thus, conditioned reinforcers can act to bridge the time delay to the ultimate goal of drug taking. An interesting effect of drugs of abuse (e.g., amphetamine) is that they can increase the responding to conditioned reinforcers (Taylor and Robbins 1984). This effect depends on the nucleus accumbens core and mesolimbic dopamine projections to the nucleus accumbens shell - emphasising the importance of dopamine structures in this behaviour (Parkinson, Olmstead et al. 1999). A strength of the model of Everitt and Robbins as a way of understanding addiction is how the concept of

conditioned reinforcers can be translated to the real world of illicit drug use. A well described aspect of addiction is that a person spends considerable amount of time seeking drugs often to the detriment of other former pleasurable activities (WHO 1992). It can be seen how conditioned reinforcers in the form of drug-associated stimuli (e.g., syringes) could reinforce the seeking of drugs for long periods of time even when there is not an opportunity (e.g., due to lack of finances) to experience the illicit drug.

Everitt and Robbins suggest that drug self administration initially involves the formation of action outcome associations but subsequently stimulus response associations. This transition could be implemented by a brain circuit comprising the nucleus accumbens shell, nucleus accumbens core and dorsal striatum (based on the circuit for primates previously mentioned in this thesis (Haber, Fudge et al. 2000)). The nucleus accumbens shell is the main site for reinforcement and could influence the dopamine input to the nucleus accumbens core. The core is important for the formation of Pavlovian related processes and could influence the dorsal striatum via dopamine projections from the substantia nigra. The dorsal striatum is the likely site of habit formation. The importance of the dopamine neurons in the circuit emphasises its role in the understanding of addiction processes. This model is consistent with ideas in the current study as the account of stimulus outcome associations in the ventral striatum and habits in the dorsal striatum relate to similar ideas described previously using fMRI paradigms (O'Doherty, Dayan et al. 2003; O'Doherty, Dayan et al. 2004).

1.8. General aims of the study

1.8.1. Rationale

There were two broad aims in the study. One aim was to use challenge phMRI to examine dopamine function in humans. Although amphetamine related compounds have been frequently used in modulation phMRI, challenge phMRI studies have been rare. The direct investigation of dopamine receptor function in humans is usually completed with resource intensive imaging techniques using radioactive agents (e.g., by PET). Challenge phMRI does not expose people to radiation and does not require invasive procedures such as insertion of arterial lines (Leslie and James 2000; Frackowiak and Jones 2003).

Assuming that activation of dopamine receptors could be detected with the challenge phMRI technique, the next aspect of the experiment was to detect the degree to which an increase in dopamine release and consequent D1 and D2 receptor activation could be counteracted by selective D2 antagonism. The choice of a D2 antagonist to counteract the effects of D1 and D2 activation was based partly on some evidence of this effect in animal studies using challenge phMRI (Dixon, Prior et al. 2005). In addition, D2 receptor blockade in humans is the main treatment for schizophrenia – a disorder characterized by the increased release of dopamine during acute episodes of illness.

Due to the relative ease of use of fMRI, there is an extensive literature on the use of fMRI to examine BOLD signal changes during cognitive tasks. Another aim of the study was to examine how modulation of dopamine receptors affects these types of fMRI paradigms. There is more experience with modulation phMRI using dopamine related agents although it is still relatively rare and the use of multiple drugs in these kinds of studies is uncommon. The N-back was chosen mainly because of the recognised role of D1 receptors in working memory and how it relates to BOLD signal changes with increased levels of dopamine. It was felt that the study might help to clarify the role of D2 receptors in working memory. A finger tapping task was used because of the long standing models of motor function comprising alternate pathways related to D1 and D2 receptors. It was felt that this could be usefully explored in the study. In addition, as mentioned previously in this thesis, a type of modulation phMRI study in recent years showed unexpected findings (Tost, Meyer-Lindenberg et al. 2006) using a motor task. The use of computational models has been increasingly important in the understanding of associative learning processes in animals. There have been relatively few studies examining how dopamine receptor manipulation affects learning of associations in humans using the framework of computational models. This was the reason why a modulation phMRI task involving a reward learning task was used. Although no computational model was to be explicitly used in the analysis of the modulation phMRI task, some of the predictions were framed in the context of simple interpretations of a computational model (TD model).

1.8.2. Hypotheses of study

There were a number of different parts of the study and each part had separate hypotheses. For the challenge phMRI technique, it was possible to examine whether amisulpride could antagonise the subjective experiences caused by methamphetamine. There are different findings between older (antipsychotics antagonise amphetamine effects) and more recent findings (antipsychotics do not antagonise amphetamine effects) related to this but the hypothesis used was based on findings from the more recent studies (Brauer and de Wit 1996; Wachtel, Ortengren et al. 2002). For the imaging part of the task, both the work in a similar animal study (Dixon, Prior et al. 2005) and based on the use of amisulpride in the treatment of schizophrenia (a disease with increased dopamine release), led to the expectation that amisulpride would antagonise the effects of methamphamine. Due to the relative novelty of this part of the study, it was difficult to specify a priori how the BOLD signal changes would relate to specific dopamine receptors. These points led to the following hypotheses for this part of the study:

1. Amisulpride would fail to antagonise the subjective effects of methamphetamine.
2. Methamphetamine would cause increases in BOLD signal in projection sites of dopamine neurons such as the caudate nucleus, putamen and orbitofrontal cortex. It was expected that decreases would occur in frontal, amygdala and enterorhinal cortical regions. Pretreatment with amisulpride was expected to attenuate the effects of both the increases and decreases in BOLD signal due to effects of methamphetamine.

For the N-back task, there could be an improvement in performance related to increased activation of dopamine D1 receptors due to methamphetamine. On the basis of studies with amphetamine, it was expected that methamphamine would result in decreased BOLD signal compared to placebo. The effects on working memory seem more related to D1 receptors so it was not expected that amisulpride would antagonise the effects of methamphetamine. This led to the following hypotheses for this part of the study:

1. Methamphetamine would decrease activation in the prefrontal cortex. This was expected to occur through activation of D1 receptors and consequent more efficient neuronal activity. There was not expected to

be marked attenuation of this effect by D2 blockade using amisulpride pretreatment.

2. It was expected that more accurate performance of the N-back task would occur in the methamphetamine group. Amisulpride would fail to attenuate this effect.

For the finger tapping task, similar to a related study (Tost, Meyer-Lindenberg et al. 2006), the model of Alexander and Crutcher (1990) was used as the basis of predicted results. This model suggests that D2 blockade could have antagonistic effects on cortical areas activated by methamphetamine. This led to the following hypothesis:

1. Methamphetamine would increase activation in the cortical motor regions through the direct motor pathway via activation of D1 receptors. This was expected to be attenuated by amisulpride pretreatment as this would inhibit the indirect motor pathway by blockade of D2 receptors.

For the reward learning task, the main predictions were based on the modelling of dopamine neuron function of animals using the TD algorithm (Montague, Dayan et al. 1996) and previous similar human studies (Pessiglione, Seymour et al. 2006; Menon, Jensen et al. 2007). As described in the introduction earlier, an increase in dopamine release due to methamphetamine would lead to the prediction that the performance in a reward learning task would be enhanced (more frequent selection of an optimal response). It was assumed that this would be antagonised by amisulpride. For the fMRI aspects of the task, using the same model, it was expected that there would be a decrease in dopamine release at the time of the outcome of a rewarding event and an increase in dopamine release at the onset of a stimulus predicting the rewarding event whilst a participant learnt a reward learning task. This alteration in dopamine levels would be matched by differences in BOLD signals. As a result there would be increased BOLD signal at the projection sites of dopamine neurons at the time of outcome of a reward event in the early part compared to the late part of a reward learning task. Similarly there would be increased BOLD signal in the projection sites of dopamine neurons at the time of onset of a stimulus predicting a reward event in the late part compared to the early part of a reward learning task. The areas of BOLD signal changes would be similar to each

other. These effects would be amplified by methamphetamine and attenuated by amisulpride pretreatment. Based on related studies, the most likely areas of the BOLD signal changes related to phasic release of dopamine were expected to be in the dorsal and ventral striatum. This led to the following hypotheses for this part of the study:

1. It was expected that the reward learning task will be enhanced in the methamphetamine group compared to the placebo group. It was expected that amisulpride pretreatment would attenuate this effect.
2. It was expected that the pattern of BOLD signal changes over time for the reward learning task would be similar to the pattern of dopamine neuron activity in animals modelled with the TD algorithm (Kakade and Dayan 2002). These effects would be amplified by methamphetamine and attenuated by amisulpride pretreatment. The main BOLD signal changes would be in the ventral striatum and dorsal striatal regions.

Due to the general effect on methamphetamine on reaction times it was felt that the reaction times for those given methamphetamine would be quicker compared to a placebo control. Due to the lack of effects by amisulpride on reaction times it seemed unlikely that the effect of methamphetamine on reaction times would be antagonised by amisulpride. This led to the last set of hypotheses.

3. Reaction times of subjects completing the phMRI task, N-back and reward learning task would be quicker in the methamphetamine group than in the placebo group. Amisulpride pretreatment would not reverse the effects on reaction times caused by methamphetamine.

1.8.3. Implications for mental illness

This study uses healthy volunteers so the implications are indirect for mental illness. However, one of the reasons that amisulpride was used to counteract the effects of methamphetamine is that selective D2 antagonism is commonly employed in the treatment of schizophrenia. In this study, the methamphetamine bolus could be regarded as a model of the symptoms of schizophrenia (Krystal, Perry et al. 2005). The attenuation of BOLD signal resulting by amisulpride could give an indication of similar changes in a schizophrenia group. This means that the current study could provide the impetus for this technique to be used in certain circumstances rather than PET

to explore dopamine function in people who have schizophrenia or who are at risk for schizophrenia. As there is more than a 20 year difference in the life expectancy of people with schizophrenia compared to the general population (Tiihonen, Lönnqvist et al. 2009), it is important that the safest research methodologies are used in this population. In addition, schizophrenia is often a long term condition (Wiersma, Nienhuis et al. 1998) so the ability to complete repeated studies safely on the same person is also an advantage for fMRI. There are a number of models of schizophrenia relating to dopamine dysfunction (some were outlined earlier). Within this study, it may not be possible to test the specific predictions in these theories but instead highlight the general impact of D2 blockade on various measurable effects resulting from excessive dopamine release.

The N-back task is frequently used in fMRI studies in people with schizophrenia due to the clear evidence of memory deficits in this group (Heinrichs and Zakzanis 1998). There has been inconsistency about BOLD signal changes in people with schizophrenia completing the N-back, with some studies showing decreases in BOLD signal changes in the prefrontal cortex (e.g., (Perlstein, Carter et al. 2001)) and increases in (perhaps better controlled) more recent studies (e.g., (Thermenos, Goldstein et al. 2005)). If decreased positive activations of BOLD signal in the PM compared to the PP group were to be detected in this study, this would reinforce the idea of improved working memory corresponding to reduced BOLD signal changes. This would support the evidence of the more recent fMRI studies examining working memory in people with schizophrenia.

This study could have useful implications for the field of addiction studies. The examination of the effect of amisulpride on the subjective ratings following methamphetamine would help to clarify some of the conflicting findings in similar studies looking at the effects of dopamine blockade on the subjective effects of stimulant drugs. Another interesting aspect of this study, in terms of the understanding of addiction processes, is whether amisulpride could attenuate the effects of methamphetamine for the reward learning task. This may help to clarify aspects of action outcome associations in man. D2 receptor levels in stimulant addicts have been shown to be lower than healthy controls

(Volkow, Wang et al. 1997). This could mean that learning processes in stimulant addicts could operate in a different manner to controls. If there are clear findings for this study in the reward learning task, this could provide a firm foundation for similar studies in people with addiction. Using the framework of addiction presented earlier (Everitt and Robbins 2005), a particular focus could then be on people in the early stages of addiction. For these people, perhaps the adaptation of action outcome associations could be usefully targeted before behaviour becomes dominated by stimulus response associations. In the longer term, this could form the basis of treatment for people in the early stages of an addition illness.

2. Methods

2.1. Subjects

This study was approved by the North Manchester Research Ethics Committee (reference 07/Q1406/36) and the Ethics of Research on Human Beings of the University of Manchester (reference number 0741) and conducted at Hope Hospital, Salford Royal NHS Foundation Trust, (Translational Imaging Unit and Clinical Trials Unit), Salford. The experiment consisted of three visits. Visit 1 was to screen for exclusion criteria and to complete baseline questionnaires (Quick Test (Ammons and Ammons 1962); Impulsiveness, Venturesomeness and Empathy questionnaire (Eysenck and Eysenck 1978); The Big Five Inventory;(John, Donahue et al. 1991); Schizotypal Personality Questionnaire-Brief (SPQ-B)(Raine and Benishay 1995); The Brief Symptom Inventory (BPRS) (Derogatis and Melisaratos 1983); The Edinburgh Handedness Inventory (Oldfield 1971)). At visit 2, drug administration and the brain scanning took place. Visit 3 was used to assess for any problems following the drug administration and for the participants to complete again the cognitive tasks done in the scanning session. There was no brain scanning in this session; instead the cognitive tests were completed using a laptop in a testing laboratory. Recruitment of male participants was by public advertisements. Participants needed to be healthy right handed men. Exclusion criteria included the use of concomitant medication (except for simple analgesia), having a history of significant mental or physical illness, current or previous illicit substance misuse. Financial compensation (£110) for time spent in the study was paid to each participant. Physical examination, history, electrocardiography, and laboratory testing was done on each participant to examine for the exclusion criteria. Urine drug-screen tests were done to rule out illicit drug use. In addition, the screening instrument Mini International Neuropsychiatric Interview (MINI) (Lecrubier, Sheehan et al. 1997) was used to exclude those with a mental illness. Collateral history was taken from a person known to the volunteer. The GP of the volunteer was informed about the study in advance of visit 2. Written informed consent was obtained from 28 individuals before enrolment. Of these 18 completed the scanning sessions and 14 completed all three sessions.

2.2. Experimental design

See Figure 2.2-1 for an outline of the protocol. This was a double blind parallel design with each subject randomised to one of three groups. This was done using the website www.randomisation.com. The randomisation was done in blocks of three. The three groups consisted of the following:

1. A group given oral amisulpride and methamphetamine bolus (AM)
2. A group given oral placebo and methamphetamine bolus (PM)
3. A group given oral placebo and placebo bolus (PP).

The effects of methamphetamine were measured by comparing its effects on magnetic resonance imaging (MRI) measures in the PM group compared with the PP group tested on a different day. The effects of amisulpride on methamphetamine responses were shown by the AM group. For each scanning session, participants had a blood sample to test for prolactin levels and then were given the pretreatment drug – either placebo or amisulpride. Following this, the BPRS was completed, blood pressure and pulse rate (lying and standing) were taken, and the cognitive tasks used in the scanner (N-back, finger tapping task, reward learning task) were explained to the participants. Intravenous cannulation (and a further blood sample taken for prolactin) was completed 90 minutes after the oral amisulpride/placebo. Subjects were then placed into the scanner. Within the scanner, a T1 weighted scan was completed to outline the anatomy of the brain followed by fMRI scans during which participants completed the challenge phMRI and various modulation phMRI tasks. The bolus (methamphetamine or placebo) was given intravenously over 1 minute. Scanning continued during the bolus time period: this was included as part of the post bolus data. The cognitive tasks and the details of the imaging parameters are described below. After completion of the scanning session, participants had their blood pressure and pulse rate checked (lying and sitting). A further BPRS was completed and a further blood sample was taken for prolactin levels. After this, a signal detection task lasting 8 minutes was completed in a laboratory adjoining the scanner. 30 minutes after the subjects left the scanner, the blood pressure and pulse rate of the participants were again checked (lying and sitting).

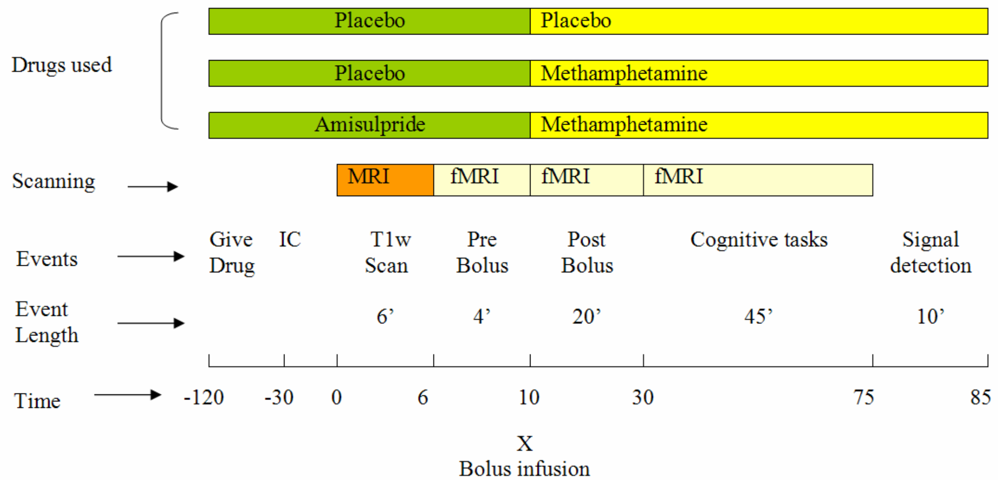


Figure 2.2-1
Outline of design of the experiment. Participants were allocated to one of three parallel groups. At the beginning of the session participants were given either placebo or oral amisulpride. After two hours the scanning sessions began. An initial structural scan was completed followed by a series of fMRI scans. 4 minutes after the initial fMRI scan began, the methamphetamine or placebo bolus was given intravenously over one minute to the participants. IC = intravenous cannulation. T1w = T1 weighted scan.

2.3. Choice of pharmacological agents

One of the motivations for this study was to understand the function of dopamine receptors, so agents with selective effects for these receptors were sought. Amisulpride is a selective dopamine D2/D3 receptor antagonist with effects only on a few other types of receptors so seemed a good choice as pretreatment. Methamphetamine was chosen as an dopamine agonist as there has been previous studies using it intravenously in MRI studies (Kleinschmidt, Bruhn et al. 1999; Völlm, De Araujo et al. 2004). In addition, the effect of amphetamine on MRI cerebral blood flow has been shown to be mainly due to dopamine rather than noradrenaline effects (Choi, Chen et al. 2006). This suggests similar effects with BOLD signal changes. There are other dopamine receptors agonists available (Kvernmo, Hartter et al. 2006). However, many of these agents were unsuitable: some (bromocriptine, apomorphine) are associated with nausea (Luciana, Collins et al. 1998; Britannia-Pharmaceutical-Limited Accessed Jan-2009) which could be difficult within a MRI scanner whereas other agents (ropinirole and pramipexole) have not been frequently used intravenously in humans.

2.4. Procedures

2.4.1. Image acquisition

Two types of fMRI sequences were used in the study on a Philips (Eindhoven, Holland) 3 Tesla scanner. Both types were T2*-weighted volumes acquired using a single-shot, multi-slice echo-planar imaging (EPI) sequence. For the challenge phMRI sequence every volume consisted of 70 contiguous axial slices (TR/TE=12000/35ms, 2 mm thickness with an in-plane resolution of 1mm x 1mm). For the modulation phMRI tasks (resting state, N-back, finger tapping and reward learning task), each volume comprised 34 contiguous axial slices (TR/TE=2000/35ms, 3.5mm thickness with an in-plane resolution of 1.8mm x 1.8mm). A T1-weighted structural scan was also acquired for each subject to exclude any structural abnormality. No abnormalities were reported for any of the 18 subjects. The reason why a different scan was used for the challenge phMRI was that this sequence had higher resolution than for the modulation phMRI tasks. This was important for the phMRI as the particular focus was relating the activations to the known anatomical distribution of dopamine receptors which would include small nuclei of the brainstem. It was not possible to use this high resolution scan for the modulation phMRI as the TR of 12000 ms is too long for meaningful analysis of the cognitive tasks. For example, in the finger tapping task the block size was 30 seconds which would only allow 2 complete volumes to be collected with a TR of 12000 ms rather than 15 volumes using a TR of 2000 ms. However, two minute blocks were used in the first line analysis of the challenge phMRI, so 10 volumes could be used for the analysis despite a TR of 12000 ms.

2.4.2. Challenge phMRI

A baseline scan was taken over 4 minutes, followed by 20 minutes of scanning to assess the direct effect of the bolus. The first 2 minute of this baseline scan was discarded prior to analysis. Subjective ratings were carried out at 2 minute intervals using items from the profile of mood states (POMS) (McNair 1971). The items used were: cheerful, carefree, energetic, gloomy, sluggish, and anxious. Ratings ranged from 1 (Not at all) to 4 (Extremely). The ratings were carried out using a right-handed button press. This was rehearsed before the scanning session. The rating took 30 seconds to complete. Following the ratings, a black screen was shown to the participants. A ten second countdown

(consisted of the digits 1 to 10 being shown in reverse order) was used prior to the presentation of the subsequent ratings.

2.4.3. Resting state

The analysis of this task is not described in this thesis. Immediately following the challenge phMRI data acquisition, subjects were asked to close their eyes and lie still in the scanner while fMRI volumes were collected for 5 minutes.

2.4.4. N-back task

The N-back task is a test of working memory. The subjects were presented with a series of letters and were asked to respond when the letter on the screen was the same as the one presented N trials earlier (N is either 0, 1, 2). With the N = 0 back condition, the participants were asked to respond to a pre-specified stimulus, this was the active control, as this does not require the manipulation of information within working memory but attention and stimulus response mapping. The task lasted approximately 7 minutes.

2.4.5. Finger tapping task

A finger tapping task using the right hand was used as a test of motor function. The subjects were asked to carry out finger to thumb opposition (tapping the tip of the thumb with the tip of a finger) sequentially beginning with the forefinger. They were asked to complete this three times for each finger. The order of the fingers opposed to the thumb was: forefinger, middle finger, ring finger and little finger. The rate of opposition was one per second. This task was rehearsed prior to entering the scanner. The participant was watched during the scanning session to ensure the task was completed correctly. Thirty seconds was allowed for the task followed by thirty seconds of rest which was repeated four times. The task took four minutes to complete.

2.4.6. Reward learning task

A learning task was used similar to those in previous similar studies (Haruno and Kawato 2006; Pessiglione, Seymour et al. 2006; Tanaka, Samejima et al. 2006). The participants were asked to associate visual stimuli with outcomes. An outline of the task is presented in Figure 2.4-1. The visual stimuli consisted of abstract pictures (shown in appendix 6.1). After presentation of the stimulus, the subject was required to press either of 2 buttons when signalled by an alteration in the stimulus (red square around it). Three image types were used:

reward stimuli, punishment stimuli and neutral stimuli. Reward stimuli were followed either by the message "+10" on one line and underneath the message "total is now 100 " (as shown in Figure 2.4-2) or the message "0" on one line and underneath the message "total is now 90" depending on which of two buttons were pressed. The upper digit represented the immediate outcome of the response and the bottom digit was a running total. Both types of messages were presented with the original stimulus. Punishment stimuli were followed either by the message "-10" on one line and underneath the message "total is now 90 " or the message "0" on one line and underneath the message "total is now 100". In a similar manner to the consequences following the reward stimuli, these effects depended on which button was pressed and both messages were presented with the original stimulus. Neutral stimuli were followed by the message "0" on one line and underneath the message "total is now 100" and a picture of the original stimulus if either button is pressed. It should be noted that the values in the above explanations (100 and 90) are used as examples of the running total. The running total varied depending on the performance of the participant. There were two types of reward, punishment and neutral stimuli used, giving a total of 6 stimuli for the task. Pressing one of the two buttons was the optimal response (caused an increase) for one type of the reward stimuli. For the alternate reward stimulus, the alternate button press was the optimal response. In a similar way, the two types of punishment stimuli also required two different responses. The timing of the task is presented in Figure 2.4-2. It can be seen that the outcome was presented 10 seconds after stimulus presentation and the subsequent stimulus occurred 10 seconds after onset of the outcome. These delays were to allow the onset and the outcome to be regarded as separate events for the fMRI analysis. The task lasted 30 minutes. To increase task difficulty, increases for reward stimuli following the optimal button press occurred on only 12 out of 15 trials (thus, if the optimal response was consistently taken the probability of a reward would be 0.80). The punishment stimuli were similarly adapted. The participants were told that the aim of the task was to increase the total. They were told that their performance on the task corresponded to an amount of money given at the end of the experiment. However, the same amount (£10) was given irrespective of performance. A dummy run was completed by the participants prior to the experiment using a different stimulus to any used within the scanner.

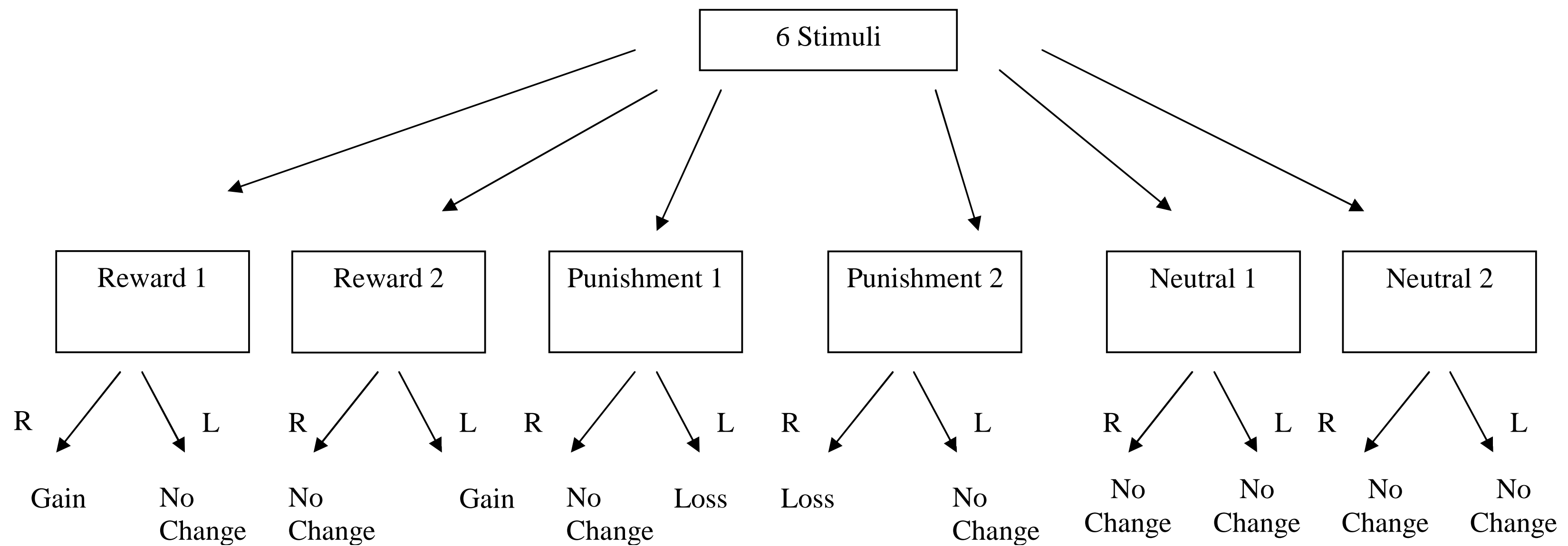


Figure 2.4-1
Outline of the reward learning task. There were 6 stimuli. For each stimulus the participant had to learn to press the button to maximise the running total. This was done by pressing buttons that resulted in gain or avoiding buttons that gave loss. For the reward image type (reward 1 and reward 2) pressing one button gave a gain of 10 in the total and the alternate button press resulted in no change in the total. For reward 1, pressing the right button gave the gain and pressing the left button resulted in no change. For reward 2, pressing the left button gave the gain and pressing the right button resulted in no change. For the punishment image type (punishment 1 and punishment 2) pressing one button gave a loss of 10 in the total and the alternate button press resulted in no change in the total. For punishment 1, pressing the left button gave the loss and pressing the right button resulted in no change. For punishment 2, pressing the right button gave the loss and pressing the left button resulted in no change. For the neutral image type (neutral 1 and neutral 2) pressing either button resulted in no change in the total. Abbreviations: R is right; L is left.

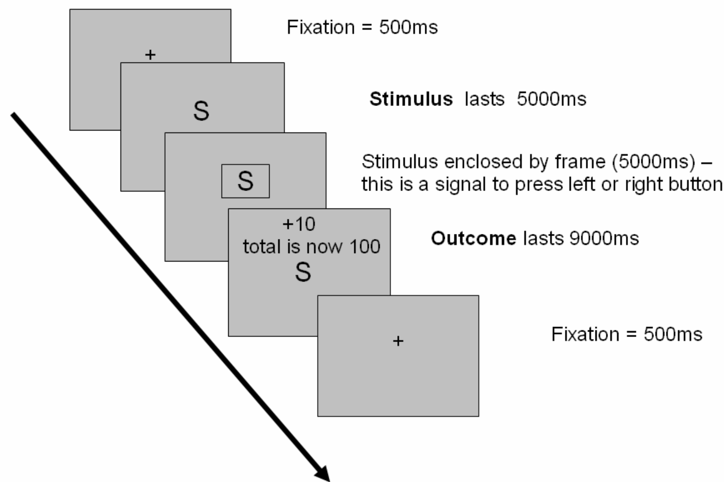


Figure 2.4-2
Outline of time frame for the reward learning task. The angulated arrow indicates timeline. The outcome was presented 10 seconds after stimulus presentation and the subsequent stimulus occurred 10 seconds after the onset of the outcome.

2.4.7. Signal detection task

The results of this part of the experiment are not reported in this thesis however, a brief description of the task is given. Subjects were asked to take part in a signal detection task after the scanning session. Volunteers attempted to detect a voice in 3-second segments of white noise. A voice was present in 60% of the total segments of white noise. In two thirds of these segments the voice was present at the threshold of auditory detection whereas in one third the voice was above auditory threshold. No voice was present in the other 40% of the total segments. This task lasted 8 minutes.

2.4.8. Scanning problems and unblinding

On one occasion there was a problem with the MRI scanner. On this occasion, the participant was unable to complete the N-back, finger tapping and reward learning task. On another occasion, software problems prevented the completion of the reward learning task. Based on the subjective experiences of the participants it was felt likely that these participants were in the PP group. The randomisation was broken for these participants and both were confirmed as being in the PP group. The participants were then invited to return but only the fMRI part of the experiment were completed for these participants as it was felt inappropriate to expose the participants again to the most invasive part

(intravenous cannulation and bolus administration) of the experiment having already acquired the dataset. Thus, the participants and experimenter were effectively unblinded for these parts of the experiment. The justification for including these datasets included the fact that due to the strong subjective effects of the drugs used, once the bolus was given, in most cases both participant and experimenter were effectively unblinded. In addition, some evidence is presented in the results section (for the N-back task) of the similarity of performance between the unblinded participant and the blinded participants suggesting that the data could be included validly for analysis.

2.5. Statistical analysis

Behavioural data were analysed using Statistical Package for the Social Sciences (SPSS 14.0). Levene's test within SPSS was used to test for equality of variance. In this test a significant difference indicates that variance was not equal between the levels of the factor (Field 2000). Mauchly's test was used to test for sphericity. If a value less than 0.05 was detected indicating a violation of sphericity (Field 2000), then the Huynh-Feldt correction was used. These tests are mentioned in the analysis only when relevant. Some graphics were completed using R (version 2.6.0). Challenge phMRI data and the fMRI data for the cognitive tasks were analysed using Statistical Parametric Mapping 5 (The FIL methods group Accessed Jan-2010).

2.5.1. Overview of analysis

This section gives an account of the general principles of analysis for the fMRI data for the challenge phMRI and the modulation phMRI tasks. As the challenge phMRI is a different type of experiment to the modulation phMRI tasks, the first line analysis for the challenge phMRI is markedly different to the other tasks. However, the principles outlined below describe the broad similarities in approach in the second level analysis for both the challenge phMRI and the other modulation phMRI tasks (reward learning task, finger tapping task, N-back task). The reward learning task was also examining different concepts compared to the other modulation phMRI tasks so additional approaches were also taken for this task in the second level analysis.

The main idea tested for the challenge phMRI and the other tasks was that the effects of methamphetamine (detected by comparing the PM group with the PP group) would be antagonised by amisulpride pretreatment. If strong antagonism occurred then the AM group would be equivalent to the PP group therefore the effects of the PM group compared with the PP group would be the same as the comparison between the PM and AM groups. In that case, the pattern of activations for the PM-PP contrast would be similar to the pattern of activations for the PM-AM contrast at a certain statistical threshold. These effects could be examined in a more robust manner by the conjunction contrast PM-PP and PM-AM which would show the common areas of activation. The separate contrasts PM-PP and PM-AM were used in addition to the conjunction contrast for the challenge phMRI. This was done on the expectation that a large number of areas could be identified in these contrasts. As it is harder to compare this part of the study to earlier work, it was hard to predict these areas beforehand. For this reason, it felt to be useful to have a general overview of the different areas identified using the separate contrasts prior to using the conjunction analysis.

As methamphetamine could have deactivating as well as activating effects, the alternate contrast PP-PM was also examined. If amisulpride antagonised these effects, then the contrast between PP-PM would be similar to the contrast AM-PM. This could be further demonstrated by the conjunction contrast PP-PM and AM-PM. As for the activating contrast, the separate contrasts PP-PM and AM-PM were used for the challenge phMRI to allow a more general exploration of common areas of deactivation.

2.5.2. Screening data and physiological data from visit 1 and visit 2

Various demographic data collected in visit 1 were analysed using a series of analysis of variance (ANOVA) statistics to identify potential underlying differences between the drug conditions. The cardiovascular data in visit 1 and visit 2 were assessed for any baseline differences and to examine for effects of drug group. The prolactin levels in visit 2 were examined using ANOVA statistics and a paired t test. The BPRS data collected in visit 2 remained essentially at baseline values throughout the experiment for all drug groups so were not analysed.

2.5.3. Preprocessing of MRI data

The preprocessing of the functional MRI data was broadly similar for all the datasets (much of the following derives from unpublished lecture notes (McKie 2008)). The images were realigned to correct for motion artifacts using the first scan as a reference. The structural scan completed on the participant was co-registered with the mean functional scan of the dataset of interest to ensure that these were in the same stereotactic space. Segmentation (classification of images into grey and white matter and cerebral spinal fluid) of the structural scan was then completed. The grey matter segmented output from this process and the standard statistical parametric mapping (SPM) grey matter template supplied by SPM were used to normalise the structural image of the participant into standard stereotactic space. The matrix used to normalise the grey matter segmented image was then applied to the functional images. Images were smoothed with a Gaussian kernel (with a Full-Width Half Maximum of x=5.4 mm, y=5.4 mm, z = 10.5 mm) for the cognitive tasks. For the challenge phMRI the Gaussian kernel used had a Full-Width Half Maximum of x=3 mm, y=3 mm, z=6 mm. These kernels were used to facilitate inter-subject averaging. Further correction to reduce movement artifact was completed on the functional images using the artifact repair toolbox (v 2.2 for SPM 5) in the SPM5 toolbox. There were problems detected with a mask used for the first level analysis in the phMRI dataset leading to data loss. The mask setting in the file spm_default.m in SPM5 was adjusted to overcome this. Brain regions and Brodmann areas were identified using the software tool Talairach client (Lancaster, Rainey et al. 1997; Lancaster, Woldorff et al. 2000).

2.5.4. Challenge phMRI

2.5.4.1. Behavioural data

Both rating data and response time data were analysed. An incomplete rating dataset was generated due to an instrument fault. Three out of the four rating response buttons worked, so data were available for ratings 1, 2 and 4 but not for 3. All non-response data were taken to represent times when persons rated themselves as 3. The data were aggregated in a manner as discussed in the results section resulting in 4 levels for the time factor. The ratings were used in a 4 x 3 mixed design ANOVA with time as a within subjects factor and drug condition as a between subjects factor. Similar to the rating data, there was an

incomplete dataset for the reaction time data. In this case it was not possible to generate data for the missing values. As described in the results sections, averages were calculated over time ranges in order to overcome this problem. The reaction time data were then used in a 4 x 3 mixed design ANOVA with time as a within subjects factor and drug condition as a between subjects factor.

2.5.4.2. fMRI data analysis

For the first level analysis, a time series analysis was completed using the pseudo-block (p-block) method in a similar way to previous studies (McKie, Del-Ben et al. 2005; Deakin, Lees et al. 2008). From previous studies using methamphetamine (Cook, Jeffcoat et al. 1993; Völlm, De Araujo et al. 2004), it was felt that peak subjective effects due to methamphetamine would have occurred within 20 minutes after the bolus. This provided a guide for the length of the scan to be used, although, the subjective response was not used as a regressor. Instead, the analysis method was a time series approach. As the first two minutes of the scan were discarded to allow the BOLD signal to stabilise, this allowed 11 time-bins, each of two minutes, to be used in the analysis. These consisted of one pre-bolus time-bin (T0) and 10 time-bins (T1 to T10) which covered the post-bolus for 20 minutes. A multiple regression was used which compared the average of each post-bolus time-bin to the average of the pre-bolus time-bin. This generated 10 contrast images (T1-T0 to T10-T0) which were subsequently passed to the second level. Temporal global normalisation was used to account for any signal changes across the whole brain that may have occurred due to movement and scanner drift. No high pass filtering was used as the temporal dynamics were not known prior to analysis. For the second level analysis, the ten images generated for each participant were used as input into a non-independent time factor with each participant being randomised into an independent drug group factor. That is, the analysis consisted of a 2 factor repeated measures random effects ANOVA with time (within subjects) and drug condition (between subjects) as factors.

The following contrasts were then used to examine for the effects of interest. The T contrast between the PM and PP levels of the drug condition (PM-PP) over all time bins was used to examine for the activating effects of methamphetamine. The alternate T contrast between the PP and the PM levels

of the drug condition (PP-PM) was used to examine for the deactivating effects of methamphetamine. The T contrasts between the PM and AM levels of the drug condition (PM-AM) over all time bins was completed so that a comparison could be made with the T contrast PM-PP. If amisulpride antagonised the effect of amphetamine then these contrasts would show similar regions. In a similar way, the T contrast between the AM and PM levels of the drug condition (AM-PM) over all time bins was compared with the contrast PP-PM to see if there were similar areas detected and to examine whether amisulpride prevented the deactivating effects of methamphetamine. These T contrasts were masked by +PM (positive effects of the PM group compared to baseline) for activating effects of methamphetamine and –PM (negative effects of the PM group compared to baseline) for the deactivating effects. This was completed to try to reduce the effects of a truncation artifact that occurred only with the challenge phMRI (described in detail below). The conjunction contrasts PM-PP and PM-AM were then used to examine in a more rigorous way whether amisulpride antagonised the activating effects of methamphamine and likewise the conjunction contrasts PP-PM and AM-PM examined in a more rigorous way whether amisulpride antagonised the deactivating effects of methamphamine.

2.5.5. N-back task

2.5.5.1. Behavioural data

This task generated accuracy and response data for the three levels of difficulty of the task. The accuracy data were set out in tables. The response data were analysed using repeated measures one-way ANOVA.

2.5.5.2. fMRI data analysis

First-level analysis was completed on the data from each subject with a general linear model using a delayed boxcar waveform to model BOLD signal changes. This generated a single mean image for each level of the factor (three levels of the N-back). Neural responses in the control blocks were subtracted from those in the active blocks, identifying areas of signal change associated with task performance. For the first level analysis these contrasts were 1-back minus 0-back and 2-back minus 0-back. These contrasts form the 2 levels of the task difficulty condition in the second level analysis. Temporal global normalisation was used for reasons outlined before. Low frequency drifts were accounted for

by a high pass filter set to two times the main repetition time (420 ms) of the task (a similar step was completed for the finger tapping task).

A two factor repeated measures random-effects ANOVA was employed for second level analysis using task difficulty (within subjects) and drug condition (between subjects) as factors. To examine for overall positive activations resulting from the doing the task, the T contrast positive effects of all conditions was used. This effectively examined the summation of all positive effects of the contrasts 2-back minus 0-back and 1-back minus 0-back across all three drug conditions**.** The F contrast main effects of task was used to examine the summation of differences between the 2-back minus 0-back and 1-back minus 0-back across all three drug conditions**.** The contrast between these levels may indicate the extra difficulty of doing the task. This was masked by the positive effect of condition in order to examine differences in positive activations only. The F contrast main effect of drugs was used to identify any differences between the three drug groups. This was masked by positive effects of all conditions to test for differences of the positive activations between the drug groups. As it was felt that there would not be widespread attenuation of the effects of PM group by the AM group only the conjunction contrasts were presented. As before, the conjunction contrast PM-PP and PM-AM examined for attenuation of the activating effects of methamphetamine by amisulpride and the conjunction contrast PP-PM and AM-PM was used to examine for the deactivating effects of methamphetamine. An interaction for drug condition and task difficulty was not completed as there were three levels in the drug condition so making the interpretation of the result difficult. If this had been a simpler (e.g., 2x2 factorial) design, this might have been done. It was also felt that the questions of interest were addressed using the conjunction analysis.

2.5.6. Finger tapping task

2.5.6.1. fMRI data analysis

The finger tapping fMRI data were analysed in a similar way to the N-back fMRI data analysis mentioned above: a general linear model using a standard delayed boxcar waveform to model BOLD signal changes was used for the first level analysis. In this case, 2 mean images were generated corresponding to the 2 levels of the task (active and rest). The neural responses in the control

block were subtracted from the active block to identify areas of signal change associated with the performance of the task. The contrast was used in the second level analysis. This was a single factor ANOVA random-effects model using drug condition as a between subjects factor. The main effects of task examined the contrast between the active and control block for all the three drug conditions. This examined the summation of the positive effects of the task across all of the drug conditions. In this case, there was interest in both the activating and deactivating effects of methamphetamine as each of these contrasts may have identified separate brain regions (activation in the cortical regions and deactivations in the striatal regions). To examine for this, two separate T contrasts were used: PP-PM (activating effects of methamphetamine) and PM-PP (deactivating effects of methamphetamine). As before, the conjunction analysis PM-PP and PM-AM was used to examine for areas of methamphamine activation that were attenuated by amisulpride. Similarly the conjunction analysis PP-PM and AM-PM was used to examine for areas of methamphamine deactivation that was attenuated by amisulpride.

2.5.7. Reward learning task

2.5.7.1. Behavioural data

The overall aims of the behavioural analysis were: first, to determine how the participants had completed the task; second, to determine whether the task was learnt differently depending on the drug group and third, to examine whether reaction times were different between the drug groups. In this task, participants were shown 6 stimuli and as described previously an optimal or suboptimal response could be made for each of the reward or punishment image types. As the outcome was the same for either of the actions for the neutral image type, data relating to this did not require analysis.

For the reward and punishment image types, an initial task in the analysis was to determine whether the participants had identified the optimal action for each reward and punishment stimulus. The participant could complete 15 button presses for each stimulus. For each stimulus the ratio of optimal responses to total responses completed was calculated. If the participant had pressed a high percentage of optimal responses, then he was deemed to have learnt the optimal response for that stimulus. If the participant had pressed a low

percentage of optimal responses, then he was deemed to have failed to learn the optimal response for that stimulus. For intermediate percentages, the pattern of responses over the course of the task was also taken into account. If a participant persistently picked the optimal response towards the end it suggested that a participant had learnt the optimal response for that stimulus.

As well as picking the optimal response persistently, a participant could also pick the suboptimal response. If the participant consistently picked the suboptimal response then it was regarded that the person had incorrectly learnt the response for that stimulus. If it was unclear whether the person had consistently picked one action more frequently than another, it was regarded that the person had not learnt a consistent response for that stimulus.

This allowed each of the punishment and reward stimuli for each participant to be classified as learnt, incorrectly learnt and not learnt. As there were 18 participants and 2 reward and 2 punishment stimuli, then in total, 72 stimuli could be split into groups of learnt, not learnt and incorrectly learnt stimuli. The assumption had been that the vast majority of the stimuli would be in the learnt group. Logistic regression was then used as a means of checking the validity of the classifications into learnt, incorrectly learnt and not learnt groups. Response was the dependent variable and trial number was the covariate. Trial number here refers to how many trials within a block that a participant had encountered a stimulus. If the classification was appropriate, the logistic regression would indicate a relationship between the response and the trial number. This would indicate that there were more optimal responses towards the end of a block of trials for the learnt group. It would also indicate that there were more suboptimal responses towards the end of a block of trials for the incorrectly learnt group. There would be no clear pattern of responses in relation to trial number for the not learnt group.

As mentioned in the section describing the modelling of dopamine neurons using the TD algorithm in the introduction, methamphetamine could reduce the number of trials required to identify an optimal action in a learning task. Amisulpride was expected to reverse this effect. To examine for this, the learnt dataset was split on the basis of reward and punishment image types. A logistic

regression was used with response as the dependent variable and trial number, drug condition and their interaction as covariates to examine for the effects of the drug conditions. For the reaction time data, a one way ANOVA was completed using reaction times as the dependent variable with drug condition as a between subjects factor. This was done separately for the reward and punishment image types.

2.5.7.2. fMRI data analysis

The main assumption for the analysis of the imaging data was that the phasic dopamine release (corresponding to the error signal (δ_t) in equation (2) in the introduction) would cause an increased BOLD signal change compared to baseline when the subject was learning the task. A further assumption used was that an increase in dopamine release with methamphetamine would cause a further increase in this BOLD signal and that this further increase would be attenuated by amisulpride pretreatment.

As described for the behavioural analysis, each reward and punishment stimulus for each participant was classified as either learnt correctly, learnt incorrectly or not learnt. Only data acquired for the learnt stimuli were used in the imaging analysis. Due to the practical problem that participants frequently failed to learn some parts of the task, by concentrating on learnt data this caused a reduction in power, so the main emphasis of the analysis was to explore the data rather than to answer definitively the hypotheses. In order to detect a change in BOLD signal during the learning of the task, these data were divided into three time bins: early, middle and late. There was a time gap of 10 seconds between onset of the stimuli and the outcome so these could be regarded as separate events. Thus, for a trial correctly learnt there were an event relating to stimulus onset and stimulus outcome. The onset and outcome events were modelled using an estimate of the haemodynamic response function (HRF) as generated in SPM5. Temporal global normalisation was used here for the same reason as for the other parts of the study. Low frequency drifts were accounted for by a high pass filter set to 1.5 times the maximum time between the events across all stimuli. Statistical parametric maps were produced for these events contrasted to baseline. These images were used in the second level analysis. Due to the nature of the task, there was a large number of factors each with a number of levels for the second level analysis.

This is presented in Figure 2.5-1. The drug factor had three levels, the stimulus presentation factor had 2 levels and both the time bin and image type factors had 3 levels resulting in a 3x2x3x3 factorial four-way ANOVA. As SPM does not allow four way ANOVAS, a number of three way ANOVAS was therefore used to examine for effects of interest.

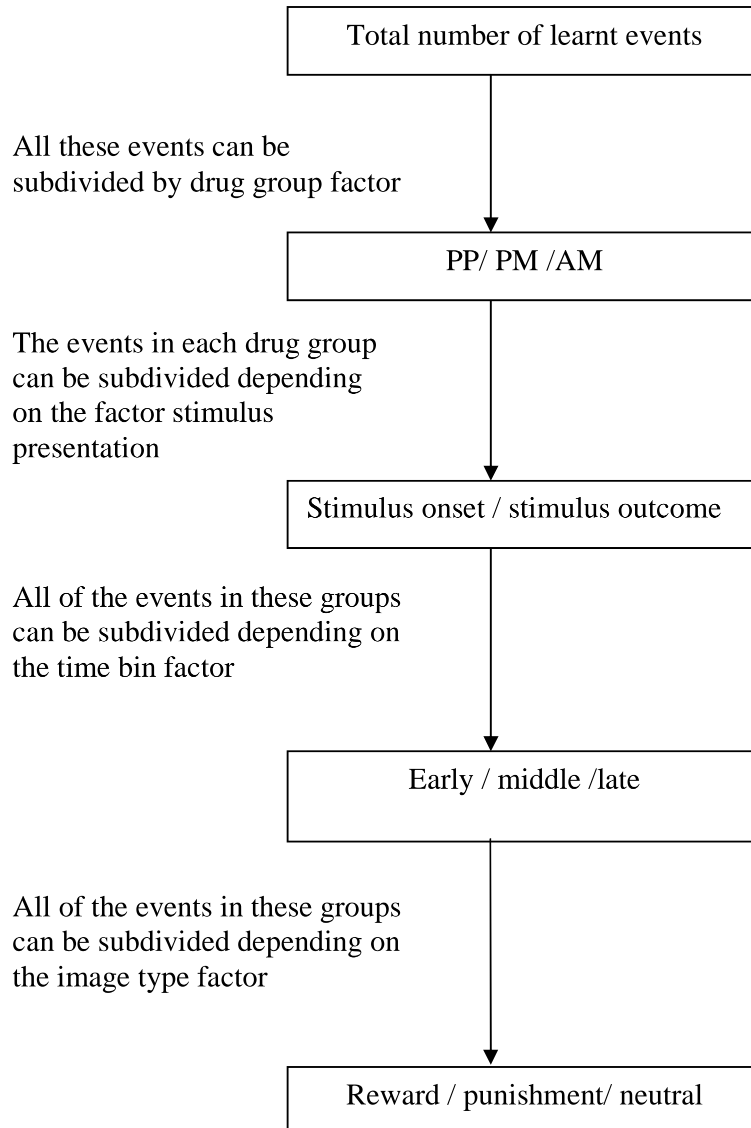


Figure 2.5-1
Overview of the factors and the levels in each factor for the fMRI analysis of the reward learning task. The drug group factor relates to drug allocation. The stimulus presentation factor relates to the occurrence of stimulus onset or stimulus outcome within a trial. The time bin factor relates to the occurrence of the event in the task. The image type factor relates to the contingencies following an action at the time of stimulus presentation.

For a number of analyses, a three way ANOVA with three within subjects factors was used. The factors comprised: image type (reward, punishment or

neutral); stimulus presentation (onset, outcome) and time bin (early, middle, late). This allowed the examination of a number of effects of interest using T contrasts. Although the main interest was the change in BOLD signal over the course of the task, it was felt useful to complete analyses to identify activations in reward and punishment areas to determine whether participants identified the stimuli in the manner expected. As there was an expectation that that the events at stimulus onset would take on the characteristics of the event at the time of stimulus outcome over the course of the task then it seemed useful to compare image types at stimulus outcome in the early time bin and stimulus onset at the late time bin.

To examine the effects of reward in the early time bin, the T contrasts reward - neutral image type and reward - punishment image type at stimulus outcome were used. To examine the effects of punishment in this time bin, the T contrast punishment – reward image type was completed at stimulus outcome. To gauge whether the stimuli at the time of onset took on the characteristics of the stimuli at outcome in the late time bin the same T contrasts (reward – neutral image type, reward – punishment image type and punishment – reward image type) were completed using the events at the time of stimulus onset. Then the corresponding areas of activations for these two sets of contrasts were compared.

One of the main aims in the use of the reward learning task was to examine the effects of time on the events. Using the model of dopamine function as described in the section describing the modelling of dopamine neurons using the TD algorithm, it was expected that there would be increased activation in target areas of dopamine neurons (especially the ventral striatum) at the time of stimulus outcome in the early bin compared to the late time bin. This was tested using a T contrast between the early and late time bins at the time of stimulus outcome for the reward image type. Using similar reasoning, it was expected that there would be increased activation in target areas of dopamine neurons at the time of stimulus onset in the late bin compared to the early time bin. This was examined using the T contrast between the late and early time bins at time of stimulus onset for the reward image type. These contrasts are summarised in Table 2.5-1. It was decided to use the reward image type only in this pair of

analyses as it was felt the role of dopamine for reward events was clearer than for punishment events.

	Stimulus onset	Stimulus outcome
Early time bin	**A (low phasic DA)**	**C (high phasic DA)**
Late time bin	**B (high phasic DA)**	**D (low phasic DA)**

Table 2.5-1
This summarises the contrasts used for the events at stimulus onset, and stimulus outcome over the time course of the reward learning task. The T contrasts used in the analysis were B-A and C-D. The expected levels of dopamine (DA) release are also shown.

Two separate three way repeated measures ANOVA models were used to explore the effects of drug group on learning. Each of the ANOVAs comprised the factors image type (reward, punishment, neutral), time bin (early, middle, late) and drug group (PP, PM, AM). One model was specified with the onset events whereas the other was specified with the outcome events. Using the model with the onset events, the T contrast late time bin – early time bin for reward images types was completed over all the drug groups. Selected voxels from the areas identified by this contrast were then plotted in a histogram to show the pattern of activation for the different levels of the drug condition. A similar histogram was plotted using the T contrast early time bin – late time bin using the model with the outcome events (for reward images types over all the drug groups). The drugs groups were examined in this way because separate models were used for onset and outcome events so there was a further reduction in the total number of events for the ANOVA model. This made it difficult to allow meaningful T contrasts to be made using the drug group factor. Hence the approach was to explore possible effects rather than definitively addressing the hypotheses about the effect of drug group on learning.

A similar problem arose when comparing pairs of contrasts above where common areas of activation could have expected to have been identified (e.g., the T contrast for early – late time bins at time of outcome compared with the T contrast late - early time bins at time of onset). It was felt that due to the reduction in the number of events, then a general overview using comparisons in sets of tables would be more useful than using a conjunction analysis.

2.6. Truncation artifact

In the challenge phMRI part of the study, a truncation artifact (also known as Gibb's Artifact) was detected ((Westbrook, Roth et al. 2005; McRobbie 2007; Stadler, Schima et al. 2007; Rakow-Penner, Gold et al. 2008). These are bright or dark parallel lines that are visible in areas close to high contrast regions where there is a change in intensity from light to dark. This effects worsens by using techniques to reduce scanning time such as undersampling in the phase-encode direction using parallel imaging techniques such as SENSE (McRobbie 2007). This results in the voxel size being too large to represent the gradient changes between the high contrast and low contrast. If this is detected at the scanning session, then the usual remedy is to increase the number of phase encoding steps. However, in this study the artifact was detected after completion of the scanning, hence it was not possible to correct this. Examining the data visually, the distortion seemed to affect relatively small areas of the datasets for the participants. As a result it was decided to continue to use the data. An image of the artifact is presented in Figure 2.6-1. The artifact was present in most of the datasets of this part of the study. It is important to note that this artifact only occurred in the challenge phMRI sequence and not in the sequence that was used for the finger tapping N-back or reward learning tasks. To try to reduce the effect of the artifact, masking was used in the second level analysis. When a contrast was used between the drug groups, this was masked by the effects of the methamphetamine drug group compared to baseline (e.g., +PM for the T contrast PM-PP). This was done to emphasize the drug effects of the PM group. This was done on the basis that due to the likely strong neuronal effects of methamphetamine, the signal for the PM group could be due to true effects rather than related to the artifact. It was not expected that this contrast would rectify the artifact but to try to reduce its effects.

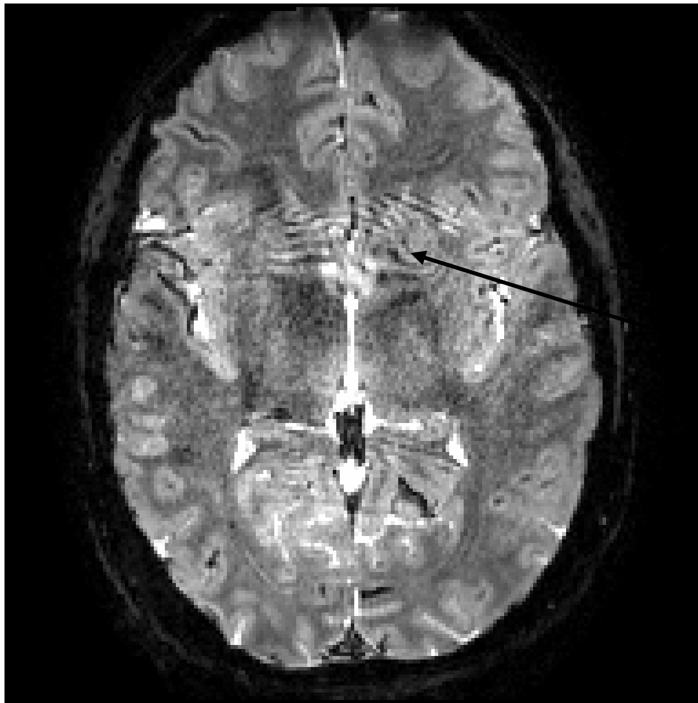


Figure 2.6-1
Image of truncation artifact in the fMRI data of a participant. There are characteristic alternating light and dark bands near areas of high contrast.

3. Results

3.1. Visit 1 and visit 2 screening and physiological data

3.1.1. Visit 1 screening data

A series of one way ANOVA models were completed on both the demographic data collected and the various questionnaires completed to assess for differences in drug conditions. The main demographic data are presented in Table 3.1-1. As can be seen in this table, there were no significant differences detected between the drug conditions for age, IQ or years of education.

Drug group	PP (n=6)	PM (n=6)	AM (n=6)
Mean Age*	25.83 (7.60)	24.17 (3.49)	28.67 (4.85)
Ethnicity	6 Caucasian	5 Caucasian 1 Asian Indian	5 Caucasian 1 Asian Indian
Employment	5 students 1 skilled manual	5 students 1 seeking employment	4 students 1 professional 1 unskilled
Mean number of years of education*	15.83 (4.31)	19.33 (1.97)	18.67 (3.33)
Mean IQ* (sd)	98.67 (4.885)	99.17(10.83)	90.50(10.15)

Table 3.1-1
Demographic data of participants classified by drug condition group. * No significant ($p<0.05$) difference detected using one way ANOVA. sd = standard deviation.

For the Big Five Inventory, none of the items (extraversion, agreeableness, openness, consciousness and neuroticism) differed by drug condition. There were no detectable differences using the total score of the Brief Symptom Inventory positive symptoms. For the impulsiveness and empathy items of the impulsiveness, empathy and venturesomeness questionnaire, a log transformation was used as Levene's test showed significant differences in the variances for the different drug groups. This transformation reduced the heterogeneity to non significant levels for the impulsiveness item but (though reduced) remained significant ($p = 0.037$) for the empathy item. There were no significant differences between the drug groups for the impulsivity item. Despite the possible heterogeneity in variances, a one way ANOVA was used for the empathy item which did not reveal any significant differences between the drug conditions. There was a difference detected between the drug conditions for the venturesomeness item ($p = 0.025$, df= (2,15), $F=4.769$) which is outlined in Table 3.1-2. For the schizotypal personality questionnaire, there were no significant differences detected between the drug conditions for the cognitive

perceptual, interpersonal and disorganized subscales. There were no significant differences for handedness using the Edinburgh Handedness Inventory.

Drug group	PP (n=6)	PM (n=6)	AM (n=6)
Venturesomeness mean (sd)	13.00 (3.35)	13.67 (1.75)	9.67 (1.75)

Table 3.1-2
Mean values of the venturesomeness items for the different drug conditions. Standard deviations in brackets.

3.1.2. Visit 1 and visit 2 cardiovascular data

For the cardiovascular data collected at Visit 1, using a series of one way ANOVAs there were no differences detected for drug group between any of the blood pressure measures (systolic blood pressure, diastolic blood pressures and pulse rates in both sitting and standing positions) The cardiovascular data from visit 2 were analysed using a series of mixed design ANOVAs with time as a within subjects factor and drug condition as a between subjects factor. One participant did not have a final blood pressure measurement so there were uneven numbers in the drug groups (PP=5; PM=6; AM=6). There were no significant differences between the levels of the drug conditions on any of the blood pressure measures collected (systolic blood pressure, diastolic blood pressures and pulse rates). There were differences detected between the levels of the time factor for sitting diastolic blood pressure, standing pulse rate, and sitting pulse rate. There was heterogeneity of variance detected (using Levene's test) between the drug groups for the first ($p=0.03$) and second time ($p=0.03$) points of the sitting diastolic blood pressure. This did not correct with log transformation so in view of the relatively high p value, the original data were used. The positive results of the analysis of the cardiovascular measures are summarised in Table 3.1-3. The plots of the means of the pulse rates are shown in Figure 3.1-1 and Figure 3.1-2.

Measure	Statistical results (p, df ,F)	Time 1 Mean (sd)	Time 2 Mean (sd)	Time 3 Mean (sd)
Sitting Diastolic BP	(p =0.001, df= (2,28), F= 8.696)	73.24 (7.085)	79.53 (8.97)	77.59 (8.94)
PR sitting	(p= 0.027, df= (2,28), F= 4.139	65.47 (10.57)	68.76 (11.08)	71.94 (11.04)
PR standing	(p =0.041, df=(2, 28), F= 3.588)	72.71 (12.48)	76.82 (14.88)	80.47 (15.33)

Table 3.1-3
Summary of main cardiovascular differences detected over time.

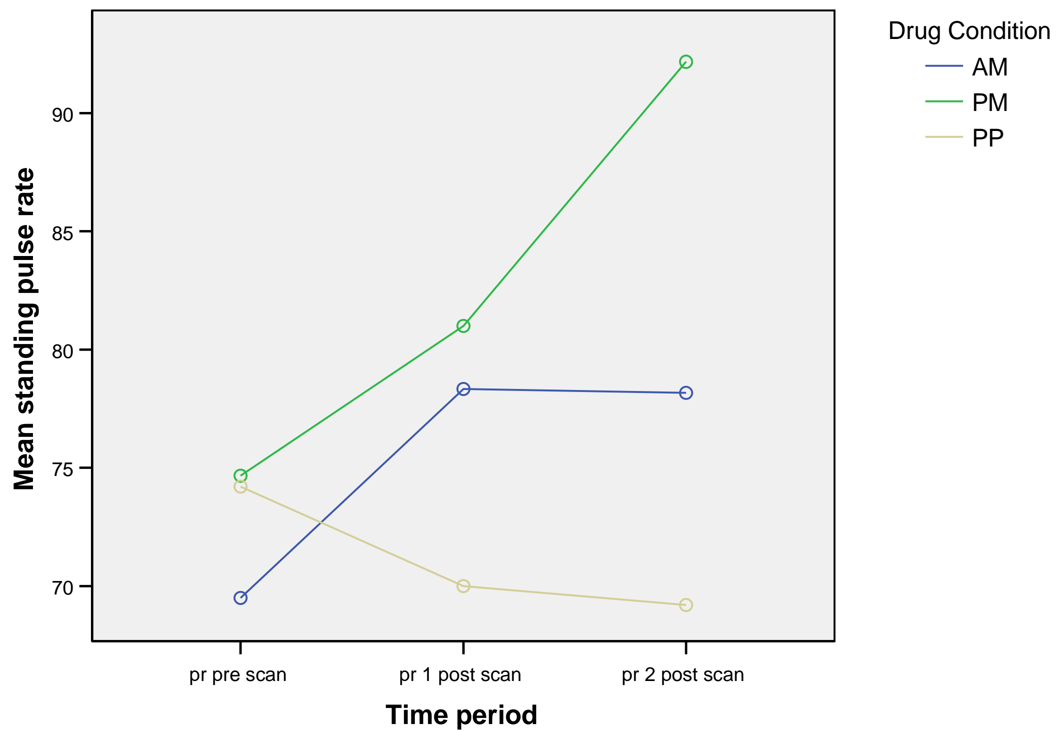


Figure 3.1-1
Plot of the change in the standing pulse rate over time. There were no significant differences between drug conditions but there were differences over time. Pr pre scan is the pulse rate prior to the scanning session. Pr 1 post scan is the first pulse rate after scanning completed. Pr 2 post scan is the second pulse rate after scanning completed.

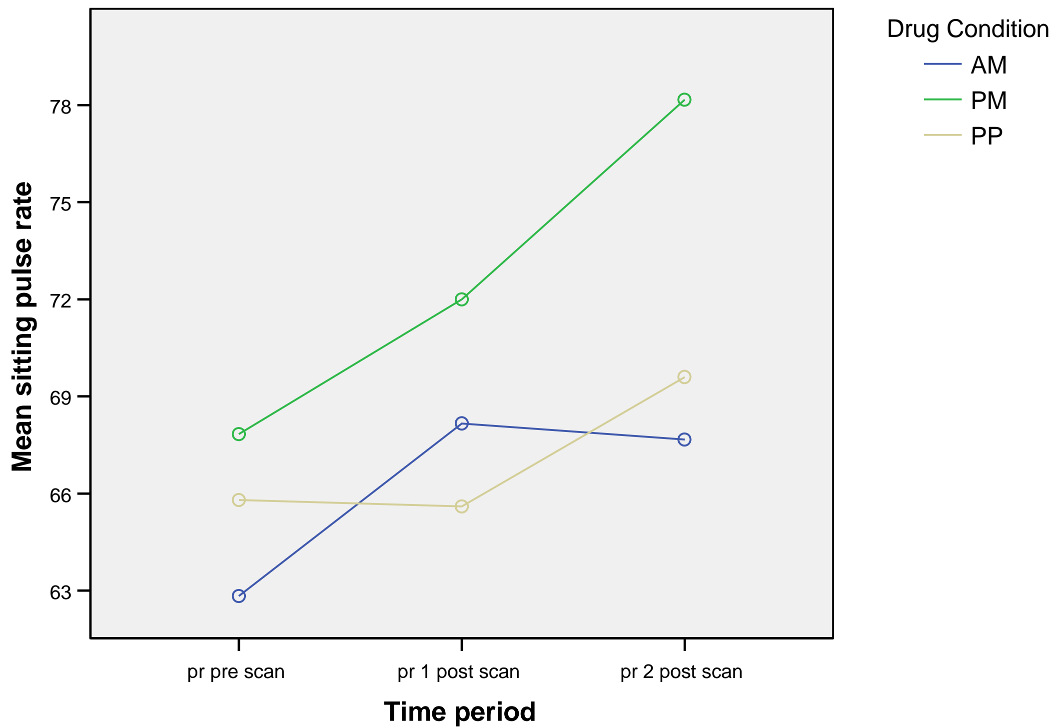


Figure 3.1-2
This is a plot of the changes in sitting pulse rate over time. As in Figure 3.1-1 there were no significant differences between drug conditions but there were differences between time points. Terminology is the same as in Figure 3.1-1.

3.1.3. Visit 2 prolactin levels

Prolactin levels were collected on three separate occasions during visit 2. There was an incomplete dataset due to difficulties with venous access and data loss in the laboratory. The data that was available for analysis is shown in Table 3.1-4.

	T1 (Pre amisulpride/placebo)	T2 (1.5 hours post amisulpride/placebo 0.5 hours prior to Methamphetamine)	T3 (3.5 hours post amisulpride/placebo 1.5 hours after Methamphetamine)
PP	4	4	2
PM	6	6	6
AM	6	6	6

Table 3.1-4
Number of participants for whom serum prolactin levels data was available at the different time periods. T1: prolactin levels at time 1; T2: prolactin levels at time 2; T3: prolactin levels at time 3.

There were a number of different analyses completed using the data collected at the time points as in Table 3.1-4. A one way ANOVA completed using data at T1 (time 1 prolactin levels) did not show any difference between the drug groups. A mixed model ANOVA was used (time was the within subjects factor and drug was the between subjects factor) to compare the PM and AM drug groups at times T1 and T2 in order to examine the effect of amisulpride. This was possible as neither the PM group nor the AM would have received methamphetamine at time T2 and only the AM group would have received the amisulpride at T1. A significant difference in the error variance between the drug conditions was detected at the time T2 using Levene's test ($p=0.031$). Following a log transformation, there were no significant differences in variances. This analysis showed a significant effect of drug condition ($p<0.001$, $df=(1,10)$, $F=45.082$). There was also an effect of time ($p<0.001$, $df=(1,10)$, $F=66.151$) and interaction between time and drug condition ($p<0.001$, $df=(1,10)$,$F=181.403$).The log mean values and the standard deviations are summarised in Table 3.1-5.

	T1	T2
AM (Log mean values (sd))	5.39 (0.21)	7.16 (0.41)
PM (Log mean values (sd))	5.32 (0.245)	4.88 (0.41)

Table 3.1-5
Table of log mean values of serum prolactin levels for two different drug conditions at two different time points. As outlined in Table 3.1-4, only the AM group would have received active medication (amisulpride). (sd = standard deviation).

A paired t test was used to compare the effects of methamphetamine by comparing serum prolactin at time points T2 and T3 in the PM group. At T2 the PM group had not received the methamphetamine whereas by T3 the participants would have had methamphetamine 1.5 hours earlier. No significant difference was detected.

3.2. Challenge phMRI

3.2.1. Behavioural data

3.2.1.1. Rating data

As outlined in the methods section due to faulty equipment, all non response data were taken as participants rating themselves as 3. A justification for this is that on other tasks that participants completed such as the N-back there were low rates of omission. This task was easier to complete than some levels of the N-back so it would seem likely that participants rated themselves on most occasions. For ease of analysis the number of data points was reduced by calculating the mean ratings for each POMS for a time bin. Three ratings were used for each time bin which corresponds to 6 minute periods (apart from the first time bin which lasted 4 minutes). Using this approach, for each POMS, initially, there were 12 ratings which were reduced to 4. This can be seen in Table 3.2-1.

Rt0	Rt1	Rt2	Rt3	Rt4	Rt5	Rt6	Rt7	Rt8	Rt9	Rt10	Rt11
arithmetic mean of Rt0 Rt1 Rt2 = At1			arithmetic mean of Rt3 Rt4 Rt5 = At2			arithmetic mean of Rt6 Rt7 Rt8 = At3			arithmetic mean of Rt9 Rt10 Rt11 = At4		

Table 3.2-1
This demonstrates how the number of ratings for each POMS was reduced. Rt0 represents rating at time 0 and similarly for Rt1, Rt2 etc. Thus, At1 is the mean of the first three ratings and similarly for At2, At3 etc. By this method, 4 ratings are generated from the original 12 ratings for each POMS for each participant.

The rating level for each item of the POMS was plotted over time and then by visual inspection (in line with a priori expectations) two aggregates were apparent as in Figure 3.2-1. Participants rated themselves in a similar manner for the POMS cheerful/carefree/ energetic; this seemed consistent with “high” mood. They also rated themselves similarly for the POMS anxiety/ sluggish / gloomy which seemed consistent with “low” mood.

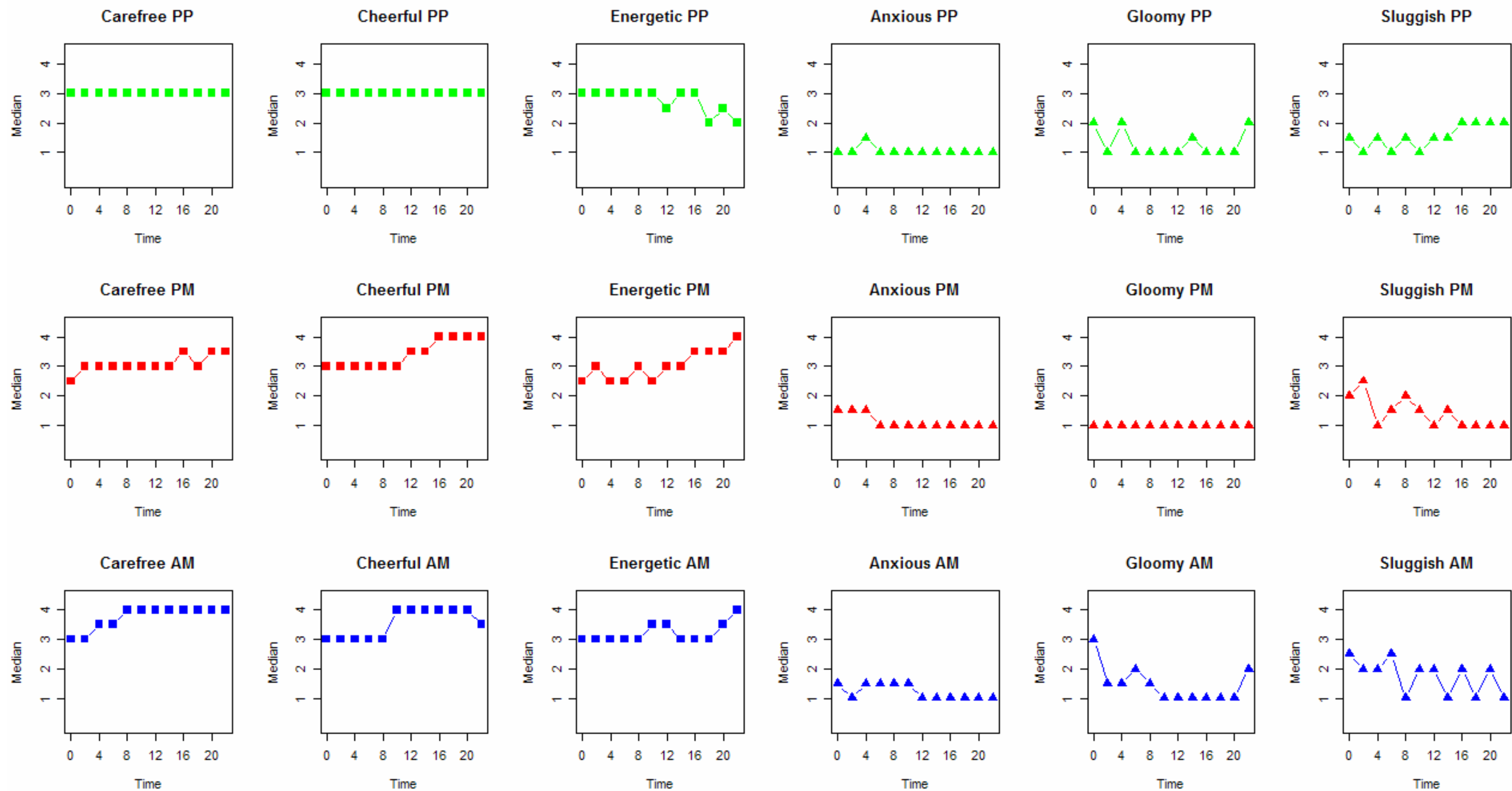


Figure 3.2-1
This figure represents the median ratings of the participants on each of the different POMS for each time point for each drug condition. The "High" POMS (carefree, cheerful, energetic) are in filled square blocks. The "Low" POMS (anxious, gloomy, sluggish) are represented using filled triangles. PP, PM, AM refer to the usual drug conditions. Methamphetamine is given at time point 4.

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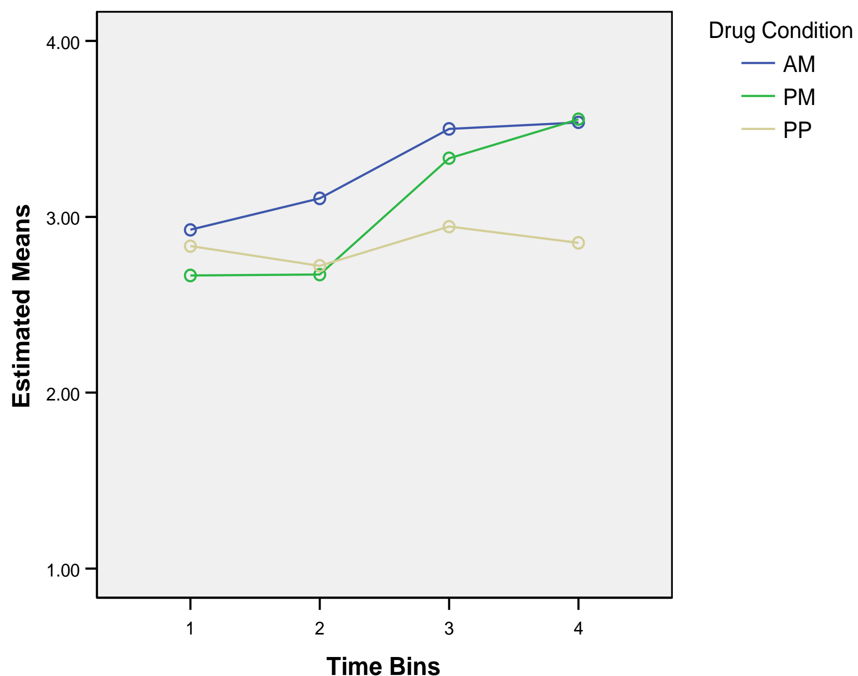


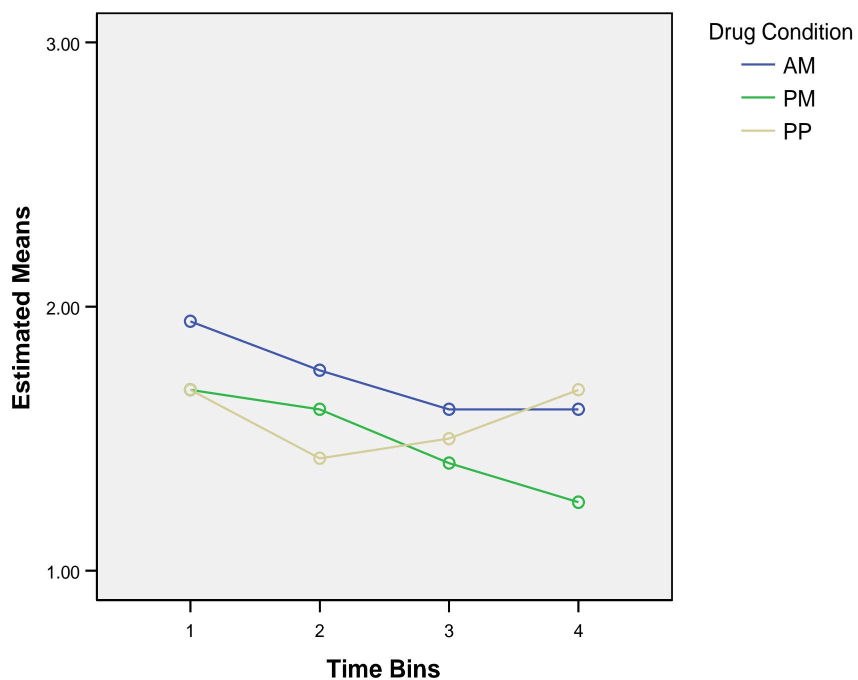
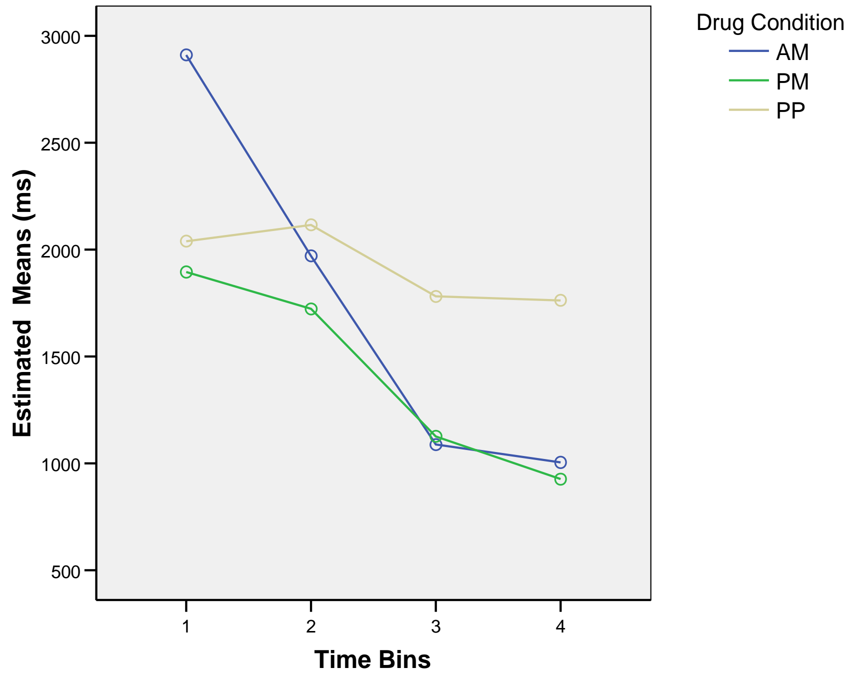


Figure 3.2-2
Plots of “high” (above) and “low” (below) emotion ratings over time. The bolus injection takes place at the end of time bin 1, so effectively, the intravenous bolus effects can first be detected at time bin 2.

As regards analysis of “high” emotion rating, Mauchly’s test showed a significant difference (p=0.012), so a Huynh-Feldt correction was used in tests for differences over time. Using this correction, there was a detectable effect of time bin (p<0.001, df=(2.332, 34.986), F=14.584) and there was a detectable effect for the interaction between time bin and drug condition (p=0.029, df=(4.665, 34.986), F=2.913). No effects of drug condition were detectable using “high” emotion rating. For analysis of “low” emotion rating, Mauchly’s test also showed a significant difference (p=0.017), so Huynh-Feldt correction was used. There was a detectable effect of time bin (p=0.031, F=3.521, df=(2.510, 37.657)) but there was not a detectable effect for interaction between time bin and drug condition. No effect of drug condition was detectable. The mean values for “high” and “low” emotion ratings are plotted in Figure 3.2-2.

3.2.1.2. Reaction time data

Due to the button box problem there were similar problems with missing data as described for the rating analysis. However, further complications arouse as it was not possible to estimate the data for the missing values. A similar method to that described above was used to generate data for the respective time bins and drug conditions in the “high” and “low” emotion groups. However, it should be noted that in this case these averages will be weighted differently depending on the number of data points available.



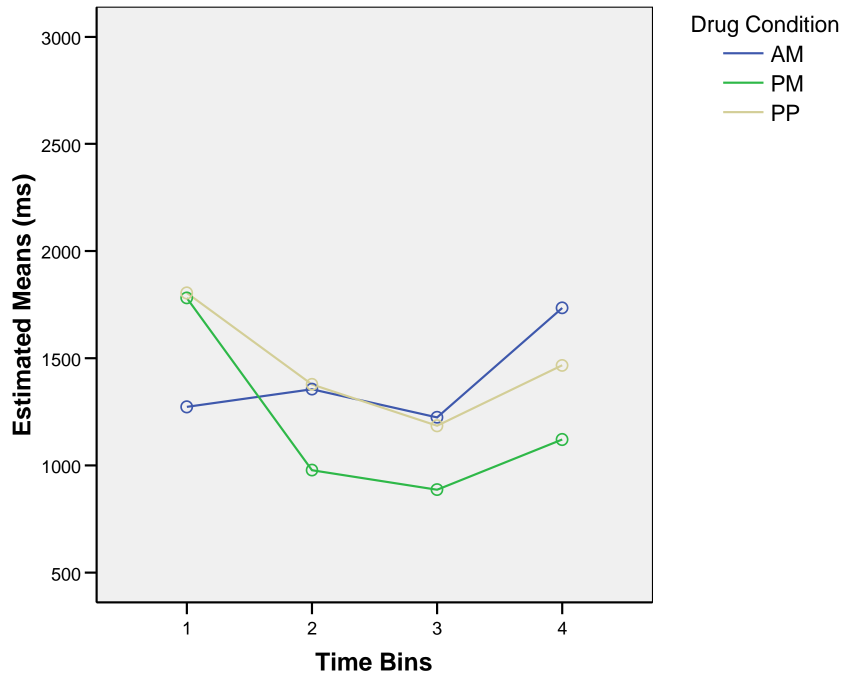


Figure 3.2-3
Plots of reaction times for both “high” (above) and “low” (below) emotion ratings over time. The bolus injection takes place at the end of time bin 1, so effectively, the intravenous bolus effects can first be detected at time bin 2.

When the reaction times for "high" ratings were examined, Levene's test indicated detectable differences in variances for some of the time bins for the drug condition. Thus, the results for drug condition effects should be interpreted with caution. There was a detectable effect of time bin (p<0.001, df= (3, 45), F= 10.716) but only approached significance for interaction between time bin and drug condition (p = 0.071, df= (3, 45), F= 2.109). There were no detectable effects for drug condition. For the reaction times for the "low" ratings, there was a detectable effect of time bin (p = 0.005, df= (3, 45), F= 4.951) but this only approached significance for interaction between time bin and drug condition (p = 0.083, df= (6,45), F= 2.015).

3.2.2. fMRI data analysis

Three main T contrasts were completed. These were done using a whole brain correction for multiple comparisons at p (false discovery rate (FDR)) < 0.05 with an extent threshold of 10 voxels. Masking was done with p(uncorrected (unc)) < 0.05. The voxels identified were the local maxima in each cluster more than 8 mm apart. The first contrast used was the comparison PM-PP over all time bins. This was masked by the positive effects of +PM in order to reduce the effects of a truncation artifact. The results of the contrast are presented in Table 3.2-2. This showed activations in a number of areas: precuneus, rectal gyrus, anterior cingulate, lingual gyrus, insula, thalamus, and cerebellum. The contrast PM-AM was used with the same masking as for the contrast PM-PP with the expectation that similar regions to the contrast PM-PP would be identified. The insula and lingual gyri were activated as in the previous contrast. The other regions activated included areas in the frontal lobe (superior frontal and precentral gyri). These results are presented in Table 3.2-3.To examine the potential deactivating effects of the PM drug group, the contrast PP-PM was used masked by –PM (negative effects of the group PM). This is presented in Table 3.2-4. These areas are in the main, dissimilar to the previous contrasts. Areas detected included: frontal lobe (superior, middle and medial gyri), parietal lobe (postcentral gyrus, supramarginal gyrus and precuneus), lingual gyrus and cerebellum. In Table 3.2-2 and Table 3.2-3, the maximum (peak) and minimal percentage signal change values are presented along with the time in which they occurred. In Table 3.2-4, data for deactivation by the PM group is shown so here peak effect size is the maximum negative percentage change for the various time bins. There were no grey matter area activations identified using

the p value and extent threshold as above for the contrast AM-PM (masked by –PM), the contrast conjunction PM-PP and PM-AM (masked by +PM) and the contrast conjunction PP-PM and AM-PM (masked by –PM).

The activation pattern (PM-PP masked by +PM) for the time bins for all the levels of the drug condition was plotted using the maxima from the clusters identified by the various contrasts of interest both in Figure 3.2-4 and Figure 3.2-7. These graphs suggest that the areas have increased signal with subjects receiving PM and that this is reduced for subjects in the other two drug groups. The latter figure may show more deactivation for subjects in the PP group. The areas of activation in Figure 3.2-4 are possibly better defined dopamine projection sites compared to the areas in Figure 3.2-7. The areas of activation for Figure 3.2-4 are shown overlaid onto slices of the brain volume supplied with SPM5 in Figure 3.2-5 and Figure 3.2-6. An overlay onto slices of a brain volume with areas featured in Figure 3.2-7 is shown in Figure 3.2-8. The deactivation pattern (PP-PM masked by –PM) over time for the different drug levels is shown in Figure 3.2-9. It indicates less of a difference between the PM and AM groups and some increase in signal over time for the PP group.

Region	BA	Side	Talairach Co-ordinates X	Y	Z	Peak % signal change	Peak at Tn	Min % signal change	Min at Tn
Precuneus	7	L	-14	-46	43	10.647	10	2.3151	1
	19		-27	-78	37	4.5336	10	0.0218	1
Insula	13	R	40	-26	14	9.2907	10	1.9762	1
			38	-12	19	11.5073	10	4.2124	1
Thalamus		R	22	-23	12	3.3327	7	1.1408	1
			6	-24	5	10.3322	6	3.1436	1
Lingual Gyrus	18	L	-5	-69	2	6.1142	7	-0.5657	1
		R	6	-69	2	8.517	6	-0.0674	1
Rectal Gyrus	11	R	8	14	-21	9.8768	9	2.9679	1
		L	-10	14	-19	5.8347	9	1.9215	1
Anterior Cingulate	24	R	2	36	7	14.3043	9	4.0104	1
Culmen (Cerebellum)		L	-21	-50	-16	6.818	6	1.6938	1
			-4	-55	-9	6.5485	6	0.4903	1

Table 3.2-2
This summarises the activations of clusters for the contrast PM-PP masked by +PM. In each case the activation of the local maximum was in a region of grey matter. The peak % signal change and the min (minimum) % signal change relate to the PM drug group.

Region	BA	Side	Talairach Co-ordinates X	Y	Z	Peak % signal change	Peak at Tn	Min % signal change	Min at Tn
Superior Frontal Gyrus	6	R	14	19	56	5.523	10	1.3546	3
Precentral Gyrus	4	L	-38	-22	53	7.1186	10	0.6149	1
Insula	13	R	45	-14	12	15.6705	9	4.3827	1
Lingual Gyrus	19	R	19	-66	-3	12.7692	10	1.855	1

Table 3.2-3
This summarises the activations of clusters for the contrast PM-AM masked by +PM. The terminology is the same as in Table 3.2-2.

Region	BA	Side	Talairach Co-ordinates X	Y	Z	Peak % signal change	Peak at Tn	Min % signal change	Min at Tn
Postcentral Gyrus	3	L	-49	-19	41	-9.5449	8	-2.1719	1
Postcentral Gyrus	2	L	-41	-24	47	-6.4317	9	-1.5077	1
Superior Frontal Gyrus	6	R	5	-4	63	-4.6934	10	-1.9017	1
Medial Frontal Gyrus	6	R	10	-8	54	-3.7472	3	-2.2354	1
Superior Frontal Gyrus	6	R	14	17	60	-4.3243	9	-0.6514	1
Postcentral Gyrus	40	R	43	-34	57	-6.9211	9	-2.6444	1
Postcentral Gyrus	1	R	64	-15	28	-7.2601	7	-2.7403	1
Middle Frontal Gyrus^{+}	10	R	36	51	1	-2.9874	6	-0.8125	10
Postcentral Gyrus	40	R	30	-35	55	-5.5754	9	-2.0329	1
Precuneus	19	R	38	-75	35	-4.5718	6	-1.6216	1
Lingual Gyrus	18	R	18	-82	-11	-7.5509	10	-2.5424	1
Declive (Cerebellum)		R	17	-66	-15	-5.6608	9	-0.2424	1
Supramarginal Gyrus	40	L	-54	-49	32	-9.956	10	-1.5264	1
Medial Frontal Gyrus	6	R	11	3	57	-3.2341	8	-1.6968	1

Table 3.2-4

This summarises the activations of clusters for the contrast PP-PM masked by -PM. For each row the activation of the local maximum was in a region of grey matter. As this table shows deactivation for the PM group, here the % signal change is the maximum negative percentage change and min (minimum) % signal change is the least negative percentage change for the various time bins. $^{+}$BA area label within 1 mm of the coordinates using Talairach daemon (no BA label given for the coordinate itself).

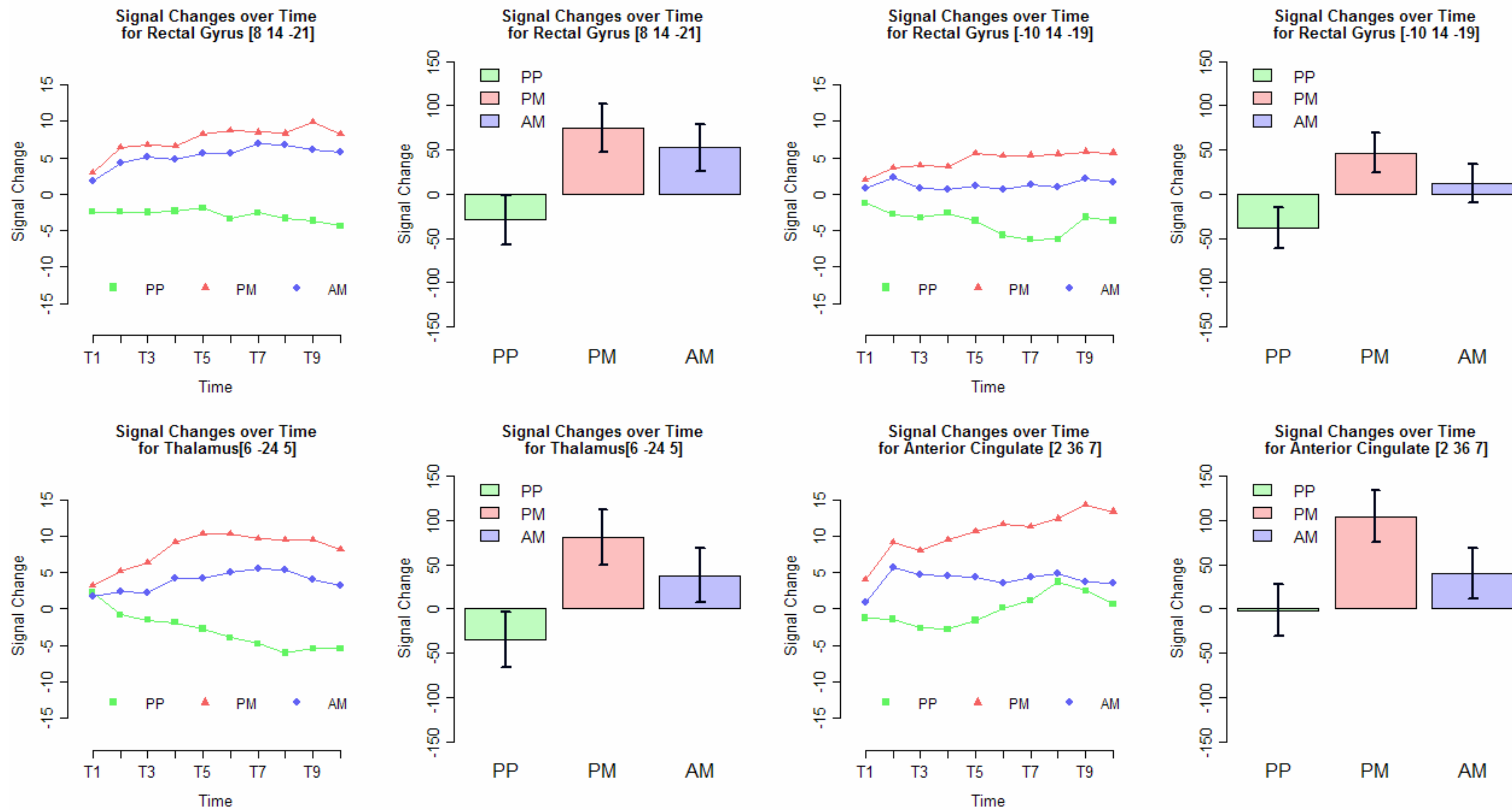


Figure 3.2-4
This figure summarises the activations of clusters for the contrast PM-PP (masked by +PM) using an events of interest F contrast. There are ten time bins each lasting two minutes. These follow the methamphetamine bolus (given during time bin T1). Maxima from 4 regions identified by the contrast are plotted. It was expected that the time bins of the PP group would be similar to those of the AM group but dissimilar to those of the PM group. The error bars are omitted to improve clarity. The barplots show the overall pattern of activations for the different drug groups. Talairach coordinates of the local maxima are in square brackets in the title of each plot (similar format used throughout thesis). The signal change refers to mean % BOLD signal change from baseline.

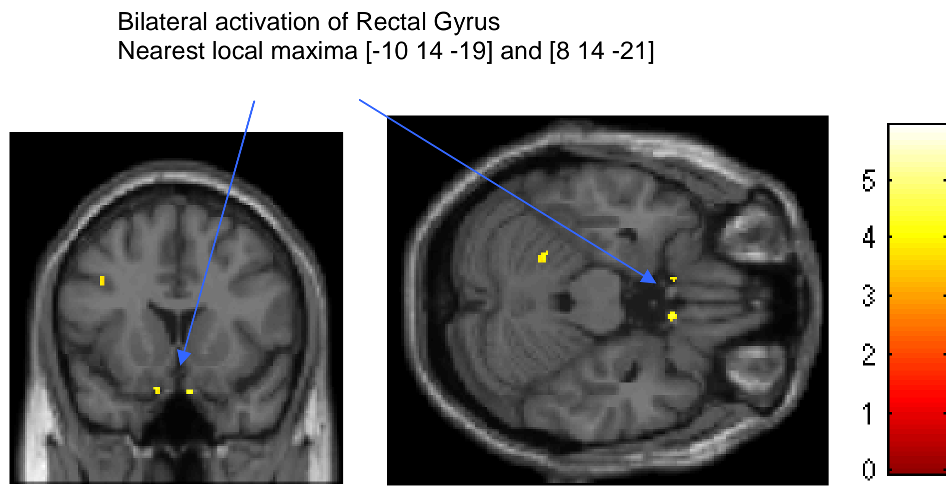


Figure 3.2-5
This figure shows the activations of the clusters for the contrast PM-PP as in Figure 3.2-4. The slices shown are taken in reference to the peak activation of the cluster in the rectal gyrus [8 14 -21]. Colour bar vertical scale relates to increasing BOLD signal.

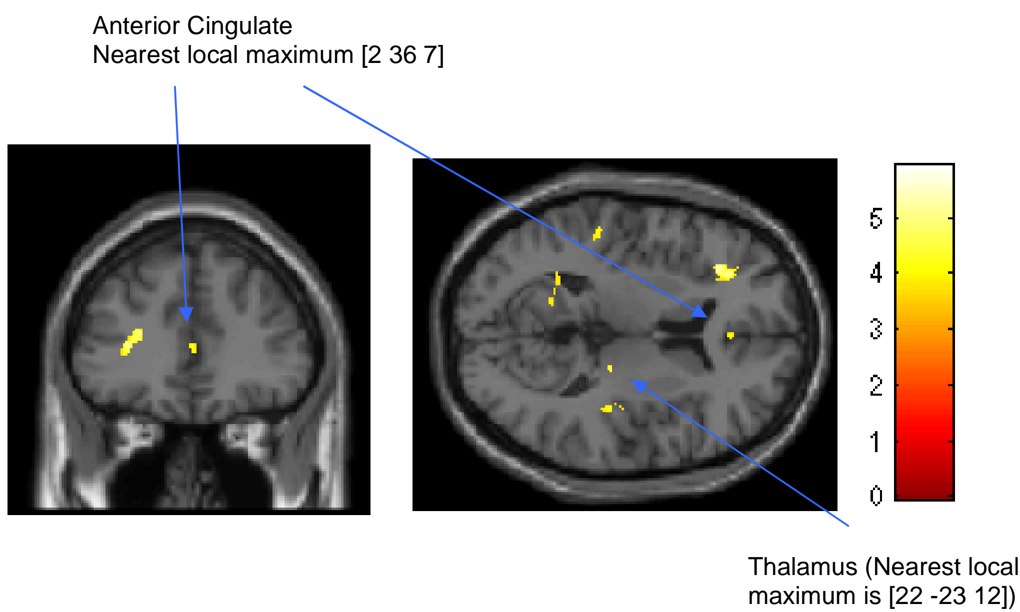


Figure 3.2-6
This figure shows the activations of the clusters for the contrast PM-PP as in Figure 3.2-4. The slices shown are taken in reference to the peak activation of the cluster in the anterior cingulate region [2 36 7]. Otherwise terminology is same as Figure 3.2-5.

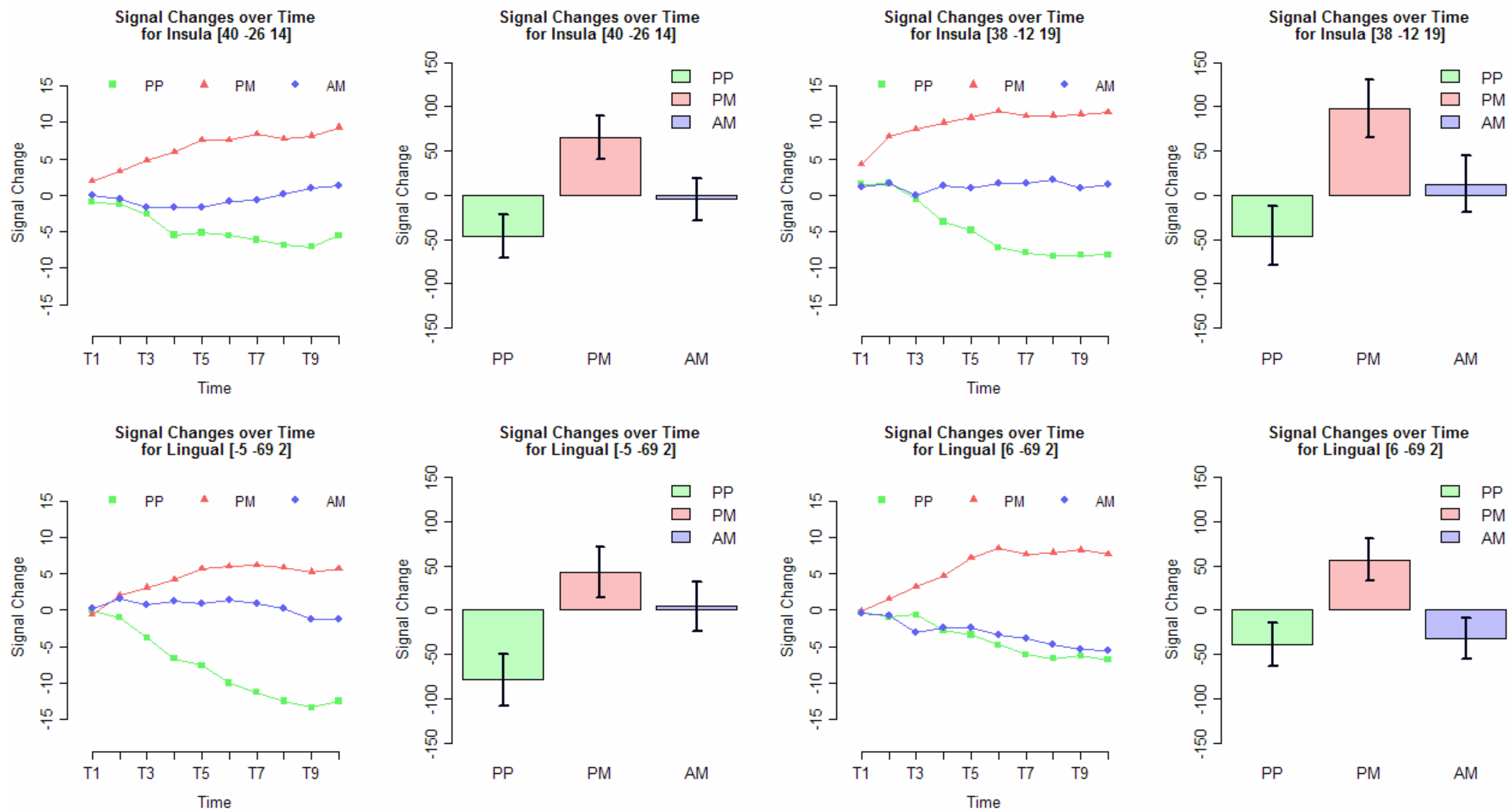


Figure 3.2-7

This figure summarises the activations of clusters for the contrast PM-PP (masked by +PM) using the events of interest F contrast. The format and terminology is similar to Figure 3.2-4 above.

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Bilateral activation of lingual gyrus.
Local maxima [-5 -69 2] and [6 -69 2]

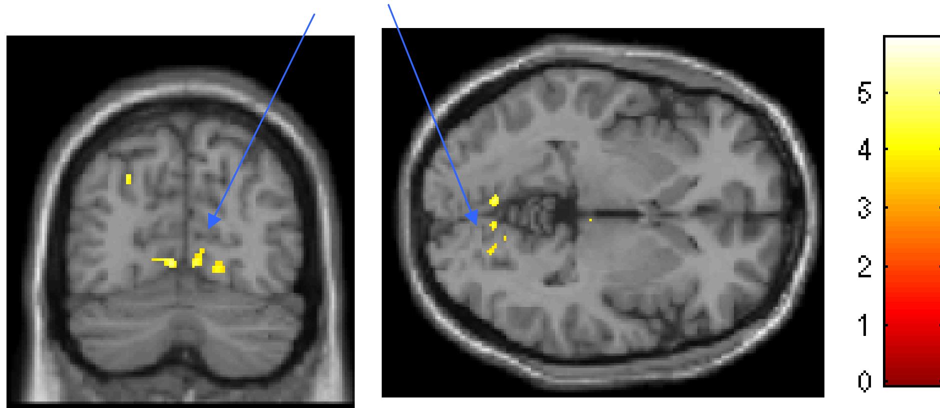

Figure 3.2-8
This figure shows the activations of the clusters for the contrast PM-PP as in Figure 3.2-7. The slices shown are taken in reference to the peak activation of the cluster in the left lingual gyrus [-5 -69 2]. Otherwise terminology is same as Figure 3.2-5.

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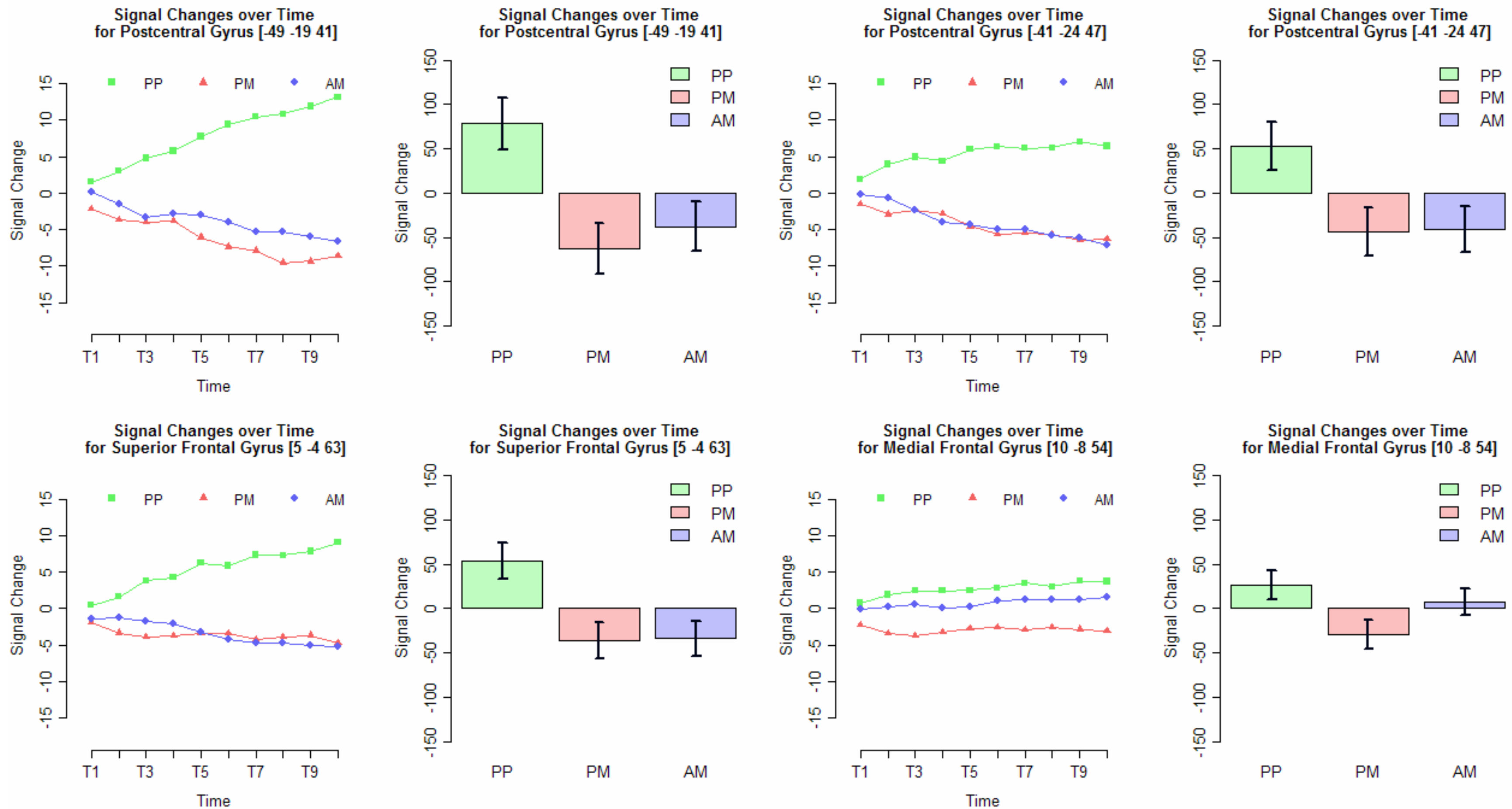


Figure 3.2-9
This figure summarises the deactivations by methamphetamine using the contrast PP-PM (masked by -PM) using the events of interest F contrast. The format and terminology is similar to Figure 3.2-4 above.

3.3. N-back task

3.3.1. Behavioural data

3.3.1.1. Performance data

The performance of the task was compared between the different drug groups. This is summarised in Table 3.3-1. This lists the number of omission and commission errors for each level of the task for each drug condition. There is a difference in the number of errors between the 2-back and each of the other two levels. Statistical analysis was completed on the 2-back level using Fisher's exact test which showed no significant difference. The table is shown in appendix 6.2.

	Omission			**Commission**		
	0-back	1-back	2-back	0-back	1-back	2-back
PP	1	0	2	0	0	4
PM	0	0	6	1	0	3
AM	1	1	4	1	0	4
Total	**2**	**1**	**12**	**2**	**0**	**11**

Table 3.3-1
Table outlining the number of commission and omission errors in each level of the N-back task. The total number of errors in each level of the task is also given.

3.3.1.2. Reaction time data

For the reaction time data, there was a significant difference between the reaction times for the N-back level ($p<0.001$, df (2,26), $F= 31.529$) but there were no significant differences in reaction times for the drug condition or significant interactions between drug condition and N-back level. A plot of the drug condition and N-back level is displayed in Figure 3.3-1. The N-back task increases in difficulty progressively (0-back to 1-back to 2-back) and this is reflected in the reaction times.

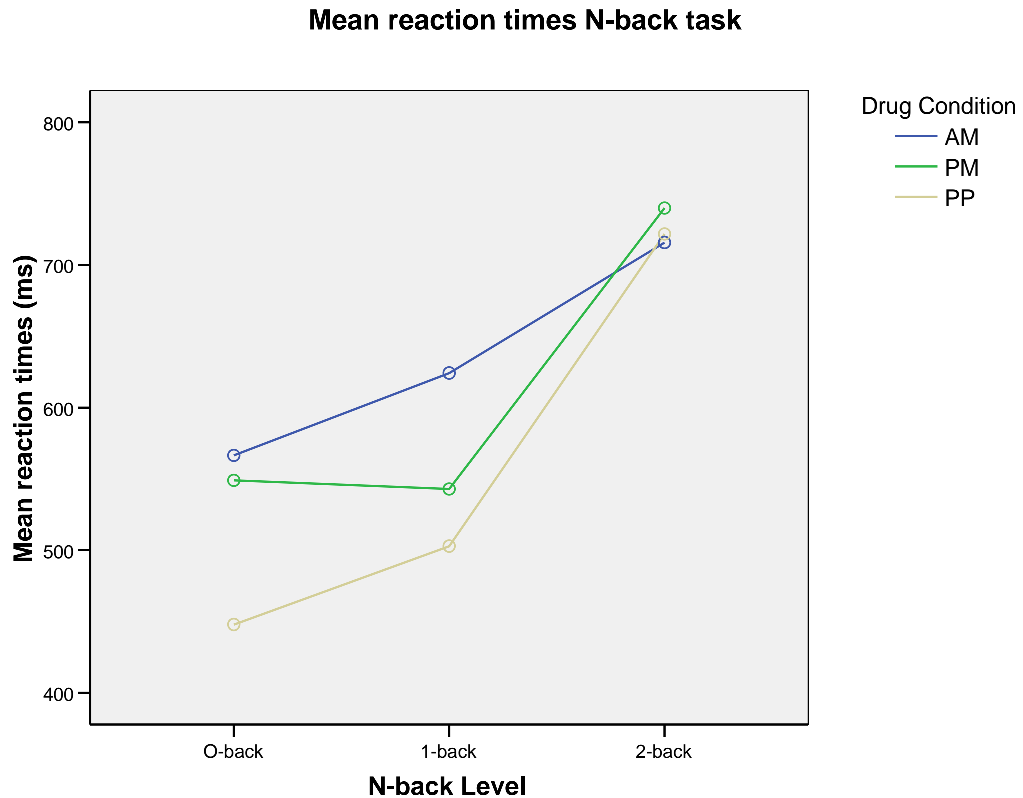


Figure 3.3-1
This is a plot of mean reaction time for each drug condition (in milliseconds) and each N-back level. There are significant differences between the levels of the N-back task as discussed in the text.

In order to assess whether unblinding one of the participants affected his performance, a comparison was made between him and the other participants in his drug condition group (PP). This participant made one omission error and no commission errors. 2 out of the 5 PP participants made the same number of omission errors and commission errors. The mean reaction times for the 0-back, 1-back and 2-back conditions for the unblinded participant were within one standard deviation of the PP participants. This suggests that his performance matched similar participants.

3.3.2. fMRI data results

Unless otherwise stated, the contrasts were completed using p(unc) < 0.01; extent threshold of 10 voxels and masking with p(unc) < 0.05.

3.3.2.1. Main effects of task

To examine the overall pattern of activation for the task, the T contrast positive effects of task was used with p(family wise error (FWE)) < 0.05. The areas identified with this contrast are presented in Table 3.3-2. These were: frontal lobe (inferior and superior frontal gyri), parietal lobe (superior and inferior lobules) and cingulate gyrus. The areas of activation are shown (rendered onto a cortical surface) in Figure 3.3-2. Areas of activation for the F contrast main effects of task masked by positive effects of task are summarised in Table 3.3-3. These areas are similar to that of the positive effects of all conditions above (inferior frontal gyrus, parietal lobe (superior and inferior lobules) and cingulate gyrus) but also identified the precuneus. The method included analysis of a single cluster (which calculates local maxima at greater than 4 mm apart) in the cingulate region. This was completed because in volume wide analysis, the coordinates of local maxima are calculated at greater than 8mm apart. However, the local maxima calculated in this way within this cluster did not correspond to grey matter using the Talairach client tool.

3.3.2.2. Main effects of drugs

There was one area of activation detected using main effects of drugs masked by positive effects of all conditions. The cluster mainly corresponded to the area BA 40 in the right parietal lobe and the data are summarised in Table 3.3-4. The activation pattern for each level of the N-back condition for each drug condition for one of the voxels is shown in Figure 3.3-3.

3.3.2.3. Conjunction of PM-PP and PM-AM

There were two areas of activation detected using the conjunction of PM-PP and PM-AM which was completed across both N-back levels. This is summarised in Table 3.3-5. The two regions detected include the inferior frontal gyrus and inferior parietal lobule. The local maxima of these clusters did not correspond to grey matter but one cluster largely corresponded to the area BA 45 in the left frontal lobe. The other cluster was largely in a region (BA 40) similar to that identified using main effects of drugs masked by positive effects of all conditions.

3.3.2.4. Conjunction of PP-PM and AM-PM

The deactivation conjunction PP-PM and AM-PM is presented in Table 3.3-6. This showed deactivation in the cuneus, lingual gyrus, cerebellum,

parahippocampal gyrus and frontal gyrus. The activation pattern for each level of the N-back condition for each drug condition was plotted using the maxima from the clusters for each of conjunctions. These plots are presented in Figure 3.3-4 (conjunction PM-PP and PM-AM) and Figure 3.3-5 (conjunction PP-PM and AM-PM).

Cluster size	Z score	Side	Area	BA	X	Y	Z
215	6.13	Right	Superior Parietal Lobule	7	25	-62	48
215	6.05	Right	Inferior Parietal Lobule	40	39	-48	51
215	5.98	Right	Superior Parietal Lobule	7	30	-55	48
217	5.94	Left	Inferior Parietal Lobule	40	-39	-52	48
217	5.66	Left	Inferior Parietal Lobule	40	-37	-45	41
117	5.91	Left	Superior Frontal Gyrus	6	-4	8	51
117	5.56	Left	Cingulate Gyrus	32	-4	20	41
134	5.69	Left	Inferior Frontal Gyrus	9	-48	10	32
134	5.56	Left	Inferior Frontal Gyrus	9	-52	20	25
28	5.47	Right	Inferior Parietal Lobule	40	43	-36	44

Table 3.3-2
These are the clusters identified using the contrast positive effects of all conditions. This was completed using p(FWE) < 0.05. The activations of the presented local maxima were all in grey matter regions.

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
940	0.02	3.86	Left	Superior Parietal Lobule	7	-37	-54	48
940	0.021	3.8	Right	Superior Parietal Lobule	7	27	-62	48
271	0.037	3.08	Right	Cingulate Gyrus*	32	5	29	28
20	0.024	3.54	Right	Precuneus	39	41	-68	36
31	0.037	3.09	Left	Inferior Frontal Gyrus	47	-39	16	-4
10	0.047	2.87	Left	Inferior Frontal Gyrus	9	-52	19	25
23	0.067	2.58	Right	Inferior Parietal Lobule	40	45	-34	47

Table 3.3-3
This summarizes data for the contrast main effects of task masked by positive effects of all conditions. This was completed using p(unc) < 0.01 with an extent threshold of 10 voxels. The mask used p(unc) < 0.05. The activation of the local maximum was in a region of grey matter in each case. * Local maximum was identified at cluster level.

Cluster size	Z score	Side	Area	BA	X	Y	Z
16	2.83	Right	Inferior Parietal Lobule	40	37	-48	51
16	2.79	Right	Inferior Parietal Lobule	40	36	-52	41

Table 3.3-4
This is the area of activation identified using the contrast main effects of drugs masked by positive effects of all conditions. This was completed using p(unc) < 0.01 and extent threshold of 10 voxels. The mask was used with p(unc) < 0.05. The activations of the local maxima were in regions 1 mm from the parietal region. p(FDR) = 0.658 for all rows in the table.

Cluster size	Z score	Side	Area	BA	X	Y	Z
5	2.76	Left	Inferior Frontal Gyrus	45	-53	22	18
3	2.68	Right	Inferior Parietal Lobule^{+}	40	39	-48	54

Table 3.3-5
Clusters identified using the contrast conjunction PM–PP and PM–AM. This was completed using p(unc) < 0.01 with an extent threshold of 10 voxels. The activation of the local maximum at [-53 22 18] was not in BA 45 region but the cluster was mainly within BA 45. The activation of the local maximum at [39 -48 54] was near to BA 40 and this area was also detected using the contrast main effects of drugs masked by positive effects of all the conditions. p(FDR) = 1 for all rows in the table.

Cluster size	Z score	Side	Area	BA	X	Y	Z
37	4.11	Left	Cuneus	18	-7	-92	14
15	3.28	Right	Lingual Gyrus	18	11	-59	3
12	2.88	Right	Cuneus	19	5	-81	30
2	2.56	Right	Parahippocampal Gyrus	34	27	4	-15
3	2.55	Right	Dentate (Cerebellum)		12	-52	-21
4	2.48	Left	Superior Frontal Gyrus	10	-23	47	23

Table 3.3-6
These are the clusters identified using the contrast conjunction PP–PM and AM-PM. This was completed using p(unc) < 0.01 with an extent threshold of 10 voxels. The activations of the presented local maxima were all in grey matter regions. p(FDR) = 0.845 for all rows in the table.

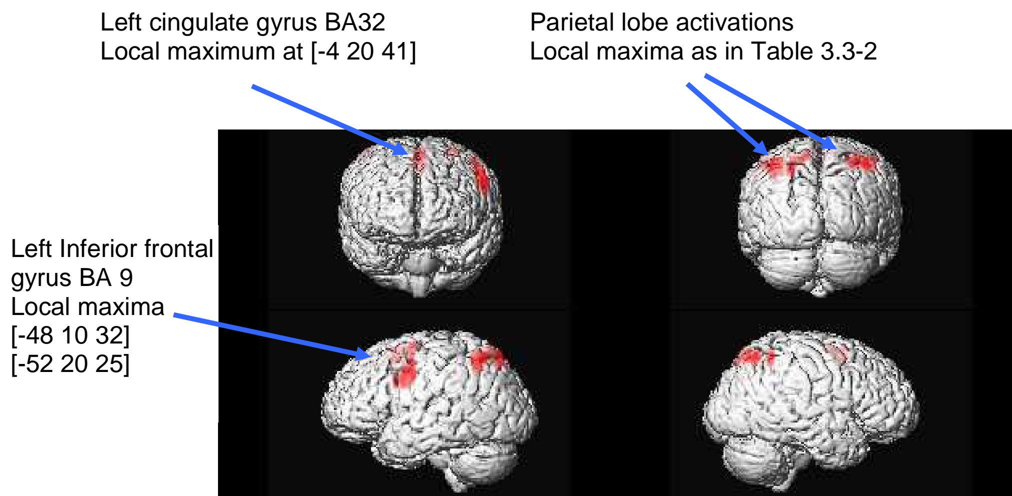


Figure 3.3-2
This show the pattern of activation for the contrast positive effects of all conditions (as in Table 3.3-2) showing expected areas of activation using p(FWE) < 0.05.

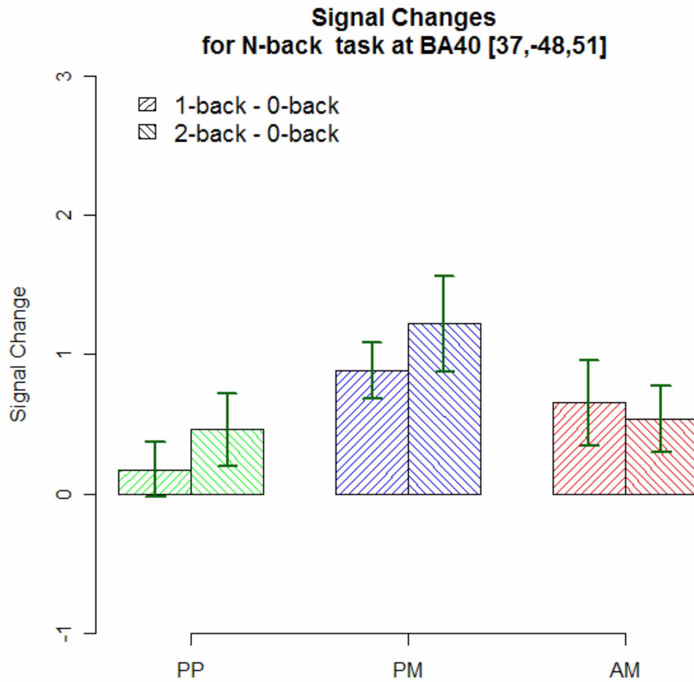


Figure 3.3-3
This figure compares the pattern of activation for each level of the drug conditions PP, PM, AM. The area of activation was identified using the contrast main effects of drugs masked by positive effects of all conditions. The details about the voxel used are in Table 3.3-4 above. Error bars are 90% confidence intervals. The signal change refers to mean % BOLD signal changes from baseline.

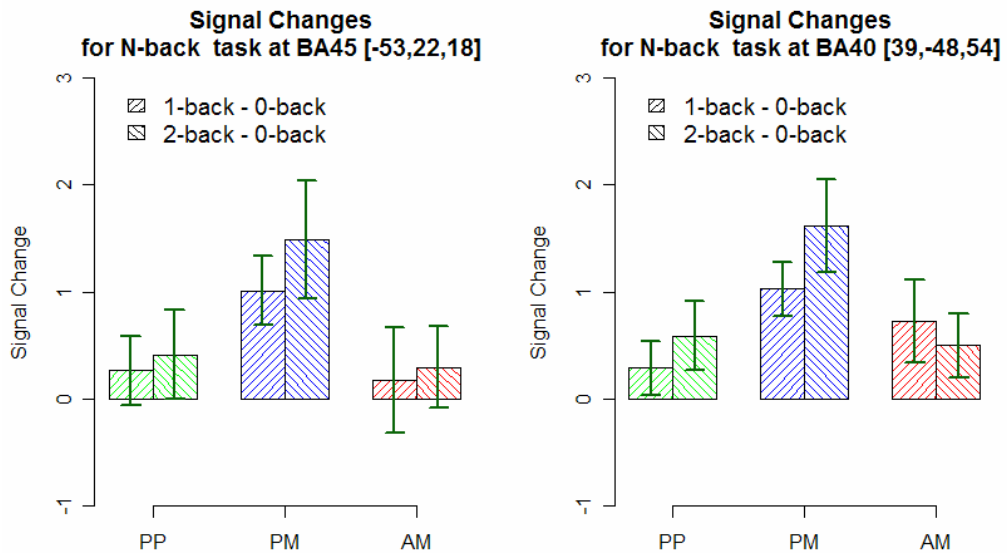


Figure 3.3-4
This figure compares the pattern of activation for each level of the drug conditions PP, PM, AM in two different areas. The areas of activation were identified using the contrast conjunction PM–PP and PM–AM. The details about the voxels used are presented in Table 3.3-5 above. Error bars are 90% confidence intervals. The signal change refers to mean % BOLD signal changes from baseline.

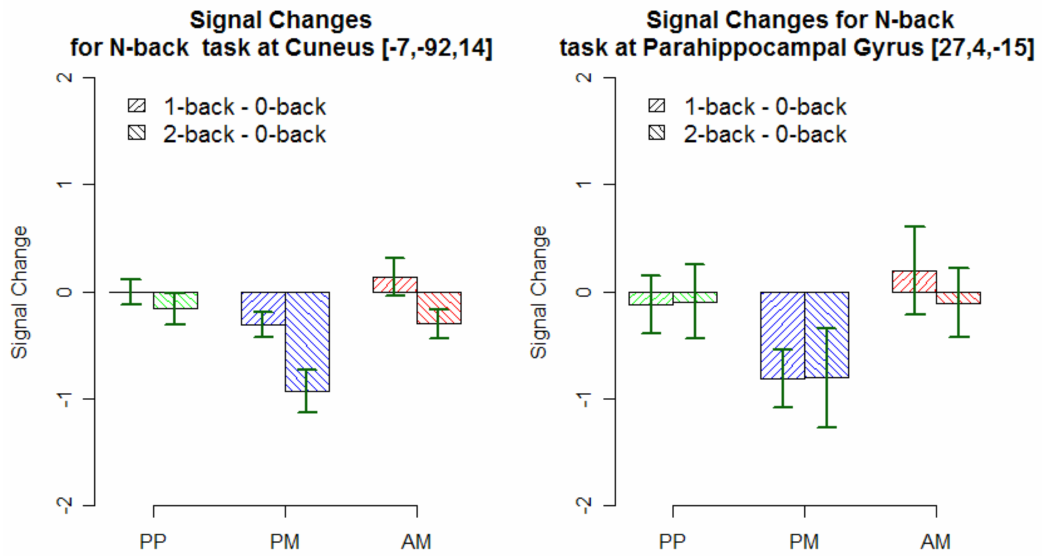


Figure 3.3-5
This figure compares the pattern of activation for each level of the drug conditions PP, PM, AM in two different areas. The areas of activation were identified using the contrast conjunction PP-PM and AM–PM. The voxels used were the local maxima of each cluster. Details about the voxels used are presented in Table 3.3-6 above Error bars are 90% confidence intervals. The signal change refers to mean % BOLD signal changes from baseline.

3.4. Finger tapping task

3.4.1. fMRI data analysis

3.4.1.1. Main effects of Task

The T contrast positive effects of task for all drug conditions was used to examine the validity of the task. This was examined using p(FWE) < 0.05. The details are summarised in Table 3.4-1. The areas of activation included regions as expected for movement of a finger: post central gyrus, precentral gyrus, medial frontal gyrus (caudal BA6), inferior parietal lobule and cerebellum. The areas of activation are shown overlaid onto slices of a brain volume in Figure 3.4-1.

3.4.1.2. Main effects of Drugs

Two T contrasts were used to examine the main effects of drugs. The T contrast PM-PP was used to examine the effects of methamphetamine and the regions identified are presented in Table 3.4-2. Regions of activation were detected in middle frontal gyrus, temporal lobe (superior and middle lobes) and cingulate gyrus. The T contrast PP-PM examined the deactivating effects of methamphetamine. This revealed a number of regions in various locations (lingual gyrus, inferior frontal gyrus, amygdala, cerebellum) distinct from the activation contrast. These regions are presented in Table 3.4-3.

3.4.1.3. Conjunction of PM-PP with PM-AM and PP-PM with AM-PM

Data in Table 3.4-4 and Table 3.4-5 indicate the grey matter activations using the conjunctions PM-PP with PM-AM (activation) and PP-PM with AM-PM (deactivation) respectively. For both conjunctions the values p(unc) < 0.01 and extent threshold of 10 voxels were used. The former conjunction showed activation in a number of areas: frontal lobe (medial, middle and inferior gyri), caudate regions and hippocampus. The latter conjunction identified regions as follows: inferior frontal gyrus, thalamus and cerebellum. The pattern of activation for the conjunction PM-PP with PM-AM is shown in Figure 3.4-2 and the pattern of activation for the conjunction PP-PM with AM-PM is shown in Figure 3.4-3.

Cluster size	Z score	Side	Area	BA	X	Y	Z
162	6.6	Right	Cerebellum (Culmen)		18	-51	-15
113	6.42	Left	Precentral Gyrus	4	-37	-20	49
42	5.48	Left	Medial Frontal Gyrus	6	-5	-6	55
42	5.3	Left	Medial Frontal Gyrus	6	-2	-3	49
51	5.43	Left	Postcentral Gyrus	2	-45	-22	49
51	5.41	Left	Postcentral Gyrus	2	-53	-21	43
51	5.04	Left	Postcentral Gyrus	2	-43	-30	56
16	5.16	Right	Inferior Parietal Lobule	40	39	-43	44
16	5.08	Right	Inferior Parietal Lobule	40	37	-46	54

Table 3.4-1
This is a summary of the activations of clusters for the positive effects of task for all drug conditions using a corrected p(FWE) < 0.05. The activation of the local maximum was in a region of grey matter in each case.

Cluster size	Z score	Side	Area	BA	X	Y	Z
58	2.7	Left	Cingulate Gyrus	31	-16	-29	40
101	3.02	Right	Middle Temporal Gyrus	19	50	-62	16
101	3	Right	Superior Temporal Gyrus	39	43	-58	19
101	2.55	Right	Superior Temporal Gyrus	39	43	-58	29
11	2.79	Left	Middle Frontal Gyrus	6	-39	-3	49
13	2.79	Left	Middle Frontal Gyrus	8	-30	14	38
14	2.73	Left	Middle Frontal Gyrus	8	-37	20	47

Table 3.4-2
This summarises the activations of clusters for the contrast PM-PP. This was completed using p(unc) < 0.01 with an extent threshold of 10 voxels. In each case the activation of the local maximum was in a region of grey matter. p(FDR) = 0.911 for all rows in the table.

Cluster size	Z score	Side	Area	BA	X	Y	Z
27	2.39	Left	Lingual Gyrus	18	-2	-82	-5
32	2.96	Left	Amygdala		-25	-4	-15
10	2.71	Left	Cerebellum (Declive)		-21	-62	-20
10	2.62	Right	Inferior Frontal Gyrus	9	57	19	22

Table 3.4-3
This summarises the activations of clusters for the contrast PP-PM. p(FDR) = 0.998 for all rows in the table. Other aspects are the same as in Table 3.4-2.

Cluster size	Z score	Side	Area	BA	X	Y	Z
6	2.98	Right	Medial Frontal Gyrus	6	20	6	51
2	2.53	Right	Caudate Head		5	11	3
2	2.46	Right	Hippocampus		29	-32	-4
2	2.41	Left	Middle Frontal Gyrus	6	-39	-3	49
3	2.38	Left	Inferior Frontal Gyrus	45	-48	25	8

Table 3.4-4
This summarises the activations of clusters for the conjunction contrast PM-PP and PM-AM. p(FDR) = 0.997 for all rows in the table. Other aspects are the same as in Table 3.4-2.

Cluster size	Z score	Side	Area	BA	X	Y	Z
4	2.64	Left	Inferior Frontal Gyrus	46	-45	47	4
2	2.56	Right	Inferior Frontal Gyrus	47	55	19	-1
4	2.54	Left	Ventral Lateral Nucleus (Thalamus)		-12	-12	13
1	2.36	Right	Declive (Cerebellum)		23	-60	-12

Table 3.4-5
This summarises the activations of clusters for the conjunction contrast PP-PM and AM-PM. p(FDR) = 1 for all rows in the table. Other aspects are the same as in Table 3.4-2.

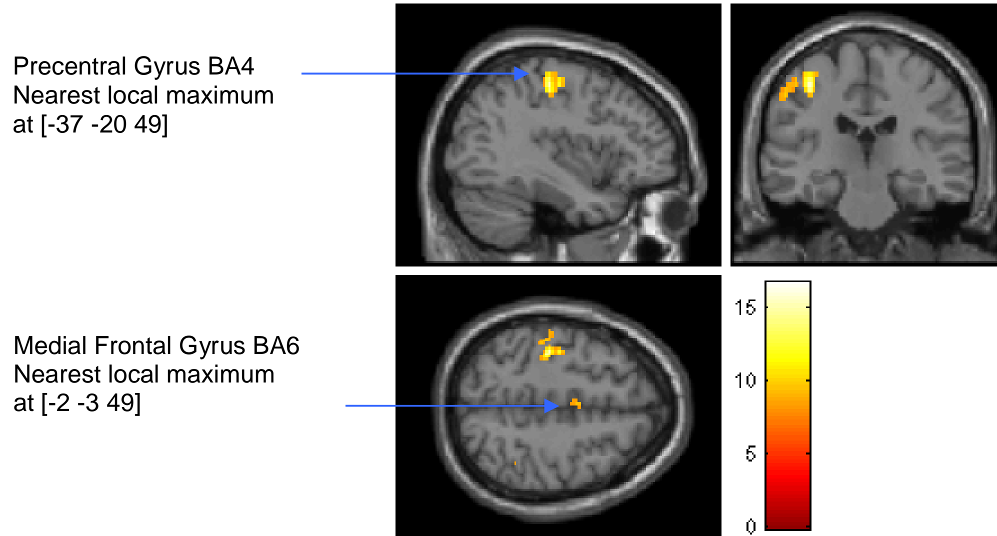


Figure 3.4-1
This shows expected patterns of activation for the contrast positive effects of task for all drug conditions. This corresponds to the data presented in Table 3.4-1. The slices shown are taken in reference to the peak activation of the cluster in the precentral gyrus [-37 -20 49]. Colour bar vertical scale relates to increasing BOLD signal.

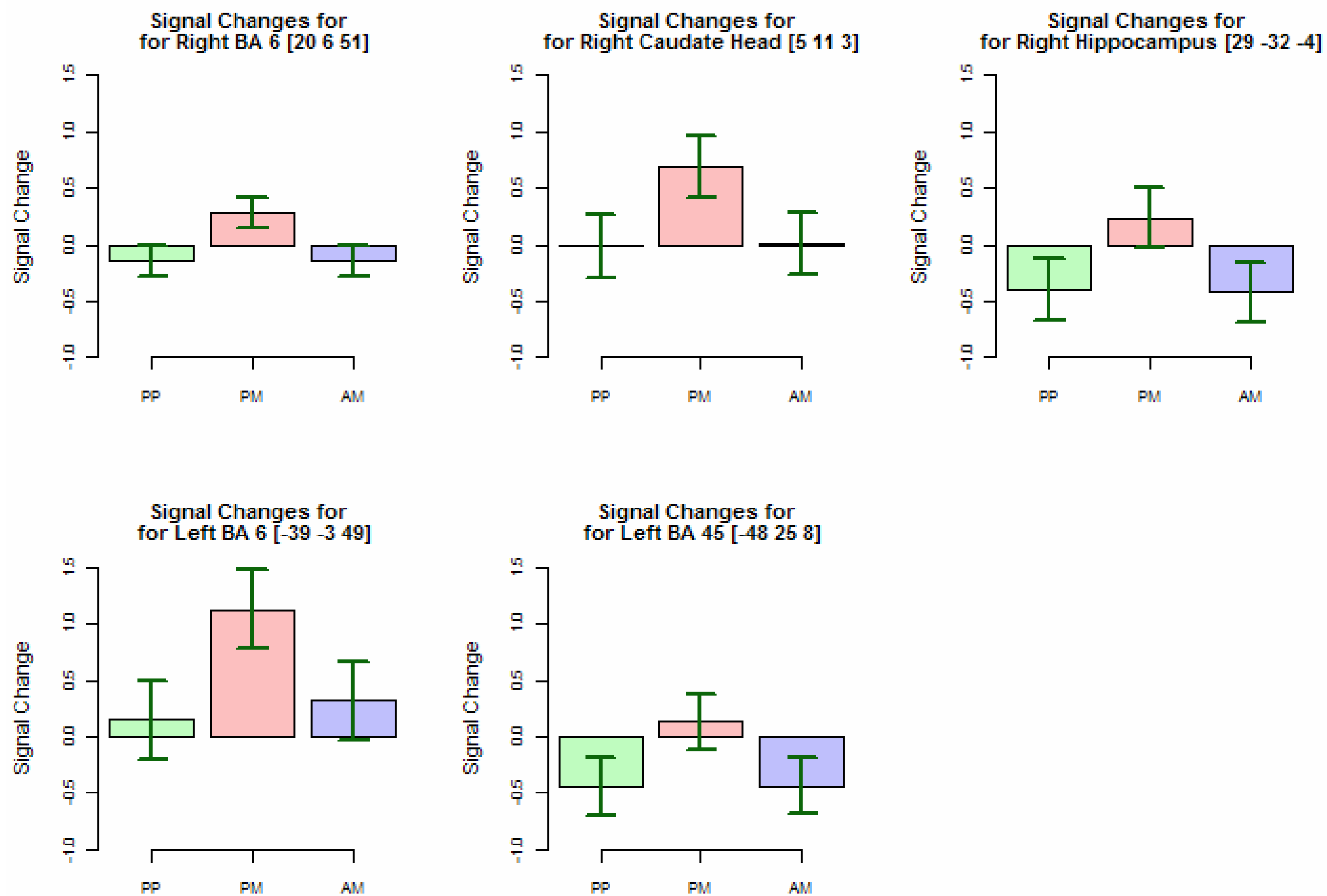


Figure 3.4-2
This figure compares the pattern of activation for each level of the drug conditions PP, PM, AM. The areas of activation were identified using the activation conjunction contrast PM-PP and PM-AM. Detailed data are presented in Table 3.4-4.

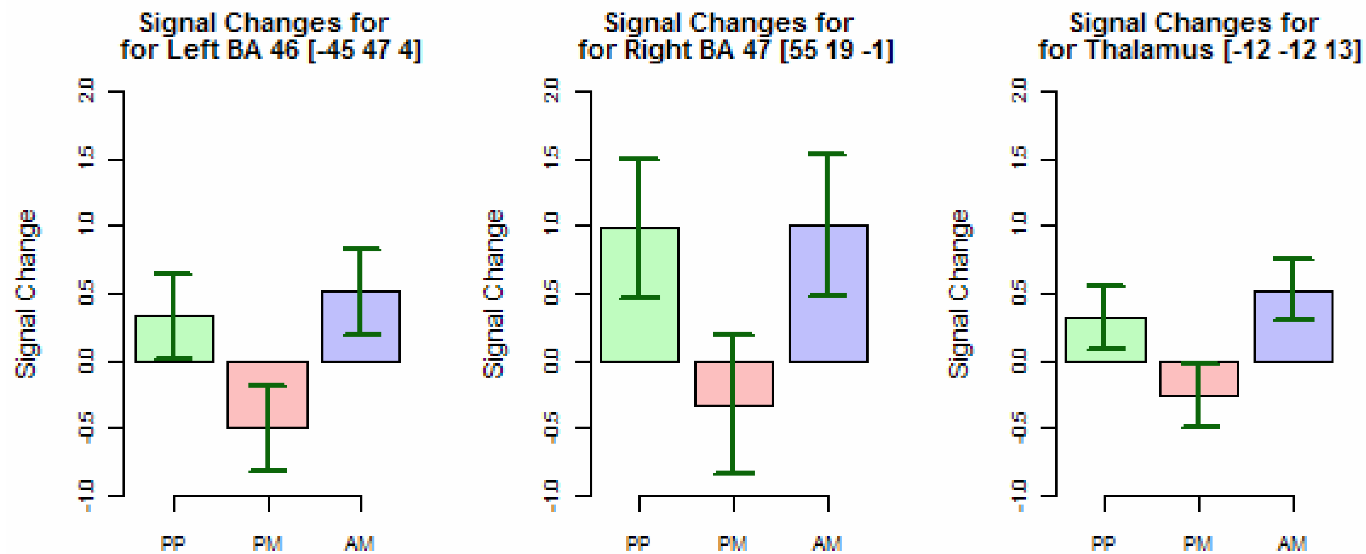


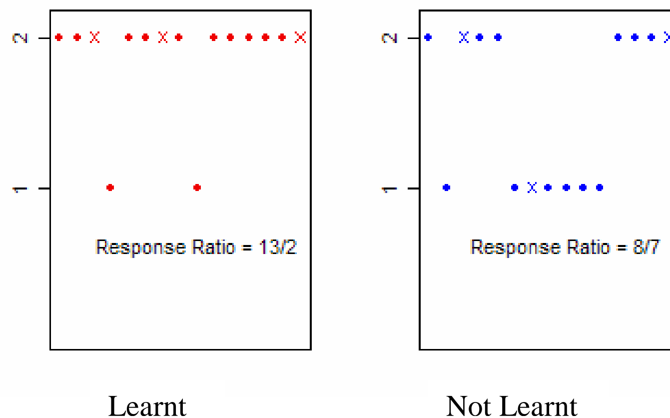
Figure 3.4-3
This figure compares the pattern of activation for each level of the drug conditions PP, PM, AM. The area of activations was identified using the deactivation conjunction contrast PP-PM and AM-PM. Detailed data are in Table 3.4-5.

3.5. *Reward learning task*

3.5.1. Behavioural data

3.5.1.1. Performance data

The first part of the analysis was finding out whether the participant learnt the optimal response for each type of reward and punishment stimulus. Preliminary analysis consisted of plotting the response made for each type of stimulus for each participant. For the reward and punishment stimuli, some responses were optimal and so if the participant had learnt the task correctly the optimal response should predominate particularly at the end of the block. This can be seen in Figure 3.5-1. Graphs of the response patterns for all the participants are given in Appendix 6.3.



Learnt Not Learnt

Figure 3.5-1
Plot of a learnt and a not learnt block of trials. The y axis refers to the response taken represented as either 1 or 2 in each case; the x axis is a plot of time of the total number of trials. The circles refer to the usual contingency between response taken and feedback given. The crosses represents occasions when this contingency is reversed. In both cases the optimal response is represented as 2. When a participant took this response the participant received a more favourable outcome. The left figure indicates that the participant immediately chose the optimal response (by chance). The participant persisted with this response for most of the sequence. In the figure on the right the participant seemed to repeatedly change his response. The pattern on the right suggests that the participant has learnt the optimal response but the participant on the left does not seem to have learnt the optimal response. The response ratio refers to the ratio of optimal responses to suboptimal responses.

However, it might be that the participant picked the suboptimal response predominately. The participant in that case would have incorrectly learnt the task. It might be that the participant had not picked a particular response consistently by the end of the block. In this case the participant was deemed to have not learnt the task. The design was such that on 3 out of 15 occasions the optimal response resulted in a suboptimal outcome. To classify whether

participants learnt the task, various cut off thresholds based on the responses were used. The first form of classification used the ratio of optimal responses to total number of responses. Two cut off points were used: an upper cut off point of 11/15 (73%) and a lower cut off point of 9/15 (60%). Participants who selected the optimal responses at a percentage greater than or equal to 73% were deemed to have learnt the task. Those who selected responses at less than or equal to 60% were deemed to have either not learnt the task or learnt the task incorrectly. The method of assessment for having incorrectly learnt the task was calculated with the same ratios but the number of suboptimal responses rather than optimal responses was used in the percentage calculation.

For blocks with intermediate values (72- 61%), the first five responses were omitted and the pattern of remaining responses was examined. This was done on the basis that the first response is entirely based on chance and as the block proceeds, the more likely that the person would respond in an optimal manner if the task had been learnt. Similarly to the first line classification, a threshold was used to classify whether the participant had learnt the optimal response for a particular block. In this case if the participant had a percentage of optimal to total number of responses greater than or equal to 70%, then they were deemed to have learnt the optimal response for that block. The overall results of the classifications are presented in Table 3.5-1.

Image type	Drug condition	Number of learnt blocks	Number of incorrectly learnt blocks	Number of blocks not learnt
Reward	PP	6	1	5
	PM	9	2	1
	AM	8	2	2
Punishment	PP	8	0	4
	PM	4	1	7
	AM	7	0	5

Table 3.5-1
This table summarises the responses on the basis of drug condition and image type. One feature is that for the punishment image type there might have been some impairment of the PM group to learn the optimal response (PM=4, PP=8, AM=7). In contrast, the PM group seemed to perform slightly better than the PP group for the reward image type (PM=9, PP=6, AM=8).

3.5.1.1.1. Logistic regression

In order to evaluate statistically whether the participants had learnt the task, binary logistic regression was used. The data were split into learnt, not learnt

and incorrectly learnt sets. For each set, a logistic regression was completed with response as the dependent variable and trial number as the covariate. The optimal response for all of the various stimuli was set as 1 and suboptimal response was set as zero. The assumption being that in the block that is learnt by a participant, the higher trial numbers (corresponding to the later part of the block) should consistently relate to the optimal response. The results of the regression for each of the three different learning sets are presented in Table 3.5-2.

	Wald	Significance	Exp(B)	Lower CI for Exp(B)	Upper CI for Exp(B)
Learnt dataset	52.238	0.000	1.220	1.156	1.288
Incorrectly learnt dataset	9.161	0.002	0.401	0.222	0.725
Not learnt dataset	.518	0.472	0.982	0.936	1.031

Table 3.5-2
Summary of results of logistic regression on different datasets. Response was the dependent variable and trial number was used as the covariate.

In Table 3.5-2, the Wald statistic enables the statistically testing (using a chi squared distribution) of the B coefficient. The Exp(B) term in each of the different datasets would seem consistent with expectations. This term designates the change in odds that results from a change of unit in the predictor (here the trial number). Thus, with B greater than one (learnt dataset), there is a relative increase in the odds of optimal response. The value of B indicates the degree of this increase. However, if the value of B is less than one (incorrectly learnt dataset) there is a relative decrease in the odds of the optimal response being taken as the trials proceed. When the confidence interval includes the value one (not learnt dataset) then it has not been possible to estimate a consistent model for the data (Field 2000).

To examine the effect of the drug condition on performance, the learnt dataset was split on the basis of reward and punishment image types. This was done as Table 3.5-1 suggested opposite effects of the PM group for each of these image types. For each of these datasets, a logistic model was used with response as the dependent variable. There was no significant effect of drug group for the punishment image type. The results of the regression for the reward image type are presented in Table 3.5-3.

	Wald	Significance	Exp(B)	Lower CI for Exp(B)	Upper CI for Exp(B)
Trial Number	22.057	0.000	1.388	1.210	1.591
Drug	6.479	0.039			
Drug(1)	0.046	0.830	1.145	0.333	3.940
Drug(2)	6.155	0.013	8.348	1.561	44.628

Table 3.5-3
Summary of results of logistic regression. Response was the dependent variable and trial number, drug condition (Drug) and their interaction were used as the covariates. The results of the interaction comparisons are omitted to reduce the complexity of the results. Drug(1) is the Helmert contrast AM-(PM+PP). Drug(2) is the Helmert contrast PM-PP.

The regression indicates that there is a significant effect of drug condition. The drug condition is represented in the model by the parameters Drug (1) and Drug (2) using Helmert contrasts. Using these contrasts Drug (2) can be used to compare effects of PM and PP. The Exp(B) value for Drug(2) is greater than one indicating that this comparison favours the optimal response. There was no statistical difference detected when the effects of AM and PM were directly compared using a different contrast suggesting that amisulpride did not attenuate the effects of methamphetamine for the learning of this task.

3.5.1.2. Reaction time data

For the reaction time data, only the learnt dataset was used. The main analysis completed was a single factor model with drug condition as a between subjects factor. This was done for both the reward image type and the punishment image type separately. Levene's test indicated there was a significant difference in the variances for the different levels of the condition for the punishment image type but log transformation did not reduce this effect. The original data was used for the analysis, but in view of the variance problem, caution should be applied in assessing the results.

For the reward image type data, there was an effect approaching significance of drug condition (p=0.077, df= (2,342), F=2.586). Pairwise comparisons indicated a significant difference between the AM and PP groups (mean difference AM-PP=181, p=0.031). There was also a difference approaching significance between the PM and PP groups on pairwise comparison (mean difference PM-PP=156, p=0.070). There was no significant difference between the AM and the PM groups. These data suggest that the PP group performed the task quicker than either of the other two groups.

For the punishment image type data, there was a significant effect of drug condition (p=0.026, df=(2,275), F=3.705). Pairwise comparison between the levels of the drug condition showed a significant difference between the AM group and the PM group (mean difference AM-PM=270, p=0.008) and approaching a significant difference between the AM group and the PP group (mean difference AM-PP=140, p=0.095). There was no significant difference between the PP and the PM groups. This suggests that the AM group performed the task slower than either of the other two groups. The reaction time data results are summarised in Table 3.5-4.

	AM-PM	AM-PP	PM-PP
Reward image type	NS	191 (p=0.031)	156 (p=0.070)
Punishment image type	270 (p=0.008)	140 (p=0.095)	NS

Table 3.5-4
This table summarises the differences in reaction times (ms) on pairwise comparison between the drug groups for the reward learning tasks. NS: not significant at p<0.10.

3.5.2. fMRI data analysis

A series of contrasts were completed to explore the data using p(unc) < 0.01 and extent threshold of 10 voxels; masking was completed using the default p(unc) <0.05. The data used were from those participants who were deemed to have learnt the various blocks using the methods in the behavioural data analysis. The data were considered in terms of early stage and late stage of learning the task. The rows in the tables are arranged with voxels in approximate order of decreasing significance (order also related to cluster effects). As there was a large number of areas detected using the various contrasts, rows with Z scores less than 3.1 are printed with italicised grey font in the main set of tables. This is done for illustration purposes only to emphasize the areas with relatively higher Z scores.This is not done for the tables where comparisons of related contrasts are set out later in this section.

3.5.2.1. Early stage of learning

For this stage of learning, the early time bins were used. To examine this, T contrasts were used between the reward and neutral and reward and punishment image types. The areas of activations are outlined in Table 3.5-5 and Table 3.5-6. The former contrast showed activations in areas as following: occipital lobe (cuneus, fusiform gyrus, superior occipital gyrus, middle occipital gyrus), frontal lobe (superior, middle, inferior gyri), middle temporal gyrus,

parietal lobe (supramarginal gyrus), anterior cingulate, cingulate gyrus, thalamus and lentiform nucleus. The latter contrast showed activations in similar regions: the cuneus, frontal lobe (middle gyrus, medial gyrus, paracentral lobule), parietal lobe (post central gyrus, supramarginal gyrus, precuneus, inferior parietal lobule), temporal lobe (superior, middle gyri), insula, putamen and subthalamic nucleus. The alternative T contrast between the punishment and the reward image type at time of outcome for the early time bin is presented in Table 3.5-7. This showed fewer areas of activation in the cortical regions compared to the previous contrasts. The areas of activations in the contrast were: precuneus, middle frontal gyrus, lingual gyrus, parahippocampal gyrus, anterior cingulate, hippocampus, thalamus, caudate regions, globus pallidus and cerebellum. The areas of activations for this contrast overlaid onto slices of a brain volume are shown in Figure 3.5-2.

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
523	0.001	5.29	Right	Cuneus*	18	12	-88	21
523	0.001	5.09	Right	Cuneus*	18	9	-90	14
261	0.033	3.5	Left	Fusiform Gyrus	19	-23	-79	-14
359	0.007	4.08	Left	Middle Frontal Gyrus	11	-43	38	-14
581	0.001	4.79	Left	Superior Frontal Gyrus	9	-18	54	26
581	0.003	4.45	Right	Superior Frontal Gyrus	9	9	57	26
539	0.005	4.22	Left	Supramarginal Gyrus	40	-57	-42	34
155	0.05	3.32	Left	Cuneus	19	-27	-82	30
155	*0.137*	*2.76*	*Left*	*Superior Occipital Gyrus*	*19*	*-39*	*-77*	*30*
53	0.01	3.95	Right	Thalamus		5	-6	10
53	*0.199*	*2.49*	*Right*	*Lentiform Nucleus*+		*14*	*-3*	*3*
73	*0.12*	*2.85*	*Right*	*Cuneus*	*19*	*32*	*-79*	*30*
63	*0.14*	*2.74*	*Right*	*Supramarginal Gyrus*	*40*	*57*	*-40*	*34*
109	0.024	3.64	Left	Anterior Cingulate	32	-5	43	-5
263	0.026	3.6	Left	Inferior Frontal Gyrus	47	-30	15	-21
30	0.045	3.37	Right	Middle Temporal Gyrus	21	66	-35	-1
60	0.048	3.33	Left	Middle Frontal Gyrus	8	-37	25	41
28	0.064	3.18	Right	Inferior Frontal Gyrus	47	55	24	-1
33	*0.089*	*3.02*	*Right*	*Middle Occipital Gyrus*	*37*	*48*	*-70*	*3*
11	*0.1*	*2.95*	*Right*	*Cingulate Gyrus*	*24*	*4*	*-5*	*45*
32	*0.172*	*2.6*	*Left*	*Superior Frontal Gyrus*	*8*	*-23*	*25*	*44*
27	*0.127*	*2.81*	*Right*	*Superior Frontal Gyrus*	*9*	*20*	*47*	*30*
27	*0.16*	*2.66*	*Right*	*Superior Frontal Gyrus*	*10*	*23*	*50*	*20*
10	*0.142*	*2.73*	*Left*	*Cuneus*	*19*	*-12*	*-86*	*27*

Table 3.5-5
This summarises the activations of clusters for the contrast reward - neutral image type at outcome of stimulus for early time bins. This was completed with p(unc) < 0.01 with an extent threshold of 10 voxels. In each case the activation of the local maximum was in a region of grey matter. *p(FWE) < 0.05.+ 1 mm from globus pallidus. In order to emphasise areas with a relatively higher Z score, rows with Z scores less than 3.1 are printed with italicised grey font. This is for illustration purposes only.

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
738	0.001	5.46*	Right	Cuneus	18	11	-88	21
738	0.004	4.58	Right	Cuneus	19	7	-79	30
256	*0.16*	*3.04*	*Left*	*Cuneus*	*17*	*-16*	*-73*	*13*
226	0.017	4.06	Left	Lentiform Nucleus (Putamen)		-21	9	6
34	0.047	3.67	Left	Middle Frontal Gyrus	9	-27	33	37
56	0.111	3.24	Left	Medial Frontal Gyrus	9	-9	39	21
89	0.126	3.19	Left	Postcentral Gyrus	3	-27	-31	50
24	*0.19*	*2.92*	*Left*	*Middle Temporal Gyrus*	*19*	*-37*	*-81*	*20*
14	0.132	3.16	Left	PreCuneus	7	-14	-47	41
92	0.137	3.13	Left	Superior Temporal Gyrus	39	-52	-61	29
54	0.139	3.13	Left	Insula	13	-37	-31	18
12	*0.15*	*3.08*	*Left*	*Superior Temporal Gyrus*	*38*	*-53*	*10*	*-18*
28	*0.155*	*3.06*	*Left*	*Supramarginal Gyrus*	*40*	*-57*	*-42*	*34*
203	*0.171*	*2.99*	*Right*	*Paracentral Lobule*	*31*	*5*	*-15*	*46*
18	*0.174*	*2.98*	*Right*	*Postcentral Gyrus*	*2*	*39*	*-24*	*46*
17	*0.198*	*2.88*	*Right*	*Subthalamic Nucleus*		*12*	*-16*	*-5*
13	*0.198*	*2.88*	*Left*	*Inferior Parietal Lobule*	*40*	*-55*	*-26*	*30*
12	*0.23*	*2.73*	*Left*	*Lentiform Nucleus (Putamen)*		*-20*	*-1*	*10*
11	*0.234*	*2.71*	*Left*	*Inferior Parietal Lobule*	*40*	*-59*	*-34*	*24*

Table 3.5-6
This summarises the activations of clusters for the contrast reward - punishment image type at outcome of stimulus for early time bins. *p(FWE) < 0.05. Other aspects are as in Table 3.5-5.

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
405	0.115	4.61	Right	Declive (Cerebellum)		21	-72	-11
405	0.161	4.11	Right	Lingual Gyrus	18	18	-79	-11
1234	0.183	3.94	Left	Declive (Cerebellum)		-23	-57	-18
1234	0.183	3.82	Right	Culmen (Cerebellum)		16	-35	-10
1234	0.183	3.81	Right	Thalamus (Pulvinar)		20	-29	8
218	0.183	3.66	Right	Middle Frontal Gyrus	46	43	39	21
121	*0.264*	*2.95*	*Right*	*PreCuneus*	*7*	*12*	*-70*	*36*
121	*0.281*	*2.84*	*Right*	*PreCuneus*	*31*	*16*	*-60*	*29*
91	0.187	3.41	Right	Thalamus (Medial Dorsal Nucleus)		5	-15	7
49	0.224	3.18	Left	Parahippocampal Gyrus	28	-20	-11	-20
49	*0.299*	*2.76*	*Left*	*Hippocampus*		*-27*	*-15*	*-17*
54	0.228	3.13	Right	Anterior Cingulate	24	5	21	-4
54	*0.303*	*2.74*	*Right*	*Caudate Head*		*11*	*19*	*2*
54	*0.306*	*2.73*	*Right*	*Caudate Body*		*12*	*11*	*6*
36	*0.241*	*3.05*	*Left*	*Thalamus (Pulvinar)*		*-18*	*-27*	*11*
36	*0.306*	*2.73*	*Left*	*Thalamus (Lateral Posterior Nucleus)*		*-18*	*-22*	*17*
36	*0.341*	*2.6*	*Left*	*Thalamus (Ventral Lateral Nucleus)*		*-14*	*-15*	*14*
17	*0.267*	*2.93*	*Left*	*Lateral Globus Pallidus*		*-23*	*-12*	*4*
21	*0.274*	*2.89*	*Right*	*Declive (Cerebellum)*		*21*	*-58*	*-15*
18	*0.288*	*2.81*	*Right*	*Anterior Cingulate*	*24*	*4*	*37*	*5*
19	*0.332*	*2.63*	*Left*	*PreCuneus*	*7*	*-2*	*-55*	*48*

Table 3.5-7
This is a table of the activations of clusters for the contrast punishment - reward image type at outcome for early time bins. Other aspects are as in Table 3.5-5.

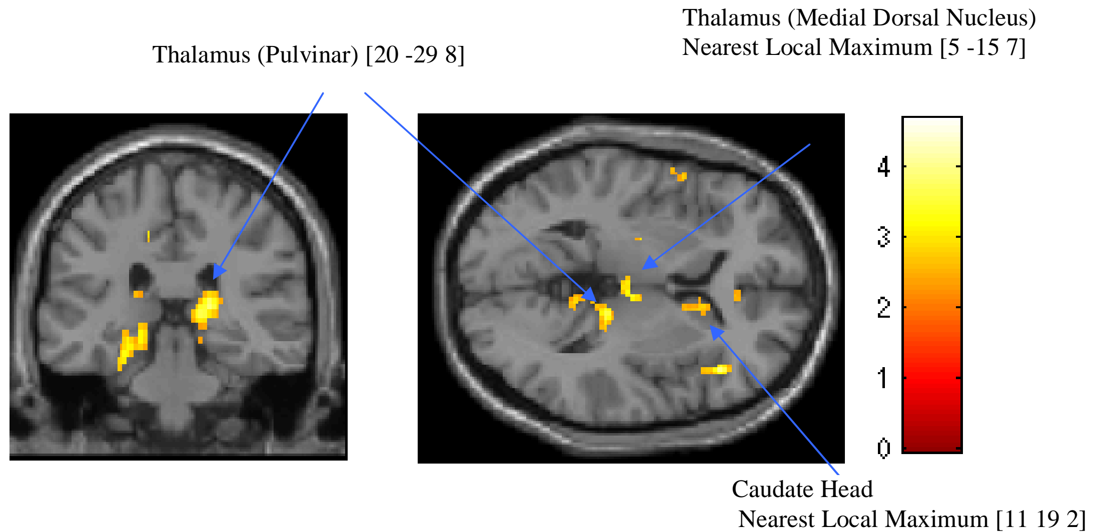


Figure 3.5-2
This show the pattern of activation for the contrast punishment – reward image type in the early time bin at outcome of the stimulus. The slices shown are taken in reference to the peak activation of the cluster in the pulvinar [20 -29 8] as in Table 3.5-7. Colour bar vertical scale relates to increasing BOLD signal.

3.5.2.2. Late learning stage

Once the task was learnt, the expectation was that stimuli at onset (predicting the outcome) would become more meaningful over time. Hence T contrasts were used between both the reward and neutral image type and the reward and punishment image type in the late time bin at the onset of the stimulus. The areas of activations for these contrasts are presented in Table 3.5-8 and Table 3.5-9. The former contrast indicated areas of activation in a number of different regions: cuneus, frontal lobe (inferior, middle, medial frontal gyri), temporal lobe (middle, transverse gyri), anterior cingulate, posterior cingulate, parahippocampal gyrus, cerebellum and nucleus accumbens. The latter contrast showed areas of activations only in three areas: parahippocampal gyrus, middle temporal gyrus and anterior cingulate. Areas of activation (which are sites of prominent dopamine neuron projection) for the contrast between the reward and neutral image type are shown in Figure 3.5-3. The alternate T contrast between the punishment and reward image type in the late time bin at the onset of the stimulus was also completed with the areas summarised in Table 3.5-10. The regions detected (many of which were also seen in the reward-neutral contrast) were: precuneus, frontal lobe (superior, inferior, medial gyri), superior temporal gyrus, parietal lobe (postcentral gyrus, inferior parietal

lobule), anterior cingulate, posterior cingulate, insula, mammillary body and putamen.

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
498	0.043	4.01	Left	Parahippocampal Gyrus	30	-9	-43	5
291	0.143	3.32	Left	Anterior Cingulate	32	-4	42	4
29	*0.239*	*2.9*	*Right*	*Middle Temporal Gyrus*	*21*	*52*	*-3*	*-20*
132	*0.312*	*2.67*	*Left*	*Cuneus*	*17*	*-2*	*-87*	*8*
57	*0.276*	*2.79*	*Right*	*Culmen (Cerebellum)*		*16*	*-34*	*-10*
103	*0.212*	*2.99*	*Right*	*Inferior Frontal Gyrus*	*47*	*34*	*13*	*-18*
83	0.119	3.45	Left	Nucleus Accumbens+		-7	5	-6
19	0.123	3.44	Right	Middle Frontal Gyrus	46	46	37	14
82	*0.272*	*2.8*	*Left*	*Culmen (Cerebellum)*		*-2*	*-58*	*-9*
19	0.165	3.21	Left	Posterior Cingulate	30	-18	-64	6
17	*0.202*	*3.03*	*Left*	*Declive (Cerebellum)*		*-20*	*-76*	*-14*
14	*0.247*	*2.87*	*Right*	*Culmen (Cerebellum)*		*2*	*-52*	*0*
16	*0.249*	*2.86*	*Right*	*Culmen (Cerebellum)*		*18*	*-39*	*-13*
23	*0.261*	*2.83*	*Right*	*Medial Frontal Gyrus*	*10*	*7*	*62*	*10*
10	*0.35*	*2.54*	*Right*	*Transverse Temporal Gyrus*	*41*	*55*	*-20*	*11*

Table 3.5-8
This table summarises the activations of clusters for the contrast reward - neutral image type at onset for late time bins. +The grey matter region nucleus accumbens was not included in the software tool (Talairach daemon) used so this area was identified by hand. Other aspects are as in Table 3.5-5.

Cluster size	P(FDR)	Z score	Side	Area	BA	X	Y	Z
370	0.714	3.66	Left	Parahippocampal Gyrus	30	-16	-35	-4
11	0.714	3.62	Left	Middle Temporal Gyrus	39	-50	-71	20
12	*0.987*	*2.85*	*Left*	*Anterior Cingulate*	*25*	*-5*	*17*	*-7*

Table 3.5-9
This is a table of the activations of clusters for the contrast reward - punishment image type at onset for late time bins. Other aspects are as in Table 3.5-5.

Cluster size	Z score	Side	Area	BA	X	Y	Z
46	*2.72*	*Left*	*Inferior Frontal Gyrus*	*45*	*-55*	*26*	*2*
90	3.87	Left	Superior Temporal Gyrus	22	-52	-57	16
90	*2.96*	*Left*	*Superior Temporal Gyrus*	*22*	*-59*	*-52*	*12*
37	3.67	Left	Putamen		-25	-9	-3
105	3.46	Right	Precuneus	7	11	-46	54
105	3.19	Right	Postcentral Gyrus	3	18	-37	60
84	3.41	Left	Insula	13	-41	-12	4
27	3.41	Right	Precuneus	31	11	-67	19
36	3.36	Left	Inferior Parietal Lobule	40	-53	-33	34
19	3.36	Left	Inferior Parietal Lobule	40	-46	-36	50
40	3.33	Left	Mammillary Body		-9	-19	4
21	3.32	Right	Anterior Cingulate	32	5	31	24
13	3.2	Left	Medial Frontal Gyrus	9	-4	48	17
20	3.17	Left	Posterior Cingulate	30	-9	-66	10
45	*2.99*	*Right*	*Inferior Parietal Lobule*	*40*	*45*	*-32*	*34*
12	*2.95*	*Left*	*Superior Frontal Gyrus*	*10*	*-27*	*53*	*17*
10	*2.89*	*Right*	*Superior Frontal Gyrus*	*9*	*21*	*57*	*26*
10	*2.88*	*Left*	*Putamen*		*-20*	*5*	*-6*
16	*2.82*	*Right*	*Inferior Parietal Lobule*	*40*	*62*	*-31*	*37*

Table 3.5-10
This is a table of the activations of clusters for the contrast punishment – reward image type at onset for late time bins. p(FDR) = 0.635 for all rows in the table. Other aspects are as in Table 3.5-5.

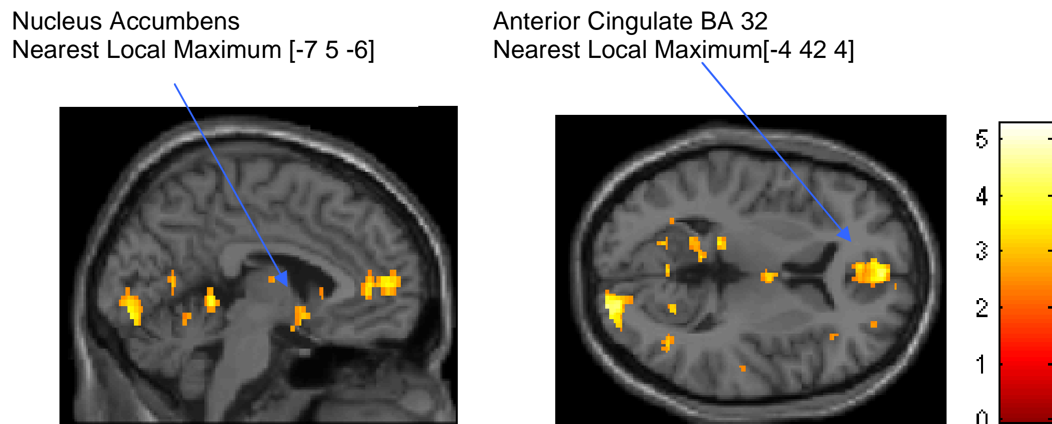


Figure 3.5-3
This show the pattern of activation for the contrast of reward – neutral image type at onset during late time bins as in Table 3.5-8. The slices shown are taken in reference to the peak activation of the cluster in the anterior cingulate [-4 42 4]. Colour bar vertical scale relates to increasing BOLD signal.

3.5.2.3. Outcome (Early versus late learning stage)

To assess the effect of time two further T contrasts were used. As the expectation was that there would be decreased activation of certain regions at the late time bin at outcome of the stimuli, a contrast was used between the

early and late time bin at outcome for the reward image type. The areas of activation identified from this contrast are shown in Table 3.5-11. These areas were: cuneus, precuneus, frontal lobe (superior gyrus, middle gyrus, inferior gyrus, precentral gyrus, paracentral lobule), temporal lobe (superior, middle, inferior gyri), cingulate gyrus, insula, cerebellum and putamen.

3.5.2.4. Onset (Late versus early learning stage)

Another way of examining the effects of time was by examination of onset stimuli. The activation for stimulus onset was predicted to be less in the early part of the task than at the later part. To explore this, a contrast between the late and early time bin for the reward image type at onset was used. The areas of activation are presented in Table 3.5-12. These regions detected were: cuneus, frontal lobe (medial, precentral gyri), middle temporal gyrus, postcentral gyrus, posterior cingulate, anterior cingulate, cingulate gyrus, hippocampus, cerebellum and lentiform nucleus. An area of activation is presented in Figure 3.5-4. Contrasts are presented in Figure 3.5-5.

3.5.2.5. Effect of drug condition

To show the effects of drugs over time the contrasts used to identify significant voxels were late time bin – early time bin at onset and early time bin – late time bin for outcome (both for the reward image type). The pattern of activation for the former contrast is shown in Figure 3.5-6 and the latter in Figure 3.5-7. The contrasts used are similar but not the same as those used to generate the data in both Table 3.5-11 (stimulus outcome) and Table 3.5-12 (stimulus onset). Whereas the factors used for the figures were time bin, image type and drug, the factors for the tables were time bin, image type and stimulus presentation (onset or outcome). Tables containing the data directly related to the contrasts in the figures are presented in appendix 6.4 (Table 6.4-2 (onset) and Table 6.4-1 (outcome)).

3.5.2.6. Common regions detected in related contrasts

A series of tables were produced to compare areas of activation detected with related contrasts. Brodmann areas and labels provided by the Talairach client tool were used to help identify similar regions. The Talairach coordinates of the maxima were also examined to help with this process. Table 3.5-13 is a summary of areas activated for the contrast reward – neutral at the different stages of learning. The areas in common included the anterior cingulate, middle

temporal gyrus and inferior frontal gyrus. Similarly, Table 3.5-14 is a summary of areas identified with the contrast reward – punishment at the different stages of learning. One region (BA 39) was similar for both of the contrasts. Table 3.5-15 is a similar summary for the areas identified for the contrast punishment – reward. Common regions detected include the precuneus and putamen/caudate head (though these were contralateral to each other). Table 3.5-16 is a summary of areas identified in the contrasts between the early and late time bins for outcome and onset of the stimuli. Similar regions include BA 22 (contralateral) and the cuneus and lentiform nucleus/putamen region. It is important to note that all the rows in the previous tables were used in the tables for comparisons.

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
972	0.035	4.47	Right	Cuneus	18	4	-85	14
972	0.051	3.95	Right	Cuneus	17	7	-76	13
626	0.045	4.16	Right	Middle Frontal Gyrus	9	45	26	34
590	0.051	3.99	Left	Putamen		-21	12	3
164	0.072	3.42	Left	Middle Frontal Gyrus	46	-52	29	18
833	0.053	3.9	Left	Cuneus	19	-27	-82	30
118	*0.108*	*2.95*	*Right*	*Inferior Frontal Gyrus*	*47*	*32*	*29*	*-10*
103	*0.114*	*2.9*	*Right*	*Superior Temporal Gyrus*	*22*	*64*	*-33*	*11*
176	0.063	3.67	Left	Declive (Cerebellum)		-30	-51	-12
126	*0.13*	*2.75*	*Right*	*PreCuneus*	*7*	*25*	*-55*	*45*
51	0.064	3.64	Left	Middle Temporal Gyrus	21	-48	6	-18
132	0.068	3.55	Left	Inferior Frontal Gyrus	46	-45	17	22
19	0.069	3.49	Left	Declive (Cerebellum)		-9	-74	-14
68	*0.154*	*2.53*	*Left*	*Superior Frontal Gyrus*	*9*	*-4*	*54*	*23*
37	0.073	3.4	Left	Middle Frontal Gyrus	11	-43	38	-14
18	0.08	3.29	Right	Middle Temporal Gyrus	39	52	-69	13
15	0.085	3.19	Right	Paracentral Lobule	6	5	-27	50
40	*0.133*	*2.73*	*Right*	*Superior Frontal Gyrus*	*9*	*9*	*54*	*30*
41	0.088	3.16	Right	PreCuneus	7	2	-59	51
79	0.093	3.11	Right	Insula	13	36	-11	20
20	*0.121*	*2.84*	*Left*	*Precentral Gyrus*	*43*	*-53*	*-10*	*10*
24	*0.121*	*2.84*	*Left*	*PreCuneus*	*7*	*-20*	*-66*	*48*
19	*0.125*	*2.81*	*Right*	*Cingulate Gyrus*	*24*	*7*	*-2*	*32*
11	*0.129*	*2.77*	*Right*	*Putamen*		*21*	*10*	*-6*

Table 3.5-11
This is a table of the activations of clusters for the contrast early – late time bins for the reward image type at outcome. Other aspects are as in Table 3.5-5.

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
4724	0	5.31*	Left	Posterior Cingulate	29	-9	-43	9
312	0	5.43*	Left	Medial Frontal Gyrus	10	-5	51	4
312	0.008	3.88	Left	Medial Frontal Gyrus	9	-16	41	14
403	0.001	4.86*	Left	Anterior Cingulate	25	-2	12	-9
70	0.004	4.18	Right	Hippocampus		34	-28	-10
32	0.022	3.41	Left	Middle Temporal Gyrus^{++}	22	-62	-45	2
21	0.034	3.2	Right	Culmen (Cerebellum)		14	-55	-18
38	*0.085*	*2.67*	*Left*	*Cuneus*	*19*	*-4*	*-77*	*30*
19	*0.051*	*2.98*	*Right*	*Cingulate Gyrus*	*31*	*14*	*-40*	*34*
15	*0.051*	*2.98*	*Left*	*Precentral Gyrus*	*43*	*-59*	*-5*	*10*
15	*0.104*	*2.54*	*Left*	*Postcentral Gyrus*	*43*	*-62*	*-8*	*17*
11	*0.052*	*2.97*	*Left*	*Lentiform Nucleus*$^{+}$		*-12*	*-2*	*3*

Table 3.5-12
This is a table of the activations of clusters for the contrast late – early time bins for the reward image type at onset. $^{+}$ 1 mm from globus pallidus. $^{++}$ Identified as being within 1 mm (as was BA 21). Other aspects are as in Table 3.5-5.

Onset late time bin Reward – neutral contrast	Outcome early time bin Reward – neutral contrast
Parahippocampal Gyrus (BA 30) **Anterior Cingulate (BA 32)** **Middle Temporal Gyrus (BA 21)** Cuneus (BA 17) Culmen (Cerebellum) **Inferior Frontal Gyrus (BA 47)** Nucleus Accumbens Middle Frontal Gyrus (BA 46) Culmen (Cerebellum) Posterior Cingulate (BA 30) Declive (Cerebellum) Medial Frontal Gyrus (BA 10) Transverse Temporal Gyrus (BA 41)	Cuneus (BA 18) Fusiform Gyrus BA 19) Middle Frontal Gyrus(BA 11) Superior Frontal Gyrus(BA 9) Supramarginal Gyrus(BA 40) Cuneus(BA 19**)** Superior Occipital Gyrus(BA 19) Thalamus Lentiform Nucleus **Anterior Cingulate(BA 32)** **Inferior Frontal Gyrus(BA 47)** **Middle Temporal Gyrus(BA 21)** Middle Frontal Gyrus(BA 8) Middle Occipital Gyrus(BA 37) Cingulate Gyrus(BA 24) Superior Frontal Gyrus(BA 8) Superior Frontal Gyrus(BA 10)

Table 3.5-13
This is a summary of the data in Table 3.5-8 and Table 3.5-5. This shows the areas activated for the contrast reward – neutral for onset of the stimulus in the late time bins (left) and outcome of the stimulus for early time bins (right). Similar regions are in bold.

Onset late time bin Reward – punishment contrast	Outcome early time bin Reward – punishment contrast
Parahippocampal Gyrus (BA30) **Middle Temporal Gyrus (BA 39)** Anterior Cingulate (BA 25)	Cuneus (BA 18) Cuneus (BA 19) Putamen Middle Frontal Gyrus (BA 9) Medial Frontal Gyrus (BA 9) Postcentral Gyrus (BA 3) Middle Temporal Gyrus (BA 19) PreCuneus (BA 7) **Superior Temporal Gyrus (BA 39)** Insula (BA 13) Superior Temporal Gyrus (BA 38) Supramarginal Gyrus (BA 40) Paracentral Lobule (BA 3) Postcentral Gyrus (BA 2) Subthalamic Nucleus Inferior Parietal Lobule (BA 40)

Table 3.5-14
This is a summary of the data in Table 3.5-9 and Table 3.5-6. Areas activated for the contrast reward – punishment for onset of the stimulus in the late time bins are shown on the left and areas for outcome of the stimulus for early time bins on the right. Similar regions are in bold.

Onset late time bin Punishment – reward contrast	Outcome early time bin Punishment – reward contrast
Inferior Frontal Gyrus(BA 45)	Declive (Cerebellum)
Superior Temporal Gyrus(BA 22)	Lingual Gyrus (BA 18)
Putamen*	Culmen (Cerebellum)
Precuneus(BA 7)	Pulvinar (Thalamus)
Postcentral Gyrus(BA 3)	Middle Frontal Gyrus (BA 46)
Insula(BA 13)	**PreCuneus (BA 7)**
Precuneus(BA 31)	**PreCuneus (BA 31)**
Inferior Parietal Lobule(BA 40)	Medial Dorsal Nucleus (Thalamus)
Mammillary Body	Parahippocampal Gyrus (BA 28)
Anterior Cingulate(BA 32)	Hippocampus
Medial Frontal Gyrus(BA 9)	Anterior Cingulate (BA 24)
Posterior Cingulate(BA 30)	**Caudate Head***
Inferior Parietal Lobule(BA 40)	Caudate Body
Superior Frontal Gyrus(BA 10)	Lateral Posterior Nucleus (Thalamus)
Superior Frontal Gyrus(BA 9)	Ventral Lateral Nucleus (Thalamus)
Inferior Parietal Lobule(BA 40)	Lateral Globus Pallidus (Thalamus)

Table 3.5-15
This summarises data in Table 3.5-10 and Table 3.5-7. It shows the areas activated for the contrast punishment - reward for onset of the stimulus in the late time bins (left) and outcome of the stimulus for early time bins on the right. Similar regions are in bold.* contralateral to each other.

Onset late – early time contrast	Outcome early - late time contrast
Posterior Cingulate (BA 29)	Cuneus (BA 18)
Medial Frontal Gyrus (BA 10)	Cuneus (BA 17)
Medial Frontal Gyrus (BA 9)	Middle Frontal Gyrus (BA 9)
Anterior Cingulate (BA 25)	**Putamen**
Hippocampus	Middle Frontal Gyrus (BA 46)
Middle Temporal Gyrus (BA 22)*	**Cuneus (BA 19)**
Culmen (Cerebellum)	Inferior Frontal Gyrus (BA 47)
Cuneus (BA 19)	**Superior Temporal Gyrus (BA 22)***
Cingulate Gyrus (BA 13**)**	Declive (Cerebellum)
Precentral Gyrus (BA 43)	PreCuneus (BA 7)
Postcentral Gyrus (BA 43)	Middle Temporal Gyrus (BA 21)
Lentiform Nucleus	Inferior Frontal Gyrus (BA 46)
	Declive (Cerebellum)
	Superior Frontal Gyrus (BA 9)
	Middle Frontal Gyrus (BA 11)
	Middle Temporal Gyrus (BA 39)
	Paracentral Lobule (BA 6)
	Insula (BA 13)
	Precentral Gyrus (BA 7)
	Cingulate Gyrus (BA 24)

Table 3.5-16
A summary of the data in Table 3.5-12 and Table 3.5-11 is presented in this table. The areas activated for the contrast late – early time bin at onset is presented on the left and the contrast early - late time bin at outcome on the right. Similar regions are in bold in both columns. *contralateral to each other.

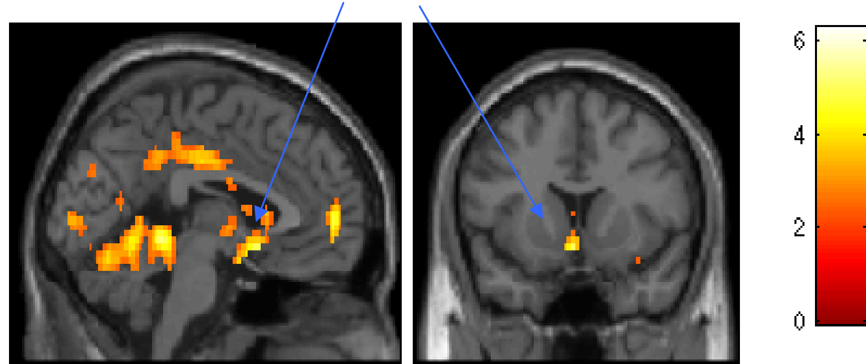


Figure 3.5-4
Late – early time bin contrast using reward image type at onset (as in Table 3.5-12). The slices shown are in reference to the local maximum in the cluster in the anterior cingulate [-2 12 -9]. Colour bar vertical scale relates to increasing BOLD signal.

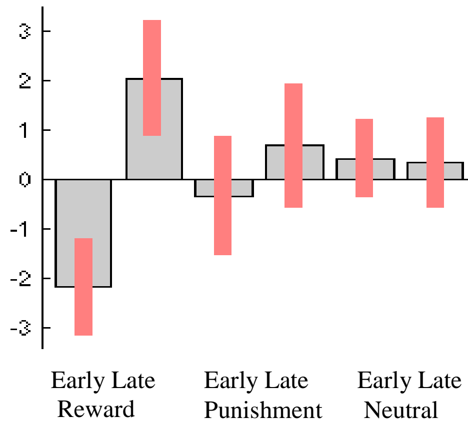


Figure 3.5-5
This figure compares the pattern of activation for reward, punishment and neutral image types at early and late time bins. The voxel used was at [-2 12 -9]. This was identified using the contrast late time bin – early time bin at stimulus onset for reward image type as in Table 3.5-12. Error bars indicate 90% confidence intervals.

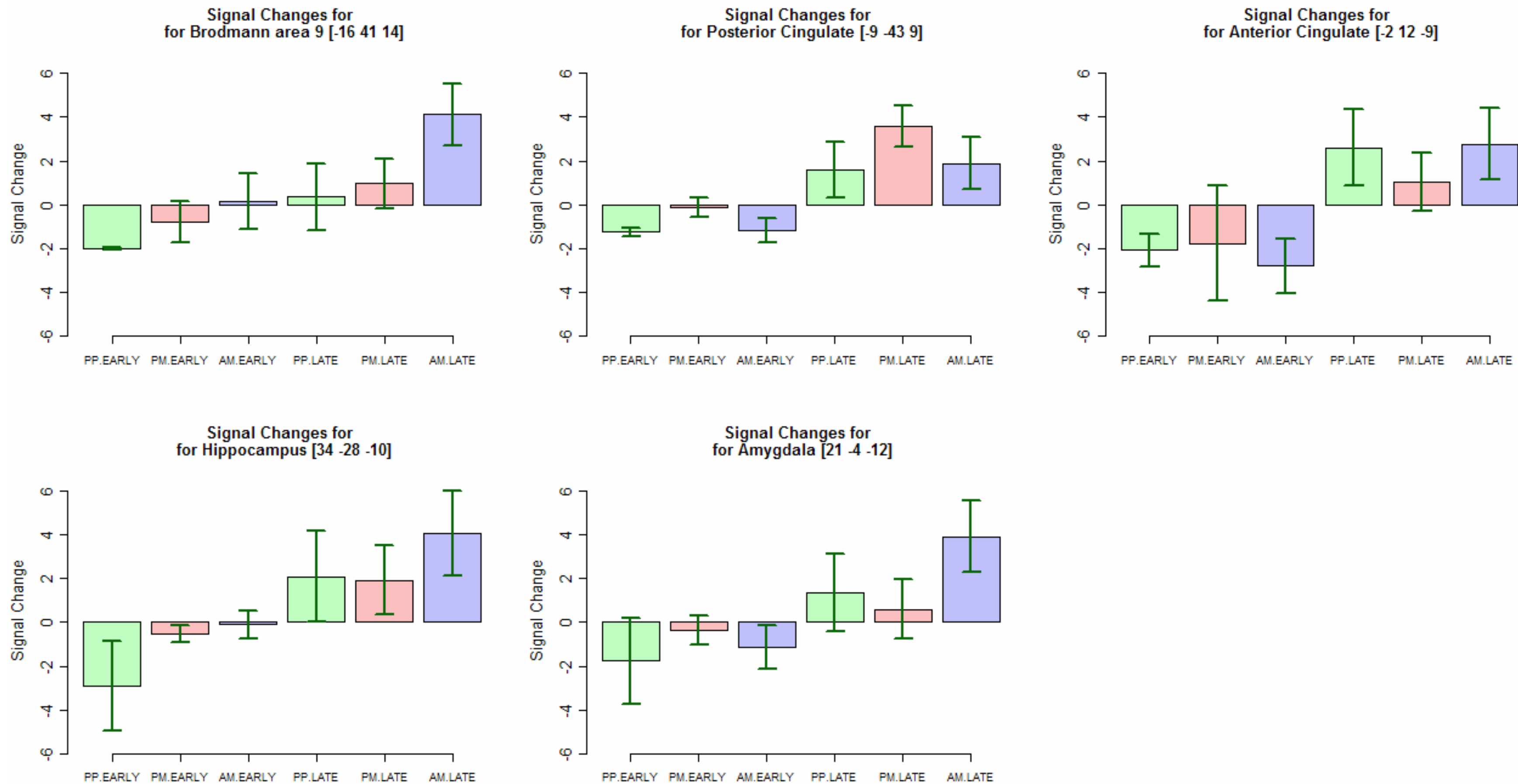


Figure 3.5-6
This figure compares activation patterns for the drug conditions PP, PM and AM. The areas of activation came from the contrast late time bin – early time bin for onset of a reward stimulus. The local maximum from each area was used in the comparisons. PP.EARLY, PM.EARLY, AM.EARLY represent the drug conditions PP, PM, AM at the early time bin, Similarly, PP.LATE, PM.LATE, AM.LATE represent the drug conditions PP, PM, AM at the late time bin. Error bars indicate 90% confidence intervals. The signal change refers to mean % BOLD signal changes from baseline. The signal change for PM.LATE had been expected to be greater than PP.LATE and AM.LATE.

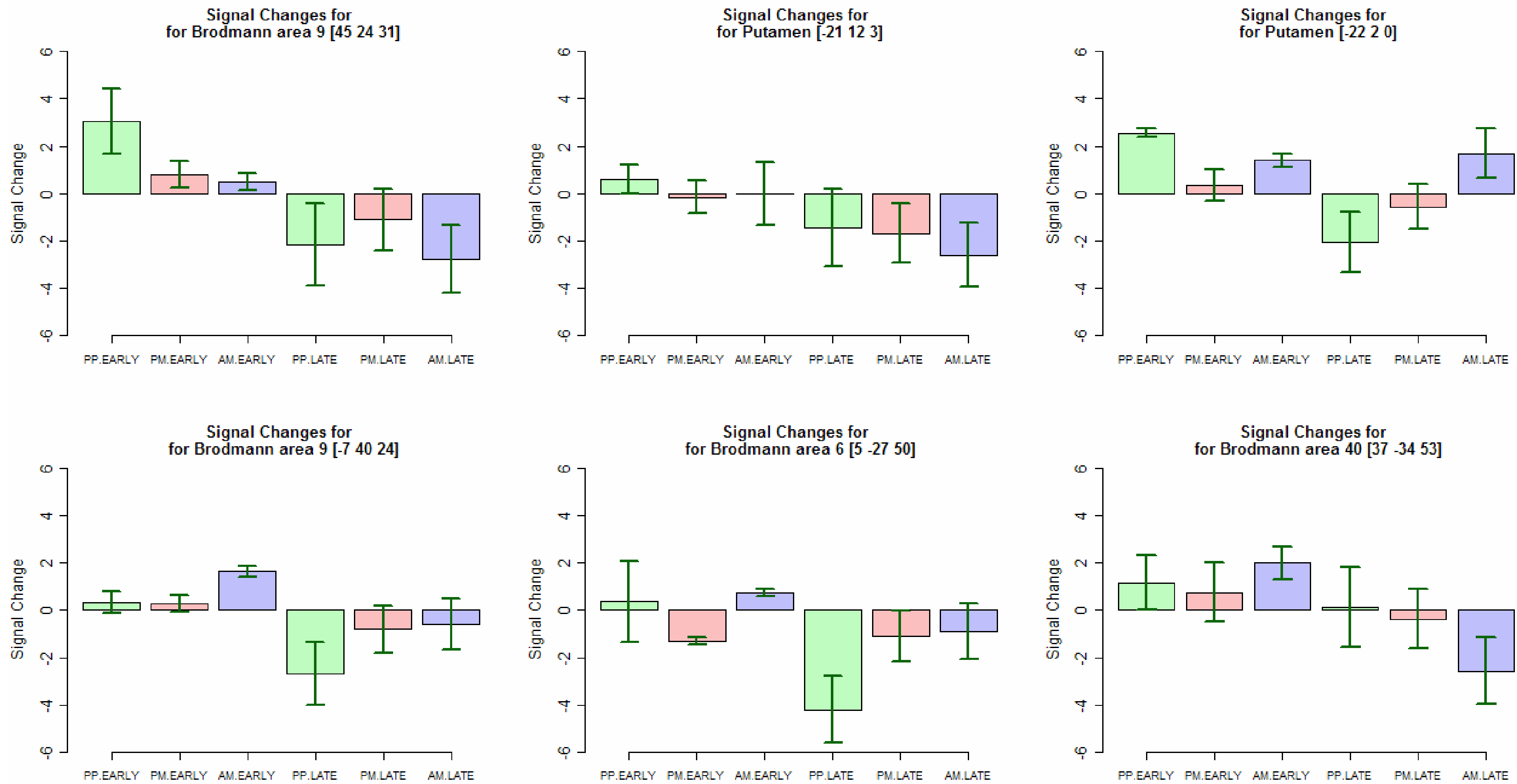


Figure 3.5-7
This figure compares activation patterns for the drug conditions PP, PM and AM. The areas of activation came from the contrast early time bin – late time bin for outcome of a reward stimulus. Terminology is the same as in Figure 3.5-6. The signal change for PM.EARLY had been expected to be greater than PP.EARLY and AM.EARLY.

4. Discussion

4.1. *Screening data*

The data at visit 1 were used mainly to assess for detectable differences between the different drug groups. The various demographic data and data from the screening questionnaires largely showed that there were very few differences between the groups. The only difference detected was the venturesomeness component of the Impulsiveness Venturesomeness and Empathy questionnaire. This was decreased in the AM group. As venturesomeness decreases with age (Eysenck, Pearson et al. 1985), this could be related to the fact that the AM group seemed slightly older (Table 3.1-1) than the other two groups. However, as this was an isolated finding it is unlikely that it signifies important differences between the drug groups. In visit 2 there were increases detected over the time course of the visit for a number of cardiovascular parameters regardless of drug group. This probably reflects the effect of methamphetamine which is known to cause increases in blood pressure and pulse rate (Cruickshank and Dyer 2009). This can be seen in both Figure 3.1-1 and Figure 3.1-2 which broadly shows the greatest changes for the PM group. However, the difference between the drug groups did not reach statistical significance. A likely reason for this is that many of the cardiovascular changes resulting from intravenous methamphetamine reduce markedly within an hour (Cook, Jeffcoat et al. 1993). In this study, the first set of measurements took place before the methamphetamine and the second set of measurements took place at least an hour after methamphetamine was given. By the time of the second set of measurements, it would seem likely that there would be a much reduced effect on the cardiovascular parameters resulting from methamphetamine.

The way the blood samples were taken allowed an examination of the effects of amisulpride on prolactin compared to placebo. The data in Table 3.1-5 indicate that there was about a 6 fold increase in concentration over time for the AM group. This would be consistent with blockade of the dopamine D2 receptors found on lactotroph cells in the anterior pituitary gland (Meltzer and Fang 1976; Paparrigopoulos, Liappas et al. 2007) and is consistent with previous findings (Samuels, Hou et al. 2006). The prolactin levels cannot be used as an index of

the effect of amisulpride on the brain as there is a dissociation between its central and peripheral effects on dopamine receptors (Kapur, Langlois et al. 2002; Natesan, Reckless et al. 2008). The relative increased peripheral effects result from low permeability across the blood brain barrier and the interaction of amisulpride with P-glycoprotein (Hartter, Huwel et al. 2003; Schmitt, Abou El-Ela et al. 2006; Natesan, Reckless et al. 2008). However, the elevation of prolactin levels in the AM group indicates that each participant of the AM group received and ingested the amisulpride tablet. There was no detectable effect of methamphetamine on prolactin over time. It is hard to compare this result convincingly with previous studies due to the low numbers used and the inconsistent results (increases and decreases detected in prolactin levels after amphetamine) from previous studies (Wells, Silverstone et al. 1978; Jacobs, Silverstone et al. 1989).

4.2. Challenge phMRI

Six items of the POMS were used for subjective ratings subsequently aggregated into two sets: "high" (carefree, cheerful, energetic) and "low" (anxious, gloomy, sluggish). Judging from the responses in Figure 3.2-1, these sets of POMS have broadly the same pattern over time within a particular drug group - justifying their aggregation. Amphetamine has been shown to increase anxiety in healthy volunteers (White, Lott et al. 2006). However, subjective ratings of anxiety (using POMS ratings) also have been shown to be the same for amphetamine and placebo (Hariri, Mattay et al. 2002). In this study, the anxiety ratings seem to have decreased over time with both the PM and AM drug groups (see Figure 3.2-1). Subjective effects of amphetamine have been shown to be related to personality traits (White, Lott et al. 2006) so the particular effect in this study could relate to the personality characteristics of participants in the PM group. However, there were no broad differences detected in the main personality measure (Big Five Inventory) used at screening between the drug groups so it is unlikely that personality effects were important.

The "high" grouping showed an effect of time but not for drug condition. There was a significant interaction between drug condition and time. The prediction had been that in comparison to the PP group, the PM group would have an increase in "high" rating over time in line with the usual effects of methamphetamine (Newton, De la Garza et al. 2005a; Newton, De La Garza et al. 2005b; Newton, Roache et al. 2006) but that this would not have occurred with the PP group. There was uncertainty about how the AM group would change over time as there is a discrepancy between older findings and recent literature (Jonsson 1972; Angrist, Lee et al. 1974; Brauer and de Wit 1996; Brauer and de Wit 1997). The more recent studies which use doses similar to this study suggest that there would be a similar effect for both the PM and AM groups but that these would differ to the PP group. Visual inspection of Figure 3.2-2 suggest an increase in the "high" rating in both the PM and the AM group but not with the PP group from time bin 2 onwards. By the end of the task, the rating for the PM group was the same as the AM group. The statistical analysis combined with Figure 3.2-2 suggest that the subjective effects of PM and the AM group changed over time but were not different from one another. This

suggests that subjective effects of methamphetamine were not attenuated by amisulpride for “high” emotions consistent with recent studies.

For the “low” emotions there was a detectable effect of time only but not of interaction or drug condition. The prediction had been that there would be an inverse picture to the “high” group with the PM group having reduced “low” ratings compared to the PP group whilst there would be uncertainty about the AM group. Combined with Figure 3.2-2, the statistical analysis in this case suggest that may have been a gradual change over time and that this was more related to the PM and AM group than the PP group but that this was less marked than for the “high” rating group. The AM group appeared more “low” than the other two groups prior to the methamphetamine – this may reflect sluggishness that is associated with amisulpride (Ramaekers, Louwerens et al. 1999) as this was one of the components of the “low” rating.

The failure of amisulpride to change the subjective effects of methamphetamine could be due to a number of factors. One factor could be that a high dose of amphetamine is needed in order to show reversal of the effects (Wise 2008). Another factor could be that amisulpride would need to be administered for a number of days prior to the methamphetamine challenge (Natesan, Reckless et al. 2008). However, if it is assumed that adequate D2/D3 blockade occurred then the results indicate that the blockade of these dopamine receptors does not attenuate the subjective effects of a drug like methamphetamine. This may indicate that blockade of receptors other than D2/D3 dopamine receptors is needed to reverse the subjective effects. It could be that D1 receptor activation was responsible for the subjective pleasure of the methamphetamine and hence this would not be blocked by amisulpride. However, another study demonstrated that haloperidol (which has affinity for D1 receptors) failed to attenuate the subjective effects of methamphetamine (Wachtel, Ortengren et al. 2002).The selective D1/D5 antagonist ecopipam has been used to try to antagonise the subjective effect of cocaine (an inhibitor of dopamine transporter). These results are mixed: an early study indicated that pretreatment reduced the subjective effects of cocaine (Romach, Glue et al. 1999) but in a subsequent study, pretreatment with ecopipam failed to alter the effects of cocaine (Nann-Vernotica, Donny et al. 2001). Considering these results along

with the challenge phMRI results presented here, it indicates that there may not be a strong link between dopamine receptor activation and subjective effects of a drug like methamphetamine.

The reaction time data were more difficult to interpret due to data loss but the “high” and “low” ratings showed an effect of time. In addition, interaction between drug and time for the “high” groups approached significance. It was expected that the PM group would have faster reaction times after the bolus compared to the PP group. It was difficult to predict what would happen for the AM group; although a previous study (Brauer and de Wit 1996) showed that pretreatment with the D2 receptor antagonist pimozide failed to change the effects of amphetamine on a visual reaction time task. The plot (Figure 3.2-3) of the reaction times of the “high” rating indicates that the PM and AM group had quicker times. Using Figure 3.2-3 and the statistical data, this suggests that methamphetamine decreased the reaction times during the “high” rating and this was not attenuated by amisulpride. The data for the “low” reaction times shows a different pattern. Here, the PM group seemed to have had the fastest times of the drug groups, but the AM group had a similar pattern to the PP group. The combination of findings for the “high” and “low” indicate that the PM group had quicker reaction times than the PP group (as expected) but the pattern of reaction times for the AM group was inconsistent.

For the imaging data, the contrast PM-PP was used to test the hypothesis that there would be BOLD signal changes in areas of high concentration of dopamine receptors such as the striatum and orbitofrontal cortex. Activations were seen for the rectal gyrus and anterior cingulate which would be broadly consistent with the hypothesis. The thalamus was also detected by this contrast and perhaps this could be regarded as an area downstream of the dopamine neuron projection areas that forms part of the basal ganglia-thalamocortical circuits (Alexander, Delong et al. 1986). The detection of cerebellar effects was not strongly expected but could be consistent with effects of methamphetamine detected in a previous study (Kleinschmidt, Bruhn et al. 1999). Although not usually considered as a dopaminergic brain region, there is evidence of dopamine neurotransmission in the cerebellum (Hurley, Mash et al. 2003). The activation of the insula region by the PM-PP contrast seemed more likely to be

due to subjective effects resulting from methamphetamine than a direct effect due to activation of dopamine receptors. This can be seen in the context of the activation of this area in a number of studies examining conscious urges to take drugs (Naqvi and Bechara 2009). It is difficult to understand the finding of increased lingual activation in this comparison. Activation of the lingual gyrus has been shown in a study investigating the effects of cocaine cues in cocaine dependent subjects (Wexler, Gottschalk et al. 2001). Perhaps in some way, the same network was activated in the current study.

The contrast PP-PM was used to test the hypotheses that there would be deactivation due to methamphetamine in areas such as the frontal cortex, amygdala and entorhinal cortex. Some areas were detected in the frontal regions (BA10, BA6, BA3, BA2) by the contrast which would be consistent with the hypothesis. There were also posterior regions and cerebellar regions detected by the contrast which perhaps relate to resting state studies. Resting state areas are those areas activated when subjects rest quietly with their eyes closed and relate to areas decreased in activity during cognitive demanding tasks (Raichle, MacLeod et al. 2001). In the comparison PP-PM there were some areas activated consistent with the resting state areas such as BA10, BA19, and BA40. This may indicate that the use of methamphetamine in this part of the task may have had some similarities to a cognitive demanding task. This is difficult to interpret as alterations of resting state functional connectivity occurs with L-dopa (Kelly 2009) and resting state abnormalities have been detected in illnesses with dopamine dysfunction such as schizophrenia (Garrity, Pearlson et al. 2007).

The effects of drugs were examined by reviewing the peak activations over time for areas identified in the contrast PM-PP (Figure 3.2-4 and Figure 3.2-7). The areas in Figure 3.2-4 were considered together as they may represent dopamine neuron projection areas more clearly than the areas in Figure 3.2-7. The data presented in Figure 3.2-4 offer some support to the hypothesis that pretreatment of amisulpride would attenuate the increased BOLD signal due to methamphetamine. This suggestion is strengthened by the fact that the temporal pattern of the PM group increases over time suggesting that the altered signal was a result of the methamphetamine. The attenuation by

amisulpride seems to occur throughout the time bins. The data are not presented with confidence intervals as there is considerable overlap between the different drug groups throughout. This probably reflects the low numbers (n=6) in each condition but emphasizes that the data need to be considered with caution. A similar effect for amisulpride can be seen in Figure 3.2-7 although in this case there was more prominent signal change in the placebo group possibly reflecting some slow signal drifts (Yan, Zhuo et al. 2009). Another possible cause for the effect in the placebo group is that the saline administration here may have been similar to the effects of placebo administration in studies of people with clinical disorders. In these studies, placebo administration leads to an expectation of clinical effects and so may act as a reward predicting cue (Egerton, Mehta et al. 2009). It could be that there was a similar type of expectations in this study with the saline administration.

The effects of the alternate contrast PP-PM can be seen in Figure 3.2-9. Here the AM group is similar to the PM group suggesting that amisulpride was not altering the pattern of activation of methamphetamine. This does not support the hypothesis that amisulpride would attenuate the deactivating effects of methamphetamine. This figure also shows an increase in signal for the PP group over time but not for the PM and AM groups. One interpretation could be that methamphetamine prevented the increase over time that occurs in the PP group (a form of deactivation). However, even if it had been a form of deactivation, this effect was not altered by the AM group.

Figure 3.2-4 gives a visual representation of the time-course of the PM group supporting the a priori prediction of an increase in the BOLD signal over the time bins for the activation contrast PM-PP. This can also be gauged from the columns “peak % signal change”, “min % signal change” and their corresponding time bins as shown in Table 3.2-2. According to this table, the minimum BOLD signals occurred in the early time bins (soon after the bolus) and the maximal BOLD signal occurred towards the end of the task. This is further support to the idea of activating effects of methamphetamine. Similarly, the deactivation pattern of the contrasts PP-PM can be seen in Table 3.2-4 using a similar method to that as in Table 3.2-2. The changes in signal for the

PP-PM look smaller than for the PM-PP again suggesting that the deactivating effects were weaker than the activating effects.

One concern for this study is that the effects over time could have been due to blood pressure changes. However, BOLD signal changes do not match the changes in blood pressure following administration of amphetamine in animal studies (Chen, Galpern et al. 1997). For human subjects, one group (Newton, Roache et al. 2005) showed that the peak cardiovascular effect following methamphetamine bolus (intravenous doses of 15 and 30 mgs used in methamphetamine dependent subjects) occurred 10 minutes post dose. The data in an earlier study on subjects familiar with the use of methamphetamine (Cook, Jeffcoat et al. 1993) indicated that cardiovascular effects occurred mainly within the first 30 minutes following methamphetamine intravenous administration. The maximum heart rate was reached at 11.8 minutes after intravenous injection. The data in Table 3.2-2 show the peak BOLD effect occurred about 16-20 minutes after the bolus indicating dissimilarity with the likely time course of the cardiovascular effects.

Taking an overview of the above account, the data here offers partial support to the hypothesis that methamphetamine would result in increased BOLD signal. The clearest evidence of this was the time series data for the rectal gyrus and the anterior cingulate in Figure 3.2-4. The areas and activation patterns due to the deactivation contrasts form less strong support for the hypothesis that deactivation of cortical regions would occur. Extrapolating from animal studies (Dixon, Prior et al. 2005) and the activating effects of dopamine D1 receptors on glutamate receptors (Neve, Seamans et al. 2004; Surmeier, Ding et al. 2007), it seemed likely that the regions of increased BOLD signal regions would be as a result of D1 receptor activation. However, given the antipsychotic effects of D2 receptors antagonists on increased dopamine release in schizophrenia, it was felt reasonable to test the hypothesis these effects could be antagonised by amisulpride. The absence of areas identified using the conjunction contrast PM-PP and PM-AM indicate an absence of marked attenuation. However, the data in Figure 3.2-4 indicate that some mild attenuation may have occurred. Similarly the absence of areas in the conjunction contrast PP-PM and AM-PM indicates that the deactivation effects

of methamphetamine were not markedly attenuated by amisulpride. This was despite the fact that the animal study suggested that deactivation seemed more related to dopamine D2 receptor activation and so might have been expected to reverse with D2 receptor antagonism.

The data here suggest that challenge phMRI can be used to detect the effects of methamphetamine using fMRI. However, it is strange that there was an absence of effects in the striatal regions as this area contains the greatest concentration of dopamine receptors. However, an animal study showed that with relative low doses of amphetamines, there were decreases in relative cerebral blood flow in the caudate and putamen region (Ren, Xu et al. 2009) whereas with high doses there were increases. Although blood flow does not exactly correspond to BOLD signal, the relatively low dose of methamphetamine used in this study may have not have allowed increased BOLD signal changes to be detected. Another difficulty in this study is the marked subjective effects that arise even with low doses of methamphetamine. There could have been a difference in BOLD signal changes between the effects detected using the method used here and BOLD signal changes corresponding to the subjective effects. It was difficult to examine for this as the subjective effects of methamphetamine have been shown to take place most strongly within 30 minutes of bolus infusion (Newton, Roache et al. 2005) which was the same time course as the model used here. Perhaps with larger numbers of participants and more reliable ratings acquisition, a regression could have been completed between subjective effects and BOLD signal changes. The areas identified could have been compared with the activation pattern detected using the method in this study.

The lack of common areas of activation for the activation contrasts (PM-PP and PM-AM) and deactivation contrasts (PP-PM and AM-PM) suggests that there was not a marked attenuation of effects of methamphetamine by pretreatment with amisulpride for either of these effects. One possible reason for the lack of strong attenuation could be the low permeability of amisulpride across the blood brain barriers and the potential need to have repeated dosing to overcome this effect (Natesan, Reckless et al. 2008). As suggested previously, it could be that BOLD signal changes from methamphetamine are due mainly to D1 receptors

so that blockade of D2 receptors (even with adequate penetration of the brain) would not reverse the effects of methamphetamine. The low numbers and consequent low power (Mumford and Nichols 2008) also need to be considered as factors for lack of detected effects. The truncation artifact must have affected the results as well, although it is difficult to quantify the nature of this effect.

4.3. N-back task

The main prediction for the N-back task was that there would be better performance with methamphetamine but that this would not be attenuated with amisulpride. This would show that the effects on performance in working memory would be mainly related to D1 receptor activation. There were no clear effects on performance related to drug group. There were more omission errors made by the PM group than the PP group but this was not statistically significant. An obvious potential cause for the lack of effects was the low number of participants in each group. However, there were aspects of the task that could have been done differently that may have helped to detect differences. If the more difficult 3-back level of the task had been included then all three groups would likely have committed more errors. In this case, the advantage of methamphetamine for performance might have been more detectable. A baseline performance can also be useful in this kind of experiment as pre-selecting those with low baseline performances may allow the additional benefit of methamphetamine to become apparent (Mattay, Callicott et al. 2000). This might not have been useful for the present study as the baseline performance in a drug group would have probably been the same before and after the drug but could have been useful if the 3-back level had been included.

The reaction time data showed an effect of task but not of drug. There was no interaction between task and drug. The reaction times for the N-back reflected the difficulty of the task as the hardest level of the task corresponded to the slowest reaction times. Again the low numbers could have been the cause of the inability to detect differences between the drug groups. However, it is not certain that there would have been a difference even with larger numbers as the plot of the reaction times for the different levels of the task in Figure 3.3-1 does not indicate that there was a marked difference between the drug conditions.

The first aim in the imaging analysis was to examine whether the performance of participants resulted in BOLD signal changes in the typical brain regions for this task. The contrast positive effects of all conditions was used to test this and the identified areas (Table 3.3-2) were consistent with the literature on the N-back task (Owen, McMillan et al. 2005). The main hypothesis for the imaging data for the N-back was that there would be decreased activation in the

methamphetamine group compared to the placebo group. It was not expected that there would be marked attenuation of this effect with amisulpride. The contrast main effects of drugs masked by positive effects of all conditions was used to examine for the main differences in positive activations between the drug groups. This contrast identified a region in the right parietal lobe and the associated pattern of activations can be seen in Figure 3.3-3. This shows an increased activation in the PM group compared to the other groups and a progressive increase for the more difficult level of the N-back task. This pattern of activation was also identified in the same region using the conjunction contrast PM-PP and AM-PM. This contrast also identified a region in the left frontal cortex with a similar pattern of activations (Figure 3.3-4).

However, the most surprising aspect of the data in Figure 3.3-3 and Figure 3.3-4 is the pattern of activation. Both the relative increase in activation in the PM group and its attenuation in the AM group is unexpected. The activation pattern is strange as amphetamine studies and studies in people with schizophrenia indicate that increased activation may represent less efficient processing. This would be opposite to what was found here. The participants committed few errors overall so the task was completed as expected. One possible explanation could be that the subjects in the PM group had polymorphisms of COMT and DAT resulting in higher dopamine levels at baseline compared to the other groups (Bertolino, Blasi et al. 2006). Hence the methamphetamine might have resulted in excessive D1 receptor activation and could have been detrimental to PFC function (Arnsten 1997). Perhaps the slightly increased number of omission errors in the PM might support this idea. However, an alternate possibility in view of the limitations of the study is that it could be an erroneous finding.

The parietal area identified in these contrasts is broadly consistent with a comparable earlier study using PET to investigate the effects of methylphenidate (a dopamine and noradrenaline reuptake blocker). In that study, using a spatial working memory task a drug by task interaction was detected in the left dorsolateral prefrontal cortex and left posterior parietal cortex (Mehta, Owen et al. 2000). Although it should be noted that the parietal area was more posterior and medial than the area identified in the present

study. There is also evidence of the left dorsal aspect of the parietal inferior lobe being modulated by load level in an N-back task (Ravizza, Delgado et al. 2004). This may mean that the dorsal aspect of the inferior parietal lobe acts as part of a frontal parietal executive system (Posner and Dehaene 1994). Though genetic studies emphasise the role of dopamine in the frontal lobe and anterior cingulate (Egan, Goldberg et al. 2001; Bertolino, Blasi et al. 2006), it may not be surprising that effects on the prefrontal cortex related to dopamine receptor manipulation could have effects in the parietal lobe. Thus, the areas of activation seem plausible even if the pattern of activation in them is puzzling.

The effects of amisulpride preventing deactivation by methamphetamine (using the conjunction PP-PM and AM-PM) for this task were not the main concern in this part of the study but some interesting areas were identified. These areas included the cuneus, lingual gyrus and dentate. These latter areas seem close to resting state areas (Raichle, MacLeod et al. 2001) although they are more medial and posterior than would be expected. Other areas identified by the conjunction contrast included the right parahippocampus and the left superior frontal region. It is difficult to understand the identification of the parahippocampal region; this region is not usually identified in the fMRI studies using the N-back (Owen, McMillan et al. 2005). However, increased left hippocampal activity has been shown to be related to load in the maintenance period of a working memory task (Axmacher, Mormann et al. 2007). It could be that methamphetamine interfered with this activation.

As outlined above, the areas identified in the contrasts for the main effects of drugs (masked by positive effects of task) and the conjunction contrasts are broadly consistent with expectations. However, it is the nature of the activations that are surprising. Some reasons have given above to explain this but it is difficult to draw strong conclusion from the results in this part of the study as the clusters contained very low number of voxels and a very liberal statistical threshold was used in the analysis.

4.4. Finger tapping task

This task did not have any behavioural data that could be used to assess performance, so the effects of the drug conditions depend on analysis of the fMRI data. As for the N-back task, the first aim in the imaging analysis was to examine whether the performance of participants seemed to result in BOLD signal changes in the typical brain areas for this task. The positive effects of task contrast identified a number of areas consistent with the literature including the primary motor cortex (BA 4), premotor cortex (caudal BA 6), somatosensory cortex gyrus (BA 2), inferior parietal lobule and cerebellum (Fink, Frackowiak et al. 1997; Mattay, Callicott et al. 1998). The activation of the parietal region is interesting and perhaps it reflects the fact that participants performed sequential movements. Increasing complexity of a motor task (sequential compared to random movements) has been shown to be related to increased ipsilateral parietal activation (Mattay, Callicott et al. 1998). Perhaps it is also consistent with the role of the parietal lobe in attention (Corbetta, Kincade et al. 2000) and as a component of the frontal parietal executive system (Posner and Dehaene 1994).

Based on a previous similar study (Tost, Meyer-Lindenberg et al. 2006), the main predicted effects of methamphetamine was increased activation of cortical regions related to motor function. This would result from activation of the direct pathway by D1 receptors and inhibition of the indirect pathway by D2 receptors in the striatum as shown in Figure 1.5-1. The contrast PM-PP identified some of the expected brain regions such as the contralateral cingulate gyrus and middle frontal gyrus. These regions probably represent premotor areas which, as outlined in the introduction, are associated with a large number of functions related to movement. The identification of a number of distinct premotor areas is not surprising as there are multiple spatially separate premotor areas (Dum and Strick 1991). The activation of the ipsilateral temporal lobe is more difficult to understand – an adjoining ipsilateral parietal region (BA 40) has been detected in previous studies (Mattay, Callicott et al. 1998) but it is not very clear how this is related to methamphetamine activation in this task.

It is difficult to explain the areas of deactivations (lingual, amgydala, declive, right inferior frontal gyrus) in the contrast PP-PM. These regions do not seem to

be clearly related to motor related areas (Fink, Frackowiak et al. 1997; Mattay, Callicott et al. 1998) or resting state regions (Raichle, MacLeod et al. 2001). Increases in activations in the inferior frontal gyrus (Willson, Wilman et al. 2004) and right middle frontal lobe (Uftring, Wachtel et al. 2001) have been identified in participants given amphetamine completing motor tasks. However, it is unclear why similar regions were deactivated in the present study.

The second main prediction for this task was that amisulpride would antagonise the effect of methamphetamine by effects on the indirect motor pathway. This was examined by the conjunction contrast PM-PP and PM-AM. The pattern of activation in the regions identified is shown in Figure 3.4-2. The activation pattern for the motor cortex (BA 6) is in line with the a priori prediction and is consistent with a previous study (Tost, Meyer-Lindenberg et al. 2006). This pattern shows an increase in activation with methamphetamine compared both to the placebo group and the amisulpride pretreatment group.

The increase in activation in the caudate is also similar to the findings of the study mentioned above (Tost, Meyer-Lindenberg et al. 2006). This had been an unexpected finding for that group but subsequently it was explained by viewing the striatum as a dynamic rather than a static network. By this view, modulation by the different dopamine receptors could change the type of activation in the network rather than changing the overall level of activity. It may also be consistent with a view of dopamine modulating the various interconnected components of the striatum (Schultz 1998) which are continuously interacting in a complex manner (as represented in recent neural network models (Cohen 2007)). This view may explain why there was not decreased activation in the striatum but perhaps does not explain the increased activation in the caudate head.

The alternate conjunction contrast (PP-PM and AM-PM) allowed the examination of the effects of D2 blockade on deactivation by methamphetamine. In a similar way to other parts of this study, some of these areas (inferior frontal gyrus) match those found in resting state studies but some of the other regions (cerebellum and thalamus) are more difficult to explain in this context. It should be noted that in all the contrasts used, there was a liberal

statistical threshold used and frequently there were low numbers of voxels in the clusters. As such, these findings should be interpreted with caution. However, some of the results seem to support the hypothesis that there would be increased activation in cortical motor regions due to methamphetamine - particularly the contrasts that examined the direct effects of methamphetamine (PM-PP) and the conjunction contrast PM-PP and PM-AM.

4.5. Reward learning task

The initial aim of the behavioural analysis was to classify blocks into learnt, not learnt and incorrectly learnt groups. There seems to be validity for this kind of classification as detection of reward prediction error using fMRI is related to whether subjects learn the task (Schonberg, Daw et al. 2007). It was difficult to use statistical techniques to classify individual blocks into learnt, not learnt and incorrectly learnt groups due to the low number of trials in a block. In addition, for a number of blocks, the optimal response was readily apparent to the participant. It was difficult to use a link function such as a logistic function for the individual blocks with this kind of data. Instead, the pattern of responses was examined for each individual block and by aggregating similar patterns, classification by learning status was made. This was then tested using logistic regression to examine the validity of the classification. As it was possible to fit a logistic regression model (using response as the dependent variable and trial number as a covariate) to the data for the learnt group, this indicated that the responses changed over time in a consistent manner. This was in line with expectations that the optimal action would be chosen more often for the learnt group as the block of trials proceeded.

Another aim was to examine the effect of drug group on performance. Focusing on data for the reward image type in the learnt group, the effect of drug condition was examined using a logistic regression model and it was found that the odds were greater for the PM group to choose the optimal response rather the PP group. This is consistent with the number of learnt blocks in each group as in Table 3.5-1 but also included the nature of the responses over time. This suggests an advantage for the PM group in picking the optimal response. This supports the hypothesis that learning would be enhanced with methamphetamine. There was no clear attenuation of this effect with amisulpride. This can be seen from Table 3.5-1 where the number of learnt blocks for the PM and AM are similar for the reward image type. In addition, there was no statistical difference in the logistic regression model when a contrast was used the between the AM group and the PM groups. It is difficult to identify with confidence the cause of enhanced learning with methamphetamine as it has enhancing effects on a wide range of cognitive tasks (Rapoport, Buchsbaum et al. 1978). It had been hoped that demonstration of increased

BOLD signal changes relating to methamphetamine during the task could provide a possible explanation for this effect. However, as will be seen later it was not possible to draw this inference from the data.

For the reaction time data, it was predicted that there would be a decrease in reaction time for the PM group and this would not be attenuated by amisulpride. The effects of drug condition for reward image type data were unexpected, as in general, the PP group performed the task quicker than either of the other two groups. It is difficult to explain why this was the case. For the punishment image type data, the AM group was broadly slower than the other two groups which was an unexpected finding. This particular result would suggest that amisulpride had a general sedating effect. This was surprising as generally amisulpride does not affect reaction times in cognitive tasks even after repeated daily administration (Ramaekers, Louwerens et al. 1999). In addition, this was the only time this effect was apparent for reaction times in the study in this thesis.

For the reward imaging task, one aim of the analysis was to see if reward and punishment areas were activated in the brain. This was examined by contrasts between the different image types for different times during the task. As outlined in a recent review (Montague, King-Casas et al. 2006), three important reward related areas include the orbitofrontal cortex, ventromedial cortex and the ventral striatum. The orbitofrontal cortex has been implicated in experience of pleasure for various sensory modalities (e.g., satiety to a foodstuff was found to be related to a region in inferior frontal gyrus (O'Doherty, Rolls et al. 2000)). Increased activation in the striatum and orbitofrontal region relate to rewards that change, increase and that are learnt over time (Montague, King-Casas et al. 2006). In contrast, the ventromedial prefrontal cortex corresponds to reward value - for example increased activation was found to occur in BA10/BA32 with reward trials outcomes compared to non rewarded trials (Knutson, Fong et al. 2001). It can be seen from the various tables (summarized in the columns of Table 3.5-13, Table 3.5-14 and Table 3.5-15) that these regions are activated in varying degree for the contrasts used. However, in what follows, the main focus of this discussion is to examine areas detected in relation to dopamine neuron activity.

The main assumption for the reward learning task was that there would have been a phasic release of dopamine at the time of stimulus outcome in the early time bin but that in the late time bin this phasic release of dopamine would have occurred at the stimulus onset. The prediction was that the contrast between the reward and neutral image type at stimulus onset in the late time bin should identify similar areas to the contrast between the same image types at stimulus outcome in the early time bin. The areas identified are shown in Table 3.5-13. In particular, it was predicted that dopamine projection sites (particularly striatal regions) would be identified in both contrasts. Of the areas identified, perhaps the anterior cingulate was the area most likely to be modulated by dopamine neurons having a relative high concentration of D1 receptors (Hurd, Suzuki et al. 2001; Abi-Dargham, Mawlawi et al. 2002) and being in a regions where it could be a target of the well known mesocortical dopamine neuron projections (Mendoza and Foundas 2007). The other areas identified (middle temporal gyrus and ventral frontal cortex) could also be modulated by dopamine.

Another method used to detect the phasic effects of dopamine was by the comparison of the effects of the stimulus onset and stimulus outcome over time. It was predicted that there would be maximal differences in phasic dopamine effects in the contrast between the stimulus outcome in the early time bin and stimulus outcome in the late time bin. Related to this there should be maximal effects between the stimulus onset in late time bin compared to stimulus onset in the early time bin. Comparing the areas of the contrasts might indicate areas that relate to phasic dopamine activation. Reward image type was used rather than punishment image type as it was felt this was more clearly related to phasic dopamine release. The left lentiform nucleus was identified as an area in common. However, this region identified in the stimulus onset contrast corresponded more to the globus pallidus (with low levels of dopamine receptors) rather than putamen. The middle temporal lobe and cuneus were other common areas identified that could be modulated by dopamine.

Figure 3.5-5 shows the differences in activations for the contrast late time bin – early time bin at stimulus onset. The plots were generated using a local maximum in the anterior cingulate for the different image types. The anterior cingulate region was used as it was an area fairly consistently activated by the

various contrasts and is an area that plausibly could reflect phasic dopamine neuron effects. This figure suggests that the effects for the reward image type for this voxel were greater than those for the punishment and neutral image types. This is consistent with the focus on the reward image type data in the above analyses.

In view of this, data for the reward image type were used to examine the effects of drugs used in the study. This is presented in Figure 3.5-6 (stimulus onset) and Figure 3.5-7 (stimulus outcome). The areas used in the plots were chosen as these were possible dopamine projection sites. The prediction here was that the BOLD signals would be increased in the PM group and these effects would be attenuated in the AM group. These effects would occur in the late part of the task for stimulus onset and for the early part of the task for stimulus outcome. Due to the loss of power as described in the methods section, only explorations of the data were feasible rather than definitively addressing the hypotheses. Presenting the pattern of activity in diagrams was a way of exploring the data.

It is difficult to draw conclusions with confidence from Figure 3.5-6, but examining the data for the late time bins only (right side of the plots), it suggests an unexpected increased BOLD signal for the AM group compared to the PM drug group at stimulus onset. Although it is less prominent, the PP group might also have had an unexpected increased BOLD signal compared to PM. The effect of methamphetamine seems to have been advantageous for the learning of the task yet the BOLD signal may be reduced compared to the other drug groups at the end of the learning task. The data for the early time bins (left side of the plots) in Figure 3.5-7 also indicate that the PM group had (mostly) an unexpected lower BOLD signal change than the PP group and the AM group at stimulus outcome.

The numerous studies that have used the TD model to reflect dopamine activity suggest that the BOLD signal (particularly in the striatal regions) relates to the reward prediction error. This suggests that improved performance could result from methamphetamine induced increased reward prediction error signals. This could be reflected by an increased BOLD signal. In this part of the study, there was some evidence that dopamine projection sites (anterior cingulate and the

striatal regions) had increased BOLD signal changes corresponding to the expected times of increased phasic dopamine release during a learning task. However, the exploratory analysis of the effects of drugs did not indicate that these effects were augmented in the expected way by methamphetamine.

The effects of drugs on the BOLD signal changes is inconsistent with previous work using computational models (Pessiglione, Seymour et al. 2006; Menon, Jensen et al. 2007). However, the results in this part of the study cannot be considered with confidence as the power in this part of the study (due to concentrating only on those who learnt the task) was even lower compared than the other parts of the study. Another problem is the possible ambiguity about the nature of the learning task used. It is unclear the degree to which the outcome was related to the action taken or to the stimulus presented previously. Thus, there was interplay of goal directed and Pavlovian aspects to the task. These aspects of behaviour may be encoded differently in the brain (Balleine and O'Doherty 2010) and it also makes it more difficult to relate the task to a conceptual framework such as the TD model.

5. Overall review of experiment

5.1. Screening data and performance data for all parts of the study

Analysis of the physiological data indicated that there were changes in diastolic blood pressure and pulse rate (sitting and standing) over time considering all the participants but there were no detectable differences between the drug groups. There were detectable effects on prolactin relating to amisulpride administration.

For the data collected during the challenge phMRI, the increase in subjective "high" ratings for the PM group following the bolus of methamphetamine during the challenge phMRI occurred as expected. Amisulpride pretreatment did not attenuate these subjective effects of methamphetamine – an effect which was also largely expected. For the N-back task, there were no clear differences in performances between the drug groups on any of the levels. However, there was an indication that as the task became more difficult, differences began to emerge between the drug groups. The data in the reward learning task were subdivided to allow focused examination of effects on the reward rather than the punishment image type. For the reward image type data, the PM group seems to have completed the reward learning task in a more optimal way than the PP group.

5.2. Reaction times

For the reaction time data completed during the challenge phMRI, there was no statistically significant difference detected between the drug groups. However, the pattern of the data plots suggested quicker reaction times for the PM and AM groups compared to the PP group for the "high" rating and quicker reaction times for the PM group compared to the AM and PP groups for the "low" rating. For the N-back task there were no clear differences between the drug groups. For the reward learning task, the PP group unexpectedly had quicker reaction times than either the PM or the AM group for the reward image type data. The AM group had slower reaction times for the punishment image type data compared to the other two drug groups which was also an unexpected result.

5.3. fMRI data

For the challenge phMRI, there was increased BOLD signal for a number of target areas of dopamine neurons (rectal gyri and anterior cingulate) over time relating to activation by the PM group. This suggested that increased dopamine release was related to BOLD signal changes. This provided a basis for investigation of phasic dopamine changes in the reward learning task. There was some indication that these effects were attenuated in the AM group.

For the N-back task, a conjunction contrast indicated that regions in the inferior frontal lobe and inferior parietal lobule were activated in the PM group and this effect was attenuated in the AM group. A region in the parietal lobe was also detected (with a similar activation pattern) using the main effects of drugs masked by positive effects of task. The pattern of activation was not in line with the hypothesis for this part of the study. For the finger tapping task, the conjunction contrast PM-PP and PM-AM identified cortical motor areas where there was increased activation for the PM group and attenuation by the AM group consistent with the hypothesis. Various contrasts were used in the reward learning task to examine for the areas of activation in dopamine projection areas consistent with activity predicted using a computational TD algorithm. Areas identified consistent with the model included the anterior cingulate and striatal regions. The exploratory analysis used to examine the effects of drugs did not correspond to the predictions of the model.

5.4. General implications for theories of dopamine function

The data from the part of the study using the challenge phMRI technique indicate that there could be BOLD signal changes directly related to the activation of dopamine receptors. This would be consistent with the relationship between post synaptic glutamatergic signalling and BOLD signal changes (Attwell and Iadecola 2002; Drake and Iadecola 2007) and the effect of dopamine receptor activation on glutamate receptors (Neve, Seamans et al. 2004). The effect of D2 blockade was not clear from this study despite a suggestion of this kind of effect in a related animal study (Dixon, Prior et al. 2005). The lack of attenuation by D2 blockade to the subjective ("high") effects of methamphetamine may support the idea that the pleasurable effects are not directly related to dopamine receptor activation.

The effect of methamphetamine on the N-back is hard to understand in this study. Neither the increased activation with methamphetamine nor the attenuation by amisulpride seems consistent with enhancement of working memory primarily by D1 receptor activation. The most likely explanation is that it was probably an erroneous result due to low thresholds used in the fMRI analysis. The finger tapping results correspond to simple models of opposing effects of D1 receptor activation and D2 blockade in cortical areas downstream of the striatum. The specific effects of dopamine receptor activation in the striatum are more difficult to fit with the simple model used. Perhaps the effect of dopamine alters the patterns of activity between the components of the striatum that would require complex models to predict (e.g., (Cohen 2007)). However, the final output from the striatum may have effects on cortical activity (Surmeier, Ding et al. 2007) which is predictable using simple models involving dopamine.

With respect to the reward learning task, the behavioural results suggested an enhanced performance with methamphetamine. This was consistent with predictions based on simple interpretations of the TD algorithm discussed in the introduction. The effects of drug group were explored to see if increased BOLD signal occurred with methamphetamine in the context of the task. This would have provided a base to explain the improved performance with methamphetamine. Exploratory analysis of the data revealed decreased activations related to methamphetamine. This did not correspond with the hypotheses and it was difficult to explain the enhanced performance in the context of this finding. However, as it was not possible to assess the effect of drug group in a rigorous way, then the findings relating to methamphetamine could have been erroneous.

5.5. Implications of results for mental illness

It was difficult to identify clear implications for mental illness from this study due to its limitations. The results of the challenge phMRI indicated that this was a relatively safe technique that could be used to examine the effects of excessive dopamine release and attenuation with D2 receptor blockade. This suggests that this technique could be employed in people with schizophrenia although

due to the artifact problems, further studies on healthy populations would need to be completed.

The results on the N-back were puzzling as it had been expected that decreased activations would have been detected in the PM group. This would have added to the evidence of improved performance relating to decreased BOLD activation. This would have helped to show that people with schizophrenia would be more likely to have increased rather than decreased BOLD signal changes compared to controls during the completion of memory tasks (Thermenos, Goldstein et al. 2005).

There were some findings from this study that can be related to addiction. One of the findings from the part of the study using the challenge phMRI technique was that D2 blockade does not markedly affect the pleasurable effects of methamphetamine. The rewarding aspect of dopamine (Wise 2008) remains an important concept in addiction research (Nutt and Lingford-Hughes 2008; Volkow, Fowler et al. 2009). The results of this part of the study add to previous work that raises doubt about this role of dopamine. It had been hoped that the results from the reward learning task would have additionally focused attention on the role of dopamine in learning processes and how this could be applied to addiction. Using the behavioural data, methamphetamine seemed to result in enhanced learning of the reward learning task- an effect that was not attenuated by amisulpride. However, it was not possible to clearly examine the possible biological mechanisms in this process making it unclear how the effect could be attenuated. However, this part of the study could still provide a platform for further investigations. If amphetamine related agents consistently enhanced reward learning tasks in a way that could be antagonised in humans, then these antagonists could have a role in the treatment of addiction.

5.6. Study Limitations

There were a number of problems encountered in this study which need consideration. As mentioned in the methods section, for 2 participants, there were problems with equipment and so a part of the experiment was completed on a separate day. This was only possible as the participants were in the PP group. As a result there was unblinding for these participants. Each of them had completed the part of the study using the challenge phMRI technique but was

unblinded for some of modulation phMRI tasks. Some results (relating to the N-back task) were given previously in this thesis which showed that the performance of one of these participants did not appear markedly different from the others in the PP group. It was also felt that the effects of methamphetamine were such, that once the bolus (methamphetamine or placebo) had been given, partial unblinding had effectively occurred for all participants. Hence, the data from the separate days were included in the analysis. However, it must be acknowledged that still this leads to problems with bias for this data.

For the challenge phMRI technique, problems with the equipment affected the validity of the performance and reaction time data. The type of MRI sequence used for the challenge phMRI contributed to the truncation artifact. It was not possible to rectify the artifact but particular contrasts were selected in the analysis to try to reduce its effects. However, the presence of the artifact is a limitation for the validity of the results for the challenge phMRI part of the study. A limitation of the finger tapping task was the absence of performance data or reaction time data. Even though the participants were viewed while they completed this task, a differently designed task may have allowed the collection of this kind of data. Consistency of performance and drug effects related to the task could then have been examined. It is possible that the version of the N-back task used was insufficiently difficult for the requirements of the study. If the 3-back version had been used then drug effects may have become more apparent, as there was a suggestion of these types of effects at the 2-back level. As regards the reward learning task, there was some ambiguity about the nature of the learning (Pavlovian or goal directed) taking place making interpretation of the results more difficult. The use of a mathematical model of the TD algorithm could have been used as an alternative approach to identify common areas of activation for the different conditions of the task. It could also have helped with the power for this part of the study. An alternative design resulting in an increased number of trials per block may have enabled more participants to learn the task correctly and so increase the amount of usable data.

The main problem for the study overall was the low power which was further reduced in the reward learning task. In the comparisons between the drug

groups there were usually only 6 per group. In fMRI studies using a block design, 18 subjects may be needed to achieve 80% power (Mumford and Nichols 2008). Perhaps with a pharmacological study, a greater effect size could be expected thereby reducing the number of subjects required. However, it would seem unlikely this would occur to the degree required in this study. Another general problem related to the use of methamphetamine as a dopamine probe. This agent also releases other monoamines so it is possible that some of the effects are due to noradrenaline and serotonin. In addition the use of methamphetamine activated both D1 and D2 dopamine receptors which may cause opposing effects thereby making interpretation of the results difficult. The advantage of using amisulpride was its high selectivity for dopamine receptors. However, the main drawback with its use is the uncertainty about the length of time for its entry into the CNS. As a result is difficult to be sure to what degree D2 and D3 dopamine receptor blockade occurred.

5.7. Future directions

For further work, the use of challenge phMRI seems to be a promising approach to explore dopamine function in humans. Perhaps future studies could use either a selective D1 or D2 receptor agonist as an intravenous bolus (e.g., ropinirole (Ramji 1999)) and compare the effects to placebo. A follow up study could examine the effects of pretreatment with a related selective dopamine receptor antagonist. The same agents could also be used in modulation phMRI studies using variations of the cognitive tasks in this study. In particular, a redesign of the reward learning task would be warranted to reduce the ambiguity of what type of learning occurred during the task.

The other avenues for further research relate to the application of these methods to clinical populations. For example, the challenge phMRI technique could be used in people with mental illnesses with dopamine dysfunction such as schizophrenia. Comparisons of BOLD signal changes between controls and people with addiction in the completion of reward learning tasks could also be a promising line of future research. Subsequent studies could then examine the different effects of dopamine manipulation between these groups.

5.8. Conclusion

In this study, different types of imaging techniques and different types of drugs were used to examine the role of dopamine in man. The results were broadly in line with expectations although there were some unexpected findings. In view of limitations of the study, the results should be regarded with caution. However, the descriptions of the procedures used and the analytical techniques employed may serve as a useful basis for future similar experiments.

6. Appendix

6.1. Fractals used in the reward learning task

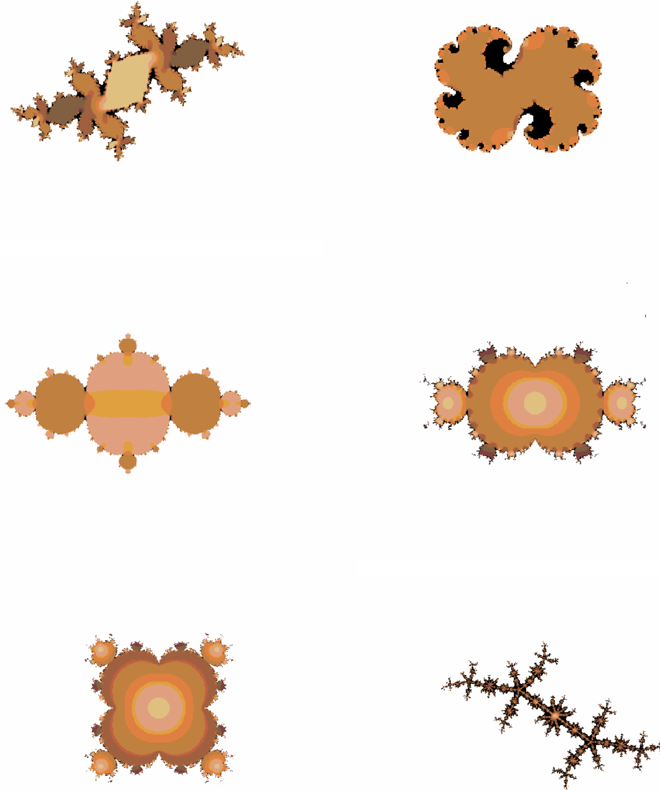


Figure 6.1-1
The fractals used in the reward learning task were generated from matlab code (Strumia Accessed May-2007) using the copper colormap.

6.2. Supplementary table for N-back performance

	Omission	Correct Responses
PP	2	43
PM	6	48
AM	4	50
Total	**12**	**141**

Table 6.2-1
This table shows the data used to examine the differences between the drug groups for the number of omissions for the 2-back level of the N-back task. Participants either completed a correct response or omitted the response. There was no difference between the drug groups using Fisher's exact test.

6.3. Plots of reward learning task

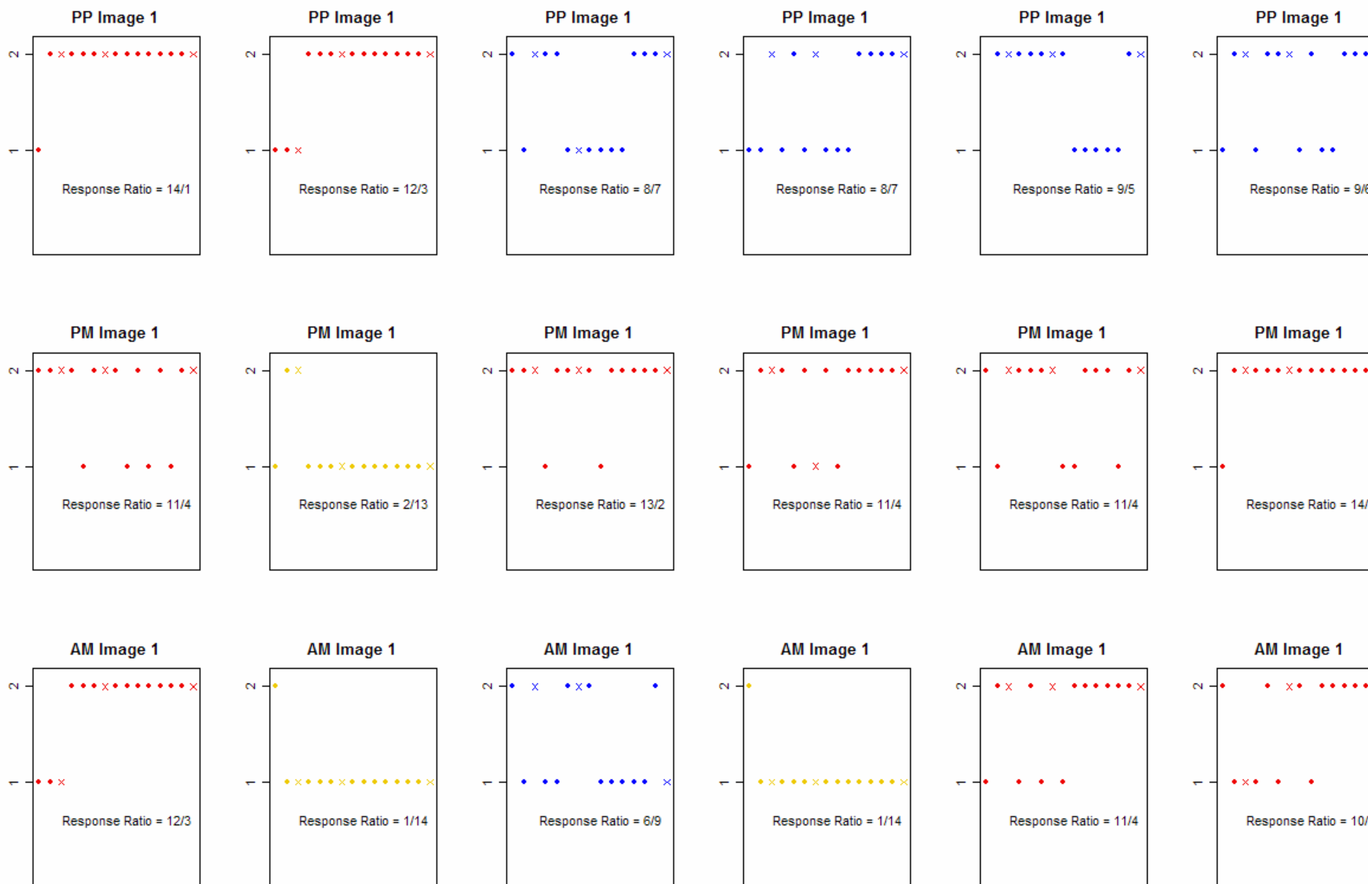


Figure 6.3-1
These are the plots for participants learning the optimal responses for stimulus reward 1. Each plot relates to a different participant. The y axis refers to the response taken represented as either 1 (suboptimal response) or 2 (optimal response), the x axis is the trial number for a block. The circles refer to the usual contingency between response taken and feedback given. The crosses represents occasions when this contingency is reversed. Plots in red represent learnt blocks; plots in blue represent blocks that were not learnt and gold plots represent incorrectly learnt blocks. The response ratio is the number of optimal to suboptimal response. Each row refers to a different drug group: PP in the first row; PM in the second and AM in the third.

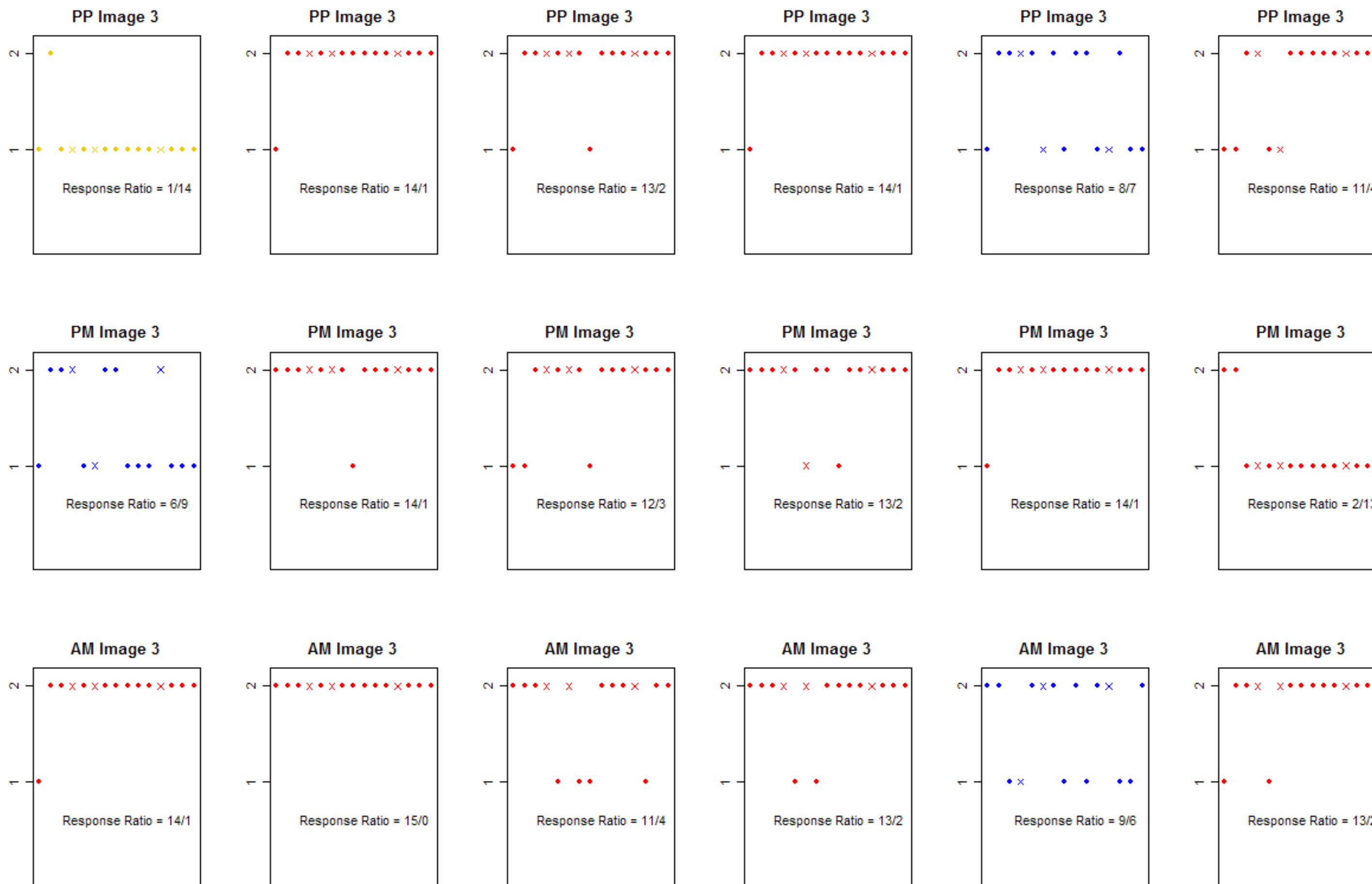


Figure 6.3-2
These are the plots for participants learning the optimal responses for stimulus reward 2. Explanation of the plot is outlined in Figure 6.3-1.

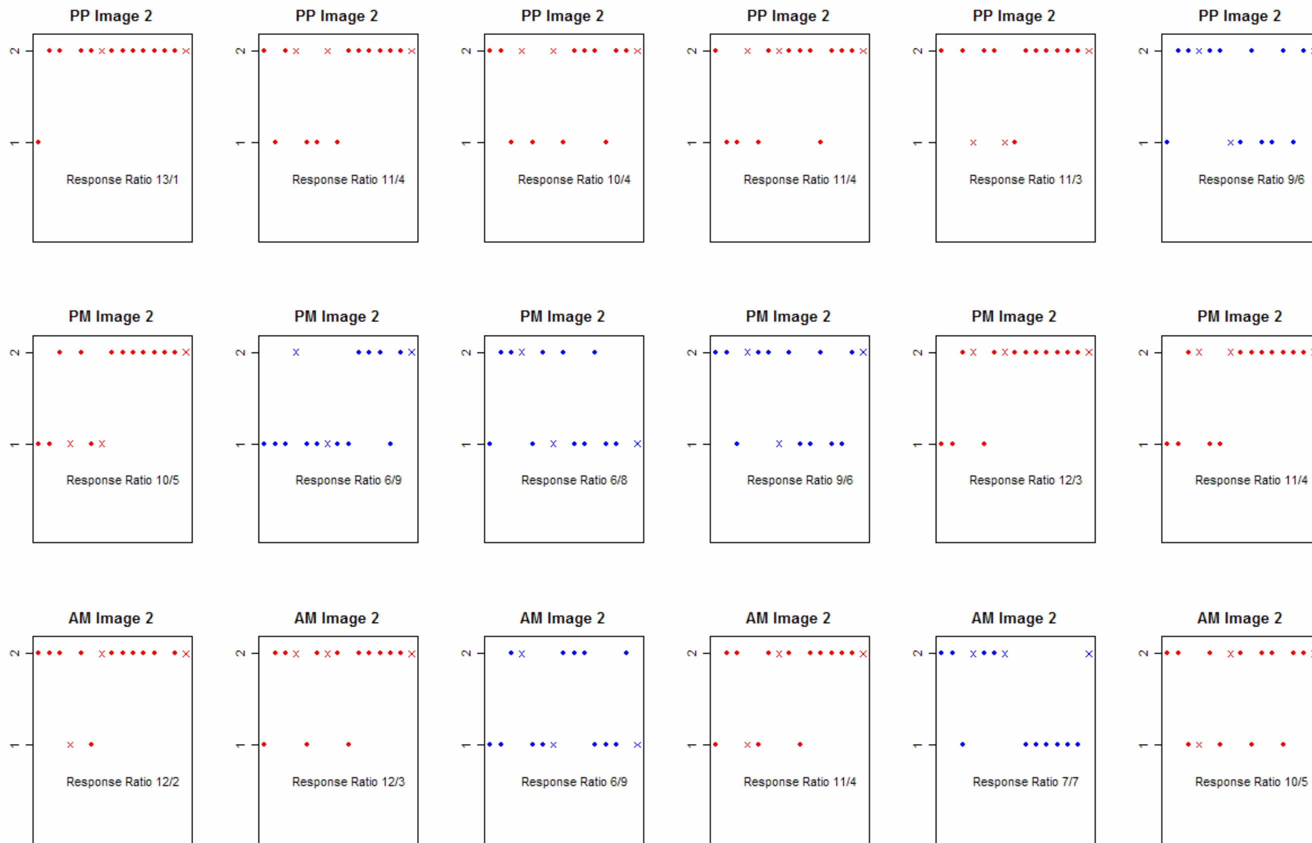


Figure 6.3-3
These are the plots for participants learning the optimal responses for stimulus punishment 1. Explanation of the plot is outlined in Figure 6.3-1.

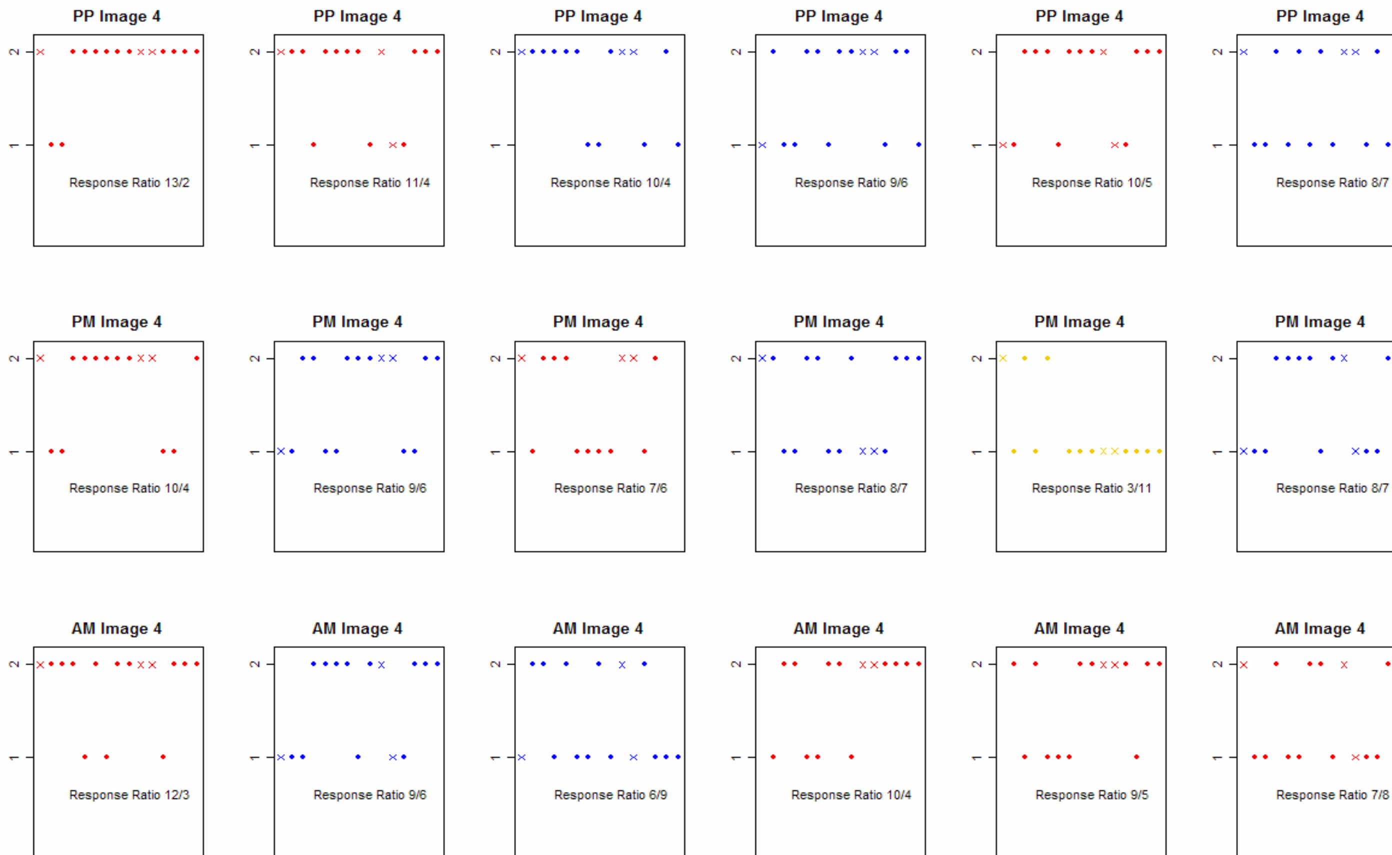


Figure 6.3-4
These are the plots for participants learning the optimal responses for stimulus punishment 2. Explanation of the plot is outlined in Figure 6.3-1.

6.4. Supplementary tables for reward learning task

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
389	0.131	3.52	Right	Middle Frontal Gyrus	9	45	24	31
461	0.105	4.38	Right	PreCuneus	19	25	-77	36
196	0.152	3.26	Right	Cuneus	17	7	-76	13
172	0.223	2.57	Right	Superior Temporal Gyrus	39	55	-60	26
151	0.111	3.99	Left	Putamen		-21	12	3
151	0.227	2.54	Left	Putamen		-21	2	0
80	0.126	3.72	Left	Declive (Cerebellum)		-39	-60	-15
92	0.2	2.82	Left	Medial Frontal Gyrus	9	-7	40	24
39	0.134	3.39	Left	Middle Temporal Gyrus	21	-48	6	-18
18	0.152	3.26	Right	Paracentral Lobule	6	5	-27	50
11	0.156	3.23	Right	Middle Temporal Gyrus	39	52	-69	13
30	0.161	3.18	Right	PreCuneus	7	4	-60	51
37	0.191	2.87	Right	Postcentral Gyrus	40	37	-34	53
88	0.165	3.13	Right	Superior Temporal Gyrus	13	48	-43	21
88	0.196	2.84	Right	Supramarginal Gyrus	40	55	-46	22
17	0.167	3.11	Left	Postcentral Gyrus	3	-25	-31	50
30	0.208	2.7	Left	Middle Frontal Gyrus	8	-25	20	47
39	0.183	2.92	Right	Superior Frontal Gyrus	9	9	54	30
13	0.239	2.43	Right	Inferior Occipital Gyrus	19	37	-77	-5

Table 6.4-1
This is a table of the activations of clusters for the contrast early - late time bins for the reward image type at outcome. This was completed using p(unc) < 0.01 with an extent threshold of 10 voxels and the activation of the local maximum was in a region of grey matter. The model used included factors for time bin, image type and drug group. This table relates to Figure 3.5-7 in the main text.

Cluster size	p(FDR)	Z score	Side	Area	BA	X	Y	Z
245	0.046	3.45	Left	Medial Frontal Gyrus	9	-16	41	14
3022	0.008	4.82	Left	Posterior Cingulate	29	-9	-43	9
194	0.008	4.76	Left	Anterior Cingulate	25	-2	12	-9
192	0.043	3.5	Right	Fusiform Gyrus	20	53	-3	-23
40	0.157	2.57	Left	PreCuneus	7	-4	-33	47
54	0.036	3.6	Right	Hippocampus		34	-28	-10
224	0.049	3.42	Left	Cingulate Gyrus	23	-2	-18	33
51	0.056	3.32	Right	Amygdala		21	-4	-12
38	0.058	3.29	Right	Uncus	28	30	4	-21
26	0.088	3.02	Left	Middle Temporal Gyrus		-62	-45	2
23	0.061	3.26	Left	PreCuneus	7	-16	-45	54
36	0.193	2.38	Left	Cingulate Gyrus	31	-4	-36	41
10	0.086	3.04	Right	Culmen (Cerebellum)		16	-39	-19
23	0.09	3	Right	Culmen (Cerebellum)		14	-55	-18
22	0.107	2.88	Right	Thalamus		2	-8	7

Table 6.4-2
This is a table of the activations of clusters for the contrast late - early time bins for reward image type at onset. This was completed using p(unc) < 0.01 with an extent threshold of 10 voxels and the activation of the local maximum was in a region of grey matter. The model used included factors for time bin, image type and drug group. This table relates to Figure 3.5-6 in the main text.

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