The Contribution Of The Brain Response To Ketamine To Understanding The Glutamatergic System And Its Role In Human Cognition

De Simoni, Sara

Awarding institution:
King's College London

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THE CONTRIBUTION OF THE BRAIN RESPONSE TO KETAMINE TO UNDERSTANDING THE GLUTAMATERGIC SYSTEM AND ITS ROLE IN HUMAN COGNITION

Sara De Simoni

Department of Neuroimaging

Institute of Psychiatry

King's College London

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ABSTRACT

A high degree of interest continues in studying the central effects of ketamine, an NMDA receptor (NMDAR) antagonist, due to its potential relevance to schizophrenia, antidepressant efficacy and analgesia. This thesis investigates the brain response to ketamine to (1) determine whether it is a valuable pharmacological tool to investigate glutamatergic dysfunction and (2) further our knowledge on the role of the glutamatergic system in human cognition.

The administration of low-dose ketamine to healthy volunteers was combined with arterial spin labelling (ASL) and blood oxygen level dependent (BOLD) imaging in both resting state and cognitive task conditions. The test-retest reliability of the ketamine response was assessed together with its effects on working memory (N-BACK) and paired associative learning (PAL) task networks. Modulatory effects of lamotrigine and risperidone, treatments thought to reduce ketamine-induced glutamate release, were also evaluated.

Ketamine induced robust and reliable effects in predicted cerebral networks with ASL and BOLD. However, risperidone and lamotrigine only attenuated the BOLD changes. Ketamine altered the load-response profile during the N-Back within the dorsolateral prefrontal cortex and subtly decreased activation in brain areas including the cingulate and thalamus. A trend towards ketamine-induced attenuation of learning-related increases in activation was found in the PAL task. Pretreatments had differential effects depending on the cognitive task, with risperidone having a more prominent role in working memory and lamotrigine in associative learning. Finally, ketamine altered associative learning-related changes in fronto-temporal connectivity.

Results suggest (1) the resting brain response to ketamine is a suitable tool to test and validate pharmacological interventions; (2) fMRI demonstrates that the function of NMDARs is implicated in both region-specific and inter-regional brain communication in working memory and associative learning contexts respectively and (3) the distinct downstream neural substrates of NMDAR antagonism are evidenced by the context-dependent reversal of its effects. The findings have implications for future ketamine-related research and its use as a model of glutamatergic dysfunction relevant to psychiatric disorders.

PERSONAL CONTRIBUTION

Together with my supervisors, Dr. Mehta and Dr. O'Daly, I was intimately involved in all phases of the studies described in this thesis, which were set up as a scientific collaboration between the Department of Neuroimaging, King's College London and Eli Lilly. The protocol and design of the studies was worked up together with my supervisors, including dose and task selection. I also worked closely with the programmer within the department, to modify and refine the design of the existing tasks, including creating novel playlists. With the support of my supervisors, I was responsible for the application for ethics and R&D approval. The study days involved the alignment of pharmacy, medical and nursing cover, scanning facilities and volunteers; the logistics of setting up the study were managed and conducted myself as well as the recruitment of volunteers and the running of all study visits. During the first year of my PhD, I attended training to allow me to better fulfil these roles such as Good Clinical Practice (GCP) and a Phlebotomy course (Maudsley hospital). Thus under the supervision of our research nurse I was also responsible for the clinical screening procedures (e.g. taking blood samples, electrocardiogram (ECG) recordings, drug testing). The use of the gamma variate function to model the ketamine response in Chapter 4 was a decision taken by my supervisor, Dr. Mehta, and Dr. Adam Schwarz (Eli Lilly). All the analyses in this thesis were conducted by myself, under the supervision of Drs. Mehta and O'Daly. The interpretation of the results are my own, arrived at through discussion with my supervisors. Chapter 4 has resulted in two first author publications.

PUBLICATIONS

De Simoni, S., Schwarz, A.J., O'Daly, O.G., Marquand, A.F., Brittain,C., Gonzales, C., Stephenson, S., Williams, S.C.R & Mehta, M.A. (2013). "Test-retest reliability of the BOLD pharmacological MRI response to ketamine in healthy volunteers", Neuroimage, 1;64:75-90.

Doyle, O.M.,* **De Simoni, S.,*** Schwarz, A.J., Brittain,C., O'Daly, O.G., Williams, S.C.R & Mehta, M.A. (2013). "Risperidone and lamotrigine attenuate the phMRI response to ketamine", Journal of Pharmacology and Experimental Therapeutics, 345(1):151-60. ***Both authors contributed equally to this work.**

LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
AMPAR	α-Amino 3-hydroxy-5-Methyl-4-isoxazoleProprionic Acid Receptor
ANOVA	Analysis of Variance
ASL	Arterial Spin Labelling
BOLD	Blood Oxygen Level Dependent
CADSS	Clinician Administered Dissociative States Scale
CANTAB	Cambridge Neuropsychological Test Assessment Battery
CASL	Continuous Arterial Spin Labelling
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
DLPFC	Dorsolateral Prefrontal Cortex
FMRI	Functional Magnetic Resonance Imaging
GLM	General Linear Model
ICC	Intra-class Correlation Coefficient
HIPP	Hippocampus
ITL	Inferior Temporal Lobe
KAR	Kainate Receptor
NMDAR	N-Methyl-D-Aspartate Receptor
mGluR	Metabotropic Glutamate Receptor
MRS	Magnetic Resonance Imaging
PAL	Paired Associates Learning task
PCC	Posterior Cingulate Cortex
PC	Parietal Cortex
PET	Positron Emission Tomography
phMRI	Pharmacological Magnetic Resonance Imaging
PK	Pharmacokinetic
PSI	Psychotomimetic States Inventory
ROI	Region of Interest
SPM	Statistical Parametric Mapping
THAL	Thalamus
VAS	Visual Analogue Scales
VLPFC	Ventrolateral Prefrontal Cortex
WM	Working Memory

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Chapter 1 INTRODUCTION

In this introductory chapter an overview of the glutamatergic system will be given first, with an emphasis on the NMDA receptor and its role in learning and memory processes. In the following section, the cognitive deficits evident in schizophrenia, particularly those in working memory (WM) and associative learning, will be summarised. The potential involvement of different neurotransmitters in the aetiology of the disorder will also be discussed, with a central role for glutamatergic dysfunction and the NMDA receptor hypofunction model of schizophrenia. A more detailed description of the behavioural and neural effects of ketamine, an NMDA receptor antagonist, will then be given in order to gain an understanding of the neurocognitive pathways regulated by the glutamatergic system which could potentially become the targets of novel cognitive-enhancing treatments, an endeavour relevant for schizophrenia and other psychiatric disorders such as depression where cognitive deficits are evident.

1.1   The Central Glutamatergic System

Glutamate is considered to be the main excitatory neurotransmitter in the central nervous system (CNS; (Curtis, Phillis, & Watkins, 1960; Fonnum, 1984; Koller & Urwyler, 2010). Glutamate neurotransmission is precisely controlled presumably due to its role in synaptic plasticity (Malenka & Bear, 2004) and excitotoxicity (Lau & Tymianski, 2010; Y. Wang & Qin, 2010). Ionotropic (iGluRs) and metabotropic (mGluRs) glutamate receptors have been identified, responsible for fast and slow synaptic transmission respectively (Ferraguti & Shigemoto, 2006; Koller & Urwyler, 2010).

1.1.1   Glutamatergic Receptors

1.1.1.1   Metabotropic Receptors

Metabotropic receptors are coupled intracellularly to G-proteins via which they are able to activate downstream second messenger systems (Lau & Tymianski, 2010; Moghaddam, 2004). Their primary role is modulatory, mediating synaptic activity and plasticity (Kew & Kemp, 2005). To date eight different mGluRs have been identified and are classified into three groups depending on their pharmacology and the G-protein to which they are coupled; Group I (mGlu 1 and 5), Group II (mGlu 2 and 3) and Group III (mGlu 4, 6, 7, and 8) (Gregory, Dong, Meiler, & Conn, 2011). All of the mGluRs consist of a homodimer composed of seven transmembrane (TM) domains with an intracellular carboxyl (C)-terminus and a large extracellular amino (N)-terminus also referred to as the

'Venus flytrap' domain that includes the ligand-binding site (see Figure 1-1; (Gregory et al., 2011; Niciu, Kelmendi, & Sanacora, 2012; Vinson & Conn, 2012). Group I mGluRs are coupled to the G-protein G_q/G_{11} whose stimulation has downstream effects on either phosphoinositide hydrolysis or diacylglycerol (DAG), leading to an increase in intracellular Ca^{2+} or activation of protein kinase C (PKC) respectively (Gregory et al., 2011; Niciu et al., 2012; Vinson & Conn, 2012). Both Group II and Group III mGluRs are coupled to the $G_{i/o}$ protein which leads to an inhibition of adenylyl cyclase (Gregory et al., 2011; Niciu et al., 2012; Vinson & Conn, 2012). Group I are predominantly located post-synaptically and Group III pre-synaptically whilst Group II mGluRs can be found in both locations. In addition, due to their placement, Group I and Group II mGluR can interact with certain ionotropic receptors, specifically the N-methyl D-aspartate (NMDA) ion channels (P. Krieger, Hellgren-Kotaleski, Kettunen, & El Manira, 2000; Lecourtier, Homayoun, Tamagnan, & Moghaddam, 2007; Vinson & Conn, 2012).
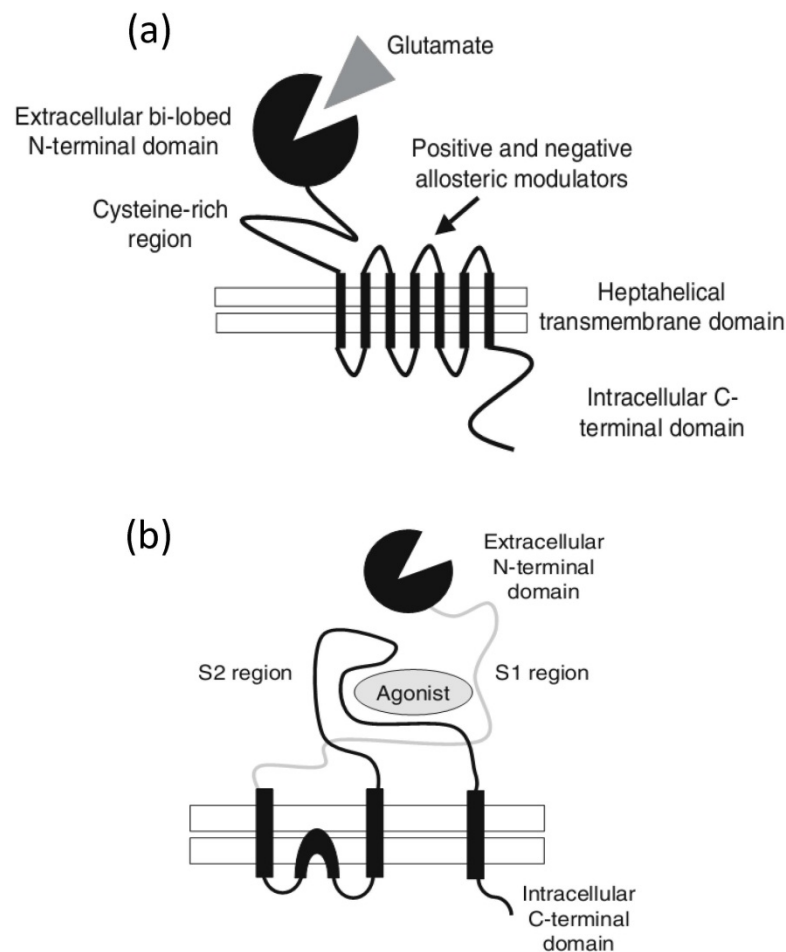
Figure 1-1 Molecular structures of metabotropic and ionotropic glutamate subunits

Adapted from Kew & Kemp (2005). Molecular structures of (a) an mGLuR and (b) one iGluR subunit (see text for more details).

1.1.1.2 Ionotropic Receptors

Ionotropic receptors are ligand-gated ion channels permeable to cations such as potassium (K^+), sodium (Na^+) and calcium (Ca^{2+}) (Stawski, Janovjak, & Trauner, 2010) and are referred to as NMDA, AMPA (α-amino 3-hydroxy-5-methyl-4-isoxazoleproprionic acid) and kainate glutamate receptors due to their affinity for certain ligands (Collingridge, Olsen, Peters, & Spedding, 2009). IGluRs have a tetrameric structure; they are composed of four subunits which together form an ion channel at their centre (Traynelis et al., 2010). As with the mGluRs each subunit has an extracellular N-terminus and a cytoplasmic C-terminus; however they have only three TM domains with

an additional domain helping to form the ion pore by creating a re-entrant loop. The ligand-binding domain resides in an extracellular loop between the second and third TM domains (S2 region) and the extracellular N-terminus S1 region (see Figure 1-1; (Kew & Kemp, 2005; Stawski et al., 2010)).

Kainate/AMPA receptors (KAR/AMPAR): Kainate subunits include GluK1 – GluK5, forming a receptor that is present on both pre- and post-synaptic neurons and maintains a mainly modulatory role in synaptic transmission (Collingridge et al., 2009; Stawski et al., 2010). AMPARs, most commonly formed from a combination of subunits (GluA1-4; (Collingridge et al., 2009)), are considered to play a primary role in the generation of fast excitatory post-synaptic potentials (EPSPs; (Niciu et al., 2012)). Furthermore, the number and placement of AMPA receptors present at the post-synaptic membrane can have significant effects on strength of the neuronal transmission ((Derkach, Oh, Guire, & Soderling, 2007); see below). The presence of the GluR2 subunit determines whether the receptor is permeable to Ca^{2+} or not, a property which has been seen to affect AMPAR-mediated synaptic transmission and plasticity (Isaac, Ashby, & McBain, 2007). The majority of AMPARs contain GluR2, which due to the positively charge amino acid arginine in the pore-forming re-entrant loop of the subunit, makes them impermeable to calcium (Kew & Kemp, 2005). However, although GluR2 lacking AMPARs are permeable to Ca^{2+}, they are still less so than NMDA receptors (Isaac et al., 2007).

NMDA receptors (NMDARs): NMDARs (Figure 1-2) are formed from a combination of seven different subunits; GluN1, GluN2A – GluN2D, GluN3A-B (Collingridge et al., 2009), whereby the presence of the GluN1/GluN2 unit is essential for the functionality of the receptor (Kohr, 2006). NMDARs are located on both pre- and post-synaptic neurons, with those on the latter more highly concentrated within the post-synaptic density (PSD) on dendritic spines (Kohr, 2006). Receptors located extrasynaptically (e.g. on dendritic shafts or cell body) can be activated by glutamate spillover, which has been found to potentiate the plasticity effects of glutamate acting at synaptic receptors (Chalifoux & Carter, 2011; Kohr, 2006).

A factor that contributes to the importance of the NMDAR in brain function is the fact that it is both "ligand-gated and voltage sensitive" ((Koller & Urwyler, 2010), p.1683). While the neuron is still at the resting membrane potential (-70mV), a magnesium cation (Mg^{2+}) blocks the ion pore, thus preventing any ion flow through the channel (Koller & Urwyler, 2010; Zorumski & Izumi, 2012). NMDAR activity thus depends on (1) binding

of glutamate at its site on the GluN2 subunit, (2) concurrent binding of the co-agonist glycine on the GluN1 subunit and (3) depolarisation of the post-synaptic cell which enables the Mg^{2+} block to be dislodged and calcium and sodium ions to flow into the cell (Ghasemi & Schachter, 2011; Koller & Urwyler, 2010; Zorumski & Izumi, 2012). The depolarisation of the cell, which reflects the activity of the post-synaptic neuron, is primarily mediated by the actions of glutamate (released from the active pre-synaptic neuron) at AMPARs (Zorumski & Izumi, 2012). Thus the NMDAR integrates information from the simultaneously activated pre- and post-synaptic neurons, earning its name as a 'coincidence detector' (Zorumski & Izumi, 2012).
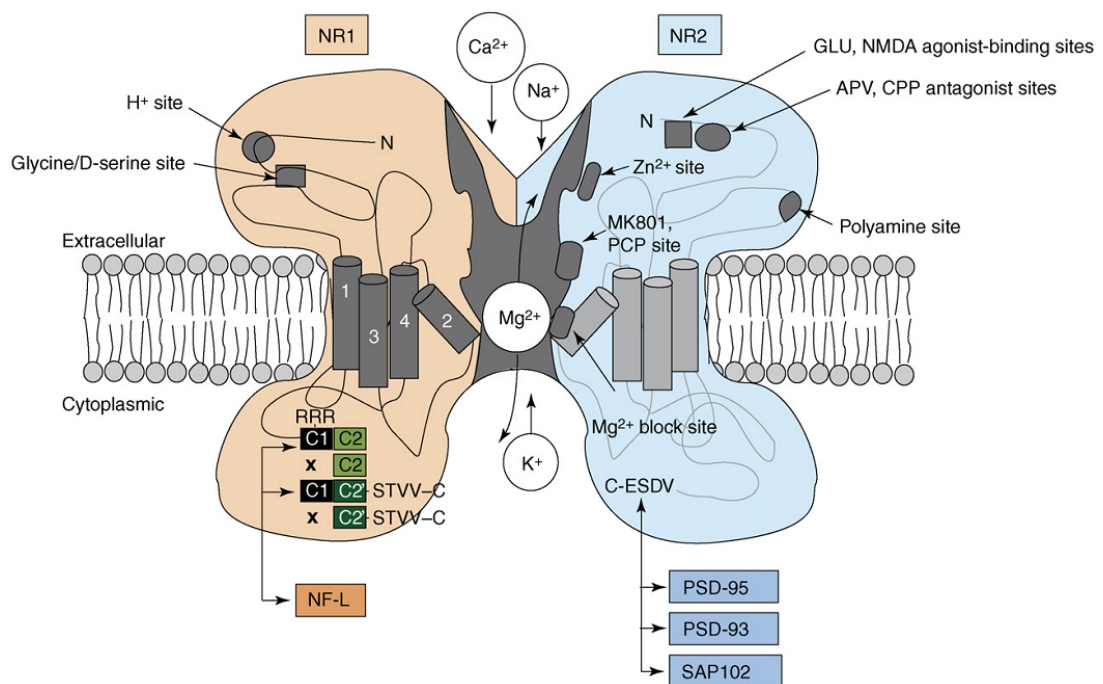
Figure 1-2 The NMDA receptor

Taken from Kristiansen et al. (2007). NR1 and NR2 subunits with the binding site for glutamate, glycine and NMDAR antagonists phencyclidine (PCP), dizocilpine (MK-801) and ketamine (at the PCP site). NF-L, PSD-95, PSD-93 and SAP102 are proteins in the post-synaptic density that bind to the C-termini of the subunits.

NMDA receptor-mediated synaptic plasticity: In the 1940's Donald Hebb proposed that the neuronal basis for learning and memory could be represented by a strengthening of the synapse through coincident detection of both pre- and post-synaptic activity (Tsien, 2000). Since then two forms of Hebbian synaptic plasticity have been extensively studied; long-term potentiation (LTP) and long-term depression (LTD) (Malenka & Bear, 2004). These forms of synaptic plasticity refer to the modification of "the strength or efficacy of

synaptic transmission at existing synapses" ((Malenka, 2002), p.147) with the potential for structural changes such as the growth or pruning of synapses (Malenka, 2002). Due to their integration abilities, NMDARs are thus the prime candidates for the mediation of these processes, and have indeed been found to play a fundamental role in most forms of LTP and LTD (Rebola, Srikumar, & Mulle, 2010). In addition, NMDARs are considered to be the mediators of slow synaptic transmission with longer periods of activity post-stimulation compared to the fast on-off mechanism of AMPARs (Edmonds, Gibb, & Colquhoun, 1995; Magleby, 2004). This property is considered to be important for a neural system to be able to integrate and maintain information over a prolonged period of time, as is required in working memory (Kantrowitz & Javitt, 2010; von Engelhardt et al., 2008; X. J. Wang, 1999).

As previously described, during baseline stimulation levels, AMPARs are the only glutamatergic channels activated, allowing K+ and Na+ ions to flow into the dendritic spine of the neuron. Through repetitive high-frequency stimulation the cell becomes depolarised, allowing the removal of the Mg2+ block and the influx of Ca2+ into the spine via the NMDAR (Malenka, 2002). Increased intracellular levels of calcium trigger the activation of kinases such as calcium/calmodulin-dependent protein kinase II (CaMKII), cyclic adenosine monophosphate-dependent protein kinase (PKA) and protein kinase C (PKC) (Rebola et al., 2010). These kinases in turn elicit the synthesis of proteins that support the insertion of AMPARs into the post-synaptic membrane (Derkach et al., 2007)(see Figure 1-3). The increase in number and conductance of AMPARs at the membrane surface is viewed as the molecular expression of LTP at the synapse, although modifications to the NMDARs themselves have also been found to contribute to LTP formation (Rebola et al., 2010). LTD of the synapse is most commonly produced following lengthy low-frequency stimulation, and is also induced by calcium inflow via NMDARs. However, compared to during LTP, the Ca2+ inflow is thought to be less and to exhibit different temporal features (Derkach et al., 2007; Malenka, 2002). Finally, LTD expression is characterised by the endocytosis (removal) of AMPARs from the post-synaptic membrane (Malenka & Bear, 2004; Rebola et al., 2010).

Although NMDA-mediated LTP and LTD have been found in the majority of excitatory synapses in the brain and in many brain areas, for example the hippocampus, thalamus and prefrontal cortex (Malenka, 2002; Malenka & Bear, 2004), it is not the only form of synaptic plasticity present (for example dopamine receptor or mGluR-mediated plasticity) thus compensatory 'learning and memory' mechanisms may exist when the activity of

NMDARs are disrupted (Jay, 2003; Kantrowitz & Javitt, 2010; Mukherjee & Manahan-Vaughan, 2012).

Figure 1-3 Long-term Potentiation
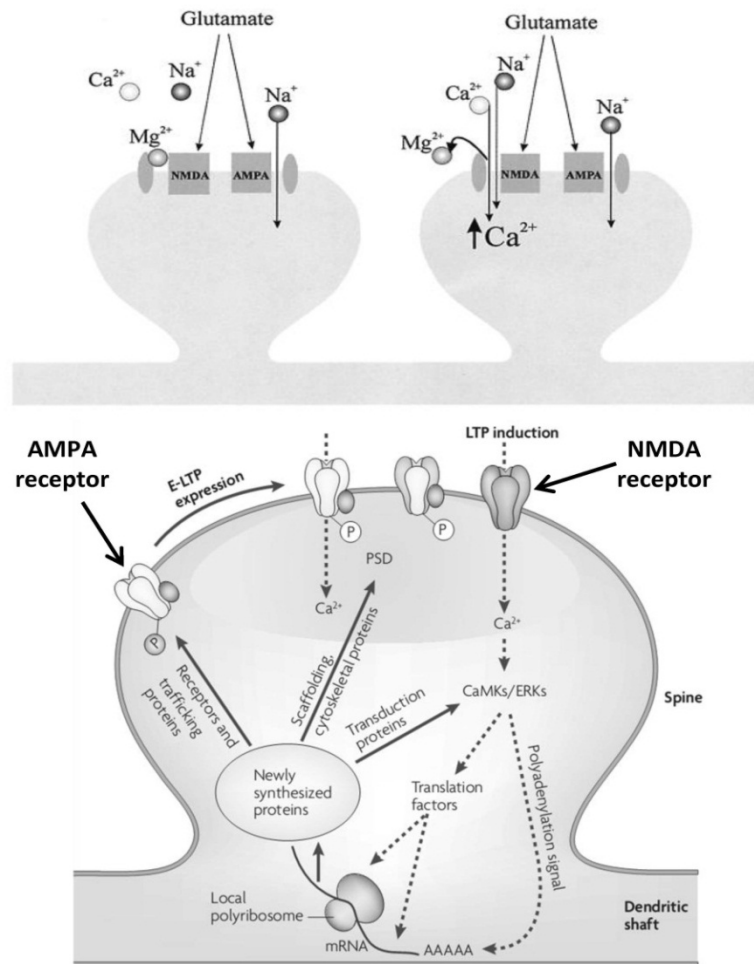
Adapted from Malenka (2002) and Derkach et al., (2007). Schematic demonstrating long-term potentiation (LTP) induction. Glutamate activation of AMPARs allows the influx of monovalent ions into the post-synaptic density (PSD). AMPAR-mediated cell depolarisation triggers the removal of the Mg^{2+} block at the NMDAR, allowing the inflow of Ca^{2+} ions. An increase in intracellular Ca^{2+} concentration leads to the activation of kinases (CaMKs = calcium/calmodulin-dependent protein kinases; ERKs = extracellular signal-related kinases) and subsequent synthesis of proteins that support AMPAR trafficking and membrane insertion (Malenka, 2002; Derkach et al., 2007).

1.1.1.3   Anatomical Distribution of Glutamate Receptors

In the mammalian brain, excitatory amino acid neurotransmission is ubiquitous in nature, with its receptors localised to multiple sites in the cerebrum (Cotman, Monaghan, Ottersen, & Storm-Mathisen, 1987; Monaghan, Bridges, & Cotman, 1989; Monaghan, Yao, & Cotman, 1984; Storm-Mathisen et al., 1983). Nonetheless, receptor expression can differ between brain regions. For example, animal studies have shown that NMDAR levels are the highest in areas such as the hippocampus (with highest expression in the strata oriens and radiatum of the CA1 region) and the cortex (with highest expression in layers I and III of the frontal, anterior cingulate and parietal cortices) (Cotman et al., 1987; Geddes, Chang-Chui, Cooper, Lott, & Cotman, 1986; Monaghan & Cotman, 1985). Lower levels have been found subcortically in the thalamus and basal ganglia with negligible levels in the cerebellum, mammillary bodies and brain stem nuclei (Cotman et al., 1987). Taken together, NMDA receptors' role in synaptic plasticity and their distribution pattern in the brain, indicates that "hippocampal and cortical regions have the machinery for highly plastic functioning"((Cotman et al., 1987),p.279)).

AMPAR distribution has generally been found to overlap with that of the NMDARs, with those located on pyramidal cells in the cerebral cortices and hippocampus containing the aforementioned calcium impermeable GluR2 subunit, in contrast to those on GABAergic (inhibitory neurotransmitter γ – aminobutyric acid) interneurons which contain a high number of Ca^{2+} permeable receptors (Geiger et al., 1995; Kew & Kemp, 2005). KARs, on the other hand, show a more complementary profile with highest densities in areas of low NMDAR expression. For example, KARs dominate in layers V and VI of the cortex whereas NMDARs are primarily located in layers I, II and III (Cotman et al., 1987). Metabotropic receptors mGlu 1,3,5 and 7 are expressed across the brain, in contrast to mGluRs 2,4 and 8 whose expression is more localised and mGlu 6 which is only found in th retina (Ferraguti & Shigemoto, 2006; Gregory et al., 2011). mGluRs 2 and 4 are found predominantly in the cerebellum, with lower densities in parts of the hippocampus and olfactory nucleus of the rat brain (Ferraguti & Shigemoto, 2006).

1.1.2   Glutamatergic Neurotransmission

In the CNS, glutamate's release, clearance and cycling occurs at the 'tripartite synapse' (Araque, Parpura, Sanzgiri, & Haydon, 1999); where the pre- and post-synaptic neurones interact with the surrounding glial cells. In the pre-synaptic neuron, glutamate is packaged into vesicles by vesicular glutamate transporters (VGLUTs) (see Figure 1-4; (Niciu et al.,

2012)). Through the influx of calcium (Ca^{2+}) ions and the actions of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment receptor) protein, the vesicles fuse with the cell membrane to release the glutamate into the synaptic cleft (see Figure 1-4; (Marsman et al., 2011)). Here it can bind to the available postsynaptic glutamate receptors or be removed from the extracellular space by excitatory amino acid transporters (EAATs) present on the adjoining astrocytic glial cells (see Figure 1-4;(C. Zarate, Jr. et al., 2010)). Once inside the astrocyte, glutamate is converted into glutamine via a reaction which requires the activity of the enzyme glutamine synthetase and the presence of adenosine triphosphate (ATP; to provide energy) and ammonia (see Figure 1-4; (Niciu et al., 2012; Tokita, Yamaji, & Hashimoto, 2012)). Glutamine is then transferred back into the extracellular space via the cystine-glutamate antiporter 'system x-C' and into the presynaptic neuron where the enzyme glutaminase re-converts it to glutamate (Niciu et al., 2012). Glutamate can also be synthesised in the pre-synaptic neuron from α-ketoglutarate, a component of the tricarboxylic acid (TCA) cycle (Marsman et al., 2011).
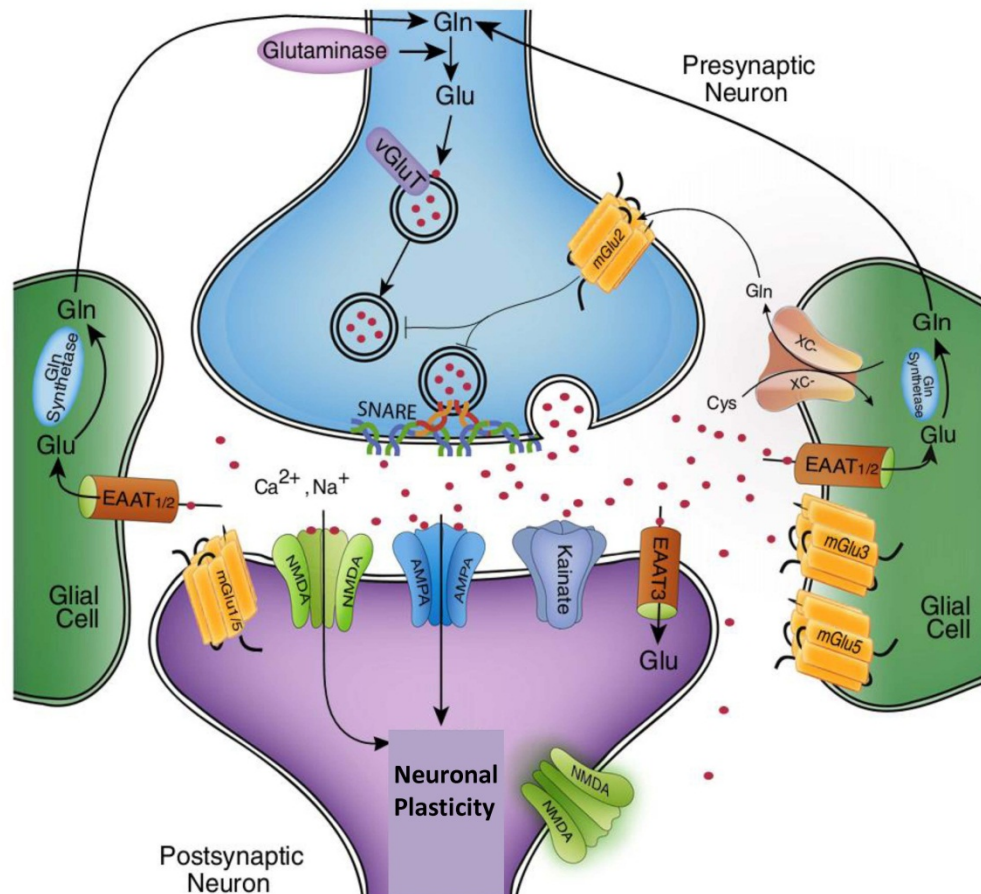
Figure 1-4 Glutamate neurotransmission in the central nervous system

Adapted from Niciu et al., (2012). A simplified schematic of how glutamate is released into the extracellular space and recycled, via astrocytes, back into the presynaptic neuron. Glutamate targets metabotropic glutamate receptors (mGluRs) and ionotropic glutamate receptors (NMDA, AMPA and kainate) located on pre- and post-synaptic sites. Glu = glutamate; Gln = glutamine; VGLUT = vesicular glutamate transporter; EAAT = excitatory amino acid transporter; SNARE = soluble N-ethylmaleimide-sensitive factor attachment receptor protein; xc- = system x-C (cystine-glutamate antiporter).

1.1.3   Interim Summary

In summary, the glutamatergic system appears to be important for the integrity of neuronal function, with a key role for the NMDAR. Furthermore, the anatomical distribution and pharmacological properties of this receptor highlight its importance for neuroplasticity and subsequent regional brain intercommunication. As described below, this may have implications for the role of NMDARs in learning and memory function.

1.2   Schizophrenia and Glutamatergic Dysfunction

Schizophrenia is a severely debilitating disease which typically becomes apparent in late adolescence, with the development of positive symptoms, characterised by hallucinations, delusions and thought disorder, together with negative symptoms including apathy, anhedonia and social withdrawal and cognitive deficits (Laruelle, Kegeles, & Abi-Dargham, 2003). For some, the positive symptoms have been seen to be the defining symptoms of the disorder (Andreasen, Flaum, Swayze, Tyrrell, & Arndt, 1990), although the pervasive cognitive dysfunction has also been considered to be a principal feature since the time of Kraepelin (Robertson, 1919). Various neurochemical disruptions have been hypothesised to underlie these deficits (Abi-Dargham & Guillin, 2007), however the focus in this thesis will be on the dopaminergic, serotonergic and glutamatergic contributions.

1.2.1   Schizophrenia & Cognition

Cognitive impairments have been found in areas of executive function, WM, attention and learning (Conklin, Curtis, Calkins, & Iacono, 2005; J. Hall et al., 2009; S. Krieger et al., 2005; Luck & Gold, 2008) and have been found to (1) precede the onset of the disorder (Cornblatt, Obuchowski, Roberts, Pollack, & Erlenmeyer-Kimling, 1999), (2) be present in first degree relatives of schizophrenic patients (Brahmbhatt, Haut, Csernansky, & Barch, 2006; Sitskoorn, Aleman, Ebisch, Appels, & Kahn, 2004; Snitz, Macdonald, & Carter, 2006), and (3) be relatively unaffected by current antipsychotic treatments (Harvey & Keefe, 2001; Mishara & Goldberg, 2004). Furthermore, these deficits are critical for the prognosis and quality of life of the patient (Green, Kern, Braff, & Mintz, 2000; Green, Kern, & Heaton, 2004). Together these findings have emphasised the need for research on cognition in schizophrenia to not only understand the neurocognitive mechanisms underlying the disorder but also to enable the development of successful treatments (Barch & Ceaser, 2012; Goff, Hill, & Freudenreich, 2011; Kuperberg & Heckers, 2000). Two aspects of cognitive function, WM and learning, identified by the CNTRICS (Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia) initiative as highly affected in schizophrenia, will be the focus of the following sections (Barch, Moore, Nee, Manoach, & Luck, 2012).

1.2.1.1 Working Memory

Working memory (WM) impairment is seen as one of the core cognitive deficits evident in schizophrenia (Barch & Smith, 2008). WM can be described as the "ability to maintain and manipulate information over short periods of time" (Barch, 2006), p.74; please see Chapter 6 for a more detailed description of WM theories). Research by Goldman-Rakic and colleagues has shown that the prefrontal cortex (PFC) is essential for WM function (Funahashi, 2007; Goldman-Rakic, 1995a, 1995b). A current view of WM supports a process-based model, whereby the maintenance of information (both spatial and non-spatial) within WM is allocated to the ventrolateral prefrontal cortex (VLPFC) and manipulation of the information to the dorsolateral prefrontal cortex (DLPFC)(D'Esposito, Postle, Ballard, & Lease, 1999; Funahashi, 2007; Owen, Evans, & Petrides, 1996); see Chapter 6 for details on the competing domain model of WM function). With the use of behavioural tasks that require phonological loop processes (maintenance) and fMRI, patients with schizophrenia have been shown to have relatively intact performance and functional activation of brain areas, such as the VLPFC (Barch, 2005). On the other hand, DLPFC activation elicited by 'central executive' (manipulation) processes (e.g. that occur during the N-BACK task; see chapter 6 for more detail) has been found to be abnormal compared to controls with additional deficits in performance measures (Callicott et al., 2003; Jansma, Ramsey, van der Wee, & Kahn, 2004; Minzenberg, Laird, Thelen, Carter, & Glahn, 2009; Van Snellenberg, 2009).

This abnormality in DLPFC activity in patients has been found to be characterised by both hyper- and hypoactivity (Ragland, Yoon, Minzenberg, & Carter, 2007). To explain why such contradictory findings occur Manoach (2003) and Callicott et al (2003) suggested that this discrepancy was due to the heterogeneity in their brain response and a shift in the inverted U-shaped response characteristic of WM brain activity in healthy volunteers in response to parametric manipulation of load (Callicott et al., 1999). Specifically, Manoach (2003) proposes that in patients this curve is shifted to the left, with increased activity compared to controls at low memory loads ('cortical inefficiency') but with decreased activity at high memory loads, demonstrating the classic 'hypofrontal' response. Importantly, circuit-level hypotheses of WM dysfunction have also been put forward, highlighting the possibility that the deficit arises from altered connections rather than a localised break down of function (Barch, 2005). In fact, by correlating brain activity during WM tasks, patients with schizophrenia show altered DLPFC connectivity with regions such as the thalamus and the parietal and temporal cortices (J. J. Kim et al., 2003;

A. Meyer-Lindenberg et al., 2001; A. S. Meyer-Lindenberg et al., 2005; Schlosser et al., 2003).

1.2.1.2 Associative Learning and Memory

The idea that schizophrenia involves disturbances in the forming of associations has been present for a long time (J. Hall et al., 2009), with many theories suggesting that these deficits underlie symptoms such as hallucinations and delusion formation (Corlett, Honey, & Fletcher, 2007; Corlett, Honey, Krystal, & Fletcher, 2011; Corlett, Murray, et al., 2007). Early studies on associative learning in schizophrenia centred upon classical Pavlovian conditioning experiments (Fanselow & Poulos, 2005; J. Hall et al., 2009; Shanks, 2010).In fact, individuals with schizophrenia have shown deficits in a variety of such learning tasks, for example aversive learning (Jensen et al., 2008), latent inhibition (N. S. Gray & Snowden, 2005), and blocking paradigms (Escobar, Oberling, & Miller, 2002) supporting the notion that the aetiology of this disorder includes dysfunction in association formation. In order to target processes involved in associative learning and memory in a more specific manner, studies in schizophrenia have also focused on the fact that this ability requires the acquisition and integration of different stimuli and the retrieval of such associations from memory (Banyai, Diwadkar, & Erdi, 2011). These studies assessing the encoding and retrieval of associations over time using visuospatial paradigms, such as the paired associates learning (PAL) task (see Chapter 7 for more detail), have demonstrated that patients show deficits in this area; with impairments in remembering object-location associations and subtle reductions in learning rates (Barnett et al., 2005; Brambilla et al., 2011; Diwadkar et al., 2008; Wood et al., 2002).

Cellular, lesion and imaging studies highlight the importance of various brain areas in associative learning and memory. With the use of a conditional motor associative learning task, cells in the non-human primate hippocampus have been shown to exhibit learning-related signals; with either, (1) a sustained decrease in activity, (2) a sustained increase in activity or (3) a transient increase in activity once the visuomotor association had been acquired. Similar learning related signals were also found in the prefrontal cortex and striatum (Suzuki, 2007a, 2007b, 2008). Studies in experimental animals have found that lesions to the hippocampus significantly impair object-location association formation (Langston, Stevenson, Wilson, Saunders, & Wood, 2010; Miyashita, Kameyama, Hasegawa, & Fukushima, 1998) and individuals with Alzheimer's disease who have a known hippocampal pathology show deficits in the paired associates learning task

(Blackwell et al., 2004; Fowler, Saling, Conway, Semple, & Louis, 2002; O'Connell et al., 2004; Swainson et al., 2001). Furthermore, prefrontal and hippocampal activation is also elicited by associative learning tasks in healthy volunteers (Bunge, Burrows, & Wagner, 2004; Law et al., 2005; J. A. Meltzer & Constable, 2005) with the hippocampus being able to anatomically differentiate between successful and unsuccessful retrieval of associations (J. Chen, Olsen, Preston, Glover, & Wagner, 2011). Imaging studies in schizophrenia have found prefrontal, striatal and hippocampal dysfunction during associative memory and learning tasks (Kircher, Whitney, Krings, Huber, & Weis, 2008; Lepage et al., 2006; Toni, Ramnani, Josephs, Ashburner, & Passingham, 2001). In addition, reduced temporal lobe volume in patients has been found to be correlated with deficits in verbal paired associates recall (Nestor et al., 1993). Connectivity studies in patients have also found reduced fronto-hippocampal coupling during associative learning tasks (Banyai et al., 2011; E. Murphy, Keshavan, & Diwadkar, 2008), a pathway seemingly important for human learning and memory.

1.2.2 Neurotransmitter Hypotheses of Schizophrenia

1.2.2.1 The Dopamine Hypothesis of Schizophrenia

The dopamine hypothesis, which postulates that a dysregulation of dopamine transmission underlies symptoms of schizophrenia, has been the predominant neurochemical model of this disorder investigated in the last 50 years (Seeman, 1987). The dysregulation in dopamine is described by a hyperactive subcortical striatal dopamine system, thought to underlie the positive symptoms of the disorder and a hypoactive mesocortical system thought to underlie the negative and cognitive symptoms (Guillin, Abi-Dargham, & Laruelle, 2007; Howes et al., 2012).

The primary evidence for a disrupted dopamine system in schizophrenia stems from the discovery of typical antipsychotic drugs and the fact that they selectively block dopamine D2 receptors ((Carlsson & Lindqvist, 1963) cited in Grace (2009)). Furthermore, a correlation exists between clinically effective doses and the potency of the antipsychotics to block D2 receptors ((Seeman & Lee, 1975) cited in Laruelle et al (1999)). In addition, drugs that induce the release of dopamine in striatal areas, such as amphetamine, have been found to provoke psychotic symptoms which closely resemble those of patients with schizophrenia (Farde, 1997). However, post-mortem studies of changes in the dopamine system have been inconsistent (J. M. Stone, Morrison, & Pilowsky, 2007), although these were not able to measure in vivo dopamine transmission. In contrast,

Laruelle and colleagues were able to directly observe abnormalities in subcortical dopaminergic neurotransmission with the use of Single Photon Emission Computerized Tomography (SPECT) imaging (Abi-Dargham et al., 1998; Laruelle, 1998; Laruelle et al., 1996). An increase in amphetamine-induced dopamine release in schizophrenics, compared to controls, was ascertained by calculating the amount of radioligand (I123) IBZM displaced from the D2 dopamine receptors by endogenous dopamine. This increase was also associated with the worsening of positive psychotic symptoms.

With regards to the mesocortical dopamine system, dopamine D1 receptors in the DLPFC have been found to be upregulated in schizophrenics, with this increase being negatively correlated with performance in an N-BACK WM task, potentially indicating lower cortical dopamine levels (Abi-Dargham et al., 2002; Abi-Dargham et al., 2012). Furthermore, Risperidone, an atypical antipsychotic known to increase dopamine levels in the PFC (Kuroki, Meltzer, & Ichikawa, 1999; Westerink et al., 2001), has been shown to increase fronto-cortical activation in a group of ten patients with schizophrenia performing a WM task (G. D. Honey et al., 1999). The importance of prefrontal dopamine in WM has been well established in primates and healthy human participants; both D1 and D2 receptors have been found to be important for WM function (Goldman-Rakic, 1995b; Goto & Grace, 2007; Mehta, Swainson, Ogilvie, Sahakian, & Robbins, 2001) and individuals carrying the high-activity allele of the COMT (cathecol-O-methlytransferase) enzyme, which metabolises dopamine, have been shown to demonstrate lower performance on different PFC-related cognitive tests (Guillin et al., 2007). Through its interactions with NMDARs, the dopamine system has also been implicated in the induction of synaptic plasticity and thus learning and memory processes, primarily through the provision of 'teaching signals' (Goto & Grace, 2007; Schultz, 2002).

The findings described above are supportive of the dopamine hypothesis, however inconsistencies with the theory have surfaced, giving rise to novel ideas on the neurochemical basis of schizophrenia. For example, Jones and Pilowsky (2002) underline the fact that numerous patients with schizophrenia are unresponsive to dopamine antagonists even though over 90% of D2 receptors are blocked. In addition, the 'new' atypical antipsychotics are clinically effective at D2 occupancies of only 10-45% compared to the 60-80% of typical antipsychotics such as Haloperidol (Seeman & Kapur, 2000). Furthermore, dopamine dyregulation alone does not appear to explain all the symptoms of schizophrenia (Jentsch & Roth, 1999) leading some to suggest that it may be more

aptly associated with only the positive symptoms of the illness (Kapur & Remington, 2001; Tsai & Coyle, 2002).

1.2.2.2 The Serotonin Hypothesis of Schizophrenia

The involvement of serotonin (5HT; 5-hydroxytryptamine) in schizophrenia was initially investigated due to the hallucinogenic effects of LSD (lysergic acid diethylamide) (Potvin, Stip, & Roy, 2005). Hallucinogens, such as LSD and psilocybin, have been found to exert their effects largely through their actions at 5HT2A receptors, as antagonists at these receptors have been found to block their hallucinogenic properties (Abi-Dargham, 2007; Aghajanian & Marek, 2000; Titeler, Lyon, & Glennon, 1988; Vollenweider, Vollenweider-Scherpenhuyzen, Babler, Vogel, & Hell, 1998). Furthermore, the atypical antipsychotic risperidone, an antagonist at both D2 and 5HT2A receptors, was discovered due to its ability to reverse the effects of LSD (Colpaert, 2003). Nonetheless, hallucinogens alone have long been considered as an inadequate pharmacological model of schizophrenia, with many drug-induced symptoms being inaccurate representations of those seen in the disorder. For example, hallucinogens induce hallucinations which are primarily visual compared to the auditory hallucinations found in schizophrenics and, furthermore, they do not induce the negative symptoms characteristic of the disorder (Potvin et al., 2005).

Animal studies have shown that stimulation of 5HT2A receptors, which are primarily located on cortical pyramidal neurons (Jakab & Goldman-Rakic, 1998), leads to enhanced 'asynchronous' glutamate release in the cortex, and thus also enhanced 'synaptic noise' (Abi-Dargham, 2007; Aghajanian & Marek, 1999, 2000). This effect has been postulated as potentially leading to processes underlying the psychotomimetic effects of 5HT2A agonists (Abi-Dargham, 2007). In addition, the beneficial effects of atypical antipsychotics have been attributed to a variety of mechanisms; (1) the release of serotonin in the frontal cortices (Hertel, Nomikos, Iurlo, & Svensson, 1996), (2) the release of dopamine in the frontal cortices(Ichikawa & Meltzer, 1999; Kuroki et al., 1999), and (3) the attenuation of glutamate dysregulation and glutamate-induced neurotransmitter dysregulations in cortical and subcortical areas (Aghajanian & Marek, 2000; H. Y. Meltzer, Horiguchi, & Massey, 2011). Thus serotonergic dysfunction is not seen as the primary deficit in schizophrenia, however it is still considered in a modulatory manner to contribute to the pathophysiology of schizophrenia due to the effects of hallucinogens and atypical antipsychotics (H. Y. Meltzer, 2003). Despite demonstrating similar clinical outcomes, the ability of atypical antipsychotics, such as risperidone, to improve certain cognitive and

negative symptoms has been shown to subtly exceed that of typical antipsychotics (H. Y. Meltzer & McGurk, 1999). Thus the mechanisms described above may represent important considerations when investigating such symptoms.

1.2.2.3 The Glutamatergic Hypothesis of Schizophrenia

In recent years an increasing number of studies (summarised below) have focused on the role of glutamate in schizophrenia and have proposed NMDAR hypofunction as the primary deficit present in the disorder (Javitt & Zukin, 1991; Olney & Farber, 1995a), although this is still a matter for debate.

Genetics: Schizophrenia has been found to be a highly heritable disorder, with many of the associated genes being found to be involved in NMDAR-mediated neurotransmission and neural plasticity mechanisms (Harrison & Weinberger, 2005). For example, genes encoding the proteins dysbindin (DTNBP1), G72 (G72 gene), DAAO (D-amino acid oxidase gene), mGluR3 (GRM3) and Neuregulin-1 (NRG1 gene) have all been found to be susceptibility genes for schizophrenia (Chumakov et al., 2002; Marti, Cichon, Propping, & Nothen, 2002; Stefansson et al., 2002; Straub et al., 2002). Dysbindin is a protein most commonly found in the PSD of synapses in the hippocampus, striatum and cortex (Balu & Coyle, 2011) and has been found to be involved in the trafficking and anchoring of NMDARs (Balu & Coyle, 2011; Harrison & Weinberger, 2005). In schizophrenic patients, expression studies have found dysbindin to be reduced in prefrontal cortical areas and the hippocampus, specifically in glutamatergic pathways (Balu & Coyle, 2011; Harrison & Weinberger, 2005). Both the function of the G72 protein, which has been found to activate DAAO, and DAAO itself, has been found to converge on the glycine binding site of the NMDAR, as DAAO metabolises D-serine, an agonist at this site (Coyle & Tsai, 2004; Harrison & Weinberger, 2005). With regards to the metabotropic glutamate receptor mGluR3, it has also been found to be important for NMDAR-related signalling as agonists at the mGlu receptor attenuate the effects of NMDAR antagonists (Moghaddam & Adams, 1998). However, much of the genetic research has focused on the function of Neuregulin-1, a neurotrophic and differentiation factor, and its interaction with the tyrosine kinase ErbB4 receptor (Buonanno, 2010)(Buonanno, 2010). Once stimulated, ErbB4 interacts with PSD-95; a protein essential for the integrity of glutamatergic synapses, which also interacts directly with the NMDAR (Buonanno, 2010). ErbB4 has been found to be predominantly located in parvalbumin (PV) expressing GABAergic interneurons receiving glutamatergic

stimulation in mice hippocampi (Balu & Coyle, 2011). The NRG1 – ErbB4 signalling pathway has been found to suppress NMDAR-mediated currents and, furthermore, has been found to be enhanced in the brains of schizophrenic patients, further supporting the notion of a glutamatergic dysfunction in the disorder (Hahn et al., 2006). Although most of these genes interact only indirectly with the glutamatergic system, alterations in the NR2A and NR2B subunits of the NMDAR itself have also been implicated in the genetic basis of schizophrenia (Balu & Coyle, 2011; Coyle & Tsai, 2004).

Post-mortem studies: In terms of receptor binding studies, KAR binding has been shown to be increased in cortical areas, such as the orbital frontal cortex (Deakin et al., 1989), and decreased in the hippocampi of schizophrenic patients (Coyle & Tsai, 2004; Harrison, Law, & Eastwood, 2003; Meador-Woodruff & Healy, 2000; Tsai & Coyle, 2002). Kainate mRNA (messenger ribose nucleic acid) subunit expression studies have also shown a decrease in the hippocampus (Meador-Woodruff & Healy, 2000). Similar studies for AMPARs have demonstrated both reductions in binding and expression, mainly in the medial temporal lobe (Meador-Woodruff & Healy, 2000; Tsai & Coyle, 2002), although this has not been confirmed by a more recent study (Beneyto, Kristiansen, Oni-Orisan, McCullumsmith, & Meador-Woodruff, 2007). Although subunit expression studies for NMDARs have been relatively inconsistent, the changes have been shown to be mostly concentrated in pathways connecting the prefrontal and anterior cingulate cortices to thalamic nuclei (Kristiansen et al., 2007). Furthermore, binding studies for the dizocilpine (MK-801) binding site and the glycine binding site have detected changes in cortical and hippocampal areas (Beneyto et al., 2007; Ibrahim et al., 2000; Ishimaru, Kurumaji, & Toru, 1994). In one of the only in vivo NMDAR binding studies, a decrease in binding was found in the left hippocampus in five medication naive patients (Pilowsky et al., 2006). Additional structural abnormalities in schizophrenia include decreased dendritic spine density, somal volume, (in areas of the cortex receiving excitatory thalamic projections) and white matter, suggesting abnormal regulation of glutamate levels in the brain (for review see (Konradi & Heckers, 2003; D. A. Lewis, Glantz, Pierri, & Sweet, 2003)).

Glutamatergic treatments: Treatments potentiating NMDAR function have targeted primarily the glycine binding site as these have been shown to be effective without inducing excitotoxic damage (Coyle & Tsai, 2004). In most of the trials, glycine, D-serine, sarcosine (a glycine transporter inhibitor) or D-cycloserine treatments were added to the existing antipsychotic treatment and were shown to have beneficial effects for negative, cognitive

and even positive symptoms, although to a lesser degree (Lin, Lane, & Tsai, 2012). In addition, positive modulators of the AMPAR, referred to as ampakines, have also been shown to improve cognitive deficits in patients (Goff & Coyle, 2001; Tsai & Coyle, 2002). Treatments targeting the mGluRs have been shown to be encouraging in preclinical schizophrenia models but, so far, the results in human studies have been inconclusive (J. R. Field, Walker, & Conn, 2011; Lin et al., 2012; Vinson & Conn, 2012).

Lamotrigine, an anticonvulsant, is typically used in epilepsy (Messenheimer, 1995) and bipolar disorder (Seo et al., 2011). Through its principal mechanism of action, the blockage of voltage-gated sodium channels (Messenheimer, 1995), lamotrigine has been shown to reduce glutamate release in the brain (Idris, Repeto, Neill, & Large, 2005; Large, Webster, & Goff, 2005). When used in conjunction with antipsychotics, studies have shown an improvement mainly in positive symptoms (Dursun & Deakin, 2001; Dursun, McIntosh, & Milliken, 1999; Kremer et al., 2004; Tiihonen et al., 2003) although additional studies have demonstrated a beneficial effect on cognitive measures in both patients (Goff et al., 2007) and healthy volunteers (Aldenkamp et al., 2002). Nonetheless, one must remember that lamotrigine is used primarily as an add-on therapy, thus conclusions with regards to neurochemical dysfunction underlying schizophrenia must be made tentatively (Theochari, Boulas, & Chaidemenos, 2007).

Glutamatergic transmission: Magnetic Resonance Spectroscopy (MRS) is an imaging method that can be used to directly measure the brain concentrations of glutamate and glutamine in vivo. In individuals at-risk of developing schizophrenia, un-medicated patients and those in the chronic phase, altered levels of glutamate or glutamine in the thalamus and anterior cingulate compared to healthy controls have been found (J. M. Stone, 2009; Theberge et al., 2003; Theberge et al., 2002); see (Marsman et al., 2011) for review). In terms of cognition, a loss of correlation between medial temporal lobe glutamate levels and activation during an episodic memory fMRI task compared to controls was found (Valli et al., 2011) and lower glutamate-glycine levels in frontal, cingulate and parietal cortices were correlated with impaired cognition in patients (Bustillo et al., 2011). Furthermore, a relationship between hippocampal glutamate levels and striatal dopamine levels (measured with positron emission tomography (PET)) absent in controls has been found in at-risk patients (J. M. Stone et al., 2010). These studies demonstrate that there is a potential disruption in glutamatergic transmission in schizophrenia with subsequent effects on other neurotransmitter systems and cognitive processes.

NMDAR antagonists and cognition: The primary evidence, however, for glutamate dysregulation in schizophrenia, and the motivation for the studies summarised above, is derived from the observation that administration of dissociative anaesthetics, such as phencyclidine (PCP) and ketamine, elicits symptoms which have been likened to those seen in the disorder (Bakker & Amini, 1961; J. Krystal et al., 1999; Luby, Cohen, Rosenbaum, Gottlieb, & Kelley, 1959; Rosenbaum, Cohen, Luby, Gottlieb, & Yelen, 1959). In addition, both agents have also been found to exacerbate the perturbations present in schizophrenic patients (Itil, Keskiner, Kiremitci, & Holden, 1967; Lahti, Holcomb, Medoff, & Tamminga, 1995; Luby et al., 1959; Malhotra et al., 1997). Studies in chronic PCP abusers have also shown a symptom profile similar to that seen in schizophrenia (Allen & Young, 1978). PCP, or 'angel dust', was first developed as an anaesthetic but due to the psychotomimetic side-effects experienced post-operatively it was quickly removed from use (Morris, Cochran, & Pratt, 2005). Ketamine on the other hand, is still used in paediatric and veterinary anaesthesia and in human psychopharmacological research due to its less potent effects, shorter plasma half-life and superior safety profile (Krystal et al., 2003; Morris et al., 2005; Perry et al., 2007); please see Section 1.3 for a detailed description of the effects of ketamine). At sub-anaesthetic doses, the dissociative anaesthetics' principal pharmacological action is that of NMDAR uncompetitive antagonism (Kew & Kemp, 2005). This property has given rise to the suggestion that a deficit in NMDAR-mediated signalling underlies the triad of schizophrenic symptom clusters; positive, negative and cognitive (Javitt & Zukin, 1991; Olney & Farber, 1995a), although an emphasis on the cognitive and negative symptoms has been proposed (Hui, Wardwell, & Tsai, 2009; J. M. Stone et al., 2008; Tuominen, Tiihonen, & Wahlbeck, 2005).

In animal studies, the primary NMDAR antagonists used have been MK-801 and PCP (Gilmour et al., 2011), thus the effects described in the following paragraph will refer to those incurred by these agents. Acute administration of NMDAR antagonists, not only disrupt LTP, the molecular expression of learning and memory (Newcomer & Krystal, 2001), but also produce locomotor deficits, representative of the positive symptoms in humans (Jentsch & Roth, 1999), and deficits in attention (Amitai & Markou, 2010), executive function (Abdul-Monim, Reynolds, & Neill, 2003), working memory (J. W. Smith et al., 2011) and learning (G. Riedel, Platt, & Micheau, 2003), all cognitive domains affected in schizophrenia (see (Gilmour et al., 2011) for review). Furthermore, chronic administration of NMDAR antagonists, a regimen suggested to be a potentially superior

pharmacological 'model' of schizophrenia (Jentsch & Roth, 1999), also induces impairments in paradigms assessing WM, such as the delayed matching to position (DMTP) task, and learning, such as conditional discrimination (Dunn & Killcross, 2006). Finally, these deficits have been shown to be reversed by glutamatergic agents, such as an mGluR 2/3 agonist (Moghaddam & Adams, 1998), lamotrigine (Idris et al., 2005), and the atypical antipsychotic risperidone (Didriksen, Skarsfeldt, & Arnt, 2007), supporting the notion of NMDAR antagonism as a predictively valid model of cognitive dysfunction relevant for schizophrenia.

The NMDAR hypofunction circuits: Olney and Farber (1995a) (1995b) were the first to suggest, that due to the effects of dissociative anaesthetics and their action at the NMDAR, that NMDAR 'hypofunction' could underlie the behavioural and cognitive profile demonstrated by patients with schizophrenia. Their primary evidence for this hypothesis was that administration of NMDAR antagonists to rodents caused neurotoxic damage in cortical and limbic areas such as the cingulate and hippocampus; a pattern of injury equated to that seen in schizophrenia (Olney & Farber, 1995a, 1995b). Through studying how these neurotoxic effects could be blocked, Olney and Farber suggested that they were the result of a disruption of 'inhibitory tone' in a 'multisynaptic circuit' (Farber, 2003); see Figure 1-5). When functioning normally, glutamate, acting at NMDARs situated on inhibitory GABAergic interneurons, promotes the inhibition of excitatory pathways, both cholinergic and glutamatergic (originating in the basal forebrain and thalamus respectively), that project to cortical regions (Farber, 2003). NMDAR antagonists thus disinhibit this circuit as the inhibitory control supplied by the GABAergic interneurons is lost, resulting in excessive activity of the excitatory projections and consequently the behavioural manifestation of this dysfunction (Farber, 2003).

Figure 1-5 The NMDA receptor disinhibition circuits

Taken from Farber (2003). Glutamate targeting the NMDARs creates a widespread disinhibtion of various neurotransmitter system, such as glutamate (Glu), noradrenaline (NE), acetylcholine (Ach) and serotonin (5HT). Retrosplenial cortex (RSC).

More recent animal studies have, in part, confirmed the involvement of such circuits in the action of NMDAR antagonists. These antagonists have been found to preferentially target NMDARs located on inhibitory GABAergic interneurons (Greene, 2001; Grunze et al., 1996; Homayoun & Moghaddam, 2007), resulting in an increase of glutamate and acetylcholine in the prefrontal cortex (Adams & Moghaddam, 1998; S. H. Kim, Price, Olney, & Farber, 1999; Moghaddam, Adams, Verma, & Daly, 1997), through the hypothesised disinhibition of cortical pyramidal neurons (Farber, 2003). The disruption in GABAergic transmission has been shown to lead to perturbed firing and synchronisation of cortical pyramidal neurons (Jackson, Homayoun, & Moghaddam, 2004), with dysregulation of glutamate release suggested as responsible for the cognitive deficits seen in schizophrenia due to its actions at non-NMDA cortical receptors (Marek et al., 2010; Moghaddam, 2004; Moghaddam & Jackson, 2003; Moghaddam & Javitt, 2012). Interestingly, acute PCP has also been found to increase dopamine and serotonin release in the PFC and striatum (Jentsch & Roth, 1999; Lopez-Gil, Artigas, & Adell, 2009;

Lorrain, Baccei, Bristow, Anderson, & Varney, 2003; Moghaddam & Adams, 1998), whereas chronic administration reduced dopamine transmission in the PFC, reminiscent of the classical 'hypofrontality' seen in schizophrenia (Jentsch & Roth, 1999). Nonetheless, dopamine release has been found to be dissociated in time from NMDAR antagonist-induced behavioural and cognitive deficits, suggesting that dopaminergic transmission may be unrelated to these acute effects (Adams & Moghaddam, 1998).

As with Olney & Farber (1995a, 1995b), Carlsson and colleagues (1999) emphasise the importance of inhibitory tone in the regulation of (1) glutamatergic pathways projecting to the cortex from the thalamus and (2) dopaminergic pathways projecting to the striatum from the ventral tegmental area. They suggest that both pathways may be controlled via a direct stimulatory (glutamatergic) pathway (the 'accelerator') and an indirect inhibitory (GABAergic) pathway (the 'brake'). In both circumstances, if the balance between these two pathways is disrupted (i.e. if the indirect pathway is weakened), the result will be an increased release of glutamate in the cortex and an increased release of dopamine in the striatum (Carlsson et al., 1999).
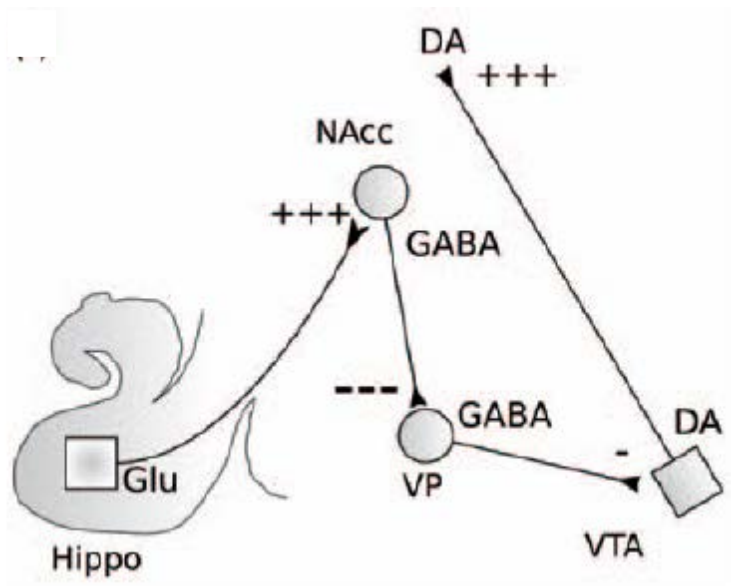
Figure 1-6 NMDA receptor antagonist disinhibition of the striatal dopaminergic system

Taken from Stone (2011). Hippocampus (Hippo); Nucleus Accumbens (NAcc); Ventral Pallidum (VP); Ventral Tegmental Area (VTA); Dopamine (DA).

Following such models, the recurrent inhibitory circuit within the hippocampus has indeed been shown to be disrupted by the administration of NMDAR antagonists

(Greene, 2001). GABAergic interneurons appear to be particularly susceptible to the NMDAR antagonists due to the fact that they have a lower threshold for generating action potentials, leading to the ability of the activity-dependent NMDAR antagonists to block these open channels (Moghaddam & Javitt, 2012). The enhanced excitatory output from the hippocampus can also lead to an increase in the number of dopamine neurons available for burst firing in the striatum (Lisman et al., 2008; Lodge & Grace, 2008); see Figure 1-6).

The studies summarised above thus suggest that that NMDAR antagonists induce their effects on behaviour and cognition through the disinhibition of excitatory transmission, resulting in a hyperglutamatergic state and disruption of additional neurotransmitter systems, such as the dopaminergic and the serotonergic system.

1.2.3   Interim Summary

The treatment of cognitive deficits, such as WM and learning impairments, is highly important due to their detrimental effects on prognosis, for example in individuals suffering from schizophrenia (Green et al., 2000). In these cognitive domains, fronto-temporal pathways appear to be particularly significant for normal functioning; brain areas that have also been found to have high concentrations of glutamatergic NMDARs, whose role in learning and memory processes has long been established (Rebola et al., 2010). Different neurotransmitters have been proposed to underlie these cognitive deficits but there is a continuing debate as to whether one is principally responsible for the pattern of neurochemical disruptions found to date (O'Carroll, 2000).

Glutamatergic dysfunction has been shown to play an important role in the pathophysiology of schizophrenia, with the use of ketamine, and other NMDAR antagonists, as a pharmacological model. The role of the glutamatergic system in *cognitive function* has thus been primarily implicated in such processes through studies investigating cognitive deficits in schizophrenia, the reason this disorder has been focussed upon within this introductory chapter. Thus the findings from such studies are an essential component of the rationale for the research summarised in this thesis, with the interpretation of the results also being considered within this framework. With regards to ketamine, due to the behavioural and neurochemical complexity of schizophrenia and the limitations of using one compound to 'model' a disorder, it may be more reasonable to consider its potential in more simple terms; as a model of glutamatergic dysfunction with accompanying effects on cognition and other neurotransmitter systems, which is *relevant*

but may not be fundamental for schizophrenia. Accordingly, it is important to acknowledge that the findings of this thesis may also be applied to disorders such as depression, whereby evidence supporting the role of the glutamate system in antidepressant activity is accruing (C. Zarate, Jr. et al., 2010). The following section, which describes the effects of ketamine, the NMDAR antagonist most commonly used in human studies, will thus be described principally from this more general perspective.

1.3 Ketamine as a Model of Glutamatergic Dysfunction

1.3.1 The Pharmacology of Ketamine

Ketamine hydrochloride (also known under the marketed name Ketalar) was first developed by Parke-Davis in 1962 (Jansen, 2001) and is a chemical analogue of PCP (Ghasemi & Schachter, 2011). Ketamine is most commonly administered as a racemic mixture, composed of its two optical enantiomers, S (+) and R (-) ketamine (Kohrs & Durieux, 1998); see Figure 1-7). It is hepatically metabolised to norketamine via the enzyme CYP3A4 (Hijazi & Boulieu, 2002; Quibell, Prommer, Mihalyo, Twycross, & Wilcock, 2011) and excreted with an elimination half-life of 2-3 hours (Craven, 2007; White, Way, & Trevor, 1982).

As previously mentioned, ketamine is an uncompetitive NMDAR antagonist, binding at the PCP site (Traynelis et al., 2010); see Figure 1-2). Due to the fact that its binding site is within the open channel pore, inhibition of receptor activity is activity-dependent. When the receptor channel closes, ketamine can become trapped, earning its name as a 'trapping blocker', which actively promotes the closed state of the receptor (Ghasemi & Schachter, 2011; Quibell et al., 2011; Traynelis et al., 2010). The kinetics of channel unblocking is slow and is only elicited by binding of the receptor agonist (Traynelis et al., 2010). Although with lower affinity and possibly only at higher doses (Breier, Malhotra, Pinals, Weisenfeld, & Pickar, 1997), ketamine can also bind to other receptors including opioid, cholinergic (nicotinic and muscarinic), monoaminergic (dopamine and serotonin) and L-type Ca^{2+} and Na^+ channels (Hirota & Lambert, 1996; Kapur & Seeman, 2002; Kohrs & Durieux, 1998).
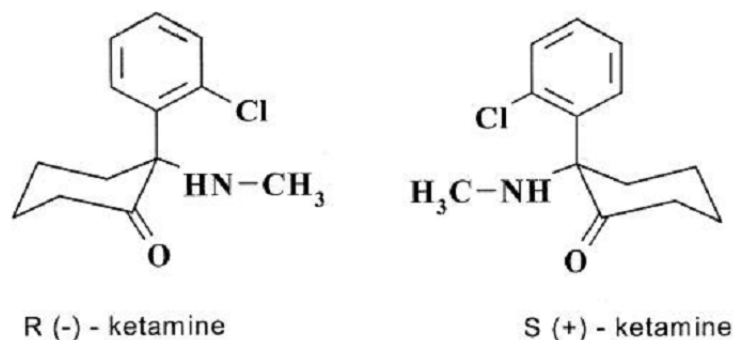
Figure 1-7 Chemical structure of the S and R enantiomers of ketamine

Taken from Kohrs & Durieux (1998). Chemical name: (S)-(+) and (R)-(-)-2-(2-chlorophenyl)-2-(methylamino) cyclohexanone hydrochloride) (C. J. Morgan & Curran, 2012).

Similarly to the neurochemical effects of NMDAR antagonists PCP and MK-801 in animals, acute ketamine has been found to increase glutamate efflux in cortical and subcortical areas such as the PFC (Moghaddam et al., 1997) and striatum (Bustos et al., 1992; Razoux, Garcia, & Lena, 2007). Specifically, glutamate release in the PFC is only elicited through systemic administration rather than direct application of the antagonist to the brain area of interest, indicating that ketamine-induced glutamate release is regulated via intermediary channels outside of the PFC such as the inhibitory GABAergic interneurons (Lorrain et al., 2003). Nuclear magnetic resonance (NMR) imaging methods have corroborated these findings; Chowdhury et al (2012) found that subanaesthetic doses of ketamine increased glutamate and glutamine cycling and neuronal metabolic activity in the PFC of rats, with a similar trend in the hippocampus. Furthermore, dopamine release in the PFC and striatum, specifically the nucleus accumbens (NAc), has also been found to be increased through ketamine administration (Lindefors, Barati, & O'Connor, 1997; Masuzawa et al., 2003; Verma & Moghaddam, 1996) although not always consistently in the latter (Rao, Kim, Lehmann, Martin, & Wood, 1989).

Human MRS studies have also found ketamine-induced increases in glutamate and glutamine (a marker of glutamate release) in cortical areas such as the anterior cingulate (Rowland, Bustillo, et al., 2005; J. M. Stone et al., 2012), although not all studies have found this effect (Taylor, Tiangga, Mhuircheartaigh, & Cowen, 2012). These increases were found to be correlated with impaired cognitive performance (Rowland, Bustillo, et al., 2005) and positive symptoms (J. M. Stone et al., 2012), suggesting that glutamate

transmission in cortical areas may mediate some of the drug-induced behavioural and cognitive effects. With regards to dopamine, some human PET studies have found increases in the release of the neurotransmitter in striatal areas (Breier et al., 1998; G. S. Smith et al., 1998). On the other hand, Kegeles and colleagues (Kegeles et al., 2002) found no changes. Nevertheless, a study using a higher dose and the more potent (S)+ enantiomer of ketamine confirmed the findings of the earlier PET studies, suggesting that ketamine-induced striatal dopamine may be dose-dependent. In addition, a priori stimulation of the dopamine system may be necessary for ketamine to exert its effects as it has been shown to potentiate amphetamine-induced dopamine release (Kegeles et al., 2000).

Overall, both animal and human studies support the notion that the subjective and cognitive effects of ketamine (see below) are mediated through the same putative mechanisms and circuits affected by PCP and MK-801 (see section 1.2.2.3).

1.3.2 Subjective Effects of Acute Ketamine

At anaesthetic doses in humans, ketamine (like PCP) is characterised by post-operative 'emergence phenomena', such as "floating sensations, vivid dreams [], hallucinations and delirium" (Reich & Silvay, 1989) p. 188), although these side-effects are less common in children (G. Honey, 2009). Since then, ketamine-induced behavioural effects have commonly been measured using the Brief Psychiatric Rating Scale (BPRS), Clinician Administered Dissociative States Scale (CADSS) and less frequently with the Psychotomimetic States Inventory (PSI) (Hetem, Danion, Diemunsch, & Brandt, 2000; Krystal et al., 1994; O. J. Mason, Morgan, Stefanovic, & Curran, 2008; Parwani et al., 2005). These and other measures have shown that ketamine induces perceptual disturbances, such as changes in the intensity of colours and sounds (Pomarol-Clotet et al., 2006) and inaccuracies in the assessment of the flow of time (Coull et al., 2011). An increase in 'positive' symptoms such as delusions of reference (Krystal et al., 1994) and 'negative' symptoms such as blunted affect and emotional withdrawal (Lahti, Weiler, Tamara Michaelidis, Parwani, & Tamminga, 2001; Micallef et al., 2003; Passie, Karst, Wiese, Emrich, & Schneider, 2005) have also been found. Illusions with regards to body image (Moore et al., 2011; H. L. Morgan et al., 2011), and aspects of thought disorder such as loose associations, derailment, blocking and poverty of speech have also been found to be heightened under ketamine (Adler, Goldberg, Malhotra, Pickar, & Breier,

1998; Adler et al., 1999; Malhotra et al., 1996). In addition, Bowdle et al., (1998) showed that many of these symptoms were highly dose-dependent.

Due to this 'psychotomimetic' symptom profile, ketamine has been extensively studied as a pharmacological model of schizophrenia. However, when looked at closely many of its effects are not entirely consistent with the disorder. For example, as with LSD, ketamine-induced hallucinations are more likely to be visual rather than auditory, the modality more commonly seen in schizophrenic patients (G. Honey, 2009). Furthermore, Pomarol-Clotet and colleagues (Pomarol-Clotet et al., 2006) were unable to confirm the symptoms of thought disorder found in previous studies.

1.3.3 Cognitive Effects of Acute Ketamine

In animal studies the effects of ketamine are largely consistent with those seen with other NMDAR antagonists PCP and MK-801. Ketamine generates deficits in attention (J. W. Smith et al., 2011), executive function (Nikiforuk, Golembiowska, & Popik, 2010), working memory (Castner et al., 2010) and learning (Dix, Gilmour, Potts, Smith, & Tricklebank, 2010). Interestingly, reversal of deficits in spatial WM and pre-pulse inhibition have been found with the administration of a GABA receptor agonist which potentiates inhibitory mechanisms (Castner et al., 2010), with lamotrigine which directly reduces glutamate release (Brody, Geyer, & Large, 2003) and a dopamine D1 agonist which facilitates NMDAR-mediated transmission (Roberts, Seymour, Schmidt, Williams, & Castner, 2010). Together these findings support the idea that ketamine exerts its detrimental effects on cognition through the disinhibitory circuit described in section 1.2.2.3.

One of the first studies to investigate the effect of acute administration of ketamine on cognition in healthy volunteers was performed by Ghoneim et al., (1985), with subsequent studies demonstrating that that it can induce deficits in areas such as attention, executive function, episodic and working memory and learning (see Table 1-1). Table 1-1 presents a comprehensive overview of the effect of ketamine on cognition and common infusion protocols. From the studies summarised it appears that inconsistencies exist, perhaps due to differences in methodology, such as sample size, cognitive measures, ketamine dose or infusion procedures used (Rowland, Astur, et al., 2005). One must also consider the fact that the tasks may also recruit various cognitive processes, such as the 'executive function' WCST task which has a strong WM component, hindering the ability to disentangle specific drug-induced deficits (G. Honey, 2009).

Irrespective of such confounds and as Table 1-1 shows, attentional processes appear to be impaired whereas executive function and response inhibition measures appear to be relatively intact. On the other hand, WM impairments emerge in the non-spatial domain, specifically when manipulation of information is required (R. A. Honey et al., 2003). However, these deficits are evident in protocols with higher target plasma levels (90-260ng/ml) (Adler et al., 1998; R. A. Honey et al., 2003) (Krystal, Abi-Saab, et al., 2005; C. J. Morgan, Mofeez, Brandner, Bromley, & Curran, 2004a), with Honey et al., (2003) reporting no impairments with lower doses (50ng/ml; Protocol K, Table 1-1). Episodic memory impairments are consistent across studies, again with protocols administering a dose with target plasma levels of above 90ng/ml. Interestingly, it has been demonstrated that the deficits in episodic memory were particularly due to a disruption in encoding processes (compared to retrieval processes) by assessing retrieval performance when encoding had occurred both prior to and during the ketamine infusion (Hetem et al., 2000; G. D. Honey, Honey, Sharar, et al., 2005; G. D. Honey et al., 2006; C. J. Morgan, Mofeez, Brandner, Bromley, & Curran, 2004b; Oye, Paulsen, & Maurset, 1992). Studies which did not separate these processes demonstrate a deficit in verbal recall and recognition ability (e.g. (J. H. Krystal et al., 1999; Krystal et al., 1998; Krystal et al., 1994) and associative memory (Harborne, Watson, Healy, & Groves, 1996; G. D. Honey et al., 2006) when under the influence of ketamine. Discrepancies exist in the literature investigating the effect of ketamine on learning processes (Anand et al., 2000; Freeman et al., 2009; Krystal, Abi-Saab, et al., 2005; Krystal, Perry, et al., 2005; C. J. Morgan, Mofeez, et al., 2004a; Parwani et al., 2005; Rowland, Astur, et al., 2005), although there are some indications that learning rate may be affected (Anand et al., 2000; C. J. Morgan, Mofeez, et al., 2004a) and that males are more vulnerable to an impairment (C. J. Morgan, Perry, Cho, Krystal, & D'Souza, 2006). Overall, WM tasks that include a manipulation component and episodic memory tasks that separate out encoding and retrieval processes appear to be particularly sensitive to the effects of ketamine. On the other hand, areas of cognitive function where the effects of ketamine have not been extensively investigated include associative learning and memory. Thus, in order to learn more about the effects of ketamine, it may be advantageous for future ketamine-centred studies to focus on such areas of function, in terms of performance but also brain response (see Section (1.3.5) for a summary of such studies so far).

From the studies summarised in Table 1-1, it appears that a dose of ketamine between predicted plasma levels of 50ng/ml (no impairments) to 90ng/ml (impairments) has not

been used in cognition-based studies. This may represent a dose low enough to not induce psychotomimetic side effects but high enough to induce cognitive deficits. However it is important to note that the conclusions with regards to dose are tentative as many studies do not report the plasma levels predicted from the dosing regimen applied. It is thus also difficult to decide which infusion protocol is the most appropriate as it cannot be corroborated with true plasma values. Nonetheless it appears that a bolus together with a maintenance infusion is the most commonly used protocol, suggesting that the use of this procedure in future studies would be advisable if the goal is to standardise ketamine infusion methodology.

Attenuation of ketamine-induced impairments in WM and verbal learning and memory have been found with the administration of an mGluR2/3 agonist (Krystal, Abi-Saab, et al., 2005) and lamotrigine (Anand et al., 2000) respectively. Such findings support the idea that a hyperglutamatergic state may underlie the cognitive deficits evident when under the influence of ketamine.

Table 1-1 Studies of ketamine and cognition in healthy volunteers

Study	Subject No (Design)	Domain	Task	Infusion Protocol	Performance
Krystal et al., (1994)	14 (WS)	Attention	CPT Task	Protocol[A].	*Impaired.* Decrease in number of correct trials, increase in number of omission and commission (false positives) errors only in the high dose condition. No effect on RT.
Malhotra et al., (1996)	15 (WS)	Attention	Verbal Memory Task (attention component)	Protocol[B].	*Impaired* (included a WM component).
Harborne et al., (1996)	12 (WS)	Attention	CAT Task	Protocol[C].	*Unimpaired.*
Krystal et al., (1998)	23 (WS)	Attention	CPT Task	Protocol[D].	*Impaired.* Decrease in the number of correct trials and increase in the number of omission errors. No effect on commission errors or RT.
Radant et al., (1998)	10 (WS)	Attention	Verbal Memory Task (attention component)	Protocol[E].	*Unimpaired.*
Newcomer et al., (1999)	15 (WS)	Attention	CPT Task	Protocol[F].	*Unimpaired.*
Krystal et al., (1999)	18 (WS)	Attention	CPT Task	Protocol[D].	*Impaired.* Decrease in the number of correct trials and increase in the number of omission errors. No effect on commission errors or RT.
Umbricht et al., (2000)	19 (WS)	Attention	CPT Task	Protocol[G].	*Impaired.* Decrease in the number of correct trials and increase in the number of commission errors. Effect on omission errors or RT not reported.
Krystal et al., (2000)	22 (WS)	Attention	CPT Task	Protocol[D].	*Unimpaired.*
Hetem et al., (2000)	28 (BS)	Attention	Digit symbol substitution and symbol cancellation tasks	Protocol[H].	*Impaired.* Decrease in scores.

Passie et al., (2005)	12 (WS)	Attention	Divided attention and Signal detection tasks	Protocol[I].	*Impaired.* Increase in omission errors significant only in high dose condition in divided attention task. Trend for decrease in number of correct trials and significant increase in RT in signal detection task.
Krystal et al., (2005)	19 (WS)	Attention	CPT Task	Protocol[D].	*Impaired.* Decrease in a measure used for vigilance and increase in a measure used for distractability.
Rowland et al., (2005)	8 (WS)	Attention	Stroop Colour-Word Interference Task	Protocol[F].	*Unimpaired.*
Knott et al., (2011)	40 (WS)	Attention	Target-detection Task	Protocol[J].	*Impaired.* Decrease in number of correct trials and increase in the number of commission errors.
Krystal et al., (1994)	19 (WS)	Executive Function	VF and WCST tasks	Protocol[A].	*Impaired.* Dose-dependent decreases in VF and increases in perseverative errors in the WCST.
Radant et al., (1998)	10 (WS)	Executive Function	VF Task	Protocol[E].	*Impaired.* Dose-dependent decreases in VF, driven by deficit in the higher dose conditions.
Krystal et al., (1998)	23 (WS)	Executive Function	VF and WCST tasks	Protocol[D].	*Impaired.* Dose-dependent decreases in VF and increases in perseverative errors in the WCST.
Adler et al., (1998)	10 (WS)	Executive Function	VF Task	Protocol[B].	*Impaired.* Decreases in VF.
Newcomer et al., (1999)	15 (WS)	Executive Function	VF Task	Protocol[F].	*Unimpaired.*
Krystal et al., (1999)	19 (WS)	Executive Function	VF and WCST tasks	Protocol[D].	*Unimpaired* VF. *Impaired* WCST with an increase in perseverative errors.
Krystal et al., (2000)	22 (WS)	Executive Function	WCST Task	Protocol[D].	*Unimpaired.* Pronounced order effect: increase in perseverative errors was only present in subjects who received ketamine on the first visit.
Honey et al., (2003)	13 (WS)	Executive Function	Tower of London Task	Protocol[K].	*Unimpaired.*

Morgan et al., (2004a)	54 (BS)	Executive Function	Trail-making Task	Protocol[L].	*Unimpaired.*
Morgan et al., (2004b)	54 (BS)	Executive Function	VF Task	Protocol[L].	*Unimpaired.*
Rowland et al., (2005)	9 (WS)	Executive Function	VF Task	Protocol[F].	*Unimpaired.*
Adler et al., (1998)	10 (WS)	Working Memory	N-BACK Task	Protocol[B].	*Impaired.* Decreases in scores in 1-back and 2-back conditions.
Newcomer et al., (1999)	15 (WS)	Working Memory	Spatial WM task/ Delayed Match to Sample Task	Protocol[F].	*Unimpaired* spatial WM. *Impaired* DMS with dose-dependent decreases in recognition performance. Effect driven by the high dose.
Honey et al., (2003)	13 (WS)	Working Memory	2 Verbal WM tasks (maintenance and manipulation conditions) 2 Spatial WM tasks	Protocol[K].	*Impaired.* Decreases in scores were significant in the manipulation conditions of the verbal WM tasks in the higher dose condition. **Spatial WM was unimpaired**.
Morgan et al., (2004a)	54 (BS)	Working Memory	N-BACK Task	Protocol[L].	*Impaired.* Significant decrease in scores in 1-back and 2-back conditions, primarily driven by high dose condition.
Krystal et al., (2005)	19 (WS)	Working Memory	Non-verbal WM Task	Protocol[D].	*Impaired.*
Rowland et al., (2005)	9 (WS)	Working Memory	Non-spatial and spatial WM tasks	Protocol[F].	*Unimpaired.*
Krystal et al., (1994)	14 (WS)	Episodic Memory	Verbal Memory (immediate recall, post-distraction recall and 10min delay)	Protocol[A].	*Impaired.* Impairment in the 10min delay condition at the high dose.
Malhotra et al., (1996)	15 (WS)	Episodic Memory	Verbal Memory Task (recall and recognition components)	Protocol[B].	*Impaired.* Deficit in both recall and recognition.
Harborne et al., (1996)	12 (WS)	Episodic Memory	Associative Verbal Memory Task	Protocol[C].	*Impaired.* Deficit in recalling words associated with faces in high dose condition.
Radant et al., (1998)	10 (WS)	Episodic Memory	Verbal Memory Task (recall and recognition components)	Protocol[E].	*Impaired.* Dose-dependent impairment in recall and recognition.

Krystal et al., (1998)	23 (WS)	Episodic Memory	Verbal Memory (immediate recall, post-distraction recall and 10min delay)	Protocol[D].	*Impaired.* Impairment in the 10min delay condition.
Krystal et al., (1999)	19 (WS)	Episodic Memory	Verbal Memory (immediate recall, post-distraction recall and 10min delay)	Protocol[D].	*Impaired.* Impairment in the 10min delay condition.
Hetem et al., (2000)	28 (BS)	Episodic Memory	EM Task (recall and recognition conditions)	Protocol[H].	*Impaired.* Decrease in recall and recognition conditions through the impaired encoding but not retrieval processes.
Morgan et al., (2004a)	54 (BS)	Episodic Memory	Source Memory Task	Protocol[L].	*Impaired.* Dose-dependent increases in source and recognition errors. Effect driven by the high dose.
Morgan et al., (2004b)	54 (BS)	Episodic Memory	Prose-recall Task	Protocol[L].	*Impaired.* Decrease in recall due to impaired encoding but not retrieval processes at both doses.
Honey et al., (2005)	13 (WS)	Episodic Memory	EM task (recognition and source memory conditions)	Protocol[K].	*Impaired.* Decrease in recognition due to impaired encoding but not retrieval processes in the higher dose condition.
Honey et al., (2006)	13 (WS)	Episodic Memory	EM task (recognition and source memory conditions)	Protocol[M].	*Impaired.* Decrease in recall and recognition conditions when using associative encoding strategies compared to shallow encoding strategies (word-stem completion).
Anand et al., (2000)	16 (WS)	Verbal Learning and Memory	Hopkins Verbal Learning Task	Protocol[D].	*Impaired.* Impaired recall of words during 3 immediate recall trials and a delayed (30min) recall trial. Learning across the recall trials is impaired.
Krystal et al., (2005)	19 (WS)	Verbal Learning and Memory	Hopkins Verbal Learning Task	Protocol[D].	*Impaired.* Impaired recall of words during 3 immediate recall trials and a delayed recall trial.
Krystal et al., (2005)	41 (WS)	Verbal Learning and Memory	Hopkins Verbal Learning Task	Protocol[N].	*Impaired.* Impaired recall of words during 3 immediate recall trials and a delayed recall trial. Learning across the recall trials does not appear to be impaired (not formally tested).
Parwani et al., (2005)	13 (WS)	Verbal Learning and Memory	Verbal Learning and Memory Task	Protocol[O].	*Impaired.* Impaired recall of words during 3 delayed recall trials. Learning across the recall trials does not appear to be impaired (not formally tested).

Rowland et al., (2005)	9 (WS)	Verbal Learning and Memory	Hopkins Verbal Learning Task	Protocol[F].	*Impaired.* Impaired encoding of new words: retrieval was unaffected only when the list of words was learned prior to drug administration. Impaired delayed (30min) recall of new words.
Morgan et al., (2004a)	54 (BS)	Procedural Learning	Serial Reaction Time Task	Protocol[L].	*Impaired.* RTs only decreased across trials in the placebo group indicative of impaired learning in the drug conditions. RTs in both dose groups were significantly increased compared to placebo.
Rowland et al., (2005)	8 (WS)	Spatial Learning and Memory	Virtual Morris Water Task	Protocol[F].	*Impaired.* Impairment only in an early acquisition/encoding phase of the learning procedure (greater distance travelled and longer RTs to arrive at platform)
Freeman et al., (2009)	47 (BS)	Associative Learning	Superstitious Conditioning Task	Protocol[P].	*Unimpaired.*
Abel et al., (2003)	20 (WS)	Response Inhibition	Prepulse Inhibition (PPI)	Protocol[N].	*Unimpaired.* Increase in PPI performance (decreased startle reflex).
Morgan et al., (2004b)	54 (BS)	Response Inhibition	The Hayling Task	Protocol[L].	*Impaired.* Increase in errors in illogical sentence completion condition (effect only significant in high dose condition).
Passie et al., (2005)	12 (WS)	Response Inhibition	Go/NoGo Task	Protocol[I].	*Unimpaired.*

Within-Subject (WS); Between-subject (BS); Verbal fluency (VF); Wisconsin Card Sorting Test (WCST); Continuous Performance Task (CPT); Continuous Attention Task (CAT); Reaction Time (RT); Working Memory (WM); Episodic Memory (EM). All studies were placebo-controlled trials apart from Radant et al., 1998.

INFUSION PROTOCOLS FOR TABLE 1-1:

A I.V. Infusion: 0.0025mg/kg/min or 0.0125mg/kg/min (predicted PK levels of 0.025-0.05mg/L (25-50ng/ml) or 0.1-0.25mg/L (100-250ng/ml)). PK levels not reported.

B I.V. Bolus+Infusion: bolus of 0.12mg/kg followed by 0.65mg/kg over 1h infusion (total dose 0.77mg/mk/hr). Approximately 90ng/ml (stated in Honey et al., 2003). PK levels not reported.

C I.M. Administration: 10mg or 25mg ketamine made up to 1ml of solution. PK levels not reported.

D I.V. Bolus+Infusion: bolus of 0.26mg/kg over 1min (or 2min for Krystal et al., 2000) followed by 0.65mg/kg/hr infusion. PK levels not reported.

E I.V. Infusion was increased in steps to achieve target PK levels of 0, 50, 100, 150 and 200ng/ml. Total dose of 0.97mg/kg over 2h. Reported PK levels were highly correlated to the target levels.

F I.V. Loading Dose+Infusion: bolus of 0.0243mg/kg, 0.081mg/kg or 0.27mg/kg over 10min followed by infusion of 0.0002025mg/kg/min, 0.000675mg/kg/min or 0.00225mg/kg/min respectively. This was done to achieve target PK levels of 13.5ng/ml, 45ng/ml and 150ng/ml.

G I.V. Bolus+Infusion: bolus of 0.24mg/kg over 5min followed by a 5min pause and then a 0.9mg/kg/h infusion. Dose was reduced by 10% every 15min. PK levels not reported.

H I.V. Bolus+Infusion: bolus followed by 0.5mg/kg infusion administered over 60min to achieve target PK levels of 150ng/ml. Reported mean PK levels of 122ng/ml and 147ng/ml at 14min and 34min post-dose respectively.

I I.V. Bolus+Infusion: 5mg over 5min followed by a 0.003mg/kg/min or 0.005mg/kg/min infusion. PK levels not reported.

J I.V. Loading Dose: 0.04mg/kg over 10 min. No maintenance dose. Mean PK levels of 25-37ng/ml.

K I.V. Bolus+Infusion: bolus for <60secs followed by an infusion to achieve target PK levels of 50 and 100ng/ml. Reported mean PK levels were 54.6 and 106.8ng/ml respectively at approximately 2hours post-dose.

L I.V. Infusion: 0.4mg/kg or 0.8mg/kg over 80min. Mean PK levels of 128.96 and 261.9ng/ml at 65min.

M I.V. Bolus+Infusion: bolus for <60secs followed by an infusion to achieve target PK levels 100ng/ml. Reported mean PK levels of 93.4ng/ml.

N I.V. Bolus+Infusion: bolus of 0.23mg/kg over 1min followed by 0.5mg/kg over 1h infusion. PK levels not reported.

O I.V. Loading Dose+Infusion: bolus of 0.27mg/kg over 10min followed by 0.12mg/kg over 50min. Reported peak PK level of 71ng/ml.

P I.V. Infusion: low dose ketamine (target PK level 75ng/ml) and high dose ketamine (target PK level 150ng/ml). Reported PK levels of 54.64ng/ml and 143.42ng/ml at 45min post-dose, respectively.

1.3.4 Effects of Acute Ketamine on Resting Brain Physiology

Early experimental animal studies demonstrated that sub-anaesthetic ketamine administration increased the rate of glucose utilization, as measured by ^{14}C-2-deoxyglucose (2-DG) uptake, in frontal, cingulate, hippocampal, thalamic and amygdalar regions of the rat brain (G. E. Duncan, Leipzig, Mailman, & Lieberman, 1998; G. E. Duncan, Miyamoto, Leipzig, & Lieberman, 2000; G. E. Duncan, Moy, Knapp, Mueller, & Breese, 1998). This is in comparison to anaesthetic levels of ketamine that induced global decreases in 2-DG uptake (G. E. Duncan, Moy, et al., 1998). The pattern of metabolic activity elicited by amphetamine and ketamine differs, whereas the pattern exhibited by MK-801 is identical to that seen with ketamine ((G. E. Duncan, Miyamoto, Leipzig, & Lieberman, 1999); Figure 1-8 (a)). This supports the idea that, although ketamine can act at various receptor types, it is its actions at the NMDAR that are primarily responsible for the changes in brain metabolism (G. E. Duncan et al., 1999). Interestingly, a more recent animal 2-DG study demonstrated that ketamine administration induced changes in connectivity between the prefrontal cortex and brain areas such as the thalamus and the serotonergic dorsal raphe nucleus (Dawson, Morris, & Pratt, 2011).

Rodent pharmacological MRI experiments (see Chapter 2 for a description of this method) with ketamine, PCP and MK-801 have shown increases in blood oxygen level dependent (BOLD) signal, blood flow and cerebral blood volume (CBV) in frontal, hippocampal and thalamic regions (Burdett, Menon, Carpenter, Jones, & Hall, 1995; Gozzi, Herdon, et al., 2008; Gozzi, Large, et al., 2008; Hackler et al., 2010; Littlewood, Cash, et al., 2006; Littlewood, Jones, et al., 2006) consistent with the previously described 2-DG work (G. E. Duncan et al., 1999). Many of these findings in anaesthesised rodents were confirmed in a cohort of awake rats, whereby BOLD signal increases were found in medial prefrontal, cingulate, retrosplenial, entorhinal, auditory and visual cortices together with increases in the CA1 region of the hippocampus and the striatum ((Chin et al., 2011); Figure 1-8(b)). Furthermore, these increases were attenuated by pretreatment with an mGluR2/3 agonist, suggesting that ketamine-induced increases in glutamate release may underlie the BOLD signal changes (Chin et al., 2011). Decreases in BOLD signal were found in either the periacqueductal gray (PAG) or in the inferior colliculus demonstrated by Littlewood et al (2006).
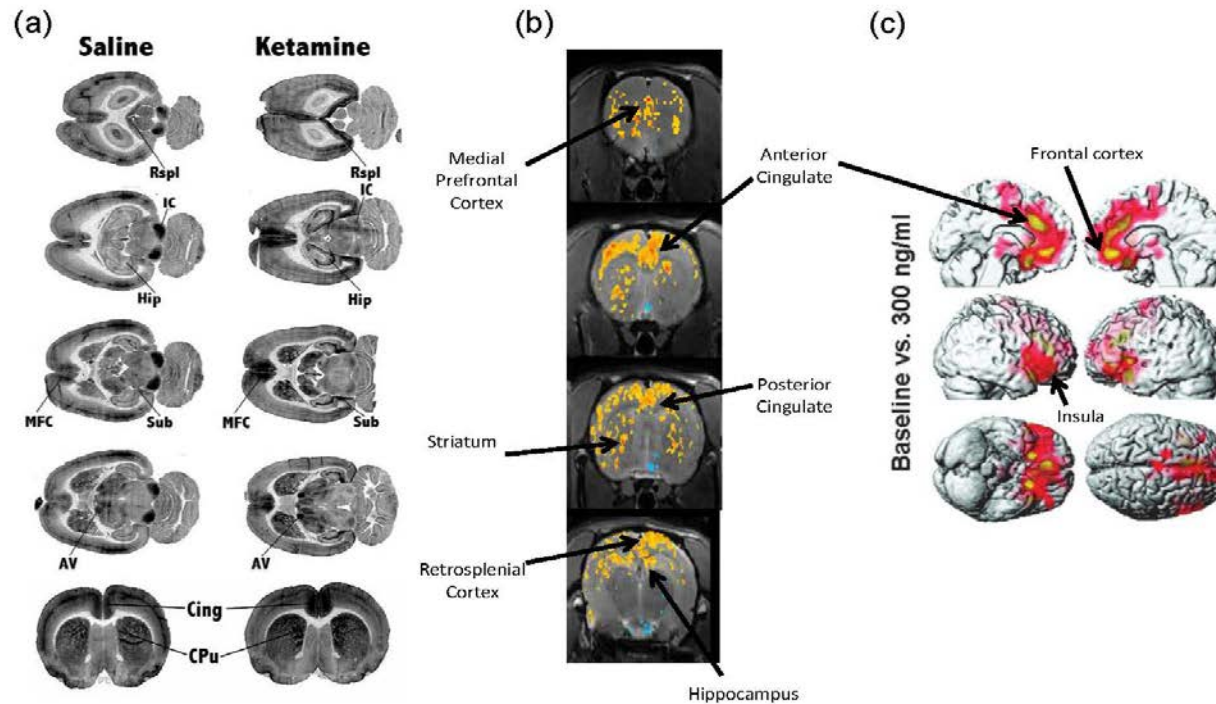
Figure 1-8 The brain response to ketamine

(a) Autoradiograms of 2-DG uptake in rats (Duncan et al., (1999); Retrosplenial Cortex (Rspl); Hippocampus (Hip); Subiculum (Sub); Medial Frontal Cortex (MFC); Cingulate (Cing); Inferior Colliculus (IC); Anteroventral Thalamic Nucleus (AV)). (b) BOLD MRI response in awake rats (Chin et al., (2011)). (c) Relative cerebral blood flow increases in humans during 300ng/ml ketamine infusion (Langsjo et al., 2003).

Studies using [^{18}F]fluorodeoxyglucose (FDG) PET to investigate ketamine-induced changes in glucose metabolism in healthy human volunteers demonstrate a striking similarity to preclinical findings. Increases in glucose utilisation were found particularly in frontal and cingulate cortices, with lesser increases in the insula, parietal and temporal cortices (Breier et al., 1997; Vollenweider, Leenders, Scharfetter, et al., 1997). Higher doses also showed significant increases in the thalamus (Langsjo et al., 2004). These findings were replicated in a study using the (S)-ketamine enantiomer, which has been shown to bind with greater affinity to the PCP site on the NMDAR (Oye et al., 1992; Vollenweider, Leenders, Oye, Hell, & Angst, 1997). In line with these findings, increases in cerebral blood flow (CBF) have been shown primarily in frontal, cingulate and thalamic regions with the use of H$_2$^{15}O PET ((Holcomb, Lahti, Medoff, Cullen, & Tamminga, 2005; Holcomb, Lahti, Medoff, Weiler, & Tamminga, 2001; Langsjo et al., 2003); Figure 1-8(c)). In the only previous study so far to investigate ketamine infusion induced BOLD phMRI signal changes in humans, increases were found in regions including the mid-posterior cingulate, thalamus and anterior temporal cortical areas, along with a decreased BOLD signal observed in the ventromedial prefrontal cortex (Deakin et al., 2008). Furthermore, many of the signal changes and associated behavioural effects were attenuated by lamotrigine, a drug known to inhibit glutamate release (Deakin et al., 2008).

Together with the preclinical findings, human ketamine imaging studies demonstrate a compatibility with the neural circuits and brain regions affected by the administration of NMDAR antagonists. With regards to patients with schizophrenia, the similarity is harder to ascertain as their metabolic differences are inconsistent, with brain regions showing both hypo- and hypermetabolism (Vollenweider, Leenders, Oye, et al., 1997; Vollenweider, Leenders, Scharfetter, et al., 1997), although increases in blood perfusion have been found (Medoff, Holcomb, Lahti, & Tamminga, 2001).

1.3.5 Effects of Acute Ketamine on Cognitive Brain Networks

As behavioural studies have found that ketamine can induce deficits in multiple areas of cognition more recent studies have investigated the neural correlates of these deficits by combining the administration of ketamine with fMRI BOLD cognitive tasks. Irrespective of cognitive domain, it appears that overall, ketamine tends to increase brain activation when compared to placebo, predominantly in frontocortical and temporolimbic system regions (see Table 1-2). Researchers have suggested that the increase in activation in cortical areas is in agreement with the NMDAR antagonist functional circuits described in

section 1.2.2.3, whereby there is an increase in glutamate and dopamine release (and thus metabolism) in cortical areas (Adams & Moghaddam, 1998; G. D. Honey, Honey, O'Loughlin, et al., 2005), with decreases in activation due to a possible disruption in the signal-to-noise ratio and the integrity of local neuronal networks (Fu et al., 2005; Musso et al., 2011). Ketamine-induced increases in activation in BOLD signal also align with the ketamine PET studies in healthy volunteers and schizophrenic patients that show increases in blood flow in prefrontal and cingulate cortices (Breier et al., 1997; Holcomb et al., 2005; Lahti et al., 1995; Medoff et al., 2001). Nonetheless, as with the behavioural studies, the different doses and infusion protocols utilised make the comparison between studies and interpretability and generalisability of the effects more difficult.

In agreement with the demonstration that the manipulation component of a WM task was impaired (R. A. Honey et al., 2003), Honey et al., (2004) found changes in brain activation during the manipulation component of the same WM task; supporting the idea that ketamine induces a manipulation-specific deficit. On the other hand, Honey et al., (2005)found changes in activation in both encoding and retrieval conditions during an episodic memory task, thus compared to behavioural studies that suggest only encoding processes are disrupted by ketamine, fMRI may also be able to detect disruption to retrieval processes. However, it must be noted that these ketamine effects were driven by the higher dose (100ng/ml plasma level). Furthermore, in some of the studies (see Table 1-2), changes in brain activation are evident in the absence of a behavioural impairment. This ensures that behavioural performance does not act as an obvious confounding factor. Taken together, these findings support the idea that fMRI may be more sensitive than behavioural measures in detecting changes in cognitive processes and compensatory strategies underlying the preserved performance (R. A. Honey et al., 2004).

The ketamine-induced deficits mirror cognitive impairments evident in patients with schizophrenia; for example, impairments in manipulation processes in WM or learning rates are evident (see section 1.2.; (G. Honey, 2009)). In addition, ketamine has been shown to affect both encoding and retrieval mechanisms, a pattern of disruption also seen in patients with schizophrenia, although a retrieval-specific deficit has been found to be the most consistent within this population (P. C. Fletcher & Honey, 2006). However, discrepancies between the effects of ketamine and those seen in patients also exist. For example, LaPorte and colleagues found no deleterious effects of ketamine administration on cognitive performance in schizophrenia patients (LaPorte, Lahti, Koffel, & Tamminga, 1996).

Table 1-2 BOLD imaging studies of ketamine and cognition in healthy volunteers

Study	Subject No (Design)	Domain	Task	Infusion Protocol	Performance	Differences in brain activation on ketamine
Daumann et al., (2008)	14 (WS)	Attention	Covert orienting of attention task (inhibition of return; IOR)	Protocol[A].	Unimpaired	*Increase* in activation in rSFG, lSTG, rMFG in IOR condition.
Honey et al., (2008)	15 (WS)	Attention	Continuous performance task (CPT; sustained attention)	Protocol[B].	Unimpaired accuracy. Significantly increased RT.	*No effect* of ketamine on brain activation.
Daumann et al., (2010)	14 (WS)	Attention	Target-detection task in both visual and auditory modalities	Protocol[A].	Unimpaired accuracy. Significantly increased RT.	*Increase* in activation in auditory cue condition in INS and pCG. No effects in visual modality.
Musso et al., (2011)	24 (WS)	Attention	Visual-oddball task	Protocol[A] (reduced by 10% every 10 min).	Impaired motor RT.	*Decrease* in activation in target vs non-target condition in MFC, ACC, TPC and OCC.
Fu et al., (2005)	10 (WS)	Executive function	VF task	Protocol[C].	Unimpaired	*Increase* in activation irrespective of task difficulty in ACC, mPFC, MFG, IFG, VLPFC, insula, IPC, ITC, OCC, CC, CAU, PUT and NAc. *Decrease* in activation in PFC and striatal areas with increasing task demand.
Nagels et al., (2011)	15 (WS)	Executive function	Continuous overt verbal fluency tasks: lexical, semantic and phonological VF tasks	Protocol[D].	Impaired lexical and semantic tasks (decrease in words generated). Unimpaired phonological task.	Lexical task: *Increase* in activation in right frontal and supramarginal regions. Semantic task: *Decrease* in activation in PREC, lMFG and PCC. Phonological task: *Increase* in activation in lTG and lSG, cluster extending from SFG, to MFG and lIPC. *Decrease* in activation in rDPHG.

Study	N	Domain	Task	Protocol	Behavioural findings	Neuroimaging findings
Nagels et al., (2011)	15 (WS)	Executive function	Phonological VF task	Protocol[D].	Unimpaired	*Increase* in task activation network (lIFG, STG, lOCC, and bilateral MC and CC).
Honey et al., (2004)	12 (WS)	Working Memory	Verbal WM task (low and high maintenance and manipulation conditions)	Protocol[E].	Unimpaired	*Increase* in activation in manipulation vs maintenance contrast (collapsed across load) in bilateral DLPFC, VLPFC, PCC and ACC. No effect of dose was evident.
Honey et al., (2008)	15 (WS)	Working Memory	N-BACK (3-back) WM task	Protocol[B].	Unimpaired accuracy. Significantly increased RT.	*Increase* in activation in THAL and BG (across load conditions).
Northoff et al., (2005)	16 (BS)	Episodic Memory	EM retrieval task	Protocol[F].	Impaired retrieval of words	*Decrease* in activation in ACC, PCC, PREC, INS, MFG and temporal regions including HIPP.
Honey et al., (2005)	12 (WS)	Episodic Memory	EM task (encoding (shallow & deep) and retrieval conditions (old and new))	Protocol[E].	Unimpaired	*Increase* in activation in lPFC during encoding for deeply encoded items. *Increase* in activation in rPFC during encoding for items correctly retrieved. *Decrease* in activation in lPFC during retrieval for deeply encoded items. *Increase* in activation in rPFC and HIPP during retrieval for incorrectly retrieved items. *Decrease* in activation in ACC during retrieval for incorrectly retrieved items. *Increase* in activation in bilateral PFC and left HIPP during retrieval for old items.
Corlett et al., (2006)	15 (WS)	Associative Learning	Causal associative learning task	Protocol[B].	Unimpaired learning.	*Increase* in activation in rPFC in response to non-surprising control items.

Design: Within-subject (WS); Between-subject (BS); reaction time (RT); hour (h); plasma levels (PK levels); working memory (WM); episodic memory (EM); verbal fluency (VF); right superior frontal gyrus (rSFG); left superior temporal gyrus (lSTG); right medial frontal gyrus (rMFG); insular (INS); precentral gyrus (pCG); medial frontal cortex (MFC); anterior cingulate cortex (ACC); temporoparietal cortex (TPC); occipital cortex (OCC); medial prefrontal cortex (mPFC); inferior frontal gyrus (IFG); ventrolateral prefrontal cortex (VLPFC); inferior parietal cortex (IPC); lateral temporal cortex (LTC); cerebellar cortex (CC); caudate (CAU); putamen (PUT); nucleus accumbens (NAc); precuneus (PREC); left middle frontal gyrus (lMFG); posterior cingulate cortex (PCC); thalamus (THAL); hippocampus (HIPP); left temporal gyrus (lTG); left supramarginal gyrus (lSG); superior frontal gyrus (SFG); left inferior parietal cortex (lIPC); right dorsal parahippocampal gyrus (rDPHG); left middle temporal gyrus (lMTG); superior temporal gyrus (STG); left occipital cortex (lOCC); motor cortex (MC); dorsolateral prefrontal cortex (DLPFC); parietal cortex (PC); basal ganglia (BG); left prefrontal cortex (lPFC); right prefrontal cortex (rPFC).

INFUSION PROTOCOLS FOR TABLE 1-2:

[A] I.V.Bolus: 0.1mg/kg over 5min; MI: 0.0066 – 0.015625mg/kg/min over 20min. PK levels not reported.

[B] I.V.Bolus: 1mg/ml solution; MI: rates maintained target PK levels of 100ng/ml for the cognitive task assessment and 200ng/ml for psychopathology assessment. Reported mean PK levels of 88 and 210ng/ml respectively.

[C] I.V.Bolus: 0.23mg/kg over 30sec; MI: 0.65mg/kg over 1h. PK levels not reported (expected 0.1-0.25mg/kg).

[D] I.V.Bolus: 8mg from 0.5mg/ml solution in 0.9% saline; MI: 0.01mg/kg/min over 1h. PK levels not reported.

[E] I.V.Bolus: 1mg/ml solution in <60secs; maintenance infusion: rates maintained target PK levels of 50 and 100ng/ml. Reported mean PK levels of 47.7 and 102.3ng/ml at 65min post-dose respectively.

[F] I.V.Infusion: 0.6mg/kg/hr over 90min. PK levels not reported.

1.3.6 Chronic Effects of Ketamine on Behaviour and Cognition in humans

Due to the ethical concerns of administering ketamine repeatedly most research on the effects of chronic ketamine has been based on individuals who use ketamine recreationally, to differing degrees. Many of the same clinical features elicited by acute administration of the drug are exhibited by chronic ketamine users (Curran & Morgan, 2000; Muetzelfeldt et al., 2008), persisting after termination of drug use (C. J. Morgan, Monaghan, & Curran, 2004) and likened to symptoms seen in the prodromal stages of schizophrenia (C. J. Morgan & Curran, 2012).

In terms of cognition, episodic and semantic memory is impaired in frequent and infrequent ketamine users when given ketamine, but only present in frequent users in a drug-free state when compared to infrequent users (Curran & Monaghan, 2001; Curran & Morgan, 2000). Furthermore, these cognitive deficits are still evident in ex-users, with improvement correlating with a decline in ketamine use (C. J. Morgan, Monaghan, et al., 2004). When compared to infrequent users, non-ketamine using polydrug users, ex-users and non-drug using controls, frequent ketamine users display impairments in measures of recognition and WM, executive function (C. J. Morgan, Muetzelfeldt, & Curran, 2009), associative learning (Freeman et al., 2009) and episodic memory (C. J. Morgan, Riccelli, Maitland, & Curran, 2004), although these impairments are not always present (C. J. Morgan, Muetzelfeldt, et al., 2009). Increasing ketamine use over time has also been shown to lead to increasingly poor cognitive performance (C. J. Morgan, Muetzelfeldt, et al., 2009). Although in the absence of any cognitive impairments compared to healthy controls, an up-regulation of dopamine D1 receptors was found in the DLPFC of chronic ketamine users, demonstrating important effects of ketamine on areas of the brain involved in WM and executive functioning (Narendran et al., 2005). Structural white matter changes have also been found in frontal and temporal cortices in ketamine abusers (Y. Liao et al., 2010).

Although not identical, chronic administration of ketamine induces a similar cognitive deficit profile to that seen with acute ketamine (C. J. Morgan & Curran, 2006). Although a chronic administration paradigm is not feasible in healthy humans, the study of ketamine abusers enables us to understand which particular behavioural or cognitive processes may be more susceptible to the effects of the drug, identifying those deficits which do not improve even after cessation of drug use. Furthermore, even though acute ketamine may

not consistently induce overt deficits in such areas, the model may still be valid when used to target processes known to be affected in long-term ketamine users.

1.3.7 Interim Summary

Animal and human studies alike, suggest that acute administration of ketamine induces a hyperglutamatergic state which, together with its downstream effects on other neurotransmitter systems, mediates the drug-induces changes in subjective experience and cognition. Both acute and chronic administration of ketamine induces a pseudo-psychotomimetic behavioural profile with accompanying deficits in particular cognitive domains. Whereas WM and encoding-retrieval impairments appear consistent, overt learning impairments seem less pronounced. The functional imaging ketamine studies that have been performed demonstrate that the cognitive brain networks elicited by such memory and learning processes are affected; however there is a lack of studies specifically targeting such functions(see Table 1-2). For example, only Corlett et al., (2006) have addressed the effects ketamine on associative learning brain networks, and only two studies conducted by Honey and colleagues have investigated ketamine-induced changes to WM networks (G. D. Honey et al., 2008; R. A. Honey et al., 2004). Both resting state and functional imaging studies describe a core brain network affected by ketamine that includes frontal and cingulate cortices, temporal lobe regions (such as the hippocampus), the thalamus and striatum.

1.4 Summary

The studies summarised above support the notion that ketamine can be used as a probe of the glutamatergic system in healthy volunteers to learn about its role in specific cognitive patterns and the neural pathways underlying these responses. Consistently across species and in terms of metabolism, neurotransmitter release or BOLD signal, ketamine appears to affect brain areas, such as the frontal and cingulate cortices, hippocampus and thalamus, which are not only intimately involved in NMDAR-mediated glutamatergic transmission but are also associated with memory and learning functions. Furthermore, the principle nodes affected by ketamine are also the key affected areas of the memory and learning neural networks in schizophrenia. Thus, while ketamine is not able to mimic all aspects of behavioural and neural deficits evident in schizophrenia, it may be suitable to investigate the glutamatergic component of the disorder.

Nevertheless, more research is needed in order to determine the validity and utility of the ketamine response with regards to the investigation of glutamatergic function in healthy humans and those suffering from schizophrenia or depression. Research into the resting brain response to ketamine, measured with BOLD MRI, has recently begun to be used to investigate novel treatments targeting glutamatergic dysfunction (Chin et al., 2011; Deakin et al., 2008). It is viewed as a potential imaging biomarker due to the non-invasive characteristics of the imaging technique and the consistency of the response across species. However, to date, only one study has characterised the brain response to ketamine using BOLD MRI in humans (Deakin et al., 2008), and none have assessed the reliability across sessions of this response, an essential goal if it is to be used in within-subjects designs to determine the true effects of modulatory compounds.

With regards to cognition, there is a growing appreciation of the role of glutamatergic signalling, particularly in the context of schizophrenia. As section 1.2.1 describes, individuals suffering from schizophrenia demonstrate prominent impairments in WM and associative learning and memory, with underlying deficits in neural networks including the prefrontal cortex, hippocampus and striatum. However, only a few studies have attempted to assess the effect of ketamine on these processes and networks (Corlett et al., 2006; G. D. Honey et al., 2008; R. A. Honey et al., 2004). These findings are essential to not only replicate but also develop, for example with the use of tasks which elicit (1) activation of brain areas affected in schizophrenia and hyperglutamatergic states and (2) NMDAR-mediated learning and memory mechanisms. In addition, modulation of ketamine's effects with compounds that target the neurotransmitter systems integral to the NMDAR hypofunction 'circuit', is important to understand the mechanisms of action at play to a greater extent. Finally, such research will further our knowledge on the role of the glutamatergic system in human cognition and also help us to determine whether the brain response to ketamine is a viable model of glutamatergic dysfunction primarily relevant to schizophrenia but also to other psychiatric disorders whose neurobiology includes a glutamatergic component.

1.5   Research Approach

The purpose of this thesis is to investigate both the behavioural and neural effects of ketamine with the aim to (1) determine whether the brain response to ketamine is a valuable and valid pharmacological tool to investigate glutamatergic dysfunction and (2) to further our knowledge on the role of the glutamatergic system in human cognition,

specifically WM and associative learning. In the hope of achieving this, ketamine was administered to healthy volunteers whilst at rest and whilst performing a set of cognitive tasks in an MRI scanner. In a first cohort of volunteers, ten individuals received ketamine on two separate occasions; this was done in order to ascertain the test-retest reliability of the brain response to ketamine using two different imaging methods, BOLD and CASL (continuous arterial spin labelling) imaging. Assessing the reliability of the ketamine response across sessions is essential for its application in repeated measures designs and also the degree of confidence with which it is used to understand the modulatory influences of other compounds. Following this initial study, a second cohort of sixteen volunteers was administered ketamine, alone and in conjunction with an anticonvulsant, lamotrigine, and an atypical antipsychotic, risperidone. The primary objectives of this larger study was to (1) describe the effects of ketamine across cognitive brain networks, specifically WM and associative learning networks, in a placebo-controlled study design using functional MRI (fMRI) and, (2) modulate the effects of ketamine using two control treatments – one mechanistic (lamotrigine) and the other of direct clinical relevance to schizophrenia (risperidone), a psychiatric disorder whose underlying neurochemical dysfunction has been proposed to include glutamatergic dysregulation (please see Chapter 3 for details on the design of these studies).

All experimental chapters included in this thesis combine aspects from both of the studies summarised above. Chapter 4 addresses the question of test-retest reliability and modulation of the ketamine response through the use of resting state BOLD MR imaging in the context of the initial response to ketamine. Chapter 5 addresses these same questions but with the use of CASL imaging under steady-state ketamine conditions. Chapters 6, 7 and 8 assess (1) whether the cognitive tasks used, in this case the PAL and N-BACK tasks, reliably elicit the same cognitive task networks across sessions, (2) whether these cognitive task networks are sensitive to ketamine administration and (3) whether the effects of ketamine on cognitive performance and brain networks are modulated by lamotrigine and risperidone, compounds that have been shown to affect neurotransmitter systems involved in the mechanism of action of ketamine. From the studies summarised in this introduction, ketamine is expected to induce alterations in brain physiology.

Specifically, ketamine is hypothesised to,

(1) alter the resting state brain response by increasing BOLD signal and CBF,

(2) to disrupt working memory and associative learning related brain networks but remain free of performance confounds due to the low-dose administered,

(3) risperidone and lamotrigine, drug treatments that are thought to directly or indirectly reduce the release of glutamate, are expected to attenuate these ketamine-induced changes.

Each chapter summarises its specific aims and hypotheses.

Chapter 2 METHODS: Imaging the Brain

A basic set of physical principles underlie the success of Magnetic Resonance Imaging (MRI). Although an in depth account of MR physics is beyond the scope of this chapter, an overview of the essential facts will be provided in order to have an understanding of how this technique is applied to imaging the brain. Different MRI methods, including the BOLD and CASL approaches will be summarised and a description of how these can be used in conjunction with both cognitive tasks and pharmacological compounds will be given.

2.1   Magnetic Resonance Imaging Physics

2.1.1   The Hydrogen Nucleus

MRI systems rely on the use of a superconductive magnet and the way the components of hydrogen nuclei, specifically protons, react to the magnetic field it generates. To understand how the MR signal is generated one must first consider some characteristics displayed by these subatomic particles.

All electrically charged spinning particles have two properties, (1) they have an 'angular momentum' which represents the force or thrust with which a particle spins round; the strength of this momentum depending on the mass and the velocity of the object, and (2) due to their spinning motion, each particle generates a magnetic field of its own with a specific amplitude and direction. This is referred to as its 'magnetic dipole moment (MDM)'. Hydrogen nuclei, which contain only one positively charged proton, exhibit such properties which are essential in the generation of the MR signal. Although other MR active nuclei exist, it is the hydrogen nucleus which is used in MRI due to the fact that it is the predominant nuclei found in the human body and that it has a comparatively large magnetic dipole moment (de Haan & Rorden, 2011; McRobbie, Moore, Graves, & Prince, 2007; Westbrook, Roth, & Talbot, 2005).

2.1.2   The Hydrogen Nucleus in a Magnetic Field

The angular momentum of the hydrogen protons, caused by the fact that they are rotating about their own axis, is referred to as their 'spin'. Due to this movement, each individual hydrogen proton creates a magnetic field, or MDM, around it. When no external magnetic field is present, the MDMs are randomly oriented, however when an external magnetic field (named B_0) is applied, a proportion of the hydrogen nuclei MDMs align in

parallel with its axis. This is what happens when the human brain is placed in an MR scanner (Brown, Perthen, Liu, & Buxton, 2007)(Figure 2-1(b/c)).

A smaller proportion of the MDMs align anti-parallel to the B_0 field, and are described as being in a high-thermal energy state (due to the fact that it takes more effort to oppose the strong external magnetic field than to fall in line with it), whereas the MDMs aligned with B_0 are in a low-thermal energy state. These are referred to as 'spin-down' and 'spin-up' nuclei respectively. In the human brain, the number of low-energy nuclei is larger than the number of high-energy nuclei, thus there is always a larger number of nuclei aligned with B_0 than not. The sum of the MDMs in these two states produces a net magnetisation (M_0) which is aligned with B_0, in the longitudinal plane (Huettel, Song, & McCarthy, 2009; Westbrook & Kraut, 1998; Westbrook et al., 2005).
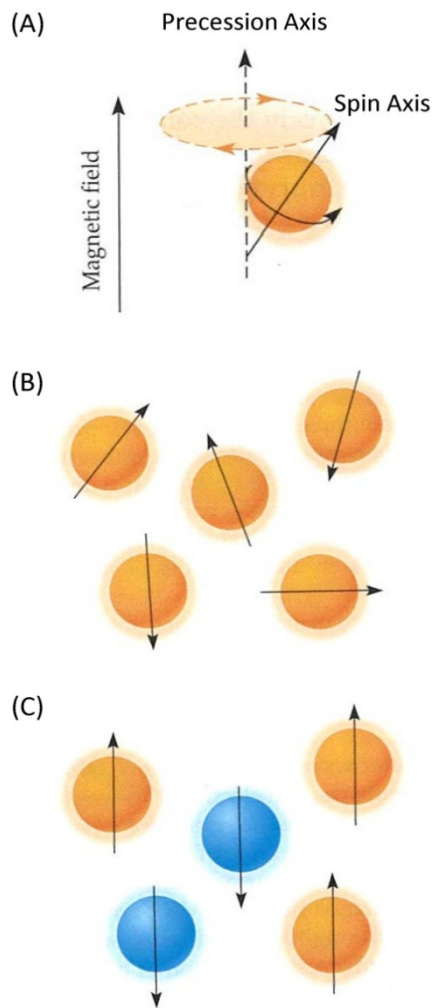
Figure 2-1 Spins

**Figure adapted from Huettel et al.,
(2009). (A) A rotating proton will rotate
about its own axis (spin) but also rotate
around the B_0 axis. (B) Randomly
oriented MDMs in the absence of B_0.
(C) Spin-up and spin-down nuclei in
the presence of B_0.**

As well as the protons spinning on their own axis, the presence of an external magnetic
field causes a second spinning motion – the MDMs of the protons begin to 'precess'
around the axis of B_0, whose strength determines the speed or frequency with which they
do this (Figure 2-1(a)). The rate at which the hydrogen nuclei precess is referred to as the

Larmor frequency and differs between the different MR active nuclei (McRobbie et al., 2007; Westbrook & Kraut, 1998; Westbrook et al., 2005).

2.1.3   How the MR Signal is Generated

Given that the net magnetisation (M_0) is the sum of the magnetic dipole moments of the individual nuclei, it also precesses around the axis of the external magnetic field at the Larmor frequency. M_0 is essential for the generation of the MR signal, but in order to measure the amplitude of this signal one must first perturb it (Huettel et al., 2009).

This is done by applying a radio frequency (RF) pulse which also oscillates at the Larmor frequency. When nuclei are exposed to a frequency of oscillation that matches their own, they gain energy or 'resonate', hence the name magnetic resonance imaging. Specifically, if an excitation pulse is applied at the Larmor frequency of hydrogen nuclei only these will resonate, as the other MR active nuclei have different precessional frequencies. By absorbing energy, more of the hydrogen nuclei transfer into the high 'spin-down' energy state and align anti-parallel to B_0. When an excitation pulse delivers enough energy to create equal numbers of spin-up and spin-down nuclei, the net magnetisation is said to lie at a 90° angle to B_0, in the transverse plane. An additional effect of the RF pulse is that the MDMs of the hydrogen nuclei which were rotating around the axis of B_0 at different points on the precessional path, become 'in-phase', or in other words, start to precess around the axis in a synchronised manner ((Westbrook et al., 2005); see Figure 2-2).

In summary, when the excitation pulse is applied the net magnetisation is tipped into the transverse plane and begins to precess in-phase at the Larmor frequency. This moving magnetic field induces an electrical voltage in a receiver coil whose frequency is identical to that of the precessional frequency of the net magnetisation. This voltage constitutes the MR signal (Huettel et al., 2009; Westbrook et al., 2005). In all MR sequences, the application of RF pulses and the signal measurement is repeated several times to obtain good quality images.

2.1.4   Different Types of MR images: Relaxation

When the RF pulse is switched off, the MR signal does not remain constant; in time the hydrogen nuclei begin to (1) relax, i.e. lose the energy that they had gained and (2) once again become out of phase with each other (see Figure 2-2). These two processes lead to hydrogen nuclei regaining their spin-up status and the net magnetisation aligning with B_0 in the longitudinal plane.
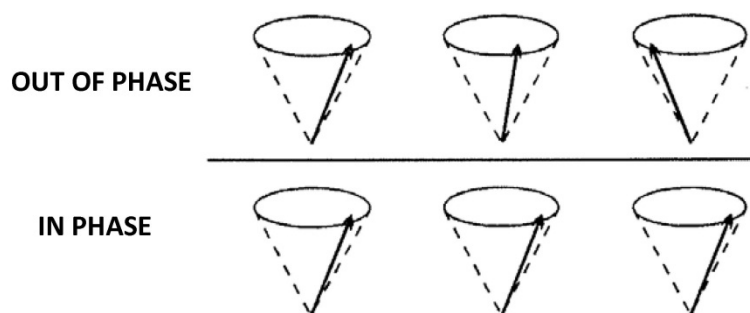
Figure 2-2 Precession Paths

Taken from Westbrook et al., (1998). Out of phase: at different points on the precessional path. In phase: nuclei precessing in coherence.

The loss of the MR signal can be defined in two ways; as a recovery of the longitudinal net magnetisation, due to the process called T1 recovery (Figure 2-3 (a)) and as the decay of the transverse net magnetisation caused by T2 or T2* decay (Figure 2-3 (b)). As previously mentioned, when the hydrogen nuclei release energy into the surroundings, the net magnetisation begins to return to its equilibrium state (more spin-up nuclei than spin-down) and tries to re-align once more with B_0. This recovery of the longitudinal magnetisation is moderately slow and occurs with a time constant named T1. With regards to the decay of the signal one must consider that, with the loss of the externally applied RF pulse, over time the precessing nuclei begin to interact with each other causing some to precess at higher frequencies than others and thus causing the said nuclei to become out of phase. These spin-spin interactions cause an exponential decay of the MR signal with a time constant termed T2. In addition, inhomogeneities in the magnetic field will cause different nuclei to experience different magnetic fields, again causing differences in precessing frequencies. The combination of spin-spin interactions and field inhomogeneities speeds up the decay of the signal even more and the rate of these combined effects is given by the time constant T2* (Brown et al., 2007; Huettel et al., 2009).

T1 and T2 time constants differ from each other and also differ depending on the type of brain tissue. Both time constants contribute to the MR signal, however the exact type of MR image obtained depends on the weight given to each measure. For clinical purposes, T1-weighted scans are acquired with specific parameters which heighten the contrast between grey and white matter, whereas T2-weighted scans highlight fluid filled regions;

both types of scans are used predominantly to capture anatomical brain images. Finally, certain scans acquired incorporate more T2* related signal (de Haan & Rorden, 2011; Huettel et al., 2009). T2*-weighted images are particularly relevant for the measurement of physiological activation within the brain, which will be the main focus of section 2.2.
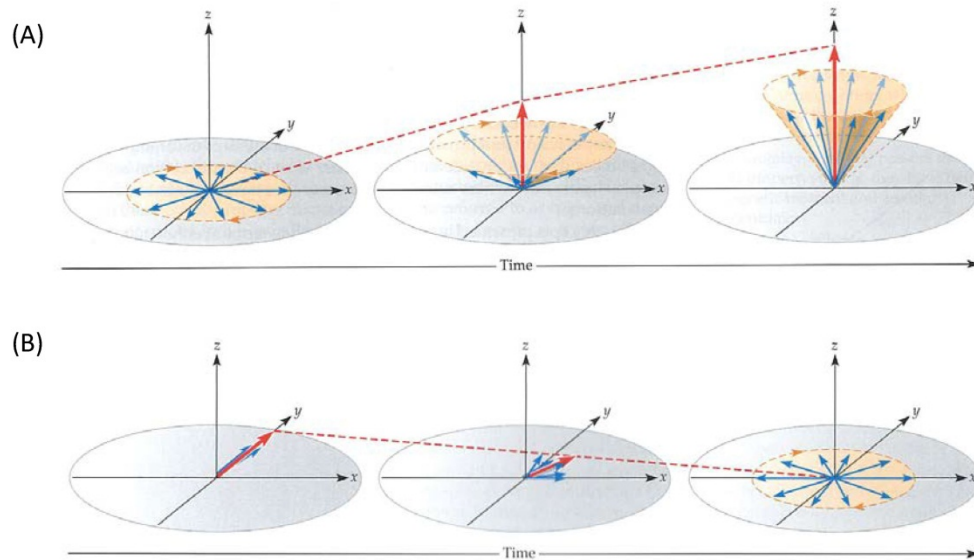
Figure 2-3 T1 recovery and T2 decay

Taken from Huettel et al., (2009). When an RF excitation pulse is applied the net magnetisation is tipped into the transverse plane. (A) T1 recovery. When the RF pulse is switched off the hydrogen nuclei release energy into the surroundings. This enables the net magnetisation to return to its equilibrium state and to re-align along the longitudinal (z) axis. (B) T2/T2* decay. When the RF pulse is switched off the hydrogen nuclei begin to precess out of phase with each other (T2 decay). Spin-spin interactions and magnetic field inhomogeneities both contribute to T2* decay. Net magnetisation [red arrow]. Hydrogen nuclei [blue arrows]. X, y and z-axes [black arrows].

2.1.5   MR Image Formation

To create an MR image which contains spatially specific information one must be able to locate the MR signal in three different dimensions. The method used to achieve this is the superposition of three gradient magnetic fields onto the main magnetic field (B_0). These gradients are usually referred to as the slice-selection, frequency encoding and phase encoding gradients, most commonly oriented along the x, y and z-axes (Huettel et al., 2009; Westbrook & Kraut, 1998).

For reasons of simplicity, MR images are usually constructed from signal obtained from numerous slices of tissue determined by the slice-selection gradient. The application of a gradient, whose strength fluctuates across space, induces different precessional frequencies in the nuclei situated at different points on the gradient's axis. Consequently, the nuclei within a specific slice will precess at different frequencies to those of adjacent slices and can be exclusively excited by an RF pulse containing the range of Larmor frequencies exhibited by the slice nuclei. A similar principle is used when the frequency encoding gradient is applied during the data acquisition phase. In this case the precessional frequencies will differ along a different gradient axis, adding spatial information to the image being created. Finally, a phase encoding gradient is also applied. This particular gradient induces a shift in the phase of the nuclei. Nuclei at different locations on the axis of the phase encoding gradient will begin to precess out of phase. When the phase gradient is switched off, although the precessional frequencies return to normal, the nuclei along this gradient remain out of phase, providing further spatial information by narrowing down the location of the signal of interest further (Westbrook & Kraut, 1998).

The order of magnetic gradient superpositions and RF pulses used to create a specific MR image is called a pulse sequence. The data acquired from this sequence to form an image is analysed using Fourier transformations, a mathematically based process (for review see (Gallagher, Nemeth, & Hacein-Bey, 2008)).

2.2   The Blood Oxygen Level Dependent (BOLD) Contrast

2.2.1   The Haemoglobin Effect

Currently, BOLD contrast is the most commonly used method for imaging functional activation of the brain and relies primarily on the magnetic properties of the haemoglobin molecule (Amaro & Barker, 2006; Ogawa, Lee, Kay, & Tank, 1990; Ogawa, Lee, Nayak, & Glynn, 1990). Haemoglobin provides oxygen around the body for aerobic energy metabolism (Brown et al., 2007). As oxygenated blood passes through the body, oxygen molecules attached to the heme molecule within the haemoglobin complex will detach themselves when required; exposing an unpaired electron (i.e. haemoglobin becomes a charged particle). The deoxygenated haemoglobin molecule thus acquires a magnetic dipole moment and, consequently, causes a magnetic susceptibility effect by distorting the local magnetic field. Due to these characteristics, deoxygenated blood is described as being paramagnetic. In comparison, oxygenated blood is described as being diamagnetic,

i.e. it is not associated with susceptibility effects due to its only very weak repulsion from a magnetic field (Brown et al., 2007; Huettel et al., 2009; Pauling & Coryell, 1936). Finally, as we know from previous sections, magnetic field inhomogeneities cause nuclei to precess out of phase, affecting the speed with which the T2* related signal decays. Therefore, the presence of paramagnetic deoxygenated blood induces a *faster* decay of the T2*-weighted MR signal (Matthews & Jezzard, 2004). Specifically, the MR signal should be weaker in brain areas with a high concentration of deoxygenated blood (Huettel et al., 2009).

2.2.2   Physiology of the BOLD Response: Neurovascular Coupling

Another important consideration in BOLD imaging, which is central to its interpretation, is the concept of neurovascular coupling. This suggests that neuronal activity is accompanied by a regional increase in CBF, which represents a source of glucose and oxygen (Huettel et al., 2009; Roy & Sherrington, 1890). One would initially assume that in an area of the brain exhibiting activity, the concentration of oxygenated blood would decrease compared to that of the deoxygenated blood due to the metabolic demands of the neurons. This is initially what is seen, termed "the initial dip". However, this is rapidly followed, on the order of 1-2 seconds, by a disproportionate increase in blood flow supplying an amount of oxygenated haemoglobin which is greater than the rate of oxygen consumption in the area (see section 2.2.4 for details)(Blomqvist et al., 1994; Fox & Raichle, 1986; Fox, Raichle, Mintun, & Dence, 1988). This leads to a marked decrease in deoxygenated blood and thus a more pronounced MR signal, typically consisting of a 0.5-5% change in regional image intensity (Detre & Wang, 2002).

Although an established process, the precise signalling mechanisms that elicit neurovascular coupling have not yet been defined and are somewhat deliberated (Arthurs & Boniface, 2002; Logothetis & Pfeuffer, 2004). For a long time it was thought that the increase in blood flow seen was due to an increase in energy demand during neuronal activity, however recent evidence suggests that it is instead a neurotransmitter-mediated effect (Attwell & Iadecola, 2002). Whether this local neurotransmitter signalling is due to neurons, astrocytes or a combination of multiple processes is also a question for debate (for review, (Attwell et al., 2010). Nonetheless, the concept of neurovascular coupling is to this day fundamental to our understanding and implementation of BOLD imaging.

2.2.3 Physiology of the BOLD response: what neural activity does the BOLD signal represent?

In order to have a conceptual appreciation of how the functional BOLD signal relates to the underlying physiology it is also essential to understand which *aspect* of neuronal activity induces a neurovascular response. Until recently, action potentials (spiking activity), the outward communicating signals of neurons, were thought to represent the critical neural response (Rees et al., 2000; (Heeger, Huk, Geisler, & Albrecht, 2000); however, Logothetis and colleagues (Logothetis, 2002; Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001; Logothetis & Pfeuffer, 2004; Logothetis & Wandell, 2004) suggest that the inputs to neurons, represented by synaptic activity, are the signals of interest (Pasley & Freeman, 2008; Raichle & Mintun, 2006). The summed synaptic activity is reflected in local field potentials, electrophysiological signals which represent "the input of a given cortical area as well as its local intracortical processing, including the activity of excitatory and inhibitory interneurons" (Logothetis & Pfeuffer, 2004), p.1521). Logically, due to the fact that axonal firing rates will be intrinsically linked to subsequent synaptic activity (Arthurs & Boniface, 2002), BOLD signal has been found to be equally correlated to both measures (Mukamel et al., 2005). On the other hand, some studies have shown this correlation predominantly with LFPs rather than spiking activity (Logothetis et al., 2001). Furthermore, when spikes and LFPs have been made to respond independently, it appears it is mainly the latter driving the BOLD signal (Mathiesen, Caesar, Akgoren, & Lauritzen, 1998; Raichle & Mintun, 2006; Viswanathan & Freeman, 2007).
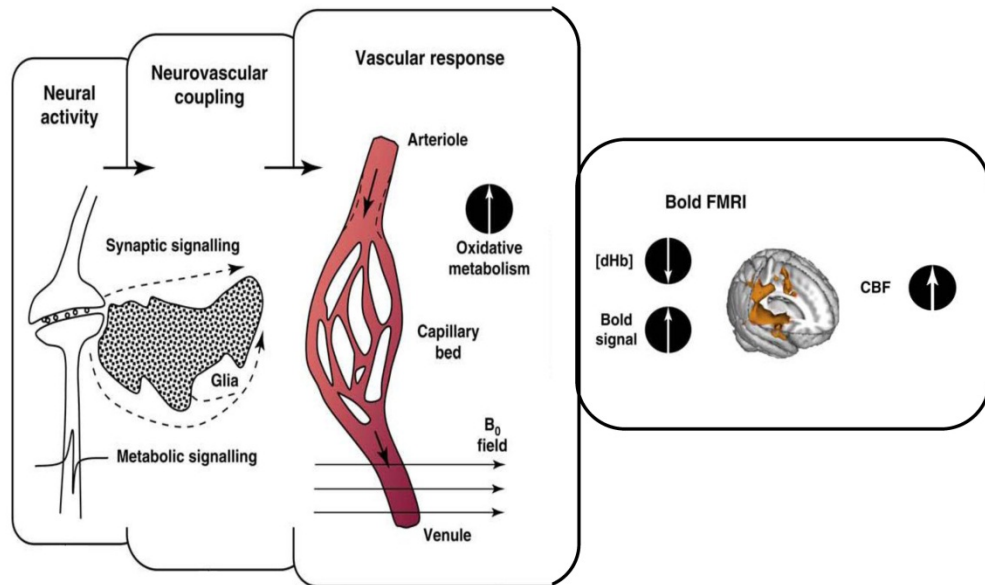
Figure 2-4 Stages in the Blood Oxygen Level Dependent (BOLD) Contrast

Signal

Adapted from Wise & Preston (2010). Neural activity leads to an increase in oxidative metabolism, followed by a greater increase in CBF. The concentration of deoxyhaemoglobin thus decreases, increasing the MRI BOLD signal.

In summary, the BOLD signal depends on the interplay between numerous physiological factors such as the rates of glucose (CMR_{glu}) and oxygen ($CMRO_2$) metabolism, CBV, CBF and neuronal specific events (Figure 2-4) (Arthurs & Boniface, 2002; Huettel et al., 2009; Matthews & Jezzard, 2004). Although studies have demonstrated that changes in BOLD signal are proportional to changes in neuronal activity (Heeger et al., 2000), it remains a surrogate measure as it relies on processes that are indirectly related to the underlying neurophysiology. Nonetheless, this method has led to countless successful functional magnetic resonance imaging (fMRI) studies that have enabled a deeper understanding of the neural correlates of different brain functions and disorders. It is unique in its ability to non-invasively measure functional activation of the brain whilst individuals perform complex cognitive tasks (Cabeza & Nyberg, 2000a).

2.2.4   Temporal Resolution: The Haemodynamic Response Function

Neuronal activity and the accompanying vascular and physiological processes trigger a change in the MR signal or 'BOLD response' which is represented as the haemodynamic response function (HRF; Figure 2-5); the exact shape and duration of this response depends on the individual, the specific brain area and on the attributes of the stimulus which induces it (Buxton, Uludag, Dubowitz, & Liu, 2004; Huettel et al., 2009).

In the first few seconds after a stimulus has been presented there is a temporary increase in deoxygenated blood giving rise to what is referred to as the 'initial dip' in the haemodynamic response mentioned above. Subsequently, due to the increase in blood flow to the area the signal increases, peaking at approximately five seconds post-stimulus and may plateau if the stimulus remains constant (Buxton et al., 2004). Following the removal of the stimulus the MR signal decreases and even undershoots before returning to baseline (Huettel et al., 2009).
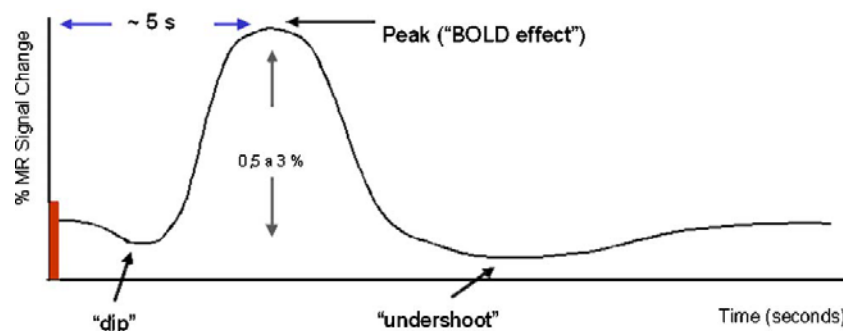
Figure 2-5 The Haemodynamic Response Function

Taken from Amaro & Barker (2006).

When more than one stimulus is presented to an individual the HRF is said to respond in a linear fashion (Boynton, Engel, Glover, & Heeger, 1996; Dale & Buckner, 1997; Heeger & Ress, 2002; Rosen, Buckner, & Dale, 1998) that is (1) an increase/decrease in the amplitude of the HRF should be proportional to an increase/decrease in neuronal activity and (2) the overall haemodynamic response to two stimuli should be the sum of the haemodynamic response to the two stimuli when presented independently. Although this system is not without its flaws (Vazquez & Noll, 1998; Wager, Vazquez, Hernandez, & Noll, 2005; Wan et al., 2006), making such assumptions enables one to model the predicted haemodynamic response to stimuli presented during tasks and thus also make

certain inferences about the underlying neural activity (Detre & Wang, 2002; Huettel et al., 2009).

As the above discussion indicates, the BOLD response is delayed with respect to the underlying neuronal activity (Menon, 2001) and it is this fact rather than the imaging techniques employed which limits the temporal resolution of fMRI (Detre & Wang, 2002). At present, fast imaging techniques are able to acquire a whole brain volume (made up of a certain number of slices) within a short time period with the use of a 2-3 second TR (time to repetition or time between two RF pulses); (Detre & Wang, 2002; Menon, 2001). This has allowed the development of more complex cognitive task designs and analysis methods, which will be reviewed in further detail in section 2.4.1 and chapter 3.

2.2.5   Spatial Resolution of the BOLD Response

In terms of spatial resolution, it has been suggested that the measured MR signal may be more diffuse than the spread of neuronal activity, as the MR signal is more pronounced in areas of low deoxyhaemoglobin levels such as in the draining veins downstream from activated regions (Detre & Wang, 2002; Huettel et al., 2009; Shibasaki, 2008). Although an inconsistent finding (Fransson, Kruger, Merboldt, & Frahm, 1998; Lindauer et al., 2001) the 'initial dip', represented by a decrease in oxyhaemoglobin, has been proposed as a more specific localiser as the oxygen extraction process should occur only in the capillaries proximal to the area of neuronal activation (Duong, Kim, Ugurbil, & Kim, 2001). In addition, some have suggested that blood flow (perfusion) may be an even better localiser of neuronal activity as this signal is more specific to the capillaries than the BOLD signal, due to the fact that the latter is influenced by many other physiological parameters (Menon, 2001). The basic unit of spatial resolution used in MRI analysis is the volume element or 'voxel', whose exact size will affect the signal to noise ratio, the acquisition time and whether or not partial volume effects (existence of multiple tissue types in the same voxel) will be present (Amaro & Barker, 2006). As with temporal resolution, the decision regarding the extent of spatial resolution needed will depend on the experimental question being asked (Huettel et al., 2009). Furthermore, spatial resolution can once again be adjusted during analysis procedures (see Chapter 3, section - smoothing).

In essence, compared to other existing imaging techniques such as PET, BOLD fMRI has superior spatial and temporal resolution. Knowledge of its limitations, however, is essential for a considered approach to the interpretation of such data.

2.3   Perfusion Imaging: Arterial Spin Labelling (ASL)

As described in the section 2.2.3. BOLD signal represents an indirect measure of neuronal activity and haemodynamic changes (S. E. Murphy & Mackay, 2010). In comparison, the arterial spin labelling (ASL) method provides an absolute measure of perfusion (regional cerebral blood flow (rCBF)) and thus, may represent a physiologically specific marker of brain function (Wong, Buxton, & Frank, 1999). Furthermore, unlike certain perfusion imaging techniques, such as PET, that require the infusion of radioactively labelled contrast agents, MR-based ASL uses RF and magnetic field gradient pulses to magnetically label endogenous blood for the non-invasive quantification of local perfusion in the brain (Detre, 2008).

Different forms of ASL techniques exist, for example pulsed ASL (PASL) or continuous ASL (CASL), however this section will focus on pulsed (or pseudo)-continuous arterial spin labelling (p-CASL) as this is the perfusion imaging technique described and utilised throughout this thesis. CASL approaches magnetically label arterial blood in a continuous manner, with the use of a constant RF pulse, as it flows through a specific labelling plane, most commonly located at the bottom of the brain (Wolf & Detre, 2007; Wong et al., 1999). On the other hand, PASL utilises one short RF pulse to invert the blood water, at times over a larger area (Wolf & Detre, 2007; Wong et al., 1999). In both techniques the magnetically labelled blood acts as a tracer and the signal it exhibits decays with the time constant T1 (Detre & Wang, 2002). Although CASL has been found to have a higher signal-to-noise ratio compared to PASL (J. Wang et al., 2002; Wong, Buxton, & Frank, 1998), it is harder to implement due to higher technological demands and practical limitations, such as magnetisation transfer (exciting the slices adjacent to the labelling slice; (Wolf & Detre, 2007) and maintaining long labelling pulses (Detre, Wang, Wang, & Rao, 2009; Golay, Hendrikse, & Lim, 2004). Thus, in order to preserve the efficiency of the CASL technique but enable an easier practical application, a CASL strategy was developed whereby the RF was applied as a sequence of pulses, instead of as a constant pulse during the labelling phase (i.e. p-CASL) (Dai, Garcia, de Bazelaire, & Alsop, 2008; Garcia, Duhamel, & Alsop, 2005).

Using this CASL sequence, perfusion images are acquired through pseudo-continuous flow-driven adiabatic inversion (see(Norris, 2002)for details on this method) of the water protons spins in arterial blood flowing upwards into the brain (see Figure 2-6). This blood is thus referred to as being magnetically 'tagged'. The tagged blood from the arteries

passes through the blood-brain barrier into capillaries where the tagged blood exchanges with the adjacent tissue magnetisation (i.e. untagged molecules). This exchange thus decreases the signal in the area and the extent to which it is reduced is a direct measure of blood flow (Golay et al., 2004). After a brief time lapse to allow the tagged blood to flow into the brain slices of interest, an image is acquired. To measure the reduction in signal control images, whereby the arterial blood has not been inverted, must also therefore be acquired. To improve the signal-to-noise ratio of the CASL method, pairs of tagged and control images are obtained and subtracted from each other. These difference images are then averaged to generate a map whose intensity is proportional to the current CBF, measured in physiological units of millilitres of blood per 100 grams of brain tissue per minute (ml/100mg/min) (Wolf & Detre, 2007; Wong et al., 1999).
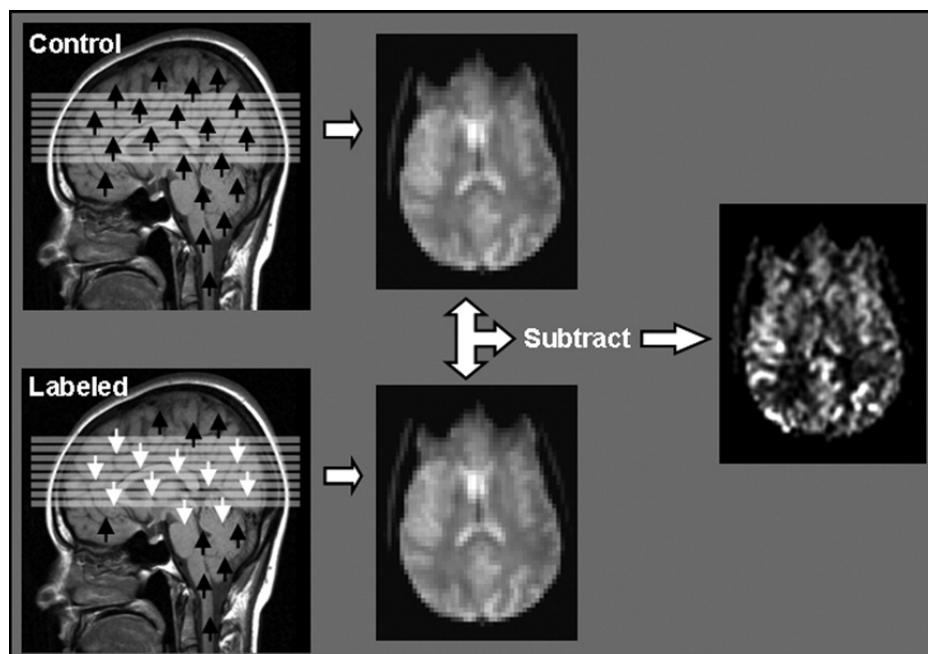
Figure 2-6 CASL Acquisition

Taken from Wolf & Detre (2007). Protons in arterial blood are magnetically tagged by RF pulses. Images are acquired under both labelling and non-labelling conditions and subtracted to obtain difference images. These are averaged to generate a map of intensities proportional to CBF.

The uses of arterial spin labelling techniques are manifold, for example it has been applied to many clinical situations (e.g. stroke; (Siewert, Schlaug, Edelman, & Warach, 1997), it is of interest in drug development studies (Bruns, Kunnecke, Risterucci, Moreau, & von Kienlin, 2009; Y. Chen et al., 2011) and in many ways may be complementary to the more

commonly acquired BOLD data. The applications of both BOLD and ASL imaging modalities will be discussed in greater detail in the following section.

2.4   Applications

2.4.1   Functional MRI

Functional MRI (fMRI) has been referred to in previous sections to describe the use of BOLD MRI in combination with cognitive tasks. This is one of the most widely used applications of MRI and is performed with the intention of measuring the functional activation of the brain in response to chosen stimuli, enabling the researcher to make certain assumptions about the changing cognitive state of the individual performing the task. In order to investigate a particular psychological theory and make certain inferences the experimental design of the task itself must be carefully constructed. The statistical power of the task, denoted by its "ability to estimate the HRF [and/or] detect significant activation" (M.A. Lindquist, 2008),p.447) also rests on its design. Two types of task design are commonly used; block and event-related.

A block design consists of grouping types of stimuli and presenting these in blocks. Blocks containing different types of stimuli are alternated so as to provoke a change in the cognitive state of the individual (see Figure 2-7). The blocks should not be too lengthy however as this would create difficulties in maintaining the same psychological state throughout. With the use of this block design it is thus possible to compare the activation differences between conditions with the use of a subtraction methodology, although this assumes that no interaction exists between the different cognitive components elicited by the task. Nonetheless, block designs have optimal detection abilities as they maximise the signal difference between conditions, due to the linearity of the HRF response (Amaro & Barker, 2006; Brown et al., 2007; M.A. Lindquist, 2008; Matthews & Jezzard, 2004). For most advantageous design, the decision regarding how many different types of blocks should be included in the task and how many times the conditions should be alternated is important. These decisions will affect whether the signal differences seen are due to the task conditions themselves and not such things as scanner drift (see Chapter 3; (Amaro & Barker, 2006; Brown et al., 2007; M.A. Lindquist, 2008; Matthews & Jezzard, 2004).

In comparison to block designs, event-related designs are more suited to the estimation of the shape of the HRF, supplying more temporal information about the response (Liu, Frank, Wong, & Buxton, 2001). Event-related designs consist of presenting a variety of

stimuli as individual trials in a randomised manner (see Figure 6), thus also allowing the detection of the BOLD response to discrete events (Amaro & Barker, 2006; M.A. Lindquist, 2008). The response from stimuli belonging to the same condition is then averaged although event–related paradigms usually require a longer run to achieve the similar detection power as block designs (Matthews & Jezzard, 2004). However, with fast event-related designs, typically the interstimulus interval (ISI) is varied (a form of 'jittering' whereby the HRF is sampled at different points in time to enable more accurate estimation of the response) with a minimum of four seconds between presentations to ensure that the individual HRF responses do not overlap too much, a preferable situation for analysis (Amaro & Barker, 2006).

In more recent years perfusion imaging has also been used in conjunction with cognitive tasks (J. Kim et al., 2011; Xu et al., 2007). This approach has been shown to be valuable in circumstances where slow continuous changes in neural activity are expected, for example in certain learning paradigms (Olson et al., 2006). However, BOLD fMRI remains the technique of choice when using experimental designs where the changes of interest are occurring at faster rates (Aguirre, Detre, & Wang, 2005).
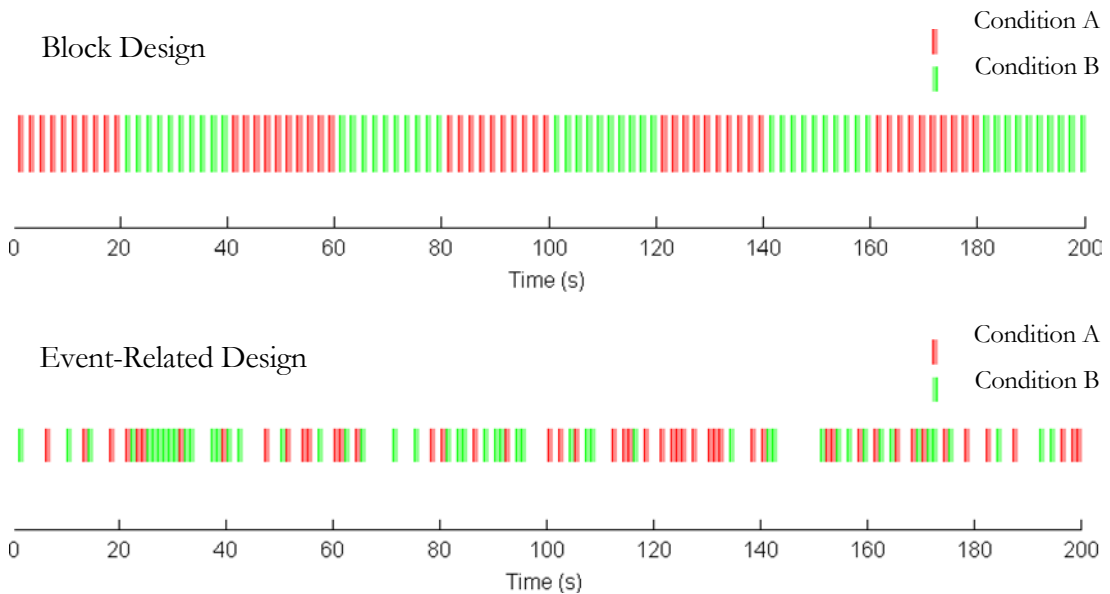
Figure 2-7 fMRI Task Designs

Adapted from Lindquist (2008). Depicts experimental block (top) and event-related (bottom) designs.

The decision whether to use (1) BOLD or perfusion imaging, (2) a block or event-related design, (3) certain stimuli rather than others and (4) the number and timings of the stimuli

presentations will always depend on the question being asked and result in a compromise between statistical power and psychological integrity.

2.4.2 Pharmacological MRI

In recent years the combination of MRI with drug administration has become increasingly popular, recognised as a method which enables researchers to map the effects of centrally acting drugs and furthermore, understand the effects of such drugs at a systems neuroscience level (Borsook, Becerra, & Hargreaves, 2006). The term used to describe this technique, pharmacological MRI (phMRI;(Y. C. Chen et al., 1997), has been coined to incorporate studies where the subject is (1) scanned 'at rest' under placebo conditions directly followed by a drug infusion and (2) performing a cognitive, perceptual, emotional or motor fMRI task during separate placebo and drug sessions (Tracey, 2001).

So far the majority of phMRI studies have investigated the pharmacological modulation of cognitive task-induced BOLD related activity, an approach which is more hypothesis driven than its 'resting' counterpart (Abel, Allin, Kucharska-Pietura, et al., 2003; Dolan et al., 1995; G. D. Honey et al., 1999; Loubinoux et al., 2002; Mattay et al., 2000; S. E. Murphy & Mackay, 2010). For example, Mattay and colleagues found that prefrontal cortical activation not only differed between high and low performing individuals on a WM task but that this activation was also differentially modulated by D-amphetamine (a dopamine re-uptake inhibitor) depending on performance levels. In a similar fashion, when investigating motor function, administration of the selective serotonin re-uptake inhibitor (SSRI), Paroxetine, a medication known to stimulate or improve motor performance, was found to modulate brain motor areas (Loubinoux et al., 2002). Furthermore, this method has been extended to the patient population; Honey et al., (1999) demonstrated that although no improvements in performance were seen, a shift from a typical to atypical antipsychotic treatment regimen in schizophrenic patients increased activity in prefrontal cortical areas during a WM task. Thus, although fMRI cannot directly visualise the effects of a drug at a fine-grained level (e.g. receptor binding concentrations), it is possible, together with the additional measurements acquired, to hypothesise about the mechanism of action of the drug and the underlying neurotransmitter systems to such cognitive functions.

A more recent application of the phMRI approach is 'resting' or 'challenge' phMRI. In comparison to task-related phMRI, this represents an unconstrained means of describing the 'fingerprint' of the drug-induced neuronal effects (Borsook et al., 2006; Wise &

Tracey, 2006). This technique, which primarily uses the BOLD contrast to detect drug-induced signal changes, has been applied extensively in animals to investigate such pharmacological models as reward and dependence, brain injury, psychosis and pain (Shah & Marsden, 2004; Steward, Marsden, Prior, Morris, & Shah, 2005). For example, a range of studies (Y. C. Chen et al., 1997; Febo et al., 2005; Marota et al., 2000) used drugs such as amphetamine and cocaine to investigate the role of the dopaminergic system in their associated behavioural effects. Other research groups investigated the actions of glutamatergic agents such as MK801 or phencyclidine (PCP), or of cannabinoid substances, demonstrating BOLD changes in hypothesised regions such as the thalamus and the cingulate respectively (Hackler et al., 2010; Houston et al., 2001; Shah & Marsden, 2004). Human phMRI studies, using analogous compounds, have found changes in brain activation similar to those seen in pre-clinical experiments (Breiter et al., 1997; Deakin et al., 2008; Stein et al., 1998; Vollm et al., 2004), demonstrating the technique's translational nature across species.

In order to appropriately characterise the drug-induced BOLD signal changes, different signal processing strategies have been applied. For example, in a study investigating neural networks related to cocaine-induced craving and euphoria in cocaine-dependent subjects the average BOLD signal in the pre-infusion imaging time block was directly compared to that of the post-infusion time block. To obtain more information about these networks, subjective ratings were also acquired and used as an input function to model the temporal variation evident in the signal (Breiter et al., 1997). Although these methods were able to detect drug-induced changes in the brain, they (1) ignore some of the temporal information available in the MR signal and (2) require the interruption of imaging data acquisition at regular intervals to collect subjective data (Stein, 2001; Tracey, 2001). Another approach, which is more sensitive to temporally related information, is to use the pharmacokinetic (PK) characteristics of the administered drug. (Bloom et al., 1999) developed a waveform analysis method that, with the use of an algorithm, identified potential brain areas underlying the drug effect by matching the PK profile of the drug to the MR BOLD signal changes (S. E. Murphy & Mackay, 2010). While being an effective method, it does require the assumption that plasma levels of the drug are directly proportional to brain penetration levels (Stein, 2001). In addition, it constrains the use of phMRI to compounds with known PK profiles. A more recent time-series approach uses a block analysis method whereby the data is modelled as one-minute time-bins (or blocks), with the pre-infusion block used as the baseline and the remaining post-infusion

time-bins as test blocks (Deakin et al., 2008; McKie et al., 2005). Significantly however, this methodology once again may overlook much of the temporal information in the signal by averaging the data within the time-bins. It is thus desirable to model the phMRI response using a single regressor (whose parameters are not based on subjective ratings or PK), to provide a single numeric value for each voxel's timeseries (see Chapter 4 for a detailed methodological description of this approach).

As described in section 2.2.3., there are some limitations to BOLD MRI; it is an indirect measure of neuronal activity and it detects relative changes in blood flow rather than offering a quantitative measure of these changes (S. E. Murphy & Mackay, 2010). Furthermore, for BOLD phMRI specifically there are some additional caveats that one must address. In phMRI, BOLD signal changes could be induced through either direct effects of the drug on neuronal activity or through non-specific effects on cerebral metabolic activity or on the vasculature itself (Wise & Tracey, 2006). To ascertain that signal changes are not due to disturbed neurovascular coupling integrity, different methods can be employed. For example, it is possible to include a 'control' task, such as a purely motor or visual paradigm, which effectively activates regions which should not be linked to the hypothesised neuronal effects of the drug. If the activation elicited by these tasks is then not modulated by the drug one may have more confidence in the idea that the drug-induced changes in your task of interest is not a global effect on CBF (Schweinhardt, Bountra, & Tracey, 2006). Differences in activation may also be monitored in 'control' brain *regions* to support this conclusion. Physiological effects of the drug, such as changes in heart rate, blood pressure and respiration may also be recorded and later correlated with the BOLD changes; if a correlation does not exist one may suggest that the changes are neuronal in origin. In addition, if correlations *are* present between subjective effects of the drug and brain activity, it is possible with greater certainty, to attribute the signal changes to neuronal events (Schweinhardt et al., 2006).

To deal with these limitations it is also possible to utilise imaging modalities such as ASL (see section 2.3), to *quantify* the changes in CBF, unconfounded by changes in CBV or $CMRO_2$ (Wise & Tracey, 2006). Although it is a technique that has a lower signal-to-noise ratio than BOLD (Jahng et al., 2005) and has less extended brain coverage (Talagala, Ye, Ledden, & Chesnick, 2004; Z. Wang, Wang, Connick, Wetmore, & Detre, 2005), ASL has been found to be reliable across sessions in healthy subjects (Jiang et al., 2010). Moreover, it has been suggested to be a better indicator of the exact location of neural activity (Pfeuffer et al., 2002) and to be more sensitive to slow changes in CBF, commonly seen

when drugs are administered (Aguirre et al., 2005; Aguirre, Detre, Zarahn, & Alsop, 2002). Controlling for global CBF changes when analysing BOLD data thus represents an important step towards identifying if the effects of the compounds administered are indeed neuronal.

PhMRI data, whether challenge or task-related, is a non-invasive technique essential for illustrating the neural networks associated with the effects of centrally acting drugs. Elucidating links between subjective or cognitive effects, brain activity and drug-related mechanisms could be extremely valuable not only in the development of novel pharmacological compounds but also in tailoring treatment to the individual (G. Honey & Bullmore, 2004).

Chapter 3 METHODS: Study Design and Analysis

The data included in this thesis is derived from two studies. The first study focuses on the reliability of the ketamine brain response (the 'reliability study') and the second focuses on the modulation of the ketamine response in both resting state and task-related contexts (the 'modulation study'). As the following experimental chapters contain data from both parts of the study, this chapter will summarise the elements of the study design specific to each part of the study and those shared across the phases. Furthermore, a more detailed overview of processes and statistical principles behind fMRI BOLD data analysis will be given. As many of concepts are shared, the specifics of the methodology involved in the ASL data analysis will be included only in Chapter 5. Finally, as the notion of test-retest reliability, in the form of the intraclass correlation coefficient (ICC), spans several thesis chapters the theory and rationale behind the use of this method will be presented.

3.1 Participants

All participants were recruited via college-wide and local web-based advertisements, in addition to contacting participants belonging to a departmental database (see Figure 3-1). Screening procedures were identical for both parts of the study.

Reliability Study: Twenty-two right-handed healthy male participants, aged 18 to 39, were recruited. Eleven participants were enrolled in the study; one participant was excluded from the phMRI analysis following a problematic cannulation on his second session (see Figure 3-1). Ten participants (mean age 25.5 years, SD = 6.5; mean IQ = 114.5, SD = 6.3) completed both sessions of the study and were included in the phMRI BOLD analysis. Weight (mean = 73.6 kg, SD = 13.9) and height (mean = 175.5 cm, SD = 7.81) of all participants was also recorded on each session as these parameters were necessary for the ketamine infusion protocol. N.B. Nine participants were included in the phMRI CASL analyses due to corrupted data for one participant.

Modulation Study: Forty-nine right-handed healthy male participants, aged 20 to 37, were recruited. Twenty participants were enrolled in the study, sixteen of whom completed all four sessions of the study (see Figure 3-1). Of the four subjects that did not complete the study, one withdrew after fainting upon cannulation at session 1, one withdrew due to nausea at Session 3 and the other two were withdrawn at session 2 and session 3 due to violating the lifestyle guidelines (see below). Imaging data from these subjects were not analyzed and they were replaced with 4 additional subjects. Only data from these sixteen

participants (mean age 26.2 years, SD = 5.9; mean IQ = 114.4, SD = 5.80) were analyzed. As with the reliability study, weight (mean = 74.3 kg, SD = 10.8) and height (mean = 176.8 cm, SD = 6.74) of all participants was also recorded on each session.

Screening procedures: Prior to being enrolled on the study, participants were excluded on the basis of a positive urine screen for drugs of abuse (SureScreen Diagnostics Ltd, 10-panel test), out of range on standard urinalysis or blood test results. In addition, those who consumed more than the equivalent of 5 caffeine drinks per day, smoked more than 5 cigarettes per day or were taking prescribed or non-prescribed drugs were excluded. Participants were also assessed, by a qualified physician, for any history of psychiatric (including substance abuse), neurological or physical illness. These procedures were supplemented by the administration of the Structured Clinical Interview for DSM-IV_TR Axis I Disorders (SCID; (First, Spitzer, Gibbon, & Williams, 2002)). All screening procedures were completed on a separate visit up to one month prior to the study sessions where demographic data including age, ethnicity and predicted verbal IQ (National Adult Reading Test, NART; (Nelson, 1982; Nelson & Willison, 1991)) were also obtained. All participants gave written informed consent and their GP was informed of their participation. The study was approved by Wandsworth Research Ethics Committee (09/H0803/48; Appendix E).

3.2   Inclusion and Exclusion Criteria

Inclusion Criteria

Participants were expected to meet all of the following inclusion criteria to be eligible for enrolment into both the reliability and modulation study:

1. Healthy right-handed male participants between the ages of 18 and 50 years, inclusive (healthy was defined as no clinically relevant abnormalities identified by a detailed medical history, full physical examination, including blood pressure and pulse rate measurement, 12-lead ECG and clinical laboratory tests).

2. Body Mass Index (BMI) of approximately 18 to 30 kg/m^2; and a total body weight 50-100 kg.

3. Evidence of a personally signed and dated informed consent document indicating that the participant was informed of all pertinent aspects of the trial.

4. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other trial procedures.

Exclusion Criteria

Participants presenting with any of the following were not be included in either study:

1. Evidence or history of clinically significant haematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease (including drug allergies, but excluding untreated, asymptomatic, seasonal allergies at time of dosing). This also included participants with previous history of epilepsy or seizures, more than one febrile convulsion, psychiatric illness, attempted suicide or suicidal ideation, or glaucoma.

2. Use of prescription or non-prescription drugs within 7 days or 5 half-lives (whichever is longer) prior to the first dose of study medication. Herbal supplements had to be discontinued 28 days prior to the first dose of study medication. At the discretion of the investigator a shorter drug free or discontinuation period may have been acceptable depending on the precise drugs/supplements taken. As an exception, paracetamol or acetaminophen was allowed to be used at doses of ≤1 g/day.

3. Treatment with a new chemical entity within the 3 months preceding the first dose of study medication.

4. Evidence or history of substance abuse (including alcohol and drugs).

5. History of sensitivity to any of the study medications.

6. History of febrile illness within 5 days prior to the first dose.

7. Any condition possibly affecting drug absorption (eg. gastrectomy).

8. 12-lead ECG demonstrating QTc >430 msec at screening.

9. Fulfilment of any of the MRI contraindications on the standard radiography screening questionnaire (for example, history of surgery involving metal implants).

10. History of claustrophobia or inability to tolerate mock scanner environment during habituation/screening session.

11. History of regular alcohol consumption exceeding 28 drinks/week (1 drink = 5 ounces (150 mL) of wine or 12 ounces (360 mL) of beer or 1.5 ounces (45 mL) of hard liquor) within 6 months of screening.

12. Use of tobacco- or nicotine-containing products in excess of the equivalent of 5 cigarettes per day.

13. A positive urine drug screen.

14. Unwilling or unable to comply with the Lifestyle guidelines.

15. Participants who, in the opinion of the investigator, had any medical or psychological condition or social circumstances which would impair their ability to participate reliably in the study, or have have increased the risk to themselves or others by participating.

Lifestyle Guidelines

1. Subjects should have water only from midnight prior to attending the site for each study period. A light breakfast will be provided on admission.

2. Subjects will abstain from alcohol from 24 hours before admission until 24 hours after dosing at each study period.

3. Subjects will abstain from caffeine-containing products for 24 hours prior to admission until the end of all fMRI and other measurements on each study period.

4. Subjects will abstain from the use of tobacco- or nicotine-containing products for 4 hours prior to admission until discharge for each study period.

5. Subjects will abstain from strenuous exercise (e.g., heavy lifting, weight training, aerobics) for 24 hours prior to admission for each study period.

6. Subjects will be warned not to drive or operate machinery from the time of dosing until 24hrs post dose at each study period.
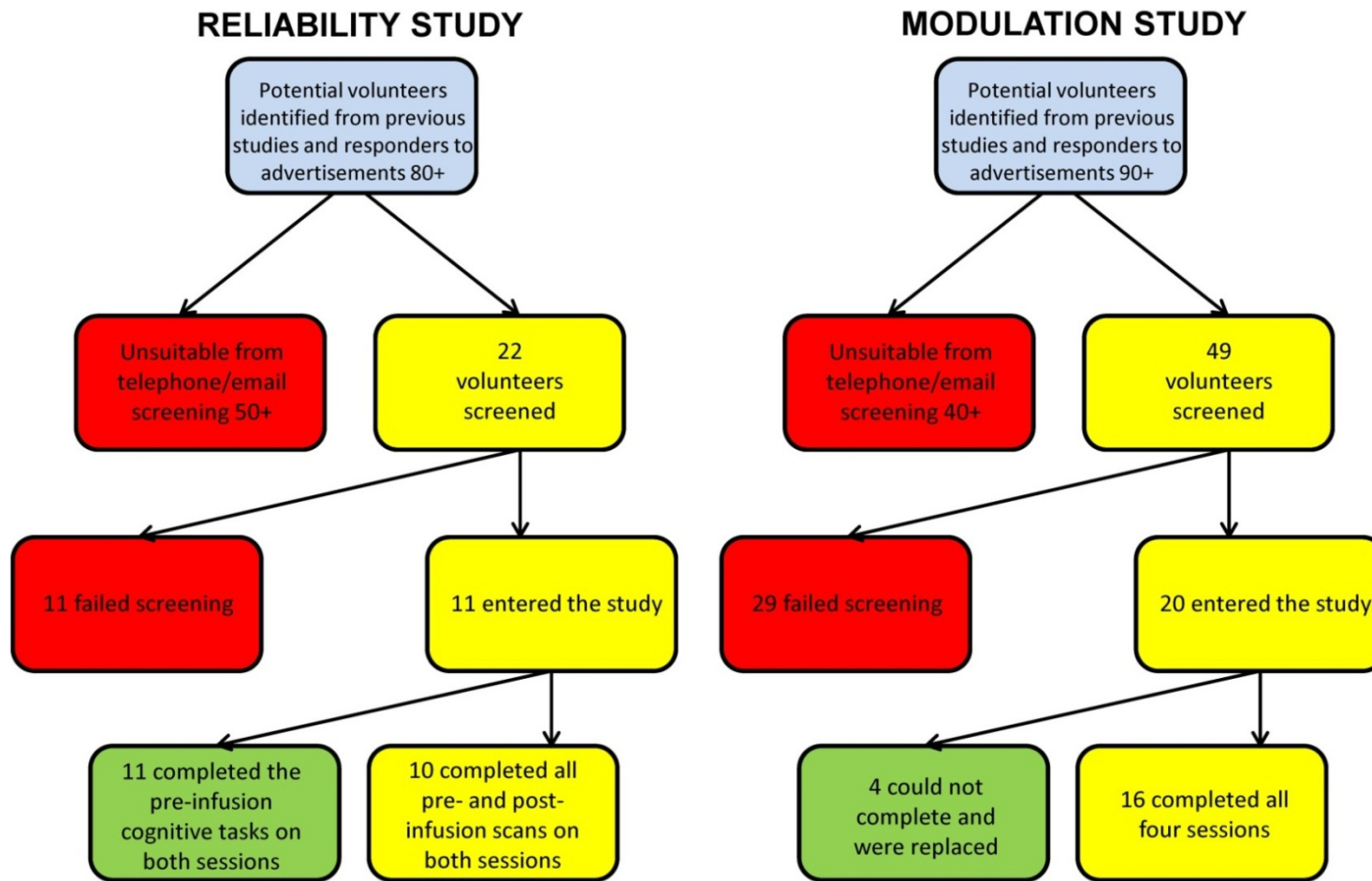
Figure 3-1 Recruitment Process for the reliability and modulation studies

3.3   Experimental Design

Reliability Study: Please see Figure 3-2 for a detailed overview of study day procedures. The study was an open-label design and MRI data were acquired from 11 healthy male participants on two separate occasions, at least one week apart, to allow for an appropriate washout period. On each occasion, participants had fMRI measurements prior to and during the ketamine infusion (please note that for this study the cognitive tasks were only acquired **pre-infusion**). Intravenous (I.V.) ketamine infusion began 5 minutes into a 15 minute BOLD phMRI scan. Subjective ratings were recorded at three timepoints during the day (see Appendix A for example questions). Each scanning visit involved a stay of approximately 5 hours at the Centre for Neuroimaging Sciences.

RELIABILITY STUDY
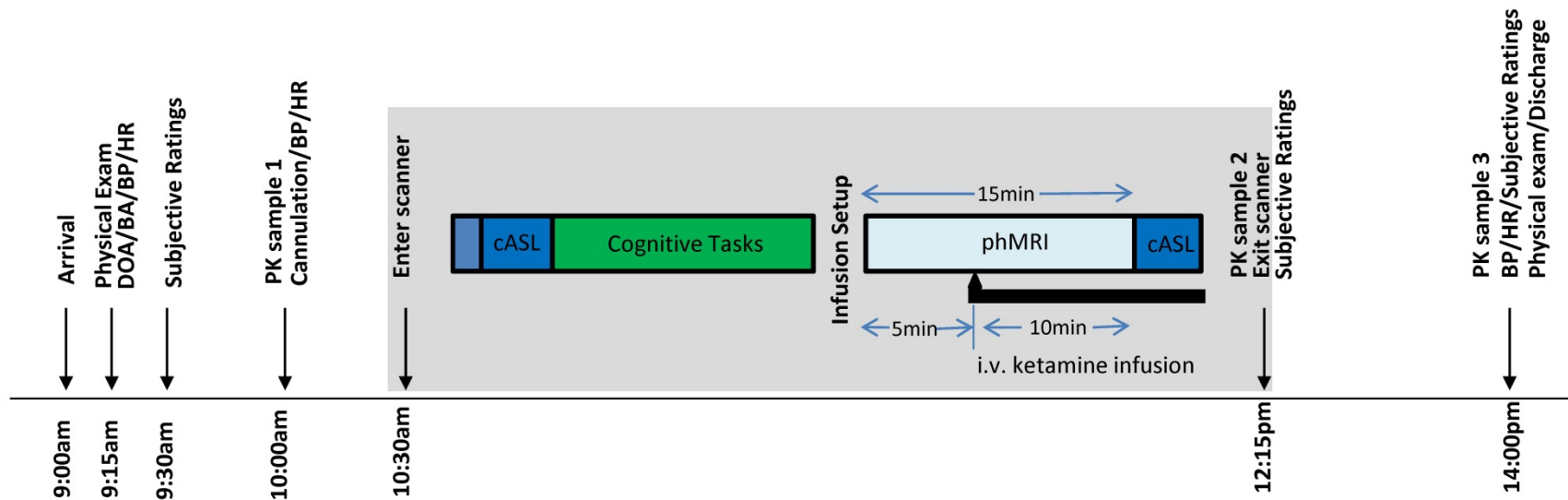
Figure 3-2 Reliability Study Day Timeline

DOA = drugs of abuse screening; **BA** = breath alcohol test; **BP** = blood pressure; **HR** = heart rate; **PK sample** = pharmacokinetic blood sample (data unusable due to corrupted samples). Subjective ratings included the Psychotomimetic States Inventory (PSI), Clinician Administered Dissociative States Scale (CADSS) and Visual Analogue Scales (VAS). Area shaded in grey includes the tasks performed while in the scanner: cASL = continuous arterial spin labelling; cognitive tasks included the **PAL, N-BACK,** sustained attention task and the breath-hold paradigm; phMRI = pharmacological MRI. Solid black arrow and horizontal line indicate the start and duration of the ketamine infusion.

Modulation Study: Please see Figure 3-3 for a detailed overview of study day procedures. The study was a randomised placebo-controlled, partial crossover design in 16 healthy male volunteers. On each scanning visit subjects received a single oral dose of one of the following treatments: placebo (2 visits), lamotrigine (300mg), or risperidone (2mg). In addition, subjects were administered the ketamine infusion on three of the four scanning days, and a saline infusion on the fourth. Thus, the four drug combinations that participants received were: oral placebo and saline infusion, oral placebo and ketamine infusion, oral lamotrigine and ketamine infusion and oral risperidone and ketamine infusion (see Figure 3-4 for how these combinations are referred to throughout the thesis). The order of these treatments was randomised and balanced within a latin-square design. The dose of lamotrigine was selected on the basis that it has been found to modulate both behavioural measures and imaging markers of ketamine effects (Anand et al., 2000; Deakin et al., 2008). The dose of risperidone was selected on the basis that, at single doses, and when compared to other antipsychotics, it can achieve high dopamine D2 occupancy (>50%) whilst being well-tolerated (Mehta et al., 2008). On each scanning visit, subjects had two scanning sessions with fMRI measurements prior to and during the ketamine infusion. As with the reliability study, I.V. ketamine infusion began 5 minutes into a 15 minute BOLD phMRI scan. Blood samples were taken at specified intervals following the oral drug administration in order to determine the plasma pharmacokinetics of the oral drug (lamotrigine or risperidone) and ketamine (see figure). Subjective ratings were recorded at five timepoints during the day (see Appendix A for example questions; see Figure 3-3). Each scanning visit was separated by a minimum of 10 days and involved a stay of approximately 8 hours at the Centre for Neuroimaging Sciences.

The scans acquired during the study sessions included: (1) four cognitive fMRI tasks, two of which are included in this thesis (the paired associates learning (PAL) task, and the working memory (N-BACK) task) and two of which are not (a sustained attention task and an offline reversal learning task); (2) the BOLD phMRI scan, (3) a perfusion CASL scan and (4) a breath-hold paradigm (also not included in this thesis). Please see figures 3-2 and 3-3 for the order in which these different scans were acquired.
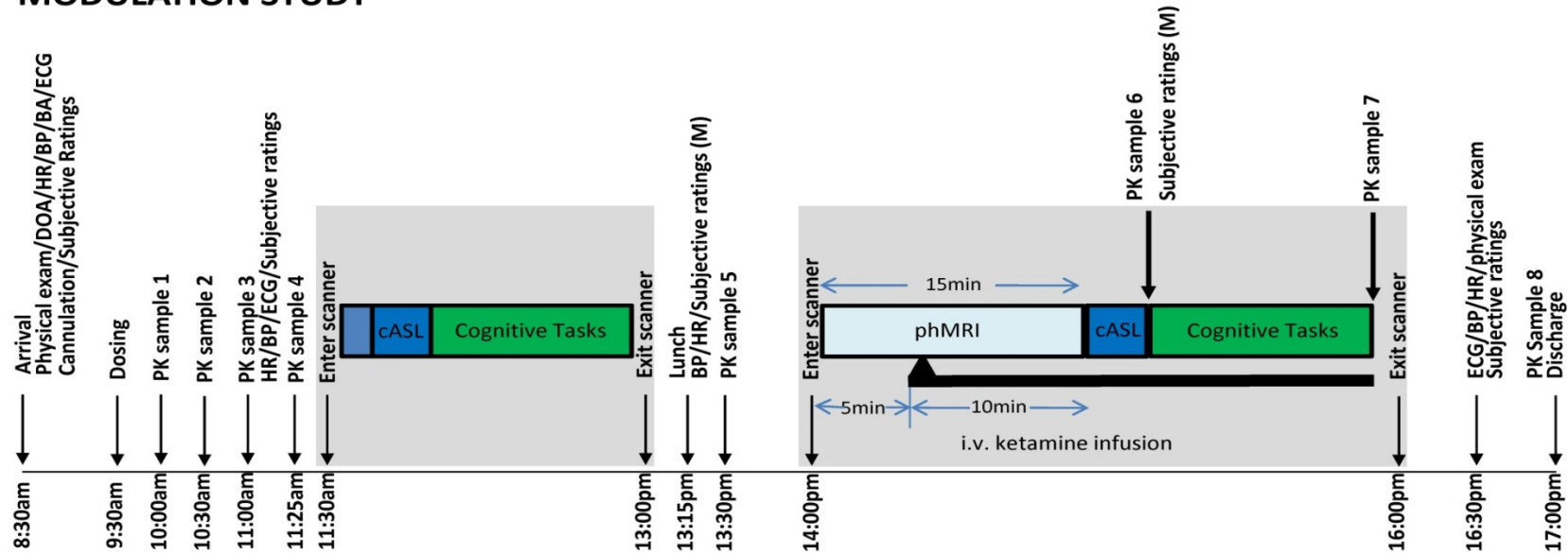
Figure 3-3 Modulation Study Day Timeline

DOA = drugs of abuse screening; BA = breath alcohol test; BP = blood pressure; HR = heart rate; PK sample = pharmacokinetic blood sample; ECG = electrocardiogram. Subjective ratings (M) indicates that only a subset of questions from the PSI, CADSS and VAS were asked. PK samples 1-4 and 8 were taken in order to measure the levels of risperidone or lamotrigine in the blood. PK samples 6 and 7 were taken to measure the levels of ketamine in the blood. Area shaded in grey includes the tasks performed while in the scanner: cASL = continuous arterial spin labelling; cognitive tasks included the PAL, N-BACK, sustained attention task and the breath-hold paradigm; phMRI = pharmacological MRI. Solid black arrow and horizontal line indicate the start and duration of the ketamine infusion.
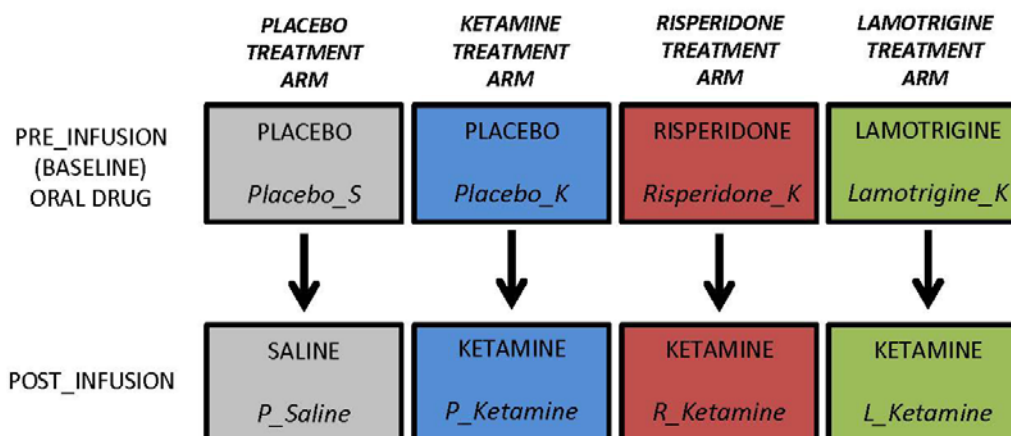
Figure 3-4 Modulation Study Treatment Arms

Diagram illustrating the drug combinations, organised into four treatment arms. Within the individual boxes, words in capitals indicate the type of treatment administered pre-infusion (in the morning) or post-infusion (in the afternoon). Words in italics indicate the labels given to the different treatment conditions for analysis purposes.

3.4   Infusion Protocol

Apart from the ketamine dose administered, the infusion protocol was identical in both reliability and modulation studies. Racemic ketamine was administered intravenously as an initial bolus followed by a continuous, near-constant, target-controlled infusion with the use of a Graseby 3400 pump located in an adjacent room and connected to the participant via tubing passed through a waveguide in the scanner room wall.

In the reliability study five participants were scanned with a target plasma level of ketamine of 50ng/ml and five participants with a target plasma level of ketamine of 75ng/ml. To achieve the target plasma levels, the ketamine doses delivered were (mean ± standard deviation) 0.08 ± 0.0022 mg/kg during the first minute followed by approximately 0.23 mg/kg/h (for the 50 ng/mL target plasma concentration) and 0.12 ± 0.0026 mg/kg during the first minute followed by 0.31 mg/kg/h (for the 75 ng/mL target plasma concentration). For each participant, the ketamine doses administered were the same on all testing sessions. The infusion parameters used were based on the Clements 250 infusion model (Absalom et al., 2007) implemented in Stanpump software (www.opentci.org) running in Windows Vista. In comparison to the Domino, Hijazi and

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Clements 125 infusion protocols, the Clements 250 model was chosen on the basis of its superior ability to predict observed low-dose ketamine plasma levels and to maintain these levels over time (Absalom et al., 2007). The doses of ketamine were chosen on the basis that they would induce only subtle subjective effects, whilst maintaining a strong phMRI response.

In the modulation study, all sixteen participants were scanned with a target plasma level of ketamine of 75ng/ml with the same dose described above. The infusion protocol for the higher dose was validated on the modulation study sample as PK samples from the reliability study were not available. On the oral placebo-ketamine session, the average plasma ketamine level was 62.7ng/mL (SD 17.6ng/mL) 15 minutes into the infusion and 72.8ng/mL (SD 20.8ng/mL) after 75 minutes infusion.

3.5   Image Acquisition

All participants were scanned at rest using a 3.0T GE HDx scanner (GE Medical Systems, Milwaukee, WI, USA). BOLD phMRI and cognitive task data were collected using gradient echo EPI. For the phMRI scan, a total of 450 image volumes of 38 slices (3mm thickness, interslice gap of 0.3mm) were acquired continuously per participant during 15 minutes (TE/TR = 30/2000ms, flip angle (FA) = 75°, in-plane resolution = 3.3mm, matrix size = 64×64, field of view = 21.1 x 21.1cm). This protocol provided voxels with isotropic resolution of ($3.3mm^3$). The paired associates learning (PAL) task and the working memory N-BACK task consisted of a total of 336 and 270 images volumes, acquired in 11 minutes 12 seconds and 9 minutes 9 seconds respectively. The slice thickness, interslice gap and additional parameters were identical to those of the phMRI scan.

A higher resolution GE-EPI "structural" scan was also acquired for each participant to assist accurate registration to a standard space template (43 3-mm-thick near-axial slices with 0.3mm gap, TR = 3 s, TE = 30 ms, FA = 90°, in-plane resolution = 3.3mm, matrix size = 128 x 128, field of view = 24 x 24 cm). An additional high resolution T2-weighted structural scan (FR-FSE) was also acquired to assist in the analysis of the ASL data (36 4-mm-thick axial slices with no gap, TR = 4.38 s, TE = 54.52 ms, FA = 90°, in-plane resolution = 4mm, matrix size = 320 x 320, field of view = 18 x 18 cm).A total of one image volume of 64 slices (3mm thickness, no interslice gap) was acquired using a pseudo-continuous flow-driven adiabatic inversion scheme during a six minute ASL scan

(TE/TR = 32.256/5500ms, flip angle (FA) = 90°, in-plane resolution = 3mm, matrix size = 64×64, field of view = 24 x 24cm).

All acquisition parameters for both studies were identical. However for the modulation study, 37 (rather than 38) slices were acquired for the phMRI and cognitive task scans as this was sufficient for whole brain coverage and prevented the loss of slices due to heating-related technical issues with the scanner experienced by other research groups.

3.6   FMRI Analysis Approach

In general, numerous whole brain volumes, each one within a 2-3 second time window, will be acquired in a BOLD fMRI experiment. Depending on the resolution applied, each volume usually contains thousands of voxels (Brett, Penny, & Kiebel, 2006), each containing a time course consisting of the fluctuations seen in the MR signal across time. FMRI data is thus "represented as a time series of three dimensional images"(Poldrack, Mumford, & Nichols, 2011), p.14. In order to generate statistical maps of the changes in brain activity, one must first subject the data to various temporal and spatial pre-processing steps in order to correct for such things as motion-related artefacts or anatomical differences between subjects. The following sections will focus on the analysis techniques employed in this thesis, all applied within the framework of the Statistical Parametric Mapping (SPM) software (version 5-update 1782 and version 8-update 4010; Wellcome Department of Cognitive Neurology, London, UK) and its associated toolboxes (see individual chapters for more detail).

3.6.1   Preprocessing

Every preprocessing step is performed at a single-subject level, in the same manner for all subjects.

Slice timing correction: During acquisition of fMRI data, the imaging volume consists of slices either acquired in a continuous manner (ascending or descending) or using an interleaved slice order. In the latter for example, the all the even slices are all acquired followed by the alternate (odd) slices. However, fMRI analysis procedures assume that all the data in one volume has been obtained from the same moment in time. Slice timing correction is thus the method used to ensure the data satisfies this assumption (Henson, Buechel, Josephs, & Friston, 1999). A common approach (and the method applied in this thesis) is to use the slice obtained mid-TR as a reference slice whose data is utilized as the standard to which all other slice data is adjusted (http://www.fil.ion.ucl.ac.uk/spm/doc/manual.pdf)

(M.A. Lindquist, 2008). This process has been shown by Sladky and colleagues (2011) to be beneficial, at the single-subject and group levels, for both event-related and block designs (Sladky et al., 2011; Van de Moortele et al., 1997).

Realignment: The integrity of fMRI data is susceptible to motion related artefacts, such as edge effects, often caused by subject head movement. For example, if a voxel is displaced from an area of the brain with low image intensity to an area with high image intensity, the subsequent statistical analysis will falsely infer that there was an increase in signal with time (M.A. Lindquist, 2008). In order to correct for the effects of head motion, each image in the time series is realigned to a reference image (usually either the first image or the mean image; (K. J. Friston, Williams, Howard, Frackowiak, & Turner, 1996)). To achieve a more accurate realignment, a two pass procedure was used in this thesis, whereby the images were initially realigned to the first image and then to their mean image (default procedure in SPM; http://www.fil.ion.ucl.ac.uk/spm/doc/manual.pdf). As part of realignment, a rigid-body transformation is applied, defined by six parameters, which translates (along x, y and z axes) or rotates (around roll, pitch or yaw) the images, minimising the positional differences between them and the target image (K. Friston, 2003b; M.A. Lindquist, 2008). The data in each image is then re-sampled to create motion corrected values (Ashburner & Friston, 2003; M.A. Lindquist, 2008).

Many a time however, movement artefacts remain post-realignment. Thus, it is common to include the six head motion parameters as nuisance regressors in the model at the single-subject stage (see section 3.5.2 for more details; (Johnstone et al., 2006)). Additional sources of motion from physiological processes such as cardiac and respiratory cycles can also be dealt with retrospectively if acquired during the scan of interest (K. Friston, 2003b; Glover, Li, & Ress, 2000; Poldrack et al., 2011). However, problems may occur if the pattern of the movement is correlated with the task paradigm; by including motion regressors at the modeling stage this may remove task-related signals as well as the unwanted motion-related signal (Poldrack et al., 2011). One should thus be very attentive to how motion is handled in the analysis stages (see chapter 4 for an example of how motion can be modeled in a different way).

There is an ongoing debate about whether realignment should be performed prior to slice timing correction or vice versa (Poldrack et al., 2011). The details of this debate are beyond the scope of this thesis, however, due to the fact that only moderate motion was present within all scans performed, realignment was performed subsequent to slice timing

correction for all BOLD imaging data in this thesis (Sladky et al., 2011). For all fMRI data reported, movement was recorded and the time-series for which the maximum detected displacement from the first scan was greater than the dimensions of one voxel (3.3mm), and rotations from the first volume over 2° in terms of pitch, roll or yaw were flagged. If movement exceeded these criteria, the decision on whether to include or exclude the scans from further analysis was based on inspection of the pattern and type of motion present.

Co-registration: Following realignment it is common to co-register the functional data to a single-subject structural scan (M.A. Lindquist, 2008). This step is performed for several reasons; (1) accurate localisation of activation can be improved if the intrinsically low resolution functional data is registered onto a high resolution anatomical image and (2) after the structural scan has been spatially normalised to the standard brain template (see normalisation section), the same normalisation parameters can be applied to the functional data if previously co-registered with the structural scan (Ashburner & Friston, 2003). In this thesis the mean image from the realignment stage was first co-registered onto the high resolution structural scan, with these registration parameters then applied to all the remaining functional time series. The co-registration step was performed using non-rigid as well as rigid body transformations due to the distortion differences between functional and structural scans (Ashburner & Friston, 2003; Studholme, Constable, & Duncan, 2000).

Spatial Normalisation: Difficulties in data integration, due to the differences in size and shape of individuals' brains, will emerge at group level analysis if no correction is applied. To ensure anatomical correspondence between brains, each subject's high resolution structural scan is thus normalised, using a combination of affine and non-linear transformations, to a standard brain template (e.g. Montreal Neurological Institute brain; (K. Friston, 2003b; M.A. Lindquist, 2008; Poldrack et al., 2011). Different normalisation methods are available, for example segmenting the white matter, grey matter and cerebrospinal fluid of the single subject's T1-weighted structural image and normalising these to the a priori tissue templates (Ashburner & Friston, 2003) is widely used. In this thesis, however, the subject's high resolution gradient echo image was used and directly normalised to the standard template. This was chosen for multiple reasons; (1) this structural scan has similar geometric distortions to the functional data (2) the processing speed of the normalisation stage is faster if this method is chosen and (3) segmentation may be more appropriate for studies where the subjects included demonstrate gross

anatomical pathology. For the studies reported in this thesis, only healthy volunteers were recruited. Furthermore, the normalisation parameters were only estimated once; a more suitable approach for repeated measures designs as the same parameters are applied to all functional images from each scanning session and run to avoid the confounding effects of drug or session comparisons by differences in normalisation parameter estimation.

Spatial Smoothing: The final step in preprocessing involves spatially smoothing (or 'blurring') the images. Although this reduces spatial resolution, smoothing is performed for multiple reasons; it (1) minimises anatomical differences between subjects left uncorrected by normalisation procedures, (2) improves the signal-to-noise (SNR) ratio for signal which extends over larger areas by removing high frequencies, and (3) renders the error in the data more normally distributed thus satisfying certain assumptions of the subsequent statistical theories applied (e.g. Gaussian random field theory; see section 3.5.4)(K. Friston, 2003b; M.A. Lindquist, 2008; Poldrack et al., 2011). Smoothing consists of convolving a Gaussian kernel with the data. The amount of smoothness applied, commonly between 4-12mm, is described by the full-width half maximum (FWHM), a measure of the width of the kernel (in statistical terms it would be referred to the standard deviation of the distribution) (K. Friston, 2003b; M.A. Lindquist, 2008; Poldrack et al., 2011). In this thesis, a smoothing kernel of 8mm for the BOLD data was chosen(Caceres, Hall, Zelaya, Williams, & Mehta, 2009).

3.6.2 Statistical Modeling: First (single-subject) Level Analysis

Following preprocessing, single subject data is analysed separately prior to being combined at the group level. Statistical analysis of fMRI data is primarily performed at the single-voxel level and is thus referred to as univariate analysis. The main aim is to generate a map of voxels whose timeseries fluctuations follow those of the experimental design to a significant degree (Matthews & Jezzard, 2004). This is achieved with the use of the general linear model (GLM) approach which, in simple terms, attempts to fit the observed data to a predicted BOLD response most commonly represented by a set of regressors (K. Friston, 2006).

When applied to imaging data, the GLM is expressed as an equation (1) whereby the observed timeseries data (Y) is described by a weighted linear combination of various explanatory variables (X) with an added error term (ε), reflecting the "residual error between the fitted model and the data" ((S. M. Smith, 2004), p.S168). C represents a constant term, which in the context of fMRI is the whole brain mean signal (K. Friston,

2006; S. M. Smith, 2004). The β (beta) term reflects an estimate of how well the particular variable (or regressor) fits the observed data (i.e. the weight assigned to each variable).

$$(1) \quad Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + c + \varepsilon$$

The β parameters are estimated with the use of the restricted maximum likelihood method (REML; for review see (Harville, 1975)), which minimises the amount of error associated with these parameter estimates as much as possible (Kiebel & Holmes, 2006).

Prior to model estimation, the explanatory variables (e.g. stimulus functions representing different psychological components of a cognitive task; see Chapter 2 for more detail) that could potentially explain part of the observed signal are convolved with a canonical HRF, typically modeled as a double gamma function (M. A. Lindquist, Meng Loh, Atlas, & Wager, 2009). This renders the stimulus function more biologically plausible and thus more likely to provide an accurate model fit (S. M. Smith, 2004). These along with the nuisance variables (e.g. motion) and constant term, which ensures at the single-subject level that the signal varies around a mean of zero, are summarised within a design matrix (see Figure 3-5). The residual error is gathered into a separate image and not included in the overall model. In order to ascertain whether the β parameter estimates are significantly different from zero, T-statistics are calculated by dividing β by its standard error (SE) and are then converted to Z-scores (S. M. Smith, 2004). Finally, in order to identify whether a regressor significantly contributes to the model several 'contrasts' can be created which, by using T- and F-tests, are used to interpret the results (Poline, Kherif, & Penny, 2003). The model estimation and statistics calculations are performed at each voxel in the brain and for every subject in preparation for group-level analysis. N.B. only the unthresholded contrasts are taken through to the second level (see section 3.5.4).
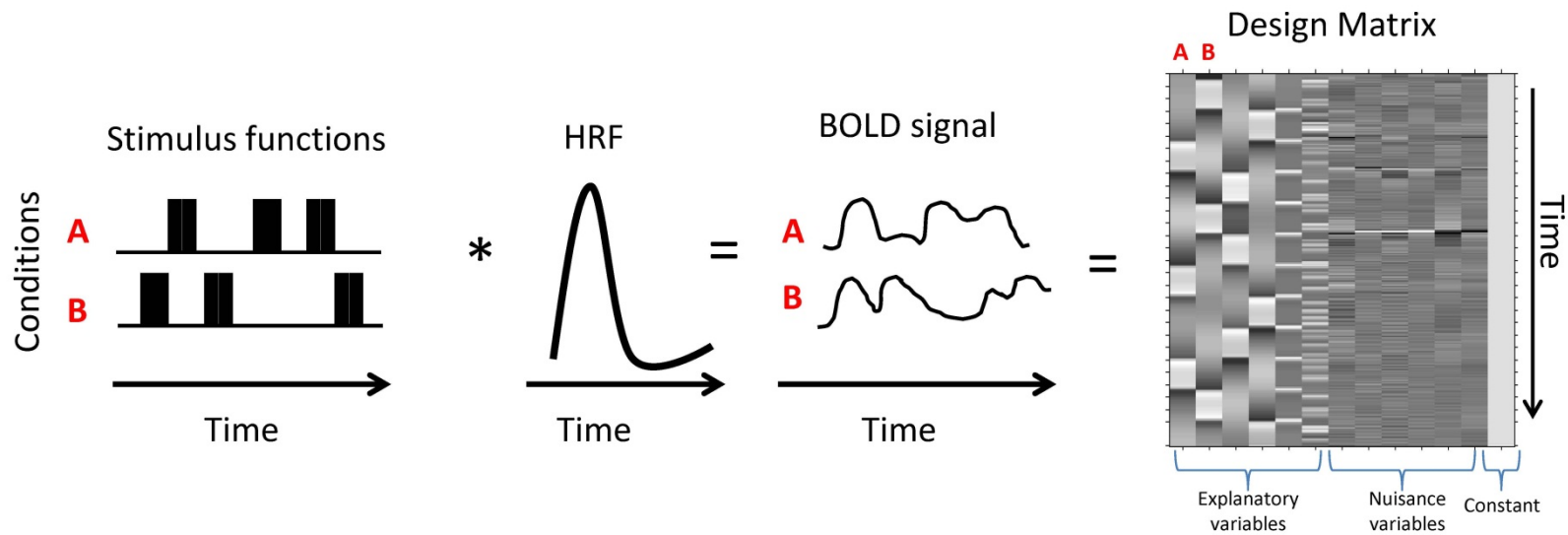
Figure 3-5 First Level Model Specification in SPM software

Adapted from Lindquist (2008). Two explanatory variables (A and B), represented as stimulus functions, are convolved with the haemodynamic response function (HRF) to generate two predicted BOLD responses. These variables are then incorporated into a single-subject design matrix along with other possible explanatory variables and nuisance variables such as motion. The mean signal (constant) is also included.

When modeling fMRI data at the first level some important considerations must be taken into account. For example, β parameter estimation can be rendered more difficult when regressors in the design matrix are highly correlated (i.e. more shared variance between regressors) and subsequently decreases the statistical power of the model (Poldrack et al., 2011). Nonetheless, ensuring that a regressor is orthogonal (uncorrelated) with respect to another is usually achieved through the experimental design itself rather than post data collection as this can lead to the arbitrary allocation of the regressors' shared variance to one particular variable (Henson, 2006). Another point to consider is how the model controls the presence of noise in the signal in the form of low-frequency drift, inevitable when acquiring BOLD data. A high pass filter is typically applied which removes all frequencies below a certain threshold, most commonly set at 128 seconds (or 0.008Hz; (Henson & Friston, 2006)). However, not all sources of noise are eliminated with the use of high pass filtering. The remaining noise can induce what is referred to as temporal autocorrelation between data points (Henson & Friston, 2006); a situation which does not satisfy the assumptions of the GLM which presumes that all data points are independent and have equal variance. The process of 'pre-whitening' is thus performed in order to remove this autocorrelation (Poldrack et al., 2011). Many of these corrections are performed as default processes in SPM with the aim to produce the most reliable β parameter estimates possible, thus these were retained while analysing the data included in this thesis.

The aim of first level analysis is to create a model which best reflects the observed BOLD fMRI timecourse, therefore minimising the amount of unexplained signal across all voxels. It is important to include all the necessary regressors which one assumes will contribute to the model; however the inclusion of redundant regressors will also lead to a decreasing number of degrees of freedom (M.A. Lindquist, 2008).

3.6.3   Statistical Modeling: Second (group) Level Analysis

Group-level analysis consists of combining the data from multiple subjects in order to increase the chance of detecting robust brain activation in response to a certain experimental paradigm. As with standard statistics, different tests can be set up at the second level such as a one-sample t-test, a paired t-test, and a repeated-measures ANOVA. Most commonly, the input to the second level analysis consists of the contrast of the parameter estimates for an effect which is of interest and the within-subject variance estimates derived from the first level analysis (although not in SPM-see below)

(Poldrack et al., 2011). However, different types of statistical approaches can be used to model the data; fixed effects, mixed effects and random effects models.

Fixed effects models only take into account within-subject variance. Thus this is the method SPM utilises to execute the first-level analysis, as at this stage only within-subject variance is available and one is only interested in subject specific effects. Fixed effects analyses can extend to the second level, however by ignoring the between-subject variance it treats all subjects as one and the same, thus it is only informative if the sample of subjects tested is the population one wishes to make inferences about (Penny & Holmes, 2006). If one wishes to generalise the findings to the population as a whole, the subjects must be treated as a random effect (i.e. having been randomly selected from the population) and this between-subject variance must be taken into account in the model. This is what is referred to as a mixed effects model which, through the inclusion of both within- and between-subject variance, allows for a more conservative but very precise estimation of the model parameters (Poldrack et al., 2011). However, in order to simplify the estimation process, SPM assumes that the within-subject variance is the same across all subjects, enabling the use of the REML estimation method (see first level analysis) which holds only if this assumption is met (Poldrack et al., 2011). Furthermore, more than one contrast of interest to be brought through to a single second level model (K. Friston, 2006) if this method is applied. This approach is described as random effects analysis in SPM and is the type of second level model employed in this thesis.

3.6.4 Thresholding and Statistical Inference

The outcome for both first and second level analyses is statistical parametric maps (SPMs) composed of T (or Z) values assigned to every voxel. Prior to obtaining these maps it is important to define an appropriate threshold at which the voxels can be said to be significantly activated (M.A. Lindquist, 2008). This significance testing occurs at every voxel in the brain, leading to a 'multiple comparison problem' (S. M. Smith, 2004). According to standard statistical theory, if the threshold of significance is set at a p-value of 0.05 (i.e. the results are considered significant if "there is only a 5% chance that the results could occur if [] the null hypothesis is true" ((A. Field, 2009), p.56)), there is also a 5% (α-level of 0.05) chance of getting a Type I error (a false positive; (A. Field, 2009)). Thus, in the context of neuroimaging, a statistical map containing 120,000 voxels will always have approximately 6000 voxels activated by chance if the voxels are considered as independent data points(Poldrack et al., 2011).

In standard statistics, to adjust for the amount of comparisons being made, a Bonferroni correction is typically applied, whereby the α-level is divided by the number of univariate tests being performed (S. M. Smith, 2004). In comparison, fMRI analysis employs a different method of correction based on Gaussian random field theory (RFT) as the Bonferroni correction is viewed as overly conservative. RFT enables the selection of a corrected threshold while taking into account the spatial correlations ('smoothness' or 'non-independence') inherent in the imaging data (K. Friston, 2006). In RFT, the greater the smoothness of the data (intrinsic smoothness of the data combined with the effect of the preprocessing smoothing kernel), the less conservative the correction must be as the number of resolution elements (resels) are decreased (Poldrack et al., 2011). The concept of a 'resel' was introduced by Worsley and colleagues (Worsley, Evans, Marrett, & Neelin, 1992) and describes a 'virtual voxel' whose size is defined by the smoothing FWHM applied in the preprocessing stages (Poldrack et al., 2011).

However, the number of resels do not represent the number of independent observations in the data. In other words the statistical correction of performing multiple tests cannot be completed by dividing the desired α-level by the number of resels. Instead, RFT applies the correction through the calculation of what is referred to as the Euler characteristic (EC) which in fact does depend on the number of resels in the data (Brett et al., 2006). This characteristic is an assessment of the topology of the data; it can be viewed as the number of activated regions present after a certain T (or Z) threshold is set (Brett et al., 2006; de Haan & Rorden, 2011). At high thresholds, the EC will be expected to be either 0 or 1 and is thus also considered as being proportional to the probability of finding an above threshold activation in the statistical map (Brett et al., 2006). Therefore, in order to minimise the number of false positives in the image an EC value of 0.05 was deemed appropriate (de Haan & Rorden, 2011). A T (or Z) threshold that gives an EC value below 0.05 (which is equated to the corrected FWE α-level available in SPM) is thus selected, as this suggests that the likelihood that one or more clusters are present at this threshold is below 0.05 (Brett et al., 2006). For a review on the more complex mathematical procedures involved in calculating the EC, please refer to (Nichols & Hayasaka, 2003). The use of a threshold corrected for multiple comparisons is ideal, although the level of correction will depend on one's prior experimental hypotheses and if they are anatomically constrained or unconstrained (please see individual chapters for the levels of correction chosen).

FMRI whole-brain analysis offers three different levels of inference. Voxel-level inference determines the level of significance at each and every voxel in the brain, in other words the activation evident in that voxel is deemed to be significant if it exceeds a certain statistical threshold (i.e. its p-value is below the set α-level) (see Figure 3-6; (Poldrack et al., 2011). This level of inference is very specific and may only be useful if one is interested in very localised brain activations. On the other hand, cluster-level inference is slightly more intuitive due to the spatial nature of the data and the anatomy of the human brain. Cluster inference defines the significance of a region by taking into account the number of contiguously activated voxels in a particular area (see Figure 3-6; (K. Friston, 2006; Poldrack et al., 2011). Finally, set-level inference takes into account the number of clusters present in the statistical map, merely informing you, if the p-value is significant, that there are a larger number of clusters present than likely to happen by chance. This level of testing is not widely used due to its non-specificity and lack of 'localising power' (Poldrack et al., 2011).
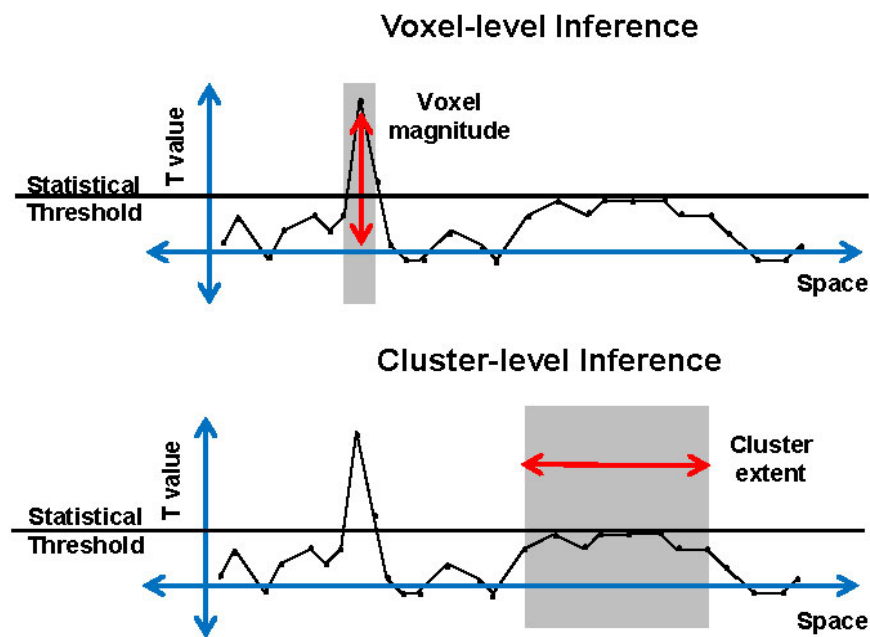
Figure 3-6 A schematic demonstrating voxel- and cluster-level inference

Adapted from Poldrack et al., (2011). TOP: At a specified statistical threshold two voxels are deemed to be significant due to their T-value exceeding this number. BOTTOM: At the same statistical threshold none of the voxels in the cluster are significant, but the cluster itself is still deemed significant due to the number of voxels present in that area (cluster extent).

In addition to whole-brain analyses, region of interest (ROI) analysis is a principled and sensitive approach to investigate pre-existing hypotheses about regional brain effects (Mitsis, Iannetti, Smart, Tracey, & Wise, 2008). In this thesis, a priori anatomical hypotheses were tested using a pre-specified set of ROIs selected on the basis of previous findings in the ketamine literature or regions known to be recruited by distinct cognitive tasks (see individual chapters for details). Importantly, a pertinent consideration when testing multiple ROIs is that of correction for multiple comparisons. While the typical bonferroni method results in the prevention of false positives, this approach is highly conservative and may mask true effects (Feise, 2002). In addition, the low-dose of ketamine used and the known diffuse nature of its effects suggest that a less stringent method may be appropriate. Thus, in this thesis, the ROI findings were not adjusted for the number of ROIs tested. This strategy was chosen to ensure that a broad understanding of the pattern of ketamine-induced effects was achieved, with the acknowlegment that a proportion of the effects seen may be merely due to chance. Without question, one must still "regard all findings as tentative until they are corroborated"((Feise, 2002), p.3), and thus the interpretation of the ROI results have been made within this framework.

3.7   Reliability Analysis

The intraclass correlation coefficient (ICC) is a widely used and accepted measure of test-retest reliability (McGraw & Wong, 1996; Shrout & Fleiss, 1979). Although the ICC has classically been described in the context of consistency or agreement between ratings given by different judges, it has also been used to assess the reliability of ratings across different testing sessions and more recently to assess the reliability of imaging methods over time (Atri et al., 2011; Bennett & Miller, 2010; Caceres et al., 2009; Freyer et al., 2009). The latter situation is relevant to this thesis as the reliability of the brain response to four different 'tasks' (phMRI, ASL, PAL or N-BACK) across two sessions is calculated (see Chapters 4, 5, 6, and 7); essentially whether the responses on the first session predict those on the second. Generally, the ICC is viewed as the ratio between "the variance of interest over the sum of the variance of interest plus error" ((Shrout & Fleiss, 1979), p.420), with ICC estimates ranging from 0 (no reliability) to 1 (complete reliability), but see (Lahey, Downey, & Saal, 1983) for a discussion on why ICC values will sometimes extend beyond this range; (Weir, 2005)). Different guidelines exist for the interpretation of the ICC (Bennett & Miller, 2010). However as a general guideline, in this thesis, an ICC

value of less than 0.40 is taken to be poor, 0.40-0.59 as fair, 0.60-0.74 as good and values exceeding 0.75 as excellent (Fleis, levin, & Paik, 2003). These terms should be interpreted with caution as they do not take into account the confidence intervals of the ICC measure.

Various different versions of the ICC exist (McGraw & Wong, 1996), however this discussion will include only the main ICCs summarised by Shrout & Fleiss (1979) termed ICC (1,1), ICC (2,1) and ICC (3,1). All of these types of ICCs are derived from the variance estimates of a repeated measures ANOVA performed on the data (A. Field, 2005). This is referred to as either a one- or two-way, random or mixed effects ICC model, depending on the sources of variance included and how the effects are defined (Weir, 2005). In the subject-judge paradigm described by Shrout & Fleiss (1979), ICC (1,1) represents a one-way random effects model and would be used in a study in which each subject is rated by a different set of judges, randomly sampled from the population, and thus the ratings cannot be separated into two distinct groups (i.e. creating unordered pairs of data). If this is viewed in the context of the reliability study described in this thesis, whereby every participant was given the same task on two different sessions, the use of this model would fit a hypothetical situation where the session from which the subject data is derived is unknown, again preventing the data to be assigned to two separate groups (one with data from session 1 and one with data from session 2). Thus, the possibility of making a prediction about whether the response on session 1 predicts the response on session 2 is impossible and only a more general statement about reliability across time is applicable.

ICC (2,1) represents a two-way random effects model and would be used in a study in which each subject is rated by the same set of judges, randomly sampled from the population. Again, in the context of the reliability study, the use of this model would fit a hypothetical situation where, although it was possible to assign the responses of the different subjects to specific sessions, the session effect is still considered as a random effect if their ordering is irrelevant to the experimental question being asked. This is in contrast to ICC (3,1) which represents a two-way mixed effects model because it assumes that the judges are not randomly selected and that they are chosen because they are the only ones of interest. In other words, the judges are considered to be a fixed effect. In the reliability study, the sessions would therefore also be considered as a fixed effect, whereby changing their order "would significantly affect the research question" ((A. Field, 2005)p. 11).

In all three ICC models the subject factor is considered as a random effect as they are assumed to be randomly sampled from the population (A. Field, 2005). Thus ICC (1,1) and (2,1) are purely random effects models with both subject and session (or judge) characterised as a random effect, whereas ICC (3,1) is referred to as a mixed effects model as it includes subjects as a random effect but session as a fixed effect. The choice of using a random or a mixed effects model depends on the design of the study in question and on the type of inferences one wishes to make about the generalisability of the reliability results (Shrout & Fleiss, 1979; Weir, 2005).

Models (1,1), (2,1) and (3,1) differ on which sources of variance derived from the respective ANOVA models are included in the ICC calculation (see Figure 3-7). Due to the design of the study to which ICC (1,1) is applied (i.e. unordered pairs of data, as described above), only one source of variance, the subject variance, is included in the estimation of the ICC value (see Figure 3-7; (A. Field, 2005; Weir, 2005). Hence ICC (1,1) is referred to as a one-way model. On the other hand, the studies to which ICC (2,1) and ICC (3,1) are applied are designed in such a way that both the variance associated with subject and session (and therefore also the residual variance) can be calculated. However, as figure 3-6 shows, the ICC (3,1) equation does not include the session variance in its calculation of the ICC (although this is not always the case, see below). By ignoring the between-session variance, the ICC is said to represent the *consistency* in the response across sessions, essentially equivalent to whether the subjects maintain the same rank order in session 1 and 2 irrespective of their change in response between sessions (Caceres et al., 2009; A. Field, 2005). Absolute agreement in reliability occurs when both subject variance and session variance are included in the calculation of the ICC, as this takes into account not only the subjects rank order but also whether their response values remain the same across sessions. It is possible for ICC (3,1) to be calculated using the equation for ICC(2,1) but retain session as a fixed effect, this would thus enable the calculation of an absolute agreement in the context of a fixed effects analysis (McGraw & Wong, 1996).

$$ICC(1,1) \qquad \frac{BMS - WMS}{BMS + (k-1)WMS}$$

$$ICC(2,1) \qquad \frac{BMS - EMS}{BMS + (k-1)EMS + k(JMS - EMS)/n}$$

$$ICC(3,1) \qquad \frac{BMS - EMS}{BMS + (k-1)EMS}$$

Figure 3-7 ICC equations

Taken from Shrout & Fleiss (1979). BMS = Between-subject Mean Squares, WMS = Within-subject Mean Squares, JMS = Between-judge (session) Mean Squares, EMS = Residual Mean Squares, k = number of sessions, n = subject number.

Although different methods have been used in reliability studies, such as the Pearson product-moment (r) correlation or coefficient of variation (CV) , the former "measures association, not agreement" ((Bartko, 1991),p.484) and the latter cannot estimate reliability for areas of zero or low activation (Bennett & Miller, 2010; Caceres et al., 2009; Jahng et al., 2005; Jiang et al., 2010). In contrast, the aim of the reliability analyses in this thesis was to "determine areas of group activation [in response to a certain drug or task] in the first session then ask if, in the same region, the rank order of subject activations will be preserved in a subsequent session"((Caceres et al., 2009), p.758). Importantly, it is not the robustness of the effect that is essential to reliability as non-significant activations can have greater reliability than the areas showing a larger effect (Caceres et al., 2009). In order to appropriately satisfy the aim of the reliability assessments and because a fixed (or mixed) effects reliability analysis is deemed suitable if it precedes a larger study wherein the drug/task under question is being used (Weir, 2005), ICC (3,1) measuring consistency was applied in this thesis.

Both ROI and voxelwise reliability estimates were calculated using ICC(3,1), corresponding to a two-way, mixed-effects model with subject as a random effect and session as a fixed effect, since each participant had two scans which were clearly ordered (Caceres et al., 2009; McGraw & Wong, 1996; Shrout & Fleiss, 1979). Estimates of ICC(3,1) were calculated in two ways. First, ICC values were estimated from the mean beta parameter estimates in each ROI. These ICCs were calculated using the ICC option in SPSS 17.0 (this was done only in Chapter 4). Second, voxelwise ICC values were

calculated using the ICC Matlab toolbox (Caceres et al., 2009), resulting in distributions of ICC values within the specified region (see Chapter 4,5,6, and 7). Median ICC values were then calculated for the whole brain, for voxels significantly responding to the either the ketamine challenge or the cognitive task network at the group level, and within ROI (see specific chapters on the details of which brain network or ROIs were assessed). These were evaluated by examining the histograms of ICC values within each specified set of voxels (only in Chapter 4) and summarised as the median and the 99% confidence intervals.

Chapter 4 Test-retest Reliability and Modulation of the Effects of Ketamine on the BOLD MRI Response

As described in Chapter 1, more research is needed in order to determine the validity and utility of the ketamine response with regards to the investigation of glutamatergic function in healthy humans. This chapter describes the resting BOLD response to ketamine, and the reliability of this response across sessions. In addition, the modulatory effects of lamotrigine and risperidone were also investigated. Overall BOLD was shown to be sensitive and to reliably detect the effects of ketamine. Both lamotrigine and risperidone were found to attenuate ketamine-induced increases in BOLD response in a number of regions including the cingulate and thalamus. These findings have implications for the use of BOLD in future phMRI studies investigating glutamatergic dysfunction.

4.1   INTRODUCTION

4.1.1   Ketamine as a Probe of the Glutamate System

The effects of uncompetitive antagonists at the N-methyl-D-aspartate (NMDA) receptor, such as ketamine and phencyclidine (PCP), have led to a growing appreciation of the potential role of glutamatergic abnormalities in psychiatric symptomatology ((Javitt, 2004); Luby et al. (1959)). Numerous studies have shown that ketamine induces behavioural and cognitive perturbations in both experimental animals and healthy human volunteers (Cilia, Hatcher, Reavill, & Jones, 2007; Gilmour et al., 2011; R. A. Honey et al., 2004; Krystal et al., 1994). At sufficiently high doses (1500 – 2000ng/ml plasma levels in humans; (Langsjo et al., 2005)) ketamine acts as a surgical anaesthetic, whereas at sub-anaesthetic doses (ca. 100-200ng/ml) it induces symptoms including thought disorder, altered integration of sensory information, and disruption in WM performance; all characteristics likened to those observed in patients with schizophrenia (Adler et al., 1999; R. A. Honey et al., 2003; H. L. Morgan et al., 2011). At still lower doses (ca. 50ng/ml), the psychotomimetic symptoms are seen to a lesser extent (Krystal et al., 1994). Given the clear limitations of using one compound to 'model' a complex disorder like schizophrenia, ketamine is utilised as a pharmacological probe of one neurochemical component, the glutamatergic system, which is relevant for psychiatric conditions where dysfunction of this system may be evident.

4.1.2   Imaging the Glutamate System

Multiple imaging techniques have been shown to be sensitive to pharmacologically-induced changes in brain physiology following acute NMDA antagonist administration in both rodents and humans (Corlett et al., 2006; G. E. Duncan et al., 2000; Gozzi, Herdon, et al., 2008; Vollenweider, Leenders, Oye, et al., 1997). These provide neuroimaging correlates of the behavioural and subjective deficits induced by the compound. Moreover, a number of studies have demonstrated that the central haemodynamic or metabolic changes evoked by acute NMDA antagonist administration can be reversed by pharmacological pre-treatment, in particular by atypical antipsychotics and group-II metabotropic glutamate receptor (mGluR-II) activators (Chin et al., 2011; Dedeurwaerdere, Wintmolders, Straetemans, Pemberton, & Langlois, 2011; Gozzi, Large, et al., 2008; Hackler et al., 2010). These findings parallel work in experimental animals demonstrating that mGluR-II agonists block NMDA receptor antagonist evoked glutamate efflux (Lorrain et al., 2003; Moghaddam & Adams, 1998). Thus, with increasing interest in the development of therapeutics designed to ameliorate glutamatergic dysfunction, the neuroimaging signal evoked by compounds such as ketamine provides a potential translational biomarker, providing a physiologically-relevant pharmacodynamic signal to confirm "mechanistic engagement" of novel therapeutic compounds and inform dose selection for subsequent studies (A. Schwarz & Tauscher, 2011). However, to enable the use and verify the validity of this imaging biomarker in within-subjects studies designed to determine the effects of modulatory compounds, further research is needed in terms of both the assessment of reliability across sessions of the ketamine brain response and the modelling approach taken.

As described in Chapter 1, early studies demonstrated that sub-anaesthetic ketamine administration increased glucose metabolism and CBF in areas such as the frontal, cingulate, thalamic and insular areas in both animals and healthy volunteers (G. E. Duncan, Leipzig, et al., 1998; G. E. Duncan, Moy, et al., 1998; Holcomb et al., 2001; Langsjo et al., 2003; Langsjo et al., 2004; Vollenweider, Leenders, Scharfetter, et al., 1997). Breier and colleagues (1997) found increased FDG uptake in the frontal cortices alone and found that these were correlated with ketamine induced changes in conceptual disorganisation. Research into drug related changes in brain responses has increasingly turned to MRI methods, motivated by the absence of ionising radiation and the superior temporal resolution. In particular, pharmacological MRI (phMRI) approaches enable the direct effect of an administered compound on "resting" brain function to be assessed via

the haemodynamic response in the absence of an experimental task (Breiter et al., 1997; Leslie & James, 2000).

4.1.3 Resting State BOLD pharmacological MRI: The Ketamine Response

PhMRI experiments commonly use the BOLD contrast to detect signal changes in a T_2*-weighted MRI time series covering both pre- (baseline) and post-compound administration conditions in a continuous acquisition (Shah & Marsden, 2004; Steward et al., 2005). As described in Chapter 1, animal phMRI experiments with ketamine have shown increases in BOLD signal in frontal, cortical, hippocampal and thalamic regions (Littlewood, Jones, et al., 2006), consistent with the previously described 2-DG and PET work. In healthy humans, ketamine- induced BOLD phMRI signal increases in regions including the mid-posterior cingulate, thalamus and anterior temporal cortical areas, along with a decreased BOLD signal observed in the ventromedial prefrontal cortex (Deakin et al., 2008). Manifestations of dissociative and psychotic subjective effects, assessed by the CADSS and the Brief Psychiatric Rating Scale (BPRS) respectively were found to be correlated with changes in BOLD activation in the same study, supporting the connection between NMDA receptor blockade and psychotomimetic symptoms.

4.1.4 Modelling Approaches to phMRI

The temporal phMRI response to acute pharmacological challenge in the brain comprises a relatively low-frequency signal change through time. As described in Chapter 2, multiple methods have been used to model phMRI BOLD responses. For example, time courses of independently measured behavioural or biological (e.g., subjective scores or neurotransmitter concentration) parameters can be used as a temporal correlate for the central phMRI signal changes; however, this approach assumes that the functional imaging changes closely parallel the reference time course and that the latter is available at a sufficient temporal resolution and signal-to-noise ratio to enable such an analysis. The more recent time-series approach applied in the Deakin et al., (2008) study to describe the ketamine BOLD response does not require these parameters but uses a block analysis method whereby the data is modelled as one-minute time-bins; however, this technique may also confer the same disadvantage with regards to temporal resolution.

Alternatively, the phMRI response can be quantified within a general linear model (GLM) framework using a regressor (signal model) chosen pragmatically based on independent data with the same pharmacological compound. This approach yields a single numeric estimate of the amplitude of response to the ketamine challenge at each voxel, analogous

to standard general linear model (GLM) functional MRI (fMRI) SPM analysis (see Chapter 3). This approach has several advantages: it enables more straightforward group analyses, the calculation of simple ROI-level summary measures and facilitates the combination of the phMRI data with behavioural, pharmacokinetic and other non-imaging variables. However, several factors can influence the measured BOLD phMRI time course and may lead to deviations from the hypothesised model: (1) phMRI often requires extended scan times compared to standard fMRI paradigms, and is thus more susceptible to artefacts such as those generated by head motion and scanner drift; (2) the pharmacological agent administered can cause physiological and/or subjective effects that may also lead to additional head motion; (3) in phMRI data there is often a degree of variation in the shape (temporal profile) of the response between subjects and brain regions (A. J. Schwarz, Whitcher, Gozzi, Reese, & Bifone, 2007). The temporal analysis of ketamine BOLD phMRI time series data within a GLM framework thus relies on the choice of an appropriate design matrix to model the phMRI response of interest and also account for drift, head motion and potentially for plausible variations in temporal profile. Moreover, the utility of phMRI in understanding the effects of ketamine, and its relationship to subjective, pharmacokinetic or genotype profiles, depends on the within-subject reliability, between-subject reproducibility and dose sensitivity of the computed BOLD response amplitude. Assessing the reliability of the ketamine response across sessions is thus essential for (1) its application in repeated measures designs and (2) the degree of confidence with which it is used to understand the modulatory influences of other compounds.

Thus the aims of the first part of this chapter were to (1) replicate the BOLD phMRI response to ketamine described by Deakin et al., (2008); (2) identify a ketamine dose that produces a robust BOLD response whilst limiting its psychotomimetic side effects (for use in studies with a focus on the cognitive effects of the drug e.g. Chapters 6 and 7); (3) assess the reliability of the ketamine response in terms of both subjective ratings and BOLD response; (4) identify a modelling approach suitable for repeated-measures interventional studies. To satisfy these aims, two different doses of ketamine (see Chapter 3) were administered to healthy volunteers during a resting state BOLD scan on two separate occasions. Due to the ubiquitous nature of glutamatergic projections in the human brain and previous imaging studies of the ketamine response, a relatively diffuse change in BOLD signal was expected, although stronger localised changes were hypothesised in areas such as the cingulate, thalamus and frontal-temporal cortices. To

test these predictions and the reliability of the response in a principled manner, an ROI-based approach was taken with a focus on anterior, posterior and subgenual cingulate and the thalamus. These ROIs were chosen on the basis of their sensitivity to the effects of ketamine determined by previous studies and the high concentration of NMDARs in these areas (Deakin et al., 2008; Holcomb et al., 2001; Langsjo et al., 2003; Olney & Farber, 1995a). Test-retest reliability of the estimated BOLD response in these ROIs was tested in a range of temporal design matrices comprising different combinations of nuisance regressors designed to model shape variance, linear drift and head motion. Reliability of subjective ratings was also assessed.

4.1.5 The Modulatory Effects of Risperidone and Lamotrigine on the Glutamate System

The second part of this chapter attempts to evaluate the sensitivity of the ketamine-induced BOLD response to pre-treatment with compounds hypothesised to modulate glutamate transmission, lamotrigine and risperidone. Thus these were administered in conjunction with ketamine during a resting-state BOLD scan.

Lamotrigine is a use-dependent (i.e. has no effect on spontaneous neurotransmitter release) inhibitor of voltage-gated sodium and calcium channels (Large et al., 2005; Messenheimer, 1995) and, in animals, has been shown to inhibit the stimulated release of glutamate in brain areas such as the prefrontal and cingulate cortices (Idris et al., 2005) and hippocampus ((Ahmad, Fowler, & Whitton, 2004); see Figure 4-1). In experimental animals, lamotrigine and metabotropic glutamate mGlu2/3 receptor agonists or potentiators, and the antipsychotics clozapine and olanzapine, have all been found to attenuate the effects of ketamine or phencyclidine on markers of brain activity (G. E. Duncan, Leipzig, et al., 1998; G. E. Duncan et al., 2000; Gozzi, Large, et al., 2008; Hackler et al., 2010; Lorrain et al., 2003). In addition, the BOLD phMRI response to ketamine was attenuated by pre-treatment with a single dose of lamotrigine (Deakin et al., 2008). Ketamine-induced increases psychotomimetic symptoms were also reduced by pre-treatment with lamotrigine, a finding consistent with a previous behavioural study (Anand et al., 2000). Given that lamotrigine inhibits the release of glutamate, it was concluded that both symptoms and the change in BOLD signal evoked by ketamine were due to an increase in glutamate release. This is consistent with rodent studies showing increased glutamate efflux in the prefrontal cortex upon acute administration of phencyclidine and ketamine (Lorrain et al., 2003; Moghaddam et al., 1997); interestingly, both of these

studies also demonstrated reversal of this glutamate efflux by pre-treatment with mGlu2/3 agonists, presumed to attenuate pre-synaptic glutamate release. Additionally in humans, the link between ketamine administration and cortical glutamate levels has recently been confirmed using MRS of the anterior cingulate gyrus (J. M. Stone et al., 2012). The aims of the second part of this chapter were thus to (1) replicate the attenuation of the ketamine-induced BOLD phMRI signal changes using lamotrigine seen in the Deakin et al., (2008) study and (2) further validate the modulation of the ketamine-induced BOLD signal changes using an existing anti-psychotic with a mechanism likely to modulate glutamate release.
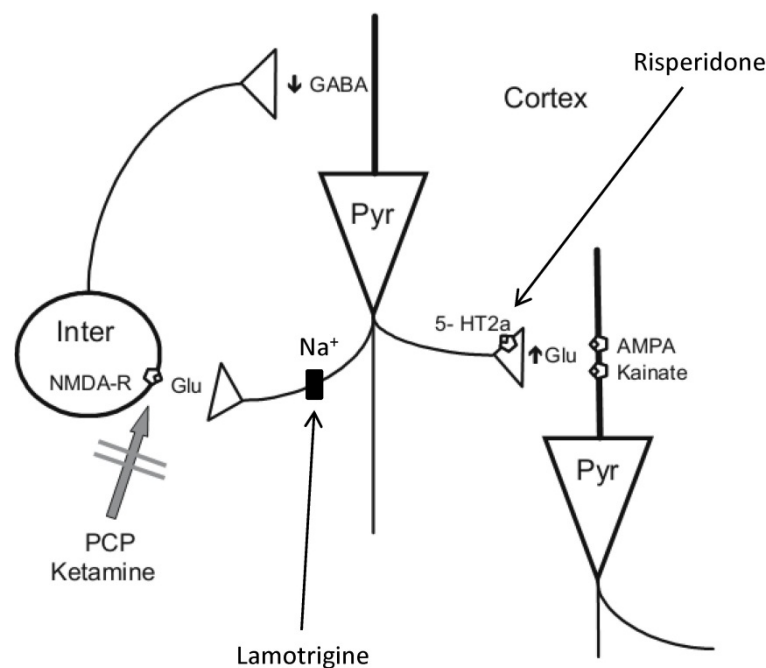
Figure 4-1 Diagram describing the potential mechanisms of action of ketamine, lamotrigine and risperidone

Adapted from Large et al., (2005). (1) Ketamine blocks the NMDAR leading to a decrease in GABA release from interneurons (Inter) and subsequent increased glutamate (Glu) release from pyramidal (Pyr) neurons; (2) lamotrigine and risperidone antagonise sodium (Na$^+$) channels and 5HT2A receptors respectively, located on pyramidal neurons, thus reducing Glu release.

The atypical antipsychotic risperidone, is a serotonin 5HT2A and dopamine D2 receptor antagonist, whose effects are thought to be primarily mediated through its antagonistic actions at 5HT2A due to the drug's higher affinity and cortical occupancy of this receptor (Farde et al., 1995; Kapur & Lecrubier, 2003; Nyberg, Eriksson, Oxenstierna, Halldin, & Farde, 1999; Nyberg, Farde, Eriksson, Halldin, & Eriksson, 1993). 5HT2A agonists have

been found to increase glutamate release in the PFC (Aghajanian & Marek, 2000), suggesting that 5HT2A antagonists may attenuate NMDAR antagonist-induced glutamate release in cortical areas ((Adams & Moghaddam, 1998; Large, 2007); see Figure 4-1), in turn contributing to therapeutic efficacy in psychoses (Large, 2007). It is thus possible to predict that risperidone will attenuate the ketamine BOLD response through its effects as a 5-HT$_{2A}$ antagonist (H. Y. Meltzer et al., 2011). In addition to its serotonergic effects, risperidone has high affinity for dopamine D2 receptors, which may have an impact on its interaction with ketamine. However, studies using selective D2 antagonists, such as haloperidol or raclopride have failed to demonstrate a modulation of the effects of ketamine or PCP (Gozzi, Large, et al., 2008; J. H. Krystal et al., 1999), suggesting that any modulation of the ketamine response seen with risperidone is unlikely to be via a D2 mechanism.

In order to test prediction that both lamotrigine and risperidone will attenuate the BOLD response to ketamine, modulation of this response was assessed in a separate cohort of volunteers within ROIs including the anterior, posterior and subgenual cingulate, thalamus, and hippocampus based on the peak BOLD response in the reliability study. Whole-brain analyses were also conducted in order to detect any treatment effects outside of the ROIs.

4.2   METHODS

Please refer to the relevant sections in Chapter 3 for details on the participants, experimental design, infusion protocol and image acquisition. All image analysis described below was conducted within SPM 5 (version 5-update 1782: Wellcome Department of Cognitive Neurology, London, UK).

4.2.1   Reliability Study

Data were acquired from 10 healthy male participants on two separate occasions, at least one week apart.

4.2.1.1   Subjective Ratings Analysis

Subjective effects were assessed at three time points:

- *Pre-ketamine*: outside the scanner, approximately 120 minutes prior to start of ketamine administration;

- *Ketamine*: outside the scanner, immediately following cessation of the ketamine infusion (participants instructed to answer questions with regards to their experience during the BOLD phMRI scan);

- *Post-ketamine*: outside the scanner, approximately 60 minutes following cessation of the ketamine infusion.

To assess such effects the following instruments were used; (1) Visual Analogue Scales (VAS; 16 scales) summarised into two factors (Alertness and Tranquility) measuring mood and subjective well-being (Bond, James, & Lader, 1974a, 1974b; Herbert, Johns, & Dore, 1976); (2) the Psychotomimetic States Inventory (PSI; 48 items), consisting of six subscales (Delusional Thinking, Perceptual Distortion, Cognitive Disorganisation, Anhedonia, Mania and Paranoia) (O. J. Mason et al., 2008); and (3) The Clinician-Administered Dissociative States Scale (CADSS; 19 subjective items), consisting of three subscales (Depersonalisation, Derealisation and Amnesia) (Bremner et al., 1998). The PSI and CADSS scales have previously been shown to be sensitive to pharmacologically-induced psychotomimetic effects (Deakin et al., 2008; O. Mason et al., 2009).

All subjective ratings data analysis was conducted in SPSS 17.0 and 20.0 (www.ibm.com/spss). Initial data screening was performed in order to assess whether assumptions for parametric analysis were met. Accordingly, exploratory analysis and Shapiro-Wilk tests were completed. PSI and CADSS data were found to violate the assumptions, thus logarithm (base10) transformations were applied. Where transformed data still did not satisfy the assumptions of parametric analysis non-parametric tests were applied to the data. VAS data were found to be normally distributed, thus parametric tests were used. Tests were applied to the subscale and total scores of each questionnaire.

Friedman's ANOVA was applied to the PSI and CADSS data to test whether there was an effect of time-point (pre-ketamine, ketamine and post-ketamine). Post-hoc Wilcoxon signed-rank matched pairs tests were then used to determine which time-points differed and also to test whether an effect of session was evident. Mann-Whitney tests were used to test for dose effects. The 16 scales of the VAS were reduced to two summary factors of alertness and tranquility for analysis (Herbert et al., 1976). VAS data were analysed using a mixed factorial repeated measures ANOVA with session (session 1 and session 2), summary factors (Alertness and Tranquility) and time point (pre-ketamine, ketamine and post-ketamine) as within-subject factors and dose (50ng/ml and 75ng/ml) as the

between-subject factor. Significant main effects and interactions were interpreted using post-hoc pair-wise comparisons with full Bonferroni correction.

Reliability analyses for the subscale and total scores of each questionnaire were calculated using the ICC corresponding to a two-way, mixed-effects model with session as a fixed effect and subject as a random effect (Shrout & Fleiss, 1979).

4.2.1.2 Image Analysis

4.2.1.2.1 Preprocessing

Slice timing correction and two-pass realignment of the time series image volumes was followed by co-registration to the high resolution gradient echo image. For each participant the high resolution image was used to determine the parameters for spatial normalisation to the Montreal Neurological Insitute (MNI) EPI template image supplied with SPM. The same parameters were applied to the time series of 450 images. Spatial smoothing (8mm FWHM Gaussian kernel) was then applied to the spatially normalised images. Due to the effects of ketamine being recorded over a 10 minute period, a high pass filter of 1200s was applied to the data to minimise the influence of very low frequency noise present in fMRI BOLD data without unduly distorting the ketamine response profile.

4.2.1.2.2 phMRI Model: First-level Regressor Definition

Gamma variate First-level modelling was performed in a GLM framework and the phMRI response to ketamine was modelled by a gamma variate (GV) regressor, defined using the following equation:

$$y(t) = \left(\frac{t}{t_{max}} \right)^{b.t_{max}} e^{(t_{max}-t)} . b \qquad (1)$$

where t_{max} (set to 240 seconds) relates to the time of peak amplitude and b (set to 0.01) is a 'shape' parameter. This was preceded by a flat baseline modelling pre-infusion. The ketamine signal model evaluated here was based on the results of the previously published ketamine phMRI study in humans (Deakin et al., 2008). The resulting curve decreases to 50% of its maximum amplitude at the end of the measured post-infusion time period (10 min), thus representing a middle ground between more peaked responses that return close to baseline within 10 minutes and more sustained responses that approach a plateau. The

beta images from the contrast of this first regressor were used in the reliability and group-level analyses.

Shape Capturer Because a degree of variation in the temporal profile of the phMRI response shape was anticipated, potential shape-variance was modelled using the method described in Pendse et al. (2010). Variations in both peak response time and shape were modelled as instances of equation (1) with b ranging from 0.002 to 0.02 while t_{max} decreased from 360s to 120s. The shape-capturer (SC) regressor was derived as the first component of a singular value decomposition of the above set of varied responses (Pendse et al., 2010).

Motion Head motion during MRI data acquisition can be a serious confound in interpreting brain activation appropriately. In phMRI experiments, this may be exacerbated by the lengthy scan times and possible physiological and/or subjective effects following administration of a pharmacological compound. Conventionally in fMRI analysis, effects of motion are dealt with in three ways: (a) realignment of each image volume in the time series to a target volume, using a rigid body transformation with six parameters (see Chapter 3) (Johnstone et al., 2006; M.A. Lindquist, 2008; Oakes et al., 2005); (b) exclusion of time series with excessive motion from further analysis; and (c) inclusion of the six head motion traces as nuisance regressors in the temporal design matrix. Method (a) was applied to the data as part of pre-processing (see above) and (b) was assessed as part of the data quality control. The inclusion of the six motion traces into the design matrix (c) was also evaluated as a mechanism to absorb any possible confounding signal changes due to macroscopic head motion during the scan.
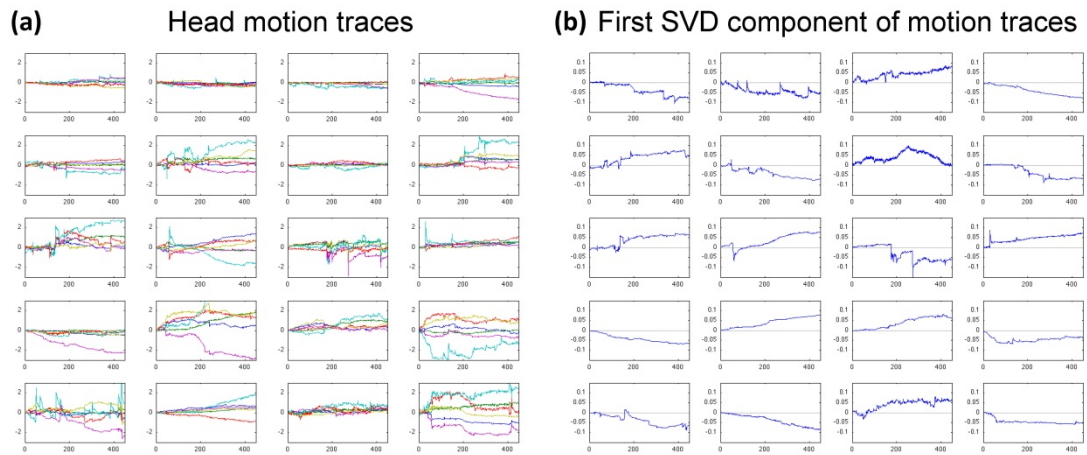
Figure 4-2 Motion Traces

(a) Head motion traces from all sessions, with rotational motion transformed into translational units. (b) The first SVD component of the six motion traces from each session.

However, inclusion of these six motion parameters in the design matrix may compromise the detection of the signal of interest, for example in circumstances where motion is highly correlated with the experimental design (in this case sharing low-frequency components in the signal (Johnstone et al., 2006; R. Liao, McKeown, & Krolik, 2006). Therefore, as a second strategy to model potential motion-induced signal changes, the performance of a single motion confound regressor was evaluated, designed to capture the predominant variance in head movement during the scan and derived as follows. Singular value decomposition (SVD) was performed on the six motion regressors (scaled to equivalent "translation" units) and the first SVD component was used as a single nuisance regressor capturing the main head motion variance (see Figure 4-2).

4.2.1.2.3 Design Matrices Evaluated

Six different design matrices were evaluated (Figure 4-3). Head motion was modelled in three ways: (1) no motion regressors, (2) all six head motion traces from the realignment output, and (3) the first SVD component derived from the normalised motion traces. For each such combination, the effect of adding the SC regressor was also evaluated. In addition, a linear drift term was included in all six models. The resulting six design matrices were as follows (see Figure 4-3):

- Model (1) includes the signal model and the linear drift regressor,

- Model (2) includes the signal model, the linear drift and the shape capturer (SC) regressor,

- Model (3) includes the signal model, the linear drift and the 1st SVD component of the motion regressors,

- Model (4) includes the signal model, the linear drift, the SC and the 1st SVD component of the motion regressors,

- Model (5) includes the signal model, the linear drift and the six motion regressors,

- Model (6) includes the signal model, the linear drift, the SC and the six motion regressors.

For simplicity, the models will be referred to by their numbers in the following text.
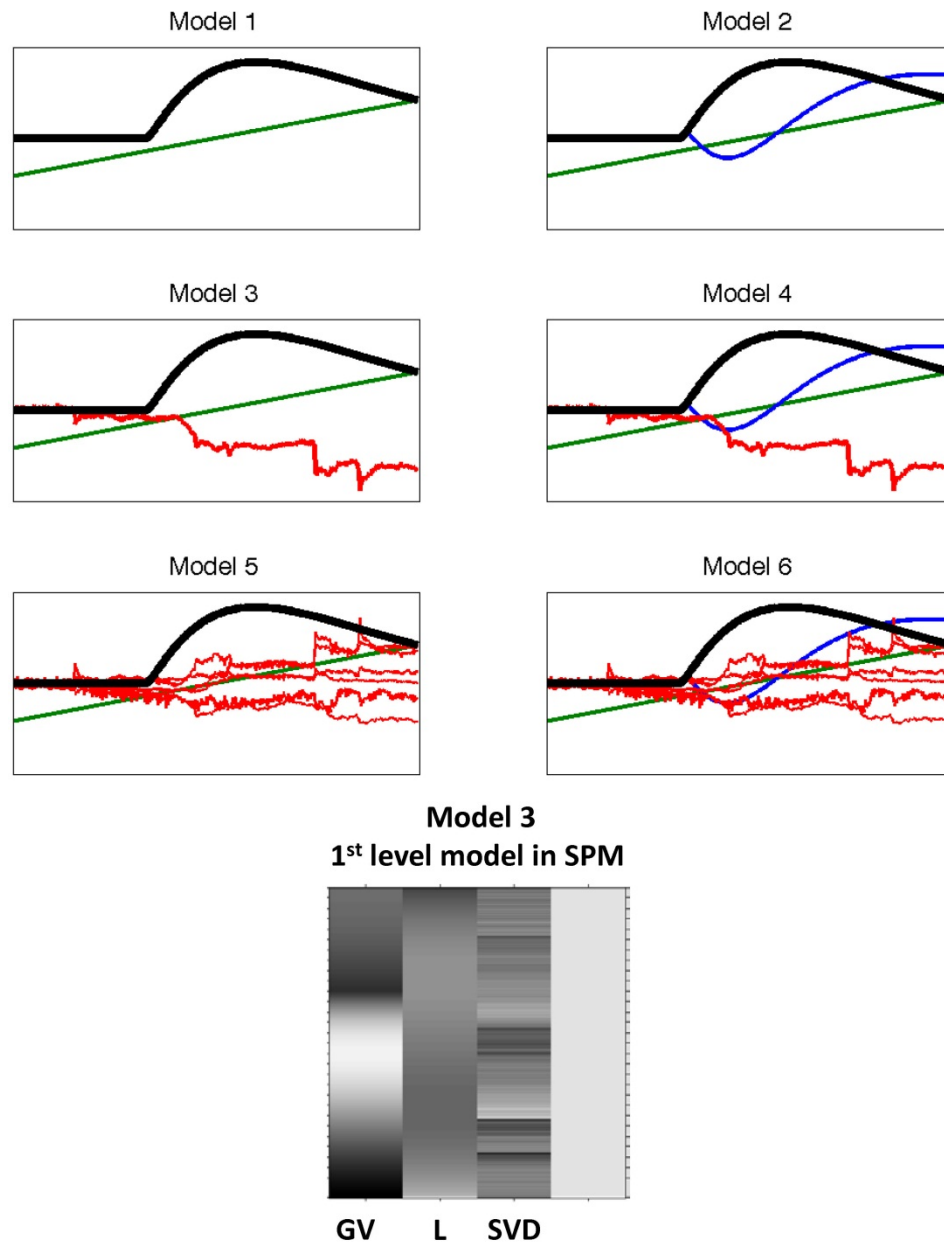
Figure 4-3 phMRI BOLD design matrices

The six phMRI design matrices investigated. All include the ketamine signal model [black; GV], a linear drift term [green; L] and a constant regressor [not shown]. Additional nuisance regressors included in each model are: (a) none in Model 1; (b) an additional regressor to capture variations in shape [blue] in Model 2, (c) an additional regressor computed as first SVD component of the six motion parameters [red] in Model 3; (d) both the shape capturer [blue] and the first SVD of the motion parameters [red] in Model 4; (e) all six head motion parameters [red] in Model 5; (f) both the shape capturer [blue] and all six head motion parameters [red] in Model 6.

4.2.1.2.4   Region of Interest Definition and Analysis

Based on the previously reported phMRI response to ketamine (Deakin et al., 2008) a focused set of ROIs were prescribed *a priori* for comparison of the different design matrices. These comprised three anatomically-defined atlas structures (anterior cingulate cortex (ACC), posterior cingulate cortex (PCC) and thalamus) and two coordinate-based ROIs. The anatomical ROIs were derived using the FSL Harvard-Oxford atlas using probabilistic regions thresholded at 25%. The coordinate-based ROIs consisted of two 5mm radius spheres centered on the Montreal Neurological Institute coordinates corresponding to the peak mid-cingulate (3, 3, 42) and subgenual cingulate (3, 39, -21) responses reported by Deakin et al. (2008). For each region and each subject/session, mean parameter estimates (beta values) for the GV regressor contrast were extracted using the SPM5 toolbox MarsBar.

4.2.1.2.5   Reliability Analysis

To evaluate modulatory effects of other compounds, a reproducible ketamine phMRI response is desirable. Accordingly, the different models were characterised with respect to within-subject test-retest reliability of the estimated signal response amplitude in the above pre-specified ROIs. Within-subject reliability was the selection criteria used to identify a phMRI model suitable for the modulation study. Both ROI and voxelwise reliability estimates were calculated using ICC (3,1) (please see Chapter 3, Section 3.6. for details on this analysis).

4.2.1.2.6   Whole Brain Analysis

Whole brain, voxel-wise analyses were performed for each of the six models evaluated, where individual t-contrasts for the response function of the GV signal model for each participant were taken forward into a three-way ANOVA model (flexible factorial model in SPM) with subject, dose and session as factors, to investigate session effects. Dose effects were investigated using a separate two-way ANOVA model excluding the subject factor. Group maps were thresholded using a voxel threshold of $p<0.001$ uncorrected, with an extent threshold chosen to ensure that only clusters surviving correction for multiple comparison (family-wise error correction $p<0.05$) were included in the resulting map.

4.2.1.2.7 Characterising the Ketamine Response using the most Reliable Model

Based on the design matrix with the highest reliability estimates, group maps for each dose (across sessions) were calculated in SPM5. In addition, beta values from the pre-defined ROIs (see above) were entered into a repeated-measures ANOVA with session (session 1 and 2) as a within-subject factor and dose as a between-subjects factor.

4.2.2 Modulation Study

Data were acquired from 16 healthy male participants on four separate occasions (both pre- and post-infusion), at least ten days apart. Please refer to Figure 3-4 (Chapter 3) for how the different drug conditions are referred to for analysis purposes.

4.2.2.1 Subjective Ratings Analysis

Subjective effects of ketamine were captured using a brief questionnaire (see Table 4-1), designed for rapid assessment, administered approximately 4h and 5h post oral dosing (just prior to ketamine infusion and again approximately 20 min following initiation of ketamine infusion respectively). The six items in this questionnaire were based on items from the PSI, CADSS and VAS that demonstrated greatest sensitivity to the administration of ketamine in the reliability study. The choice of questions to be used was based on plots demonstrating the effect sizes (Cohen's D) of each question in each session (see Appendix A) and each questionnaire. For questions across the VAS, PSI and CADDS that passed these criteria, but overlapped in their content, only one was taken forward.

Table 4-1 Brief questionnaire to index subjective response to ketamine

Item	Scale
Alert – Drowsy	VAS
Muzzy – Clear Headed	VAS
Do you feel more sensitive to light or the colour or brightness of things?	PSI
Is your experience of time unnaturally fast or slow?	PSI
Do you feel as though your head, limbs, or body have somehow changed?	PSI
Do things seem unreal to you as if you are in a dream?	CADDS

All performance data analysis was conducted in SPSS 20.0 (www.ibm.com/spss). Initial data screening was performed in order to assess whether assumptions for parametric analysis were met. Accordingly, exploratory analysis and Shapiro-Wilk tests were completed. VAS data were found to be normally distributed, thus parametric tests were used; a multifactorial repeated measures ANOVA with time (pre- and post-infusion) and treatment arm as within-subject factors for both VAS subscales separately was used. Significant effects of treatment were explored using pairwise comparisons. The PSI questions were scored on a scale of 0 to 3 and the CADSS question on a scale of 0 to 5 with 0 implying the effect was not experienced and 3/5 implying that the effect was experienced very strongly.   For these scales, most participants scored zero and so scores of greater than one were sparsely distributed across subjects. Due to the nature of the data statistical tests were not applied but instead the median, interquartile range, mean and standard deviation are presented to give an insight into the data.

4.2.2.2   Image Analysis

Preprocessing of the data was identical to that of the reliability study. The first level modelling was determined using the regressors from Model 3.

4.2.2.2.1   Region of Interest Analysis

Based on the peaks of the BOLD response to ketamine found in the reliability study (with the use of Model 3) on a separate cohort of participants, a set of ROIs were defined *a priori* for the assessment of treatment effects. These comprised nine coordinate-based ROIs, consisting of 5mm radius spheres centered on the Montreal Neurological Institute coordinates corresponding to the anterior/mid-cingulate (right: [2,14,42]; left [-8,26,32]), posterior cingulate cortex (PCC; right: [6, -42, 6]; left [-4, -42, 32]), thalamus (right: [6,-30,6]; left [-6,-16,-2]), hippocampus (right: [34,-18,-8]; left [-32,-12,-12]) and subgenual cingulate cortex [2, 30, -6]. For each region, beta values were extracted using the SPM5 toolbox MarsBar.

All ROI data analysis was conducted in SPSS 20.0 (www.ibm.com/spss). Initial data screening was performed in order to assess whether assumptions for parametric analysis were met. Accordingly, exploratory analysis and Shapiro-Wilk tests were completed. ROI data were found to be normally distributed, thus parametric tests were used. ROI data were analysed with a repeated measures ANOVA including ROI and treatment as within-

subject factors. A significant effect of treatment was then interpreted with the use of post-hoc paired t-tests for each ROI to determine which treatment was driving the effect.

Movement of three participants exceeded the criteria defined in Chapter 3. Thus analyses were performed with and without these participants.

4.2.2.2.2   Whole-Brain Analysis

For second level group analyses, the contrasts for the GV regressor were taken through to a multifactorial repeated measures ANOVA (flexible factorial model in SPM). This included subject as a random effect between-subjects factor and treatment as a within-subject factor. As described above, group maps were thresholded at $p<0.001$ uncorrected at the voxel level and cluster corrected at $p<0.05$.

4.3   RESULTS

4.3.1   Reliability Study

4.3.1.1   Subjective Ratings

4.3.1.1.1   The Effects of Ketamine

Psychotomimetic States Inventory (PSI)

As Figure 4-4 shows, overall scores while on ketamine were found to be significantly higher compared to both pre- and post-ketamine scores.

PSI total scores indicated a main effect of time-point for both session 1 and 2 ($x^2(2) = 11.73$, $p<0.01$; $x^2(2) = 11.70$, $p<0.01$), driven by significantly higher ketamine scores compared to both pre-ketamine (session 1, $z= -2.312$, $p<0.05$; session 2, $z= -2.527$, $p<0.05$) and post-ketamine scores (session 1, $z= -2.490$, $p<0.05$; session 2, $z= -2.677$, $p<0.01$). Subscale scores also demonstrated a significant effect of time-point on both sessions (Perceptual Distortion (session 1 ($x^2(2) = 12.10$, $p<0.01$); session 2 ($x^2(2) = 14.00$, $p<0.01$), Cognitive Disorganisation (session 1 ($x^2(2) = 10.52$, $p<0.01$); session 2 ($x^2(2) = 9.77$, $p<0.01$), Anhedonia (session 1 ($x^2(2) = 9.45$, $p<0.01$); session 2 ($x^2(2) = 8.62$, $p<0.05$)). Post-hoc analysis demonstrated that the ketamine scores were significantly higher ($p<0.05$) compared to both pre- and post-ketamine, for all three subscales on both sessions (apart from Anhedonia pre-ketamine vs. ketamine in session 1).

No effect of session was found on the ketamine and post-ketamine scores. An effect of session was found for the pre-ketamine scores ($z = -2.814$, $p<0.01$). This effect of session

was driven by the Cognitive Disorganisation subscale ($z = -2.264$, $p<0.05$) and the Mania subscale ($z = -2.209$, $p<0.05$), with both subscales resulting in lower scores in session 2.

No significant differences between dose groups were found at any time point, on either session on the total scores (Session 1: pre-ketamine (U=10, z=-0.525, p=0.599); ketamine (U=10, z=-0.525, p=0.599); post-ketamine (U=9, z=-0.780, p=0.435)/Session 2: pre-ketamine (U=9, z=-0.754, p=0.451); ketamine (U=5.5, z=-1.471, p=0.141); post-ketamine (U=11, z=-0.335, p=0.737)).
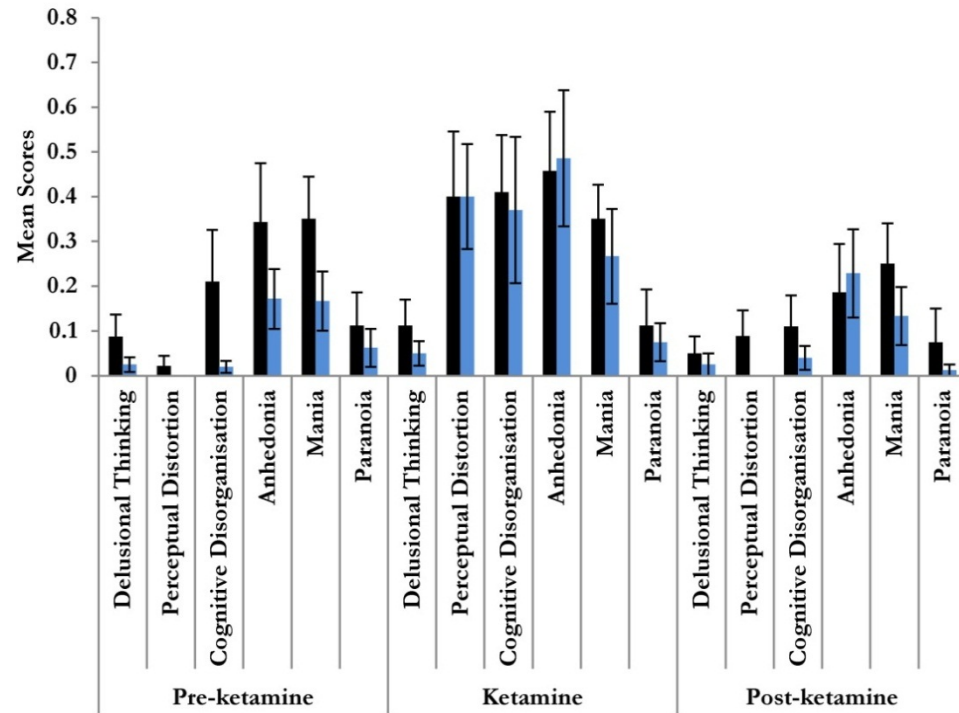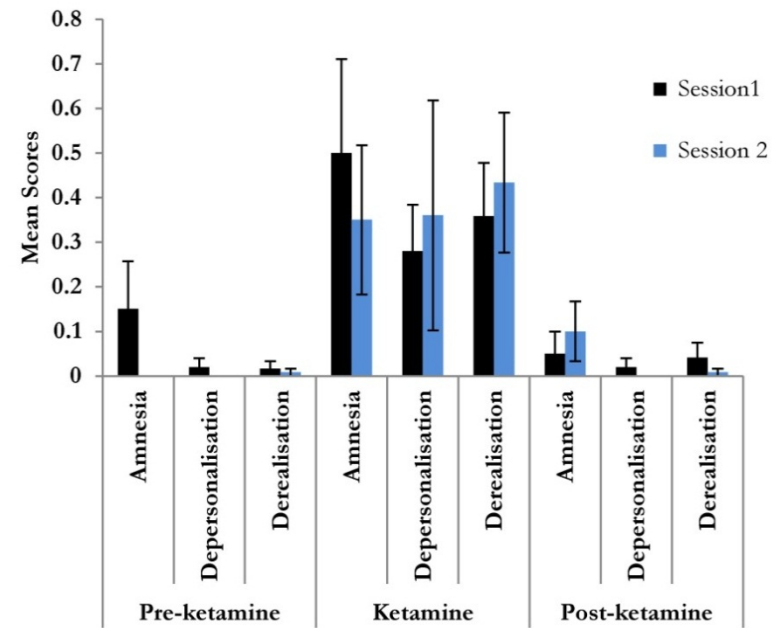
Figure 4-4 Effects of ketamine on subjective ratings

Scores have been adjusted for the number of questions in each subscale for visualisation purposes.

Clinician Administered Dissociative States Scale (CADSS)

CADSS total and subscale results showed that, overall, scores while on ketamine were found to be significantly higher compared to both pre- and post-ketamine scores (see Figure 4-4). No effect of session was found.

CADSS total scores indicates a main effect of time-point for both session 1 and 2 (($x^2(2)$ = 13.07, p<0.01); ($x^2(2)$ = 13.13, p<0.01)), driven by significantly higher ketamine scores compared to both pre-ketamine (session 1, z= -2.310, p<0.05; session 2, z= -2.371, p<0.05) and post-ketamine scores (session 1, z= -2.668, p<0.01; session 2, z= -2.366, p<0.05). Individual subscales also demonstrated a significant effect of time-point on both sessions (Depersonalisation (session 1 ($x^2(2)$ = 12.00, p<0.01); session 2 ($x^2(2)$ = 6.00, p<0.05); Derealisation (session 1 ($x^2(2)$ = 15.08, p<0.01); session 2 ($x^2(2)$ = 12.78, p<0.01); Amnesia only on session 1 ($x^2(2)$ = 7.05, p<0.05)). Ketamine scores were significantly higher (p<0.05) compared to both pre- and post-ketamine on both sessions for Derealisation, but only on Session 1 for Depersonalisation. Amnesia ketamine scores were significantly higher compared to post-ketamine scores (p<0.05).

No significant differences between dose groups were found at any time point, on either session, on the total scores (Session 1: pre-ketamine (U=12, z=-0.1.490, p=0.881); ketamine (U=7.5, z=-1.048, p=0.295); post-ketamine (U=12, z=-0.149, p=0.881). Session 2: pre-ketamine (U=10, z=-1.000, p=0.317; ketamine (U=4.5, z=-1.697, p=0.090); post-ketamine (U=12, z=-0.149, p=0.881)).
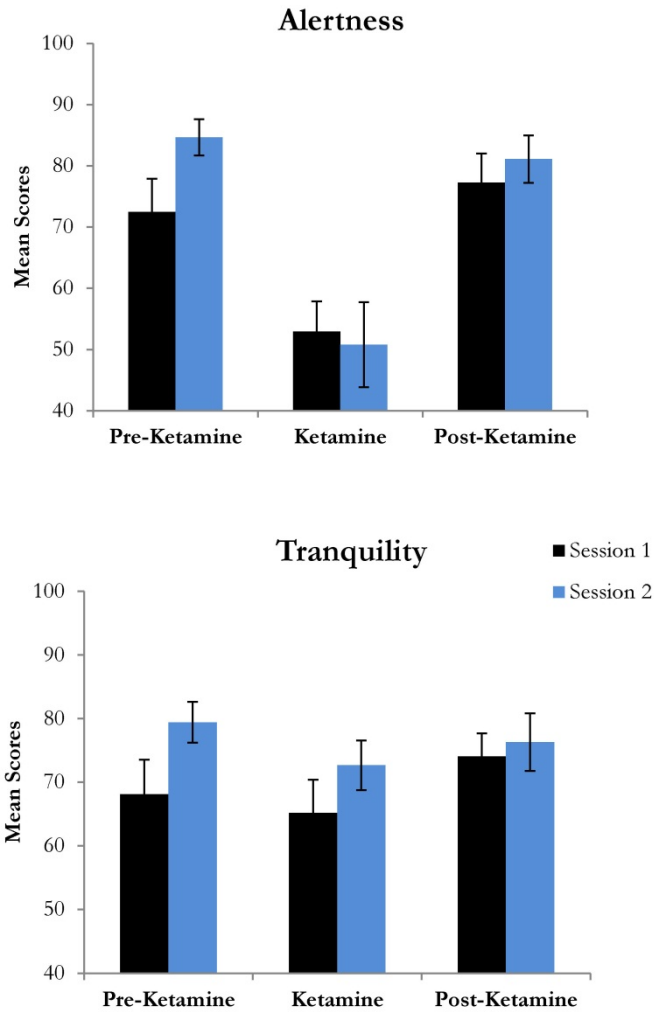
Figure 4-5 Effect of ketamine on the Visual Analogue Scales separated by factor

Errors bars represent the standard error of the mean. The higher the score the more alert/tranquil the participants felt.

Visual Analogue Scales (VAS)

Across factors, a significant main effect of time-point was found due to a decrease in scores on ketamine compared to both pre-and post-ketamine scores ($F_{(1,16)} = 14.06$, $p<0.01$). A significant interaction between summary factor and time-point ($F_{(2,16)} = 24.98$, $p<0.01$) however, demonstrated that this decrease was only significant for the Alertness factor (see Figure 4-5). A trend towards a main effect of session, with scores numerically higher on session 2, was found ($F_{(1, 8)} = 4.66$, $p = 0.06$). No significant main effect of dose was found ($F_{(1,8)} = 0.03$, $p = 0.86$).

4.3.1.1.2   Reliability

As Table 4-2 shows, ICC reliability estimates for the PSI scores were found to be high for the ketamine time point (0.93 for total score, 0.55-0.87 for subscales), indicating that the PSI response to ketamine is reliable within-subject. In contrast, ICCs for the CADSS total scores were substantially lower with only derealisation pre-ketamine showing a high ICC value (0.80) but with the caveat that this measure comprised very low absolute score values. The Alertness factor for the VAS showed reasonable to high ICC estimates (0.66 pre-ketamine, 0.60 ketamine). Total scores were not calculated for the VAS due to the factor structure of the questionnaire.

Table 4-2 Reliability for the subjective ratings

ICC(3,1) +/- SE		Timepoint		
Questionnaire	**Subscale/factor**	**Pre-Ketamine**	**Ketamine**	**Post-Ketamine**
PSI	Delusional Thinking	0.33 (0.17)	0.55 (0.14)	0.87 (0.06)
	Perceptual Distortion	0.00 (0.19)	0.55 (0.14)	0.00 (0.19)
	Cognitive Disorganisation	0.14 (0.18)	0.87 (0.06)	0.47 (0.16)
	Anhedonia	0.30 (0.17)	0.86 (0.07)	0.68 (0.12)
	Mania	0.70 (0.12)	0.77 (0.09)	0.80 (0.09)
	Paranoia	0.75 (0.10)	0.66 (0.12)	-0.04 (0.19)
	Total Score	**0.52 (0.15)**	**0.93 (0.03)**	**0.73 (0.11)**
CADSS	Amnesia	0.00 (0.19)	0.54 (0.15)	0.64 (0.13)
	Depersonalisation	0.00 (0.19)	-0.12 (0.18)	0.00 (0.19)
	Derealisation	0.80 (0.09)	0.39 (0.17)	0.06 (0.18)
	Total Score	**0.11 (0.18)**	**0.28 (0.18)**	**0.62 (0.13)**
VAS	Alertness	0.66 (0.12)	0.60 (0.14)	0.38 (0.17)
	Tranquility	0.48 (0.16)	0.01 (0.19)	0.43 (0.16)

4.3.1.2   Image Analysis

4.3.1.2.1   Reliability

Overall, models 1, 3 and 6 yielded the highest ICC values for the ROIs specified *a priori* (Table 4-3). In particular, Model 3 resulted in the highest values for the posterior cingulate and thalamus regions, and second highest in the anterior cingulate regions, for both ROI-mean and voxelwise analyses. ROI-mean ICC values ranged from 0.46 to 0.74 and voxelwise median ICC values from 0.28 to 0.72. Model 3 also yielded the highest ICC values across the whole brain (ICC_{median} = 0.26) and in voxels responding positively to the ketamine challenge (ICC_{median}= 0.27). Voxelwise ICC values for the subgenual cingulate cortex region were not available due to thresholding criteria in SPM leading to insufficient data overlap in this area across all volunteers and sessions; thus reliability of this ROI across models was assessed on the ROI-mean values alone which were not subject to any thresholding. Histograms of ICC values, across the whole brain, the activated network and two ROIs (the thalamus and posterior cingulate cortex), for each of the models are shown in Figure 4-6. The ROI distributions representing the ICC values from Model 1 and 3 are negatively skewed, indicating that a higher proportion of voxels within the thalamus and posterior cingulate have higher ICC values compared with the remaining four models. Model 3 is the only model with consistently higher values across all the ROIs (Table 4-3).

Table 4-3 Reliability of the BOLD response to ketamine for all models

ICC(3,1) +/- SE	Motion regressors:	NO MOTION REGRESSORS		SVD REGRESSOR		x6 MOTION REGRESSORS	
	Shape-capturer:	No Shape-Capturer	Shape-Capturer	No Shape-Capturer	Shape-Capturer	No Shape-Capturer	Shape-Capturer
	ICC Derivation	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Anterior Cingulate Cortex	**Voxel**	0.31 (0.008)	0.13 (0.007)	0.40 (0.006)	0.18 (0.006)	0.27 (0.005)	0.44 (0.008)
	ROI	*0.33 (0.21)*	*0.11 (0.22)*	*0.52 (0.20)*	*0.20 (0.22)*	*0.40 (0.21)*	*0.61 (0.20)*
Posterior Cingulate Cortex	**Voxel**	0.28 (0.006)	0.03 (0.005)	0.28 (0.007)	0.11 (0.005)	0.15 (0.009)	0.14 (0.006)
	ROI	*0.33 (0.21)*	*-0.13 (0.22)*	*0.46 (0.20)*	*0.09 (0.22)*	*0.11 (0.22)*	*0.08 (0.23)*
Thalamus	**Voxel**	0.42 (0.005)	-0.08 (0.007)	0.46 (0.005)	0.01 (0.005)	0.17 (0.006)	0.11 (0.005)
	ROI	*0.50 (0.20)*	*-0.16 (0.22)*	*0.55 (0.20)*	*0.03 (0.23)*	*0.22 (0.22)*	*0.08 (0.23)*
Mid-cingulate cortex (3,3,42)	**Voxel**	0.71 (0.010)	0.51 (0.038)	0.72 (0.020)	0.53 (0.026)	0.41 (0.015)	0.74 (0.021)
	ROI	*0.73 (0.15)*	*0.54 (0.19)*	*0.74 (0.15)*	*0.56 (0.20)*	*0.45 (0.20)*	*0.71 (0.15)*
Sub-genual Cingulate Cortex (3,39,-21)	**Voxel**	-	-	-	-	-	-
	ROI	*-0.35 (0.21)*	*0.33 (0.21)*	*-0.27 (0.22)*	*0.40 (0.21)*	*0.26 (0.22)*	*0.66 (0.17)*
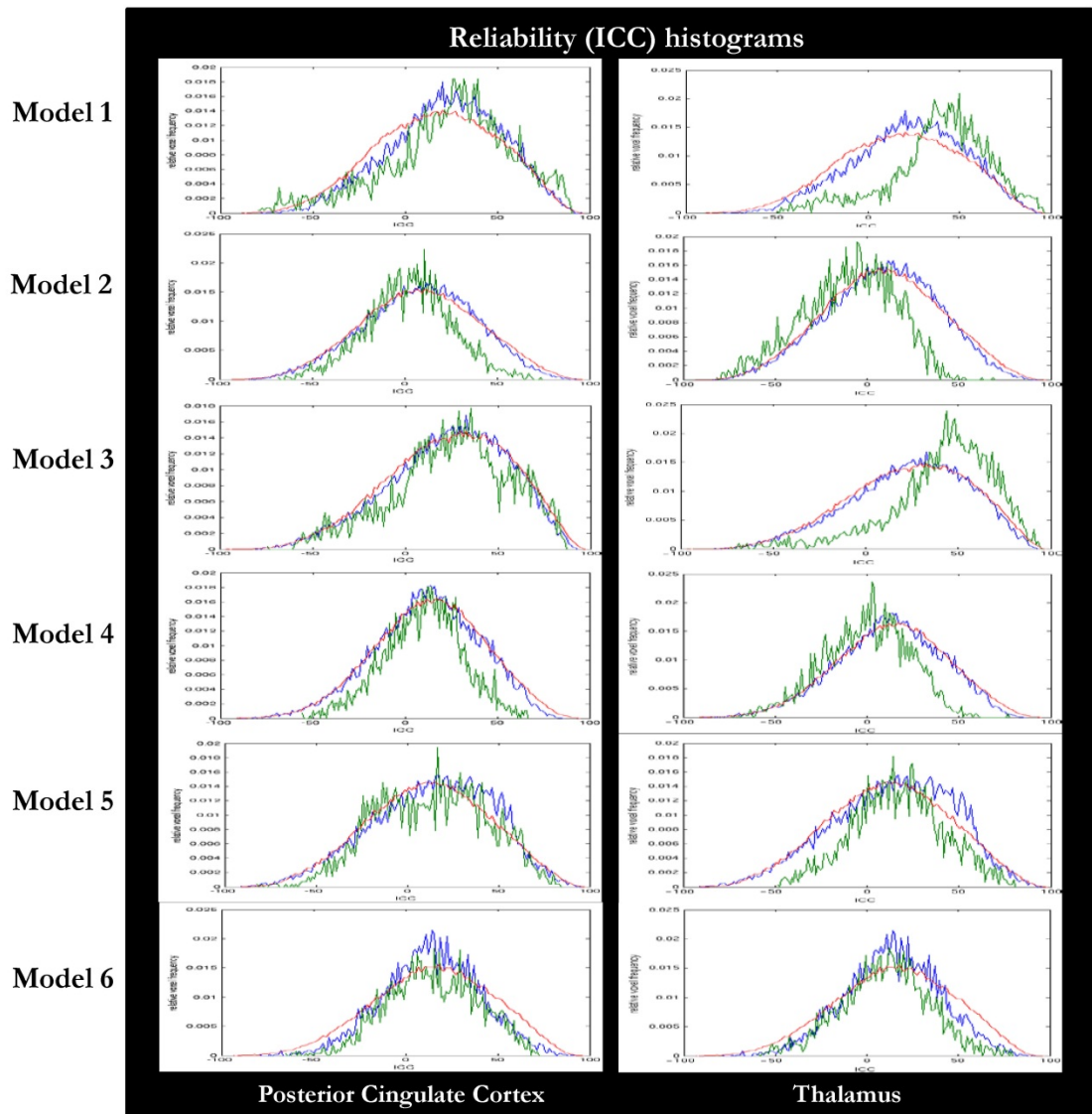
Figure 4-6 ICC histograms

Posterior cingulate cortex (PCC) and thalamus ICC histograms for six of the models investigated. The histograms display the distribution of ICC values across all voxels within either the whole brain (red curve), the network of activated areas (blue curve), or an ROI (green curve). X-axis denotes the ICC value and the Y-axis denotes the relative voxel frequency. The PCC and thalamus were chosen specifically as these ROIs emphasise the differences between the models most effectively.

4.3.1.2.2   Whole Brain Analysis

Figure 4-7 shows the group maps of the ketamine BOLD response, for each of the six models investigated, for session 1 and session 2 (both doses combined). In all models, increases in BOLD signal were evident in the anterior cingulate, mid-posterior cingulate

and paracingulate cortices, hippocampal and parahippocampal areas, bilateral insula, dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, thalamus, supplementary motor area and bilateral operculum. Models 1 and 3 yield the most extensive signal increases, with those obtained using Model 3 slightly more consistent across the two sessions. Furthermore, Model 3 was the only model in which a signal decrease in the subgenual cingulate, albeit at a lower statistical threshold of $p<0.01$, was observed in both sessions.
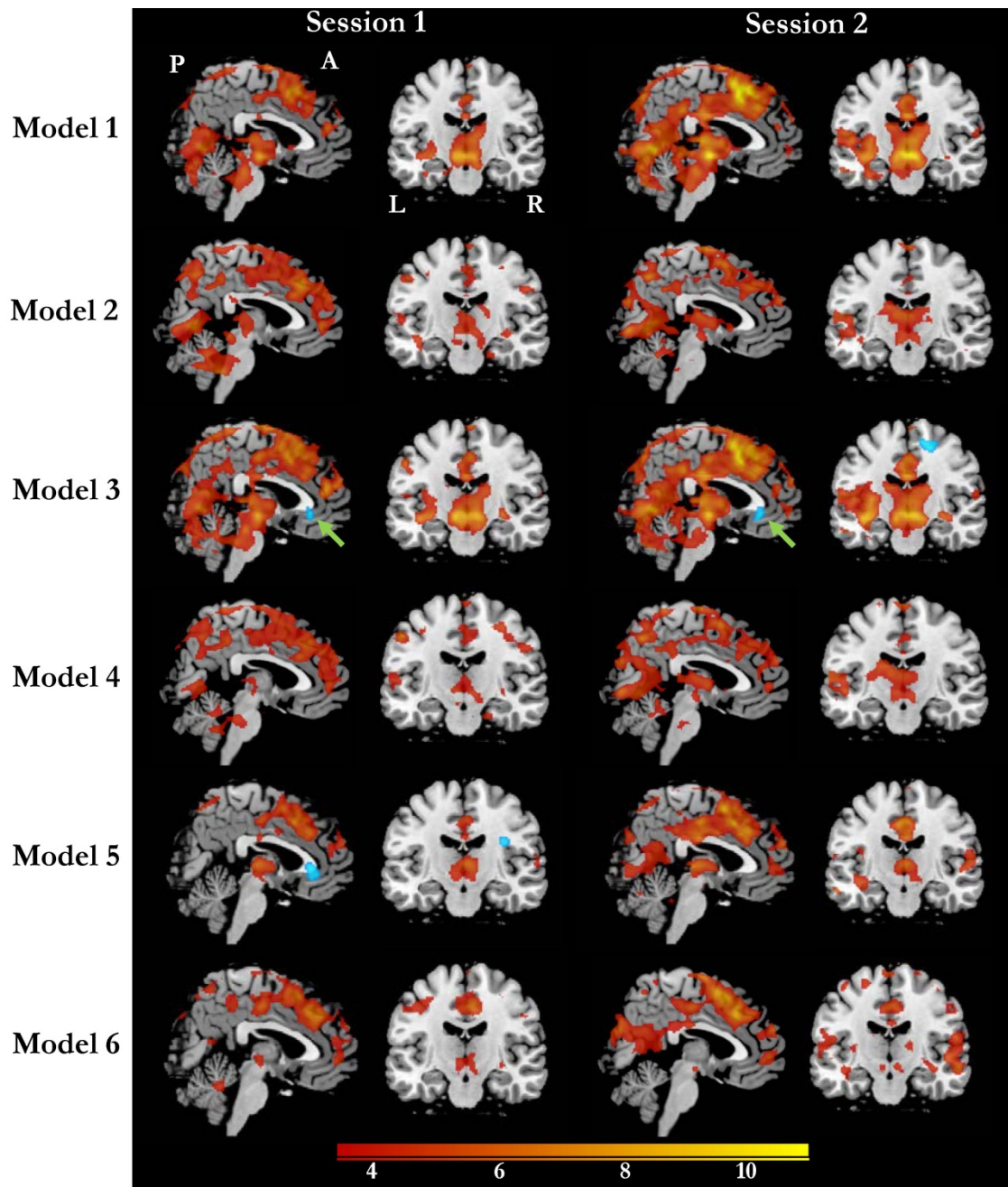
Figure 4-7 The ketamine BOLD response for each model

Group maps (N=10) of modeled ketamine BOLD response for each session, for six of the design matrices investigated (red activations: p<0.001 uncorrected, cluster corrected at p<0.05; blue deactivations p<0.01 uncorrected). Arrows indicate the negative response in the subgenual cingulate cortex, observed in both sessions with Model 3. Orientation of the slices specified in the top row [A – anterior; P - Posterior; R - Right; L – left]. Activation overlaid on a sagittal (MNI x-coordinate=3) and coronal section through the thalamus (MNI y-coordinate = -15).

4.3.1.2.3 Characterising the Ketamine Response using the Most Reliable Model (Model 3)

Based upon the above considerations and the aims of this study, Model 3 was chosen to describe the ketamine BOLD phMRI response more fully. Results obtained with this model yielded strong and reliable responses at the ROI-level, and consistent group activation maps. The overall ketamine response (across both doses and both sessions) as detected using this modelling approach is illustrated in Figure 4-8. The overall response pattern comprised strong midline responses including anterior and posterior cingulate, paracingulate gyrus, supplementary motor area, precuneus, medial occipital lobes/cerebellum, thalamus and brainstem. Bilateral foci of strongly responding voxels are also apparent in the anterior insula/operculum areas, and responses were also observed in lateral prefrontal and parietal grey matter.
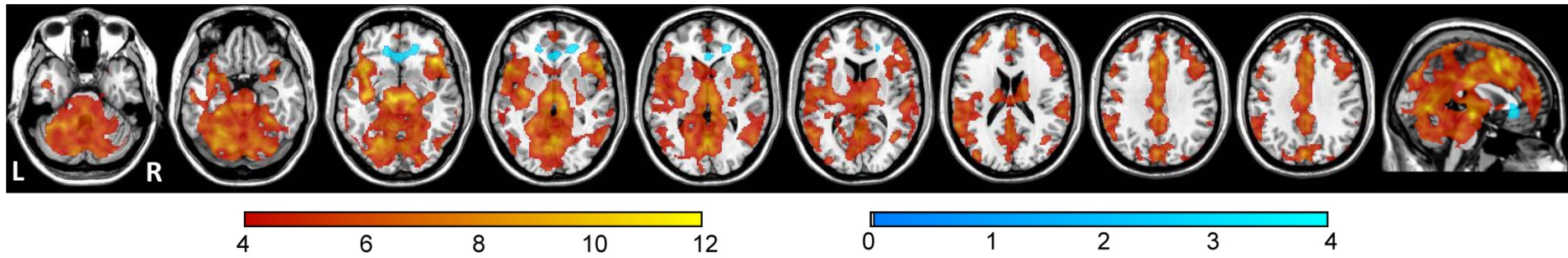
Figure 4-8 The ketamine BOLD response - Model 3

Map of ketamine effect across both doses and both sessions, using Model 3 (red activations: p<0.001 uncorrected, cluster corrected at p<0.05; blue deactivations p<0.01 uncorrected, cluster corrected at P<0.05).

No significant main effect of session or dose was found for the pre-defined ROIs. No significant interactions were found. Please refer to Table 4-4 for all relevant statistics.

Table 4-4 Dose and Session Effects on pre-defined ROIs for Model 3

ROIs	ANOVA			
	Main Effect of Session		Main Effect of Dose	
	F[1,8]	P	F[1,8]	P
ACC	0.137	0.721	0.044	0.839
PCC	1.536	0.250	1.317	0.284
Thalamus	0.080	0.784	1.260	0.294
Mid-Cingulate Cortex (3,3,42)	2.255	0.172	0.530	0.487
Sub-genual Cingulate Cortex (3,39,-21)	0.722	0.420	0.132	0.740

Although no statistical differences were found between doses in the predefined ROIs or at a whole-brain level (at a corrected threshold), the dose-dependent BOLD signal changes were stronger at 75ng/mL than 50ng/mL, at both the ROI (Figure 4-9(a)) and voxel scale (Figure 4-9(b)). Univariate activation maps of the ketamine response revealed robust effects at both doses (Figure 4-9 (b)), with the activation pattern more widespread, and the supra-threshold T values larger, at the 75ng/mL dose level.
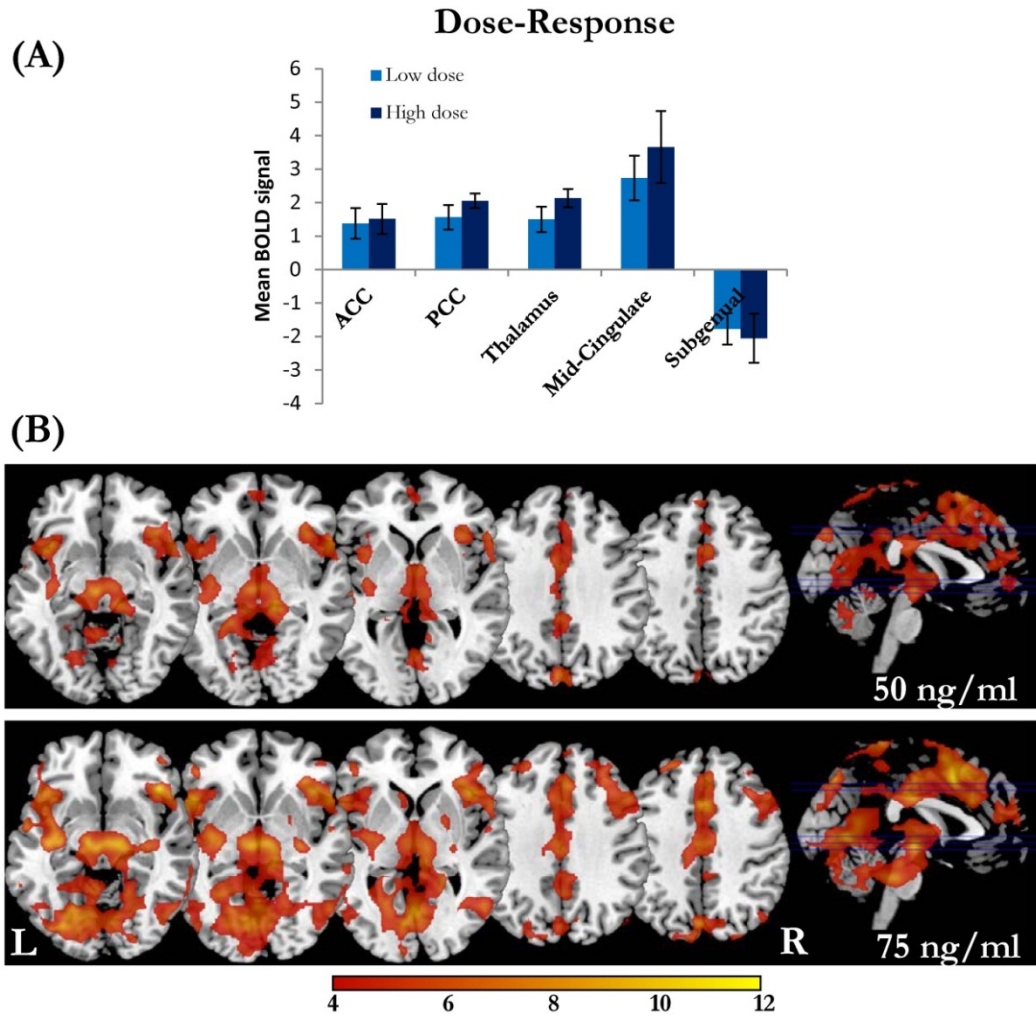
Figure 4-9 Dose-response to ketamine infusion

(a) BOLD signal dose-response profile in the predefined ROIs. (b) Univariate maps of ketamine response at each dose (p<0.001, cluster corrected at p<0.05). Errors bars represent the standard error of the mean.

4.3.1.2.4   Relationship of the phMRI data to Subjective Ratings

Within SPM5, multiple regression models were performed to combine the subjective ratings (subscale and total scores) with the phMRI imaging data (Model 3). While there were some correlations between phMRI responses and subjective ratings on individual sessions, there were *no* significant correlations present in *both* sessions. Therefore, these results are not reported. The relatively low doses of ketamine used in the study may have contributed to the inconsistency in the findings, because many volunteers scored very low in a number of subscales which might have influenced the ability of the regression models to detect any significant correlations.

4.3.2   Modulation Study

4.3.2.1   Ketamine Plasma Levels

Table 4-5 Ketamine Plasma Levels (ng/ml) in each Treatment Condition

	PLA-KET		RIS-KET		LAM-KET	
Subject	15min	75min	15min	75min	15min	75min
1	71.7	M	29.3	91	84.2	M
2	86.6	108.5	78.7	83.7	86.2	90.4
3	59.8	M	63.9	180.9	72.7	86.3
4	51	62.3	54.9	M	105.2	143.8
5	46.5	51.6	44.7	64.3	62.5	59.1
6	69.8	84.6	40.7	60.7	53.2	68.3
107	93.3	96.4	80.4	107.6	94.3	109.9
108	45.4	45.8	35.3	36.1	47	55.1
9	35.1	45.9	39.2	44.2	48	45.7
10	54.5	79.6	38.2	52	35.9	48.9
11	M	84	42.2	68.9	44.7	67.3
112	62.4	59.8	50.3	61.9	72.6	57.9
113	43.1	46	29.2	41.6	41.3	64.7
14	58	76.2	42	59.5	49.6	75.6
15	78.2	86.2	61.9	63	76.9	84.8
16	85.1	92.6	60.9	62.3	83.4	109

*M = Missing

The mean and standard deviation of plasma concentration of ketamine per-treatment was (in ng/mL): PLA-KET 62.7 ± 17.6, LAM-KET 66.1 ± 22.1 and RIS-KET 49.1 ± 15.9 after 15 minutes of infusion (concentration of KET was significantly different for RIS-KET compared to PLA-KET, p=0.02, Wilcoxon rank sum), and PLA-KET 72.8 ± 20.8, LAM-KET 71.9 ± 35.5 and RIS-KET 77.8 ± 26.9 after 75 minutes of infusion.

4.3.2.2   Subjective Ratings
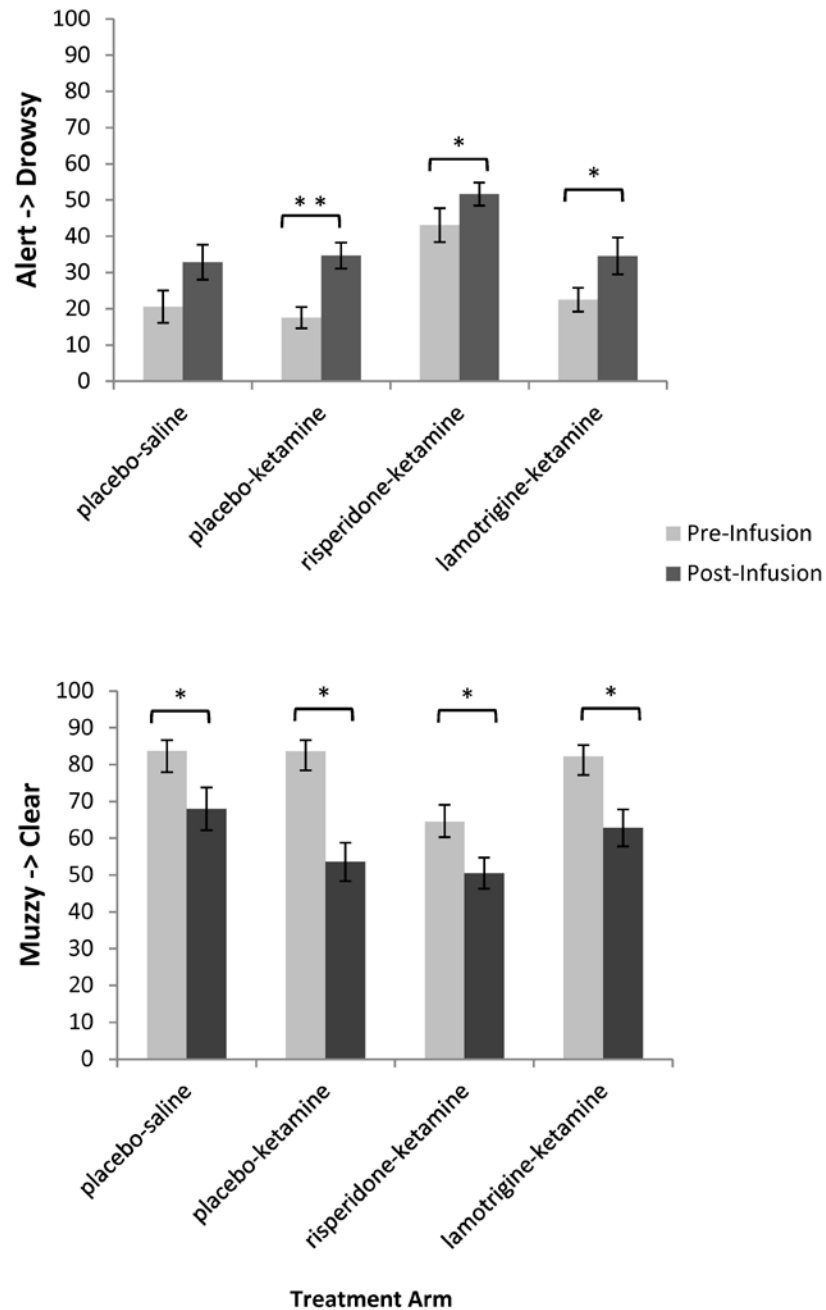
Figure 4-10 Effect of ketamine and modulation on the VAS

Scales (Alert-Drowsy and Muzzy-Clear) included in the brief questionnaire (*: p < 0.05, **: p <0.01). Error bars represent the standard error of the mean.

As Figure 4-10 shows, analysis of VAS data demonstrated a significant effect of time for both the alert-drowsy and muzzy-clear scales ($F(1,12) = 16.283$, $p<0.01$; $F(1,12) = 32.868$, $p<0.01$). Pairwise comparisons indicate that this is driven by a significant increase in

drowsiness post-ketamine infusion in all ketamine treatment arms and a significant decrease in clarity post-infusion in all treatment arms. No time by treatment interactions were found for either subscale, indicating that no modulation of ketamine-induced changes in subjective effects by risperidone or lamotrigine occurred (F(3,36) = 1.281, p=0.296; F(3,36) = 0.565, p<0.641).

Table 4-6 Subjective scores from the PSI and VAS questions

You feel more sensitive to light or the colour or brightness of things. (0 to 3)

	PLA	P-SAL	PLA	P-KET	LAM	L-KET	RIS	R-KET
Median	0	0	0	0	0	0	0	0
IQR	0	0	0	1	0	0	0	1
Mean	0.07	0.13	0.07	0.44	0	0.31	0.01	0.56
Std Dev	0.26	0.35	0.258	0.63	0	0.70	0.26	0.81

Your experience of time is unnaturally fast or slow. (0 to 3)

	PLA	P-SAL	PLA	P-KET	LAM	L-KET	RIS	R-KET
Median	0	0	0	0	0	0	0	0
IQR	0	0	0	1	0	1	0	1
Mean	0	0.07	0	0.38	0.06	0.50	0.07	0.44
Std Dev	0	0.26	0	0.50	0.25	0.82	0.26	0.81

You feel as though your head, limbs or body have somehow changed. (0 to 3)

	PLA	P-SAL	PLA	P-KET	LAM	L-KET	RIS	R-KET
Median	0	0	0	0	0	0	0	0
IQR	0	0	0	0	0	1	0	0
Mean	0	0	0	0.25	0.06	0.31	0	0.13
Std Dev	0	0	0	0.58	0.25	0.48	0	0.50

Do things seem unreal to you as if you are in a dream. (0 to 5)

	PLA	P-SAL	PLA	P-KET	LAM	L-KET	RIS	R-KET
Median	0	0	0	0	0	0	0	0
IQR	0	0	0	1	0	0	0	0
Mean	0	0	0	0.31	0	0.25	0.07	0.06
Std Dev	0	0	0	0.48	0	0.58	0.26	0.25

4.3.2.3   Image Analysis

4.3.2.3.1   Region of Interest Analysis
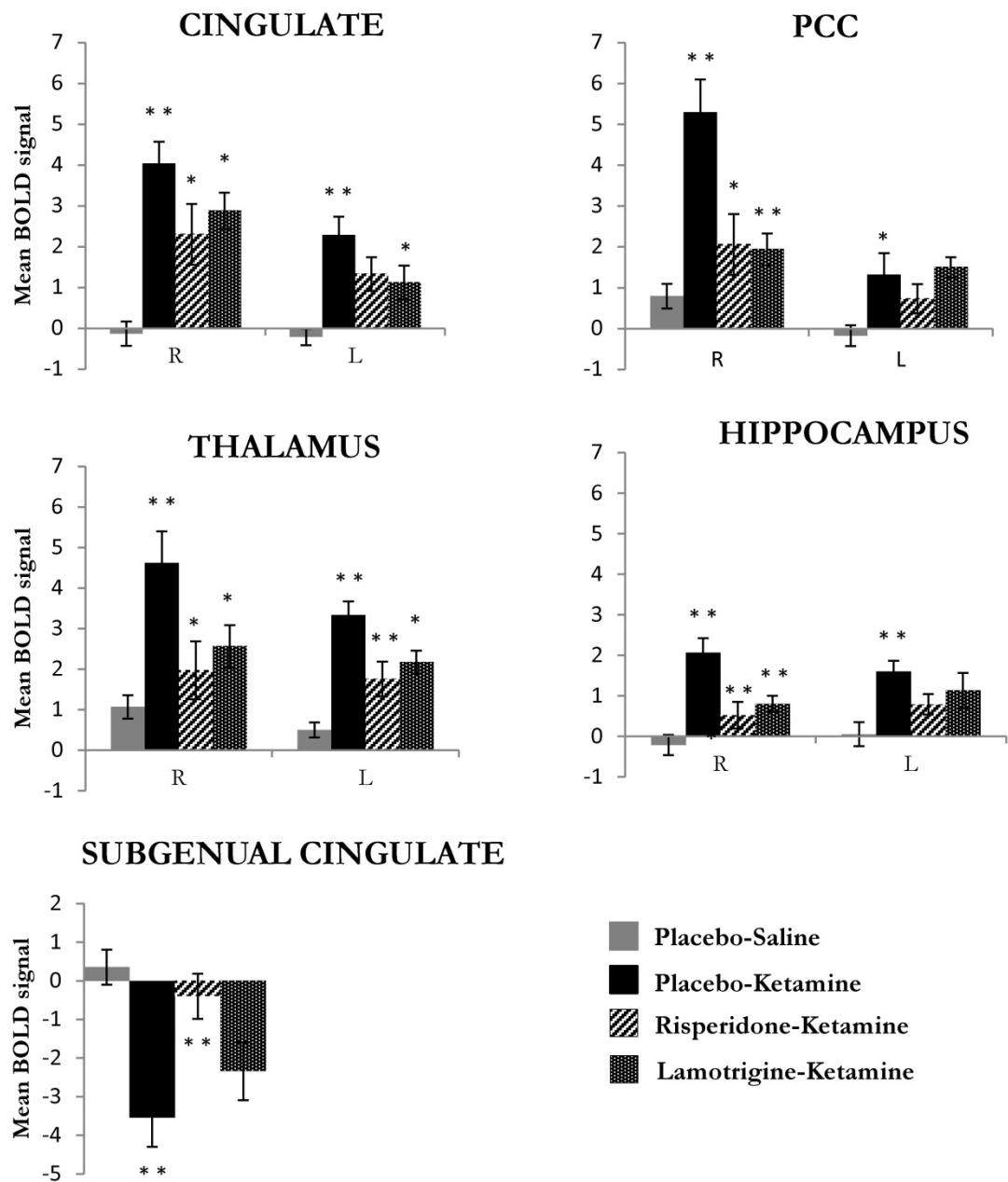
Figure 4-11 Mean BOLD response by treatment condition for all pre-defined ROIs

Statistical significance of the placebo-ketamine arm represents the outcome of a comparison with the placebo-saline arm. Significance estimates of the risperidone-ketamine and lamotrigine-ketamine arms are in comparison to the placebo-ketamine arm. Error bars represent the standard error of the mean. (R = Right; L = Left) (*: $p < 0.05$, **: $p < 0.01$).

An overall significant effect of treatment across ROIs was found $(F_{(3,45)}=12.438, p<0.01)$. A significant effect of ROI was also found $(F_{(8,120)}= 24.642, p<0.01)$ suggesting that the degree to which the individual regions respond to the treatments given differs. A significant ROI by treatment interaction $(F_{(24,360)}=8.216, p<0.01)$ is driven by the positive BOLD response found in the cingulate, PCC, thalamus and hippocampus compared to the negative BOLD response in the subgenual cingulate.

As Figure 4-11 shows, post-hoc paired t-tests demonstrate that the treatment effect is driven by (1) a significant ketamine-induced increase in BOLD signal across all ROIs and (2) an attenuation of this effect by both lamotrigine and risperidone, although the extent to which this occurs differs between ROIs.

In order to assess whether any order effects were present in the data a repeated-measures ANOVA was carried out with treatment and ROI as within-subjects factors and order as a between-subjects factor. No main effect of order was found $(F_{(3,12)}=0.463, p=0.714)$. No significant interaction was found between treatment and order $(F_{(9,36)}=0.774, p=0.641)$ or treatment, order and ROI $(F_{(126,504)}=0.819, p=0.913)$. In order to more fully account for variability due to treatment condition ordering effects, a mixed effects model with treatment (fixed), session (fixed) and subject (random) as model factors was carried out per ROI in Statistical Analysis Software (SAS) by C. Bennett at Eli Lilly. This model confirmed the treatment effects obtained in SPSS.

The results were unaffected when participants who demonstrated motion outside of the accepted criteria were excluded, thus the results presented here include all particpants.

4.3.2.3.2 Whole-brain Analysis

As expected under ketamine conditions, increases in BOLD signal were seen in the prefrontal cortices, anterior and posterior cingulate, thalamus and temporal lobe at an uncorrected threshold of $p<0.001$, family-wise corrected for multiple comparisons at $p<0.05$ on the basis of cluster extent (see Figure 4-12(a)). At the same statistical threshold, attenuation of the ketamine-induced BOLD response was also found in certain regions with both lamotrigine and risperidone, although the extent of attenuation appears to be more pronounced with the latter (see Figure 4-12(b/c)).

To investigate order effects at a whole-brain level, a full factorial with order and treatment as within-subject factors was employed. No significant main effect of order or order by treatment interaction was found.

(A) Ketamine response compared to placebo

(B) Lamotrigine pre-treatment ketamine response compared to ketamine

(C) Risperidone pre-treatment ketamine response compared to ketamine

Voxelwise T-Value
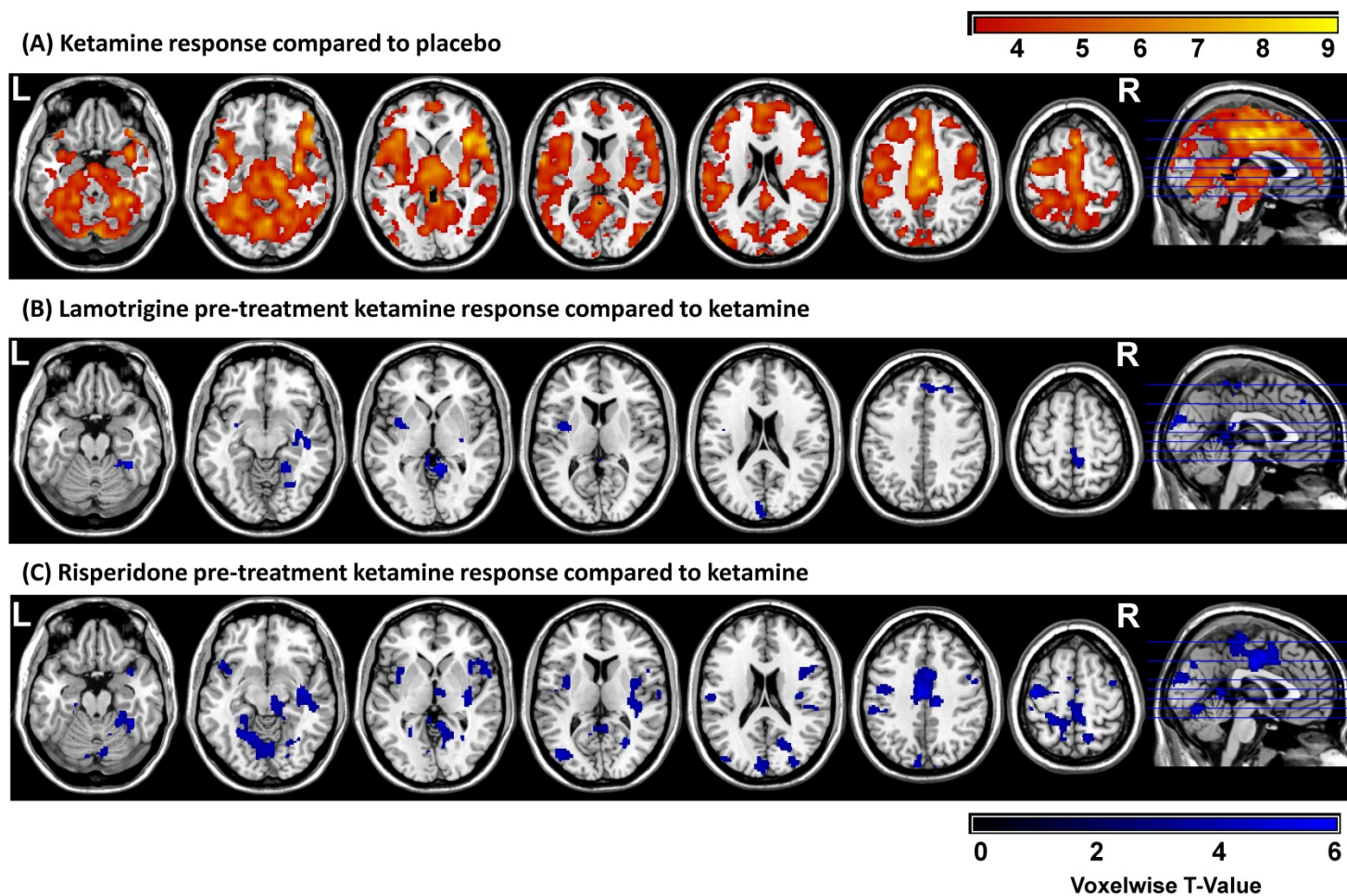
Mass univariate contrasts of the ketamine response following placebo pretreatment compared to the placebo pre-treatment saline response (A), lamotrigine pre-treatment ketamine response (B) and risperidone pre-treatment ketamine response (C).

Increases in activation (red-yellow) represent areas that respond to a greater extent to ketamine compared to placebo. Decreases in activation (blue) represent areas that respond to ketamine administration but are attenuated by either lamotrigine or risperidone pre-treatment. T- maps are thresholded at whole brain cluster significance of p<0.05, voxel threshold of p<0.001.

Figure 4-12 Whole brain modulation of the ketamine response

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4.4 DISCUSSION

4.4.1 Summary of Main Results

Administration of low-dose ketamine was found to induce robust and reliable effects in widespread and predicted cerebral networks across two sessions. The effects of ketamine in the brain were extensive, most likely due to the ubiquitous nature of NMDA receptors and the mechanism of action of ketamine at these receptors which leads to a disinhibition of excitatory transmission (Moghaddam et al., 1997; Olney & Farber, 1995a). The anatomical distribution of these effects was consistent with results of previous PET and MRI studies in humans (Deakin et al., 2008; Langsjo et al., 2003; Langsjo et al., 2004) with significantly increased responses in regions including the cingulate cortex, thalamus and temporal lobe structures. The consistency with phMRI activation patterns seen in experimental animals (Chin et al., 2011; Littlewood, Jones, et al., 2006) demonstrates the translational utility of this paradigm. Since ketamine-challenge phMRI is an assay of substantial interest for pharmacological blockade studies (Chin et al., 2011; Gozzi, Herdon, et al., 2008; Gozzi, Large, et al., 2008) a focus was placed on an analysis method which maximised reliability to the effects of ketamine. Subjective experience questionnaires used in this study were also shown to be sensitive to the effects of ketamine at the doses administered, although only two of the measures, the PSI and the VAS (Alertness summary factor) were found to be reliable across sessions.

A replication of the attenuation of the BOLD response to ketamine with lamotrigine was also achieved, in a placebo-controlled within-subject design, within the anterior-posterior cingulate, thalamus and hippocampus, together with a similar level of attenuation with risperidone in all these ROIs and the subgenual cingulate. No modulation of subjective responses occurred with these compounds.

4.4.2 Assessing the Ketamine Response

To develop the phMRI analysis methodology several different design matrices were compared, including different combinations of nuisance regressors to account for potentially confounding variance in the signal. Statistically significant BOLD signal changes were evident in all models, with strong changes present in many regions including the anterior cingulate, mid-posterior cingulate and paracingulate cortices, hippocampal and parahippocampal regions, bilateral insula, dorsolateral prefrontal cortex, ventrolateral prefrontal cortex, thalamus, supplementary motor area, bilateral operculum, precuneus

and medial occipital lobes. BOLD signal decreases were found in the subgenual cingulate cortex, although this was only present in both sessions using Model 3.

4.4.3 Role of Motion

For fMRI analysis in general, motion correction is a significant concern. For BOLD phMRI this is exacerbated by the necessity of the drug period to follow the baseline period and the length of scan typically employed. Two different ways of accounting for motion explicitly in the design matrix were thus examined.

Common practice in fMRI analysis is to include six head motion regressors as covariates of no interest in the model. This method was introduced in order to control for motion-related signal variations unaddressed by realignment (K. J. Friston et al., 1996). However, inclusion or exclusion of these parameters can have an important impact on the sensitivity of the analysis method used (Johnstone et al., 2006). For example, removing spurious motion-related activations may also affect true 'task'-related activations if these are correlated (Freire & Mangin, 2001; Johnstone et al., 2006).

As an alternative means of modelling participant head motion in the design matrix, the performance of a single motion confound regressor, designed to capture the major variance in head movement during the scan, was also evaluated. The ketamine phMRI model that was found to demonstrate the highest reliability estimates was found to include this component consisting of the first SVD component of the individual participant head motion traces (Model 3).

It is important to note that using the SVD regressor alone to model motion artefacts may confer some disadvantages. For example, modelling only the primary component of the motion automatically leads to unmodelled movement-related variance and thus may not capture, as well as modelling all six motion parameters (e.g. Model 5 and 6), the variety of different effects on fMRI signal which head motion may cause. Furthermore, if this residual motion is correlated with the regressor of interest (GV) it may influence parameter estimation and reliability values. However, multiple analyses were carried out (data not shown) and demonstrated that residual motion (1) was not correlated with BOLD signal in any of the primary ROIs responding to ketamine and (2) did not contribute to the reliability estimates in these regions, suggesting that in the context of the dataset at hand use of the SVD is justified.

4.4.4 Shape Variation

In addition to head movement, variation in the shape of the ketamine phMRI response was also anticipated. Importantly, design matrices including the shape capturer regressor did not result in the *most reliable* signal estimates, both in ROI and voxel-wise analyses. These findings, and the high concordance between the anatomical distribution of ketamine response observed in this study and that reported by other groups (Deakin et al., 2008; Langsjo et al., 2003; Littlewood, Jones, et al., 2006) suggest that the gamma variate regressor used to model the ketamine response is reliably capturing most of the temporal variance of interest present in the data.

4.4.5 Assessing the Reliability of the Brain Response to Ketamine

A core aim of this chapter was to determine the reliability of the ketamine response across sessions and participants. The outcome of this assessment will have a significant impact on the use of ketamine in future repeated measures designs, including studies to understand the mechanism of action of compounds modulating the effects of ketamine, and the confidence with which ketamine is used as a tool to model glutamatergic dysfunction. Reliability of the ketamine response was described within six different models, representing different modelling approaches. Due to the fact that a model eliciting a highly reliable ketamine response would be more suitable for repeated-measures studies, the model demonstrating the highest reliability estimates within the predefined ROIs and a consistent group-level response was chosen to more thoroughly characterise the response to ketamine across the whole brain. This was found to be Model 3, comprising the GV signal model describing the BOLD response to ketamine infusion as the regressor of interest, along with the first component of a singular-value decomposition of the six head motion realignment parameters, a linear drift term and a constant offset.

Overall, with the use of Model 3, the ketamine response in the brain was shown to have reasonable reliability estimates in areas previously found to respond to ketamine in terms of increased glucose utilisation, increased blood perfusion or BOLD signal, on single sessions (Deakin et al., 2008; G. E. Duncan, Moy, et al., 1998; Langsjo et al., 2003).The variance and reliability of the BOLD response in these strongly-responding ROIs can then be used to design future studies in which novel compounds are tested to modulate the ketamine BOLD response (Chin et al., 2011).Increases in the extent of effect in the group maps and ROI BOLD signal (although not significantly) were observed in the

higher dose group from the phMRI data. Together with the fact that the higher dose group did not exhibit increased subjective effects, this suggests that the use of the 75ng/mL target plasma dose of ketamine provides a robust BOLD phMRI response while maintaining minimal accompanying psychotomimetic symptoms, which did not appear to affect adherence to study procedures.

It is important to emphasise that using reliability as the model 'selection' criteria is applied with the aim to use ketamine BOLD phMRI as an imaging marker in repeated-measures designs, representing a paradigm suited to detect modulation. Furthermore, the conclusions drawn from the results are specific to the pre-defined ROIs chosen. Models providing a fuller fit to the data (e.g. Models 5 or 6) are (1) also effective at describing the ketamine response and (2) may be more useful in contexts where group-level replicability of the results is sufficient, for example if single group characterisation of the ketamine response is required.

4.4.6   Reversal of the Ketamine-induced Changes in BOLD Signal

The attenuation of the BOLD response to ketamine by lamotrigine demonstrated in this chapter is in keeping with previous studies in experimental animals and humans. In animals, acute administration of lamotrigine produced widespread inhibition of the relative CBV response to the NMDA receptor antagonist PCP in all activated regions (Gozzi, Large, et al., 2008). When administered prior to a ketamine challenge in healthy volunteers, lamotrigine has been shown to reverse ketamine's effects on behavioural and cognitive measures and reduce the ketamine-induced changes in the BOLD signal (Anand et al., 2000; Deakin et al., 2008). This effect has been hypothesised to be due to inhibition of ketamine-induced glutamate release, but in healthy human volunteers, lamotrigine has also been found to increase the cortical release of GABA, an effect which could also counteract the proposed neurochemical effects of ketamine (Kuzniecky et al., 2002).

This is the first study to investigate pre-treatment with risperidone on the BOLD signal response to ketamine in healthy volunteers. Amongst other regions, risperidone was found to attenuate the effects of ketamine in the anterior and posterior cingulate, supporting the proposal that antagonism at 5-HT2A receptors is the dominant mechanism underlying the risperidone-induced attenuation of the ketamine BOLD response, as 5HT2A receptors have been found to be densely distributed in cingulate cortical regions (H. Hall, Farde, Halldin, Lundkvist, & Sedvall, 2000; Varnas, Halldin, & Hall, 2004). It will be important to confirm that risperidone does not modulate the signal

in striatal regions, as this area is low in 5HT2A receptors but high in dopamine D2 receptors (H. Hall et al., 2000; Nyberg et al., 1993; Varnas et al., 2004), a receptor type also targeted by risperidone. In addition, risperidone blocked the subgenual cingulate cortex response to ketamine, whereas lamotrigine did not significantly attenuate this ketamine-related deactivation. Interestingly, the subgenual prefrontal cortex has a particularly high innervation of 5-HT neurons indicated by binding of [3H]citalopram, suggesting serotonergic rather than glutamatergic mechanisms may play a role in the effects of acute ketamine within this region (Mantere et al., 2002; Varnas et al., 2004). Due to the role of the subgenual in emotional processing and that of serotonin in depression, this finding may also have relevance for understanding ketamine's antidepressant effects (R. M. Berman et al., 2000; Cryan & O'Leary, 2010; Drevets, Savitz, & Trimble, 2008; Paul & Skolnick, 2003; C. A. Zarate, Jr. et al., 2006).

The modulatory effects of lamotrigine and risperidone, particularly for the positive ketamine phMRI response, appear to be similar across ROIs with both compounds produce a widespread attenuation. This indicates that these pre-treatments achieve a common endpoint despite known differences in their pharmacological mechanisms of action. Nonetheless, characterisation of the changes in response using other, more selective compounds aimed at modulating glutamate and other systems would be required to fully characterise the ketamine-induced changes in BOLD signal.

It is possible however that factors external to the brain may contribute to the effect of the pre-treatments given. For example, the enzyme cytochrome P450 3A4, which metabolises risperidone, is also known to metabolise ketamine (Fang, Bourin, & Baker, 1999; Hijazi & Boulieu, 2002). Thus, it is possible that the attenuation of the ketamine effect is via an increased enzymatic activity induced by risperidone, resulting in a lower delivered dose of ketamine. Thus it would be important to ascertain that the degree of attenuation is not correlated with the plasma levels of ketamine following risperidone pre-treatment, in order to ensure that the effect is not based purely on lower levels of ketamine entering the brain.

4.4.7   Caveats & Limitations

Ketamine also has effects outside of its antagonist activity at NMDA receptors; at mu-opioid receptors, serotonin and noradrenaline reuptake sites, cholinergic and sigma receptors and dopamine D2 receptors (Hirota & Lambert, 1996; Kapur & Seeman, 2002; Kohrs & Durieux, 1998). Although such effects have been described at higher doses and

in relation to analgesia, it remains possible that non-NMDA effects contributed towards the changes observed in this chapter.

An accepted assumption in fMRI research is that, in the brain, there exists a tight coupling between neuronal activity and rCBF (Golanov & Reis, 1996). However, in pharmacological MRI, and specifically with NMDA receptor antagonists, this neurovascular coupling has been shown to be disrupted (Langsjo et al., 2005; Nehls, Park, MacCormack, & McCulloch, 1990) and thus may render the interpretation of the effects of ketamine more difficult (Iannetti & Wise, 2007). Nonetheless, it has been shown that subanaesthetic doses of ketamine in humans in the range we have used do not appear to produce a disturbed coupling between CBF and metabolism (Langsjo et al., 2004; Schwedler, Miletich, & Albrecht, 1982) indicating that BOLD changes seen in phMRI studies are likely to correspond to underlying neuronal activity. Studies in experimental animals demonstrating increased glucose metabolism in response to ketamine administration in areas similar to those seen in BOLD studies (G. E. Duncan et al., 1999; Littlewood, Jones, et al., 2006) together with the fact that ketamine induces primarily focal and task-dependent BOLD changes (Abel, Allin, Kucharska-Pietura, et al., 2003) support the idea that the effects seen are not purely vascular in origin. Finally, both increases and decreases in the BOLD signal response to ketamine were observed, which is difficult to understand in a purely vascular framework. Risperidone could also have an impact on the vasculature due to its effects on the dopamine system (Krimer, Muly, Williams, & Goldman-Rakic, 1998). However, a vascular account would predict differential effects based on the distribution of pharmacological targets. Instead, the attenuation of the ketamine-induced response to lamotrigine and risperidone was highly similar.

Different confounding variables could have affected the reliability of the ketamine brain response. For example, the sample size in this study and physiological noise may have influenced the reliability calculated due to undue influence of outliers and impaired variance estimates. In addition, reliability could be affected by the development of a tolerance to the effects of ketamine (Pouget, Wattiez, Rivaud-Pechoux, & Gaymard, 2010) although the results in this study, such as the lack of session effects in both behavioural and imaging data, suggest that tolerance did not occur. Furthermore, exclusion of females from the sample may also influence the generalizability of the results, as gender has been shown to affect the response to ketamine (C. J. Morgan, Perry, et al., 2006). Thus, although no statistical outliers were present in our data set, confirmation of

these findings with larger sample sizes and investigation of gender-specific effects remain outstanding.

Finally, the analyses of this chapter were limited to addressing the localised effects of ketamine in the brain. Through the nature of its effects at the NMDA receptor, ketamine may target multiple circuits within the brain, future work investigating the reliability of changes in connectivity within the network of brain regions responding to ketamine challenge would be an interesting and useful addition to the results presented in this chapter.

4.4.8 Conclusions

This thesis chapter describes the first investigation into the test-retest reliability of the ketamine phMRI response. Strong and reliable BOLD responses were observed even at the relatively low ketamine doses employed, although the reliability and the consistency of the modelled responses were dependent upon the design matrix employed. Together with the PSI and the VAS, this imaging tool thus provides a sound methodological foundation to test and validate pharmacological interventions, as has been confirmed with the evident modulatory effects of risperidone and lamotrigine. Furthermore, the drug-induced changes in BOLD signal seen also support, although not unequivocally, the proprosed mechanism of action of all three compounds described in the introduction to this chapter. In conclusion, the bolus ketamine BOLD phMRI scan represents a technique which could be prospectively applied as a model of glutamatergic dysfunction in neuropsychiatric disorders, such as schizophrenia and depression.

Chapter 5 Test-retest Reliability and Modulation of the Effects of Ketamine on Cerebral Blood Flow: Measured with Arterial Spin Labelling

Following from Chapter 4, this chapter describes the resting CBF response to ketamine, measured with pseudo-CASL, and the reliability of this response across sessions. In addition, the modulatory effects of lamotrigine and risperidone were also investigated. Overall ASL was shown to be sensitive and to reliably detect the effects of ketamine, although this was not found to be altered by lamotrigine or risperidone. These findings have implications for the use of ASL in future phMRI studies investigating glutamatergic dysfunction.

5.1   INTRODUCTION

5.1.1   Pharmacological MRI with ASL

Resting state phMRI studies, as described in Chapter 4, are most commonly performed with the use of BOLD MRI (D. J. Wang, Chen, Fernandez-Seara, & Detre, 2011). However, the BOLD signal is marred by the fact that it represents an indirect assessment of neuronal activity, relying on multiple variables such as oxygen utilisation, CBF and CBV (Liu & Brown, 2007). In contrast, ASL, as described in Chapter 2, can provide a quantitative measure of CBF and can thus be evaluated in physiologically meaningful units rather than as a qualitative percentage change in signal (Borogovac & Asllani, 2012). This renders ASL a technique ideal for multi-centre repeated measures studies (Zelaya, Mehta, Black, Fernandez-Seara, & Williams, 2012). Furthermore, due to the tight coupling between CBF and neuronal activation, perfusion data is also considered to be a more accurate localiser of drug-induced neuronal effects (Pfeuffer et al., 2002). This is in comparison to BOLD which is influenced by deoxygenation within large draining veins and may therefore be more representative of downstream effects (Griffin et al., 2010; Petersen, Zimine, Ho, & Golay, 2006).

Due to the manner in which it is acquired, ASL has also been found to be insensitive to low frequency signal drifts, an important issue in BOLD MRI, and is resistant to susceptibility artefacts, which commonly lead to signal drop-out in areas such as the orbitofrontal cortex (Liu & Brown, 2007; D. J. Wang et al., 2011). In addition, ASL is less susceptible to physiological noise like respiration due to its insensitivity to oxygen levels and, when analysing ASL data, it is possible to control for systemic effects such as heart

rate as this may be reflected in global changes in perfusion (D. J. Wang et al., 2011). Finally, ASL does not require a priori assumptions of how the brain is going to respond to the drug for the analysis to be completed (Khalili-Mahani et al., 2011; Zelaya, Zois, et al., 2012) and it is more sensitive than BOLD to slow changes in brain function, an advantage in phMRI when considering that certain compounds may exert their effects over longer periods of time (D. J. Wang et al., 2011). As such, ASL may represent a valuable complementary technique that has the potential to either describe a separate facet of the central drug response or verify the pattern of activation seen with BOLD, increasing interpretability of any drug-induced effects.

5.1.2 The Effects of Ketamine on Cerebral Blood Flow

H_2O^{15} PET has been the standard choice to investigate drug-induced changes in CBF, however it is costly and requires a radioactively labelled tracer which may be hazardous to an individual's health (Zelaya, Mehta, et al., 2012). In comparison, ASL is a fast, non-invasive and cost-efficient method which also provides higher spatial resolution and is thus more suitable for repeated-measures pharmacological studies (Khalili-Mahani et al., 2011; Zelaya, Zois, et al., 2012).

ASL has previously been used in both animal and human phMRI studies to investigate the effects of compounds such as alcohol, cocaine, methylphenidate, propofol, psilocybin, cannabis, metoclopramide and fentanyl (Carhart-Harris et al., 2012; Fernandez-Seara et al., 2011; Griffin et al., 2010; Luo, Schmidt, Fox, & Ferris, 2009; O'Gorman et al., 2008; Tolentino et al., 2011; van Hell, Bossong, Jager, Kahn, & Ramsey, 2011; Zelaya, Zois, et al., 2012). Only one study however has used ASL to investigate the effects of NMDAR antagonists, ketamine and PCP, on CBF in animals (Bruns et al., 2009). Furthermore, the effects of ketamine were only assessed within the striatum. Dose-dependent effects of PCP on the other hand were found in the striatum and the thalamus, with decreases and increases in perfusion respectively. In humans, the effects of sub-anaesthetic levels of ketamine on CBF have so far only been investigated with H_2O^{15} PET and have demonstrated increases in areas such as the prefrontal, orbitofrontal, and cingulate cortices, and thalamic regions in both healthy volunteers and schizophrenic patients (Holcomb et al., 2005; Holcomb et al., 2001; Lahti et al., 1995; Langsjo et al., 2003; Rowland, Beason-Held, Tamminga, & Holcomb, 2010). Ketamine-induced increases in blood flow in frontal and cingulate cortices have been shown to be correlated with increases in psychotic symptoms, as assessed by the Brief Psychiatric Rating Scale (BPRS)

in both schizophrenic and healthy individuals suggesting that blood flow measures are not only useful to assess neuronal effects of drugs but are also functionally relevant (Holcomb et al., 2005; Holcomb et al., 2001).

In order to ascertain whether the ASL perfusion response to ketamine is a viable marker of glutamatergic dysfunction and whether it is suitable as a pharmacodynamic imaging tool to test and validate modulatory compounds, test-retest reliability of the phMRI response to acute ketamine challenge was assessed. Ketamine-induced increases in perfusion in frontal, orbitofrontal, cingulate and thalamic areas were expected and due to the established reliability of the ASL technique (also within pharmacological contexts; (Fernandez-Seara et al., 2011; Jiang et al., 2010; Zelaya, Zois, et al., 2012) it was hypothesised that the response would also be reliable.

5.1.3 The Effects of Risperidone and Lamotrigine on the Glutamate System and Cerebral Blood Flow

In order to assess whether this imaging marker of ketamine challenge is sensitive to modulation by compounds hypothesised to modulate the glutamatergic system, ketamine was administered in conjunction with both risperidone and lamotrigine during resting-state ASL.

Lamotrigine has been shown to attenuate neuronal activity through a decrease in the frequency of action potentials (and associated glutamate release) and evoked excitatory post-synaptic potentials (Calabresi, Centonze, Marfia, Pisani, & Bernardi, 1999; Kida, Smith, Blumenfeld, Behar, & Hyder, 2006; Leach, Marden, & Miller, 1986). Thus, suppression of activity does not appear to occur during 'resting' states, due to the fact that lamotrigine inhibits sodium channels by "stabilising the inactivated state of the channel *after* depolarisation" ((Kida, Hyder, & Behar, 2001), p.589). As a result, lamotrigine has the effect of decreasing CBF values in animals but only subsequent to neuronal stimulation (Kida et al., 2001; Kida et al., 2006). Thus it is possible to hypothesise that in healthy humans, when administered alone, lamotrigine will have no effects on CBF but when administered in conjunction with ketamine it will attenuate any induced increases in CBF.

Hypotheses with regards to risperidone, which acts as an antagonist at both serotonin 5HT2A and dopamine D2 receptors, are harder to formulate due to the complexity of its mechanism of action and the lack of studies on the effects of risperidone on CBF in animals or healthy humans. With the use of PET, a handful of studies have investigated the effects of long-term treatment with risperidone on CBF in schizophrenic patients and

demonstrated that the drug induced minimal decreases in frontal regions and greater decreases in the cerebellum (D. D. Miller et al., 2001), although increases in CBF in frontal cortices have also been found (Brewer et al., 2007). An ASL study in healthy volunteers with antipsychotics haloperidol and aripiprazole demonstrated that these drugs increased perfusion in areas such as the putamen and hippocampus; it is possible therefore that due to a similar mechanism of action (i.e. D2 antagonism), risperidone could have similar effects on perfusion (Handley et al., 2010). With regards to the effect an acute dose of risperidone may have on ketamine-induced changes in blood flow, 5HT2A antagonists have been hypothesised to attenuate NMDAR antagonist-induced glutamate release in cortical areas (Adams & Moghaddam, 1998; Large, 2007), it is thus possible that risperidone may attenuate increases in CBF seen with ketamine. In contrast, dopamine-related vascular dilation may also have an impact on the results (Krimer et al., 1998).

Within this chapter the effect of sub-anaesthetic ketamine on CBF in healthy humans is assessed, with the use of pseudo-continuous ASL, a perfusion imaging technique described in Chapter 2. Reliability of this effect was also determined within the ketamine response network and pre-defined regions of interest (ROIs), including the ACC, PCC and thalamus, similarly to Chapter 4. This is followed by the modulation of the ketamine effect on blood flow within pre-defined ROIs defined from the peak response to ketamine in the ASL reliability study. It is important to note that this perfusion data was acquired during steady-state ketamine conditions in contrast to the BOLD scan (Chapter 4) which describes the initial brain response to ketamine.

5.2   METHODS

Please refer to the relevant sections in Chapter 3 for details on the participants, experimental design, infusion protocol and image acquisition. All image analysis described below was conducted within SPM 8.

5.2.1   Preprocessing

A single-subject T2-weighted anatomical scan is required to preprocess CBF maps. In order to ensure that the co-registration and normalisation steps were optimised to align to brain tissue only, voxels containing non-brain content (e.g. skull, eyes etc.) were removed by applying the Brain Extraction Tool (BET) algorithm contained with the FSL software package. This produced a skull stripped T2 image and a binary mask of the 'brain only'

region. The raw CBF map was then co-registered onto the stripped T2 image producing a CBF map inside the individual subject's T2 space. The co-registered CBF map is then multiplied by the T2 mask. This produces a stripped CBF map in T2 space which ensures that no data outside the brain is taken through to the group analysis stage. The stripped T2 image is then normalised onto a stripped MNI T2 template (slice thickness - 2mm), and these normalisation parameters are applied to the stripped CASL. Finally, the stripped CASL is smoothed with a 10 x 10 x 10 mm Gaussian smoothing kernel. These preprocessing steps were performed for all CBF maps acquired prior to and post ketamine infusion in the reliability and modulation studies.

5.2.2   Reliability Study

Data were acquired from 10 healthy male participants on two separate occasions, at least one week apart. One subject had to be excluded from the reliability analyses due to corrupted acquisition of the T2-weighted anatomical scan.

5.2.2.1   Region of Interest Analysis

Perfusion values for each ASL scan were extracted from pre-defined anatomical ROIs (atlas defined ACC, Thalamus, and PCC, together with two 5mm spheres centred on the peak mid-cingulate (3, 3, 42) and subgenual cingulate (3, 39, -21) responses reported by Deakin et al., 2008; please see Chapter 4, Section 4.2.1.2.4 for details on how these were defined). For each ROI, these were entered into a repeated-measures ANOVA with time (pre- and post-infusion), session (session 1 and 2) as within-subject factors and dose as a between-subjects factor.

5.2.2.2   Whole-brain (voxel-wise) Analysis

Whole-brain, voxel-wise analyses were conducted in order to investigate any ketamine-induced effects outside of the regions of interest. A random effects three-way ANOVA analysis (flexible factorial model in SPM; Figure 5-1 (a)) with subject, time (pre- and post-infusion) and session as factors was performed in order to assess the main effect of ketamine and the main effect of session. An absolute threshold of 150 was applied in order to remove any perfusion values below 15 ml/100mg/min which are more indicative of perfusion within the white matter. A grey matter mask was also included to ensure that all data analysed was within the grey matter. This model was run with and without global perfusion values as a covariate. Global perfusion values were also extracted and analysed in SPSS 20.0 with a 2x2 repeated measures ANOVA with session and time (pre- and post-infusion) as factors to determine whether global perfusion differed between placebo

and drug scans. As a rule of thumb, if global perfusion values differ between treatments (or sessions) these are included in the model in order to ensure that localised effects are not masked. However, to ensure that these localised effects are not artefactual/false positives it is important to investigate whether they are also present in the models excluding global signal (Black et al., 2002). Statistically relevant results for the CBF maps were defined at an uncorrected threshold of $p<0.01$, family-wise corrected for multiple comparisons at $p<0.05$ on the basis of cluster extent.

SECOND LEVEL FLEXIBLE FACTORIALS
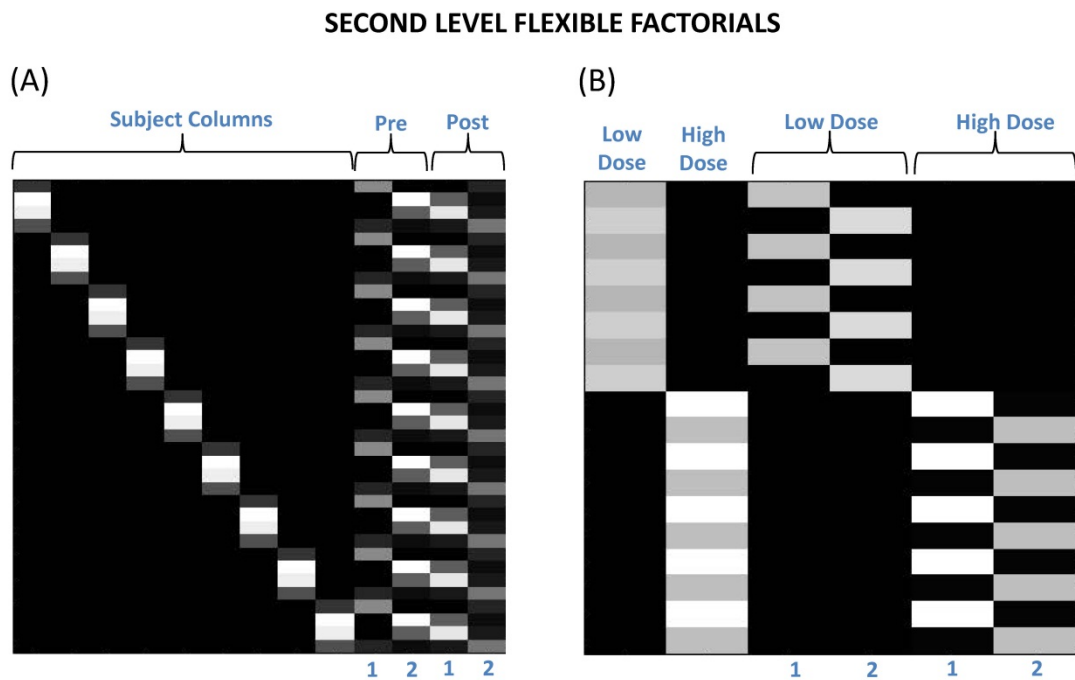
Figure 5-1 Second level design matrices for ASL flexible factorials

Pre-infusion (pre) and post-infusion (post) scans are modelled for both Sessions 1 and 2 in (A). Low (50ng/ml) and high (75ng/ml) ketamine doses are modelled separately in (B) across sessions and for Sessions 1 and 2 separately.

Difference images representing the change in perfusion with ketamine were then created by subtracting the pre-infusion image from the post-infusion image (all images were thresholded between 15 and 90ml/100mg/min using the Image Calculator within SPM 8 in order to retain only biologically plausible perfusion values). These were entered into a second three-way ANOVA analysis (flexible factorial model in SPM; Figure 5-1 (b)) with subject, dose and session as factors to investigate dose effects. These difference images were also used for the reliability analyses.

5.2.2.3   Reliability Analysis

Difference images from Session 1 only were entered into a one-sample t-test to derive the response networks used in the reliability analysis. Voxelwise ICC values were then calculated using the ICC Matlab toolbox (Caceres et al., 2009; see Chapter 3, Section 3.6 for details). ICC values represent the median (with 99% confidence intervals) of the ICC distribution across voxels within the CBF networks. Reliability of perfusion values within pre-defined ROIs (ACC, Thalamus, PCC, mid-cingulate, subgenual cingulate) was also calculated.

5.2.3   Modulation Study

Data were acquired from 16 healthy male participants on four separate occasions (both pre- and post-infusion), at least ten days apart. Please refer to Figure 3-4 (Chapter 3) for how the different drug conditions are referred to for analysis purposes.

5.2.3.1   Region of Interest Definition and Analysis

Based on the peaks of the CBF response to ketamine reported in the reliability study (a separate cohort of participants), a set of ROIs were defined *a priori* for the assessment of treatment effects. These comprised six coordinate-based ROIs, consisting of 5mm radius spheres centered on the Montreal Neurological Institute coordinates corresponding to the anterior cingulate (right: [12,36,-6]; left [-14,36,20]), subgenual cingulate (right: [12,18,-18]; left [-10,4,-20]), and the medial prefrontal cortex (right: [12,48,26]; left [0,68,26]). For each region, perfusion values were extracted using a departmental ASL analysis toolbox and the SPM8 toolbox MarsBar.

All ROI data analysis was conducted in SPSS 20.0 (www.ibm.com/spss). Initial data screening was performed in order to assess whether assumptions for parametric analysis were met. Accordingly, exploratory analysis and Shapiro-Wilk tests were completed. Perfusion data were found to be normally distributed, thus parametric tests were used. Perfusion data were analysed with two one-way repeated measures ANOVAs in order to assess the treatment effect pre- and post-ketamine infusion. In addition, in order to assess the post-infusion ketamine modulation effects while taking into account the pre-infusion effects of the oral drugs, a 2x4 multifactorial repeated measures ANOVA with time (pre- and post-infusion conditions) and treatment arm as within-subject factors was conducted. Furthermore, three 2x2 repeated measures ANOVAs were performed in order to investigate (1) any significant interaction effects found in the 2x4 model and (2) whether

any significant interactions were present when the treatment comparisons of interest were isolated. This addresses the question of whether the pre-infusion effects of placebo, risperidone and lamotrigine affect the interpretation of any treatment effects seen with ketamine. Significant main effects and interactions were interpreted using pair-wise comparisons, Bonferroni corrected for multiple comparisons.

5.2.3.2   Whole-brain Analysis

For second level group analyses, perfusion data were taken through to three multifactorial repeated measures ANOVAs (flexible factorial model in SPM). Two were conducted with subject as a random effect between-subjects factor and treatment as a within-subject factor to assess any treatment effects pre- and post-ketamine infusion separately. To investigate the post-infusion ketamine modulation effects while taking into account the pre-infusion effects of the oral drugs, an additional flexible factorial model with subject as a random effect between-subjects factor and time and treatment arm as within-subject factors was conducted. Models were run with and without global perfusion values as a covariate. Global perfusion values were also extracted and analysed in SPSS 20.0 with two one-way repeated measures ANOVA (for pre- and post-infusion conditions separately) with treatment as a within-subject factor to determine whether global perfusion differed between treatments. Group maps were thresholded at $p < 0.01$ uncorrected at the voxel level and cluster corrected at $p < 0.05$.

5.3   RESULTS

5.3.1   Reliability Study

5.3.1.1   Region of Interest Analysis

Please refer to Table 5-1 for all relevant statistics. No significant main effect of ketamine, session or dose was found for the pre-defined ROIs. No significant interactions were found.

Table 5-1 The effect of ketamine on pre-defined ROIs

ROIs	ANOVA					
	Main Effect of Ketamine (time)		Main Effect of Session		Main Effect of Dose	
	F[1,7]	P	F[1,7]	P	F[1,7]	P
ACC	1.858	0.215	0.291	0.606	1.825	0.219
PCC	0.506	0.500	0.055	0.821	0.000	0.993
Thalamus	1.115	0.326	0.011	0.921	0.064	0.808
Mid-Cingulate Cortex (3,3,42)	0.349	0.573	0.032	0.863	0.606	0.462
Sub-genual Cingulate Cortex (3,39,-21)	0.208	0.662	2.033	0.197	0.463	0.518

5.3.1.2 Whole-brain Analysis

The model excluding the global covariate was chosen to define ketamine and session effects, as a repeated-measures ANOVA of the global values found no significant difference between pre- and post-infusion (p=0.19) or session (p=0.85). Furthermore, it is possible that due to the expected widespread effects of ketamine controlling for global perfusion values may in fact remove neuronal effects of the drug.

As Figure 5-2 (a) shows, ketamine- induced increases in CBF are evident in the anterior and subgenual cingulate, prefrontal and orbitofrontal regions at a cluster-corrected threshold of 0.05 (uncorrected voxel-wise threshold of 0.01). No decreases in blood flow at this threshold were found. Similarly, no whole-brain effect of session or dose was found.

5.3.1.3 Reliability Analysis

As Table 5-2 demonstrates, reliability of the perfusion data was conducted for certain ROIs. Figure 5-2 (b) demonstrates voxel-wise reliability values across the whole brain.

Table 5-2 Reliability of the ketamine-induced changes in CBF

	ICC (3,1) +/- SE
Perfusion Network	0.41 (0.004)
ACC	0.61 (0.003)
PCC	0.38 (0.005)
Thalamus	0.07 (0.005)
Mid-Cingulate Cortex (3,3,42)	0.51 (0.007)
Sub-genual Cingulate Cortex (3,39,-21)	0.71 (0.016)

Table 5-2 Reliability of the ketamine-induced changes in CBF
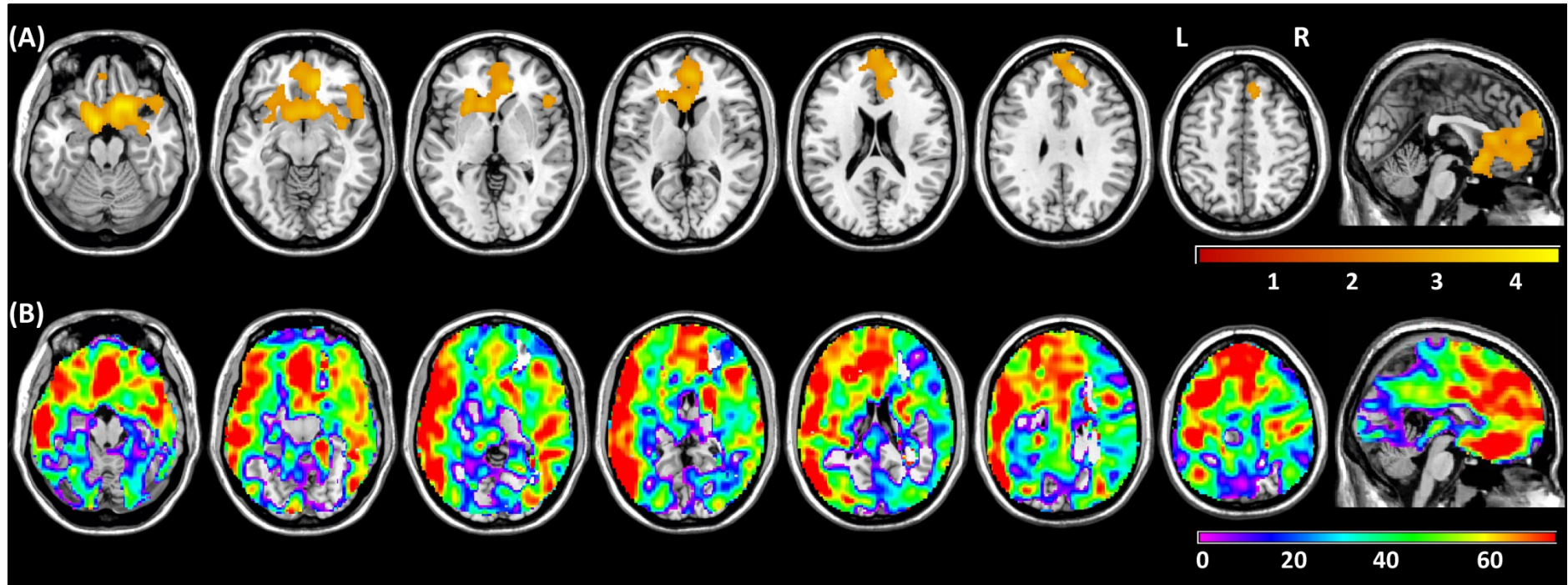
Figure 5-2 Perfusion response to ketamine

(a) Increases in CBF with ketamine across both sessions overlaid on a T1-weighted image (significant at 0.01 uncorrected, corrected for cluster extent at p<0.05; colour scale depicts T-scores); (b) whole-brain reliability of the response overlaid on a T1-weighted image (colour scale depicts ICC scores multiplied by 100).

5.3.2   Modulation Study

5.3.2.1   Region of Interest Analysis

5.3.2.1.1   Effects of Treatment

Pre-Infusion Please refer to Table 5-3 for all relevant statistics. A main effect of treatment was found for the left anterior cingulate and right medial prefrontal cortex (p<0.05). Bonferroni corrected pairwise comparisons indicate that this is due to a decrease in perfusion with lamotrigine (Lamotrigine_K) compared to placebo (Placebo_S) in both regions and a decrease in perfusion with risperidone (Risperidone_K) compared to placebo (Placebo_K) in the medial prefrontal cortex (see Appendix B).

Post-Infusion Please refer to Table 5-3. for all relevant statistics. No main effect of treatment was found for any of the ROIs.

Table 5-3 Pre- and post-ketamine infusion treatment effects

	Perfusion			
ROIs	**Pre-Infusion One-Way ANOVA**		**Post-Infusion One-Way ANOVA**	
	Main Effect of Treatment		Main Effect of Treatment	
	F[3,45]	P	F[3,45]	P
rACC	2.063	P=0.119	0.030	P=0.993
lACC	2.917	P<0.05	2.072	P=0.117
rSubG	1.410	P=0.252	1.844	P=0.153
lSubG	1.374	P=0.263	0.157	P=0.925
RmPFC	3.671	P<0.05	0.309	P=0.819
LmPFC	2.099	P=0.114	0.931	P=0.433

5.3.2.1.2   The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects

A 2x4 repeated measures ANOVA was conducted to describe the effects of time and the interaction between time (pre- and post-infusion conditions) and treatment arm (see Appendix B; Table B-3 for all relevant statistics). A significant main effect of time was found in all ROIs (p<0.05). This was found to be driven by an increase in perfusion in post-infusion conditions for all treatment arms. No time by treatment arm interactions were found. Three separate 2x2 ANOVA models were conducted in order to investigate

whether any significant interaction effects were present when the treatment comparisons of interest were isolated. No significant time by treatment interactions were found in any ROI, in any model (see Table 5-4).

Table 5-4 Treatment-specific effects

	Perfusion					
ROIs	**PLA-KET 2x2 ANOVA**		**KET-RIS 2x2 ANOVA**		**KET-LAM 2x2 ANOVA**	
	Time x Treatment Interaction		Time x Treatment Interaction		Time x Treatment Interaction	
	F[1,15]	P	F[1,15]	P	F[1,15]	P
rACC	0.216	P=0.649	0.202	P=0.659	1.311	P=0.270
lACC	2.626	P=0.126	0.004	P=0.953	0.251	P=0.624
rSubG	0.979	P=0.338	0.434	P=0.520	0.830	P=0.377
lSubG	0.254	P=0.622	0.511	P=0.486	2.772	P=0.117
RmPFC	0.012	P=0.916	0.753	P=0.399	1.242	P=0.283
LmPFC	0.803	P=0.384	0.055	P=0.817	0.357	P=0.559

5.3.2.2 Whole-brain Analysis

As expected under ketamine conditions, increases in CBF were seen in the prefrontal cortices, anterior and subgenual cingulate although this was only evident at an uncorrected threshold of 0.01. Increases in CBF were also seen in the thalamus (see Figure 5-3).

Figure 5-3 Ketamine-induced increases in perfusion compared to placebo in the modulation study

Overlaid onto a T1-weighted image in MNI space and displayed at an uncorrected threshold of 0.01.

Whole brain analyses of the perfusion data were conducted for exploratory purposes, in order to detect any treatment effects outside of the pre-selected regions of interest. Treatment effects were investigated with the use of two separate flexible factorials for the pre-infusion and post-infusion conditions separately, to examine the main effects of the oral drugs and their subsequent effects on the ketamine response. As figure 5-4 shows, statistical analysis of global perfusion values indicated that, at pre-infusion, a significant difference existed ($p<0.05$), thus these were included in the pre-infusion model to ensure that localised effects were not masked. In contrast, global perfusion did not differ significantly between post-infusion conditions and was thus not included in this model. These analyses were followed by a larger flexible factorial analysis combining pre- and post-infusion conditions in order to investigate ketamine modulation effects while taking into account the effects of the oral drugs. The result presented is from the model including the global perfusion values.
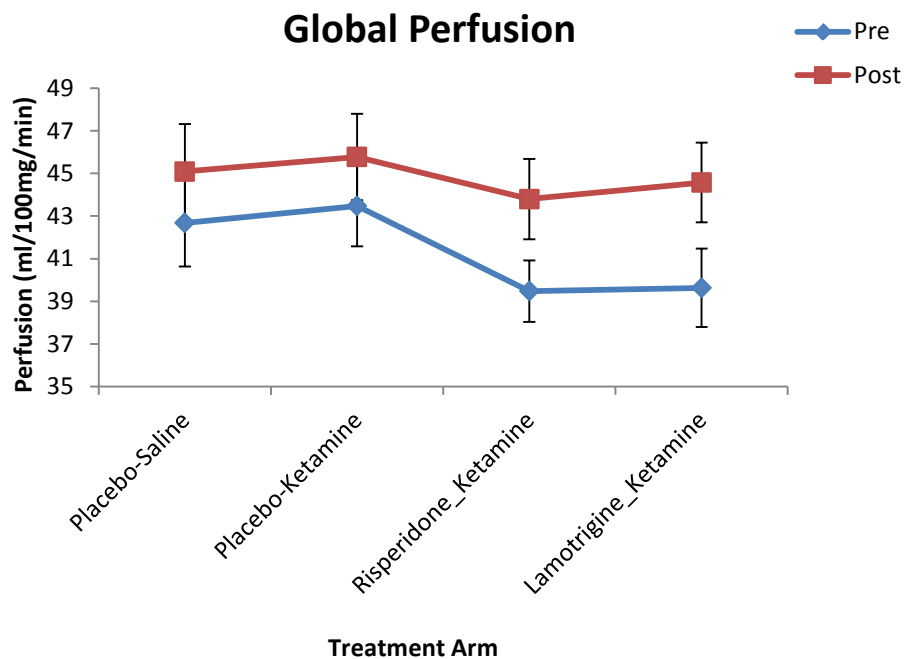
Figure 5-4 Global perfusion values for each treatment arm, pre- and post-infusion

Errors bars represent the standard error of the mean.
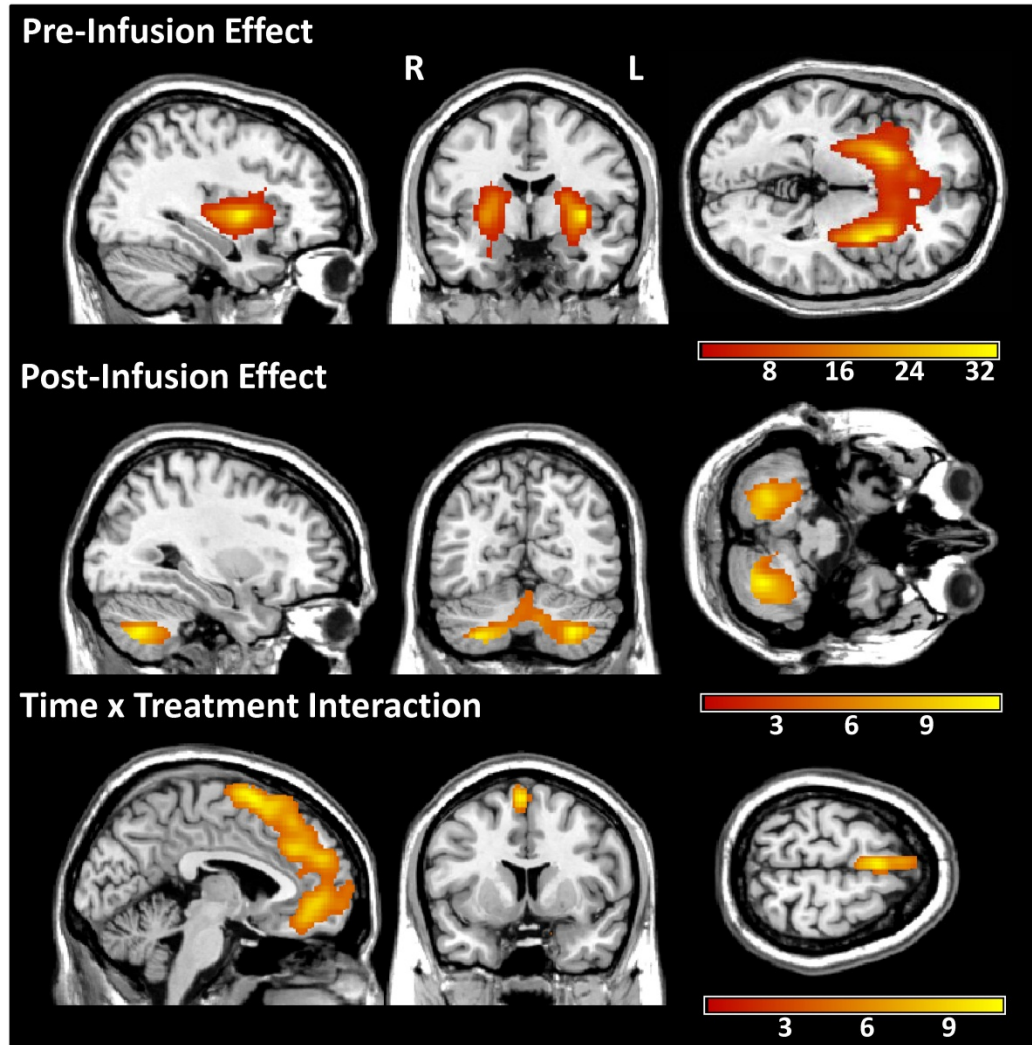
Figure 5-5 Whole-brain treatment effects

Whole-brain effects of treatment at pre-infusion and post-infusion (centred on MNI coordinates (34,-2,0) and (30,-64,-44) respectively). The time by treatment interaction is centred on MNI coordinate (-4,8,66). All coordinate peaks are significant at an FWE (0.05) corrected threshold.

As Figure 5-5 shows, a pre-infusion main effect of treatment was found in bilateral putamen and a post-infusion main effect of treatment was found in bilateral cerebellum. Both effects were significant on the basis of peak amplitude, family-wise corrected for multiple comparisons at $p<0.05$, and were present in the models both including and excluding global signal. Post-hoc T-tests within the flexible factorials demonstrated that the pre-infusion effect was due to a risperidone-induced increase in perfusion compared to both placebo sessions (significant at 0.01 uncorrected, FWE corrected at 0.05 on the basis of cluster extent) and the post-infusion effect was due to a decrease in perfusion in

the R_Ketamine condition compared to both placebo and ketamine conditions (only present at an uncorrected threshold).

The flexible factorial analysis including both pre- and post-infusion conditions demonstrates a significant time by treatment interaction in the left supplementary motor area (SMA), significant on the basis of peak amplitude, family-wise corrected for multiple comparisons at $p<0.05$ (see Figure 5-5). The direction of the perfusion response in each treatment condition suggests that this effect is driven by an interaction between placebo and ketamine treatment arms. A 2x2 interaction F-contrast within the flexible factorial confirmed this interpretation, whereby the SMA is still found to be significant at an FWE corrected threshold (on the basis of peak amplitude). This effect is driven by a decrease in perfusion at post-infusion in placebo conditions whereas an increase in perfusion occurs with ketamine.

5.4   DISCUSSION

5.4.1   Summary of Main Results

Whole-brain analyses demonstrated that pseudo-continuous ASL is sensitive to ketamine-induced increases in CBF, although this was only shown in the whole-brain analysis and not for the summary values extracted from the a priori ROIs chosen. Nonetheless, reliability of the ketamine response was found to be high, specifically within the anterior and subgenual cingulate. Lamotrigine and risperidone were found to have effects on CBF when administered alone, but did not demonstrate any modulatory effects on the ketamine response.

5.4.2   The Effects of Ketamine on Cerebral Blood Flow Measured with ASL

The distribution of the ketamine-induced increases in CBF, in areas such as the anterior and subgenual cingulate, prefrontal and orbitofrontal cortices, and thalamus is analogous to that seen in previous ketamine perfusion studies (Holcomb et al., 2005; Holcomb et al., 2001; Lahti et al., 1995; Langsjo et al., 2003; Rowland et al., 2010), confirming the utility of ASL in phMRI studies of glutamatergic dysfunction. Furthermore, the pattern of CBF increases is also in accordance with known NMDA receptor distributions (see Chapter 1). However, the effects of ketamine were not found to be significant within the pre-defined ROIs tested in the reliability or modulation study; this may be due to (1) their larger size

(in comparison to sphere-based ROIs) and thus lack of statistical power to detect drug-induced changes or (2) differences in peak response between the two studies.

As expected, reliability of (non-significant) ketamine-induced changes in CBF was found to be adequate, with higher values in the anterior and subgenual cingulate and lower values for the thalamus. Indeed thalamic increases in CBF were only present in the second cohort of participants tested. These results suggest that ASL represents a suitable alternative to BOLD for future interventional repeated-measures studies. However, the CBF response to ketamine was more restricted and present at a lower statistical threshold compared to BOLD (see Chapter 4) suggesting this methodology may be less sensitive to ketamine-induced changes, although it is important to remember that the ASL scan was acquired after the BOLD scan when ketamine levels were at a steady-state.

5.4.3 Physiological Basis of Ketamine-induced Changes in Cerebral Blood Flow

As described in Chapter 2, regional increases in CBF have been found to accompany a rise in local neuronal activity (Huettel et al., 2009), although the precise mechanisms that elicit this 'functional hyperaemia' have not yet been established (Attwell et al., 2010). Recently evidence suggests that a neurotransmitter-mediated process is central to the regulation of CBF, with a prominent role for the glutamatergic system (Attwell et al., 2010; Attwell & Iadecola, 2002). It has been found that activation of neuronal NMDARs and astrocytic mGluRs together with the ensuing influx of calcium ions, triggers the release of nitric oxide (NO), adenosine and arachidonic acid, molecules that indirectly regulate vasodilation or vasoconstriction (Attwell et al., 2010; Attwell & Iadecola, 2002). In animal studies it appears that NMDAR-mediated release of NO is important for vasodilation (Fergus & Lee, 1997; Meng, Tobin, & Busija, 1995), although NO has also been shown to affect vasoconstriction (Attwell et al., 2010). It is thus possible that ketamine-induced increases in glutamate and subsequent NO release in cortical areas may be responsible for associated increases in blood flow, although this may be an oversimplification as this ignores glutamatergic effects on astrocytes and the fact that the impact of glutamatergic vasoactive mechanisms differ between brain regions (Attwell et al., 2010; Kimura-Kuroiwa et al., 2012).

5.4.4 Reversal of Ketamine-induced Effects on Blood Flow

In contrast to the hypotheses stated in the introduction to this chapter and previous animal studies (Kida et al., 2001; Kida et al., 2006), lamotrigine was found to decrease

perfusion in the anterior cingulate and prefrontal cortices suggesting that inhibition of sodium channels may have an effect on 'resting' CBF in humans. This may be due to effects of lamotrigine on constitutive neuronal activity, or it is also possible that humans, especially within the environment that they were placed (inside a relatively noisy scanner), never entirely disengage cognitively, thus excitatory processes may still be suppressed with compounds targeting the glutamatergic system. On the other hand, no modulatory effects of lamotrigine were found on the CBF response to ketamine.

With regards to risperidone, decreases in perfusion were found in the medial prefrontal cortex and cerebellum and an increase in perfusion was found in the putamen. Such findings are similar to those seen in previous studies (Handley et al., 2010; D. D. Miller et al., 2001), and are unlikely to be due to non-specific vascular effects of the drug (e.g. dopamine-induced vasoconstriction; (Krimer et al., 1998) as these effects were present with and without controlling for global changes in perfusion. As with lamotrigine however, no modulatory effects of risperidone were found on the CBF response to ketamine.

It is possible that no modulatory effects of risperidone were detected due to the choice of a priori ROIs. These did not include the striatum, a region much more likely to be affected by risperidone due to its effects at dopamine D2 receptors (Nyberg et al., 1993). However, no modulatory effects of lamotrigine were found either, even though regions sensitive to glutamatergic changes, including the prefrontal cortex, were chosen (Idris et al., 2005; Lorrain et al., 2003; Moghaddam et al., 1997). Nonetheless, effects of these compounds were seen when administered alone suggesting that it is perhaps the effect of steady-state low-dose ketamine that does not elicit a robust change in blood flow that is susceptible to modulation.

5.4.5 Caveats and Limitations

Although no significant global changes in perfusion were detected with ketamine, it is still possible that systemic effects of the drug, such as increases in heart rate, contributed to the regional increases in CBF. However this is unlikely as any drug-induced changes in heart rate had stabilised prior to ASL acquisition. Nevertheless, in future analyses, it would be important to ensure, with for example a regression analysis, that physiological effects of ketamine do not significantly contribute to the drug-induced variations in CBF (Khalili-Mahani et al., 2011).

A further limitation of this study is that the ASL data could not be directly compared to the BOLD data described in Chapter 4, which restricts the interpretation of ketamine-induced changes in brain response. In order to improve this and crosstalk between different modalities, simultaneous acquisition of BOLD and CBF data with use of techniques using a double-echo acquisition such as QUIPPS II (Luh, Wong, Bandettini, & Hyde, 1999; Wong, Buxton, & Frank, 1997), may be beneficial (Liu & Brown, 2007; D. J. Wang et al., 2011).

5.4.6 Conclusions

This study is the first to describe the effect of ketamine on CBF with the use of ASL and to assess the reliability of this response. Overall, ASL appears to sensitively and reliably detect ketamine-induced increases in CBF. However, these effects were not detected within the pre-defined ROIs chosen nor were modulatory effects of lamotrigine or risperidone found. Due to previous research it is unlikely that the absence of modulatory effects is due to the fact that the mechanism of action of such drugs does not interact with that of ketamine (Brody et al., 2003; Deakin et al., 2008; H. Y. Meltzer et al., 2011). On the other hand this suggests that with low-dose steady-state ketamine, ASL may not represent the most suitable technique for future interventional studies. Nonetheless the high reliability of the response indicates that ASL has immense potential in glutamatergic phMRI studies, but that more research is needed to improve the context in which it is acquired.

Chapter 6 The Effect of Ketamine on the Working Memory N-BACK task in Healthy Volunteers: Modulation with Risperidone and Lamotrigine

As described in Chapter 1, the role of glutamatergic signalling in cognitive function, and specifically WM, has gained prominence. This chapter describes how ketamine, a probe of the glutamatergic system in healthy volunteers, affects WM function and its neural correlates in the context of an fMRI N-Back task. In addition, the modulatory effects of lamotrigine and risperidone, compounds expected to counteract the effects of ketamine, are also described. Overall fMRI imaging was shown to be sensitive to the subtle effects of ketamine on cognitive brain networks responding to increasing WM load, specifically within the dorsolateral prefrontal cortex (DLPFC). No significant modulatory effects of lamotrigine were found. The effects of risperidone were more pronounced, with modulation of ketamine's effects apparent in the hippocampus. These findings have implications for the role of NMDAR hypofunction in WM processing and the utility of fMRI, and specifically the N-Back task, in detecting the effects of low-dose ketamine.

6.1   INTRODUCTION

6.1.1   The Neural Basis of Working Memory

Working memory is a psychological construct which describes the processes used to maintain and manipulate information on a short-term basis, which is then used to guide behaviour (Baddeley, 1992; Manoach et al., 1997). Baddeley and Hitch's (1974) WM framework has been one of the most prominent models used to describe this function, and consists of four components; the phonological loop (a short-term store for verbal information, retained by articulatory rehearsal and representations), the visuo-spatial sketch pad (a short-term store for visual information), the central executive (an attentional control system that coordinates the two storage components to manipulate and monitor the information within) and finally the episodic buffer (a temporary storage that combines information from different sources and links WM to long-term memory storage systems) (Baddeley, 2000; Barch & Smith, 2008; G. D. Honey & Fletcher, 2006). The functions of the phonological loop and the visuo-spatial sketch pad are more restricted whereas the central executive is attributed a variety of processes including manipulation of the stored information, interference control from additional information, temporal sequencing and

updating of the contents held in WM, and preserving goal representations 'online' (Barch, 2006; Barch & Smith, 2008).

As described in Chapter 1, electrophysiological primate studies have shown that the lateral surfaces of the prefrontal cortex play a critical role in WM function (Goldman-Rakic, 1995b). Initially, Goldman-Rakic and colleagues proposed that this area of the brain, much like the visual system, was functionally segregated by domain; the ventrolateral regions engaged in non-spatial WM processes whereas the dorsolateral regions were engaged with spatial processes (McCarthy et al., 1996; Owen, 2000). In comparison, the WM model developed by Petrides (2000) through animal lesion studies suggests that WM is organised in a process-specific manner; in fact he attributes storage and rehearsal processes to parietal and temporal cortices, comparison and selection processes to the ventrolateral prefrontal (VLPFC) regions and manipulation and monitoring processes to the dorsolateral prefrontal cortex (DLPFC) (Owen, 2000; Petrides, 2000). Several PET and fMRI imaging studies in humans have supported this process-specific view, by demonstrating that DLPFC is primarily recruited or shows increased activity during tasks that require monitoring and manipulation processes to be performed regardless of domain (D'Esposito et al., 1999; Owen et al., 1996; Owen et al., 1999; Owen et al., 1998). On the other hand, the role of the DLPFC in WM per se has been questioned with some suggesting that its function is primarily that of response representation and selection rather than the actual maintenance or manipulation of this information (Rowe, Toni, Josephs, Frackowiak, & Passingham, 2000).

Although the specific function of these WM brain areas are still a matter of debate, a core brain network, which is recruited in healthy humans whilst performing WM fMRI tasks, has been identified. Activity is most commonly found in cortical regions such as the prefrontal, parietal, cingulate and cerebellar cortices, and subcortical structures such as the thalamus, hippocampus and caudate (Cabeza & Nyberg, 2000a, 2000b; S. J. Lewis, Dove, Robbins, Barker, & Owen, 2004; Ranganath & D'Esposito, 2001; Rypma & D'Esposito, 1999; Rypma, Prabhakaran, Desmond, Glover, & Gabrieli, 1999). The parietal cortex has been implicated in both storage and manipulation processes (Champod & Petrides, 2010; Jonides et al., 1998) whereas the cingulate is involved in error detection and conflict monitoring (Carter, Braver, et al., 1998). The thalamus and caudate are part of the frontostriatothalamic loops, whose integrity has been implicated in the executive dysfunction, specifically manipulation deficits, evident in Parkinson's disease, a disorder characterised by depleted dopamine levels (S. J. Lewis et al., 2004; Monchi, Taylor, &

Dagher, 2000). The hippocampus on the other hand has been shown to be recruited during the active maintenance of novel information (Ranganath & D'Esposito, 2001).

6.1.2 The Neural Basis of the N-Back Task in Healthy Volunteers

A variety of tasks have been used to assess WM function in healthy volunteers. Some, like the Sternberg task, assess maintenance processes whereas tasks including reordering or planning abilities assess the manipulation component of WM (P. C. Fletcher & Henson, 2001). A task that combines both maintenance and manipulation processes is the N-Back task, a method first devised by Gevins and colleagues (Gevins, 1990; Gevins & Cutillo, 1993), whereby participants are presented with a sequence of letters (in the verbal version of the N-Back task) and are required to maintain, monitor and update the contents of WM in order to respond appropriately when the letter presented matches that of N presentations previous (P. C. Fletcher & Henson, 2001). The brain network recruited with this task includes activation in the DLPFC, VLPFC, inferior and medial parts of the parietal lobe, the cingulate, premotor areas, medial and lateral areas of the cerebellum and the thalamus (Owen, McMillan, Laird, & Bullmore, 2005).

In order to systematically investigate WM processes, the N-Back task parametrically manipulates task load (most commonly between zero and three back), whereby the number of items the participant is expected to maintain and manipulate in WM is increased in each condition (Braver et al., 1997; Carter, Perlstein, et al., 1998). In fMRI, this affords the advantage of being able to study a particular cognitive process without relying solely on subtraction with the control condition (E. E. Smith & Jonides, 1997). Studies varying WM load show not only that the DLPFC is recruited in higher memory load conditions in maintenance studies (Rypma et al., 1999) but that activation within this region increases as a function of WM load (Manoach et al., 1997), mirroring the linear relationship between reaction time and load (Sternberg, 1969). This linear pattern in activation with respect to WM load has also been found with the use of N-Back tasks, in certain nodes of the WM brain network such as the DLPFC, VLPFC, posterior parietal and cingulate cortices (Braver et al., 1997; Jonides et al., 1997).

On the other hand, additional N-Back studies have highlighted that at times the brain response to WM load may either plateau between the two-back and three-back, with the increase in activation occurring mainly between the 1-back and 2-back conditions or follow an inverted-U shape (Callicott et al., 1999; Cohen et al., 1997). In fact, Callicott and colleagues (1999) demonstrated that when WM capacity was exceeded, activation in

certain brain areas, such as the DLPFC and thalamus, decreased, whereas other areas, such as the cingulate, demonstrated a capacity 'unconstrained response' with activation continuing to increase with load. Although the highest WM load administered was the three-back condition; the authors acknowledge that the inverted U–shaped response seen in the DLPFC may be due to an increased difficulty of the task in comparison to that used in previous studies (Braver et al., 1997; Jonides et al., 1997). Thus, a parametric task design allows the construction of a WM 'load-activation curve', determining (1) in what manner and to what extent brain regions are involved in WM processes (Braver et al., 1997) and (2) when the system has reached capacity (Callicott et al., 1999).

6.1.3   The Role of the Glutamatergic System in Working Memory

Elucidating the neural correlates and neurobiological basis of WM has become an important goal of cognitive neuroscience not only because it is central to the integrity of many higher order cognitive processes but also due to the prominence of WM deficits in disorders such as schizophrenia (G. Honey, 2009), where research into this area has been given priority (Barch & Smith, 2008). As chapter 1 describes, deficits in manipulation processes and aberrant activation patterns in frontal cortices are characteristic of WM deficits in schizophrenia (Conklin et al., 2005; Van Snellenberg, 2009). Furthermore, patients with schizophrenia have been hypothesised to reach working memory capacity at lower WM loads than healthy controls, characterised by an increase in activity (relative to controls) at lower memory loads and a decrease in activity at higher memory loads, specifically in the DLPFC (Callicott et al., 2003; Jansma et al., 2004; Manoach, 2003).

The dopaminergic (DA) and noradrenergic (NA) neurotransmitter systems have established roles in WM function (Arnsten, 1998, 2011). Similar to WM load-related BOLD signal, these neuromodulatory systems, via dopamine D1 receptors and adrenergic αA2 and αA1 receptors respectively, demonstrate an inverted U-shaped relationship with prefrontal-mediated cognitive functions, with either too little or too much stimulation impairing performance (Arnsten, 2011). On the other hand, optimal stimulation of these receptors is thought to promote the stability of recurrent excitation within networks of PFC pyramidal neurons, the hypothesised electrophysiological basis of maintaining information or representations 'online' (Arnsten, 2011; Castner & Williams, 2007; Williams & Castner, 2006). Specifically, optimal D1 stimulation has been shown to reduce neuronal firing to irrelevant stimuli and thus enhances 'tuning' of the network, whereas αA2 stimulation strengthens the signal within the representational network itself (Robbins

& Arnsten, 2009; Vijayraghavan, Wang, Birnbaum, Williams, & Arnsten, 2007). This persistent excitatory activity characteristic of WM function is thought to require cortical glutamatergic NMDARs, due to their voltage-gated mechanism and resulting slow, stable kinetics (Arnsten, 2011; Compte, Brunel, Goldman-Rakic, & Wang, 2000; Lisman, Fellous, & Wang, 1998; X. J. Wang, 1999). Thus, although D1/αA2-NMDAR interactions are important to WM function, the effects of the DA and NA systems are considered to be primarily modulatory, with the fundamental basis of WM left in the hands of the glutamatergic system (Arnsten, 2011; Castner & Williams, 2007).

Furthermore, in recent years, evidence has accumulated suggesting that glutamate dysregulation, mediated through NMDAR hypofunction, represents a significant contributor to the deficits seen in schizophrenia (see Chapter 1 for details). Taken together with the the neurochemical basis of WM function described above and the pronounced WM deficits seen in schizophrenia, it is thus possible that glutamatergic abnormalities may lead to WM dysfunction in healthy humans.

6.1.4   The Effects of Ketamine on Working Memory

These assertions have indeed been corroborated by the finding that the administration of ketamine, a well-tolerated NMDAR antagonist (Perry et al., 2007), can lead to WM deficits in both animals and healthy human volunteers at a performance (C. J. Morgan & Curran, 2006; J. W. Smith et al., 2011) and electrophysiological level (Ahn et al., 2003). In healthy humans, ketamine-induced WM performance impairments appear to be more pronounced within verbal WM and when manipulation processes are tested (see Chapter 1, Table 1-1). Specific impairments in the N-Back task have also been found, however only at doses above predicted plasma levels of 90ng/ml (Adler et al., 1998; C. J. Morgan, Mofeez, et al., 2004a). Importantly, WM deficits have been shown to be reversed in both non-human primates and humans, with the use of compounds that promote the inhibition of glutamate release (Krystal, Abi-Saab, et al., 2005; Roberts, Shaffer, et al., 2010).

In terms of fMRI, few studies have investigated the effect of ketamine on WM in healthy volunteers, with only one using the N-Back task (G. D. Honey et al., 2008; R. A. Honey et al., 2004). Although performance was not impaired, Honey et al., (2004) demonstrated that ketamine increased activation in the DLPFC, VLPFC, parietal cortex, anterior cingulate, caudate and putamen when manipulation processes were required, under low load conditions. On the other hand, Honey et al., (2008) found that ketamine increased

activation in thalamus, caudate and putamen, across all load conditions in the N-Back task. These effects were seen with the use of relatively low doses of ketamine (100ng/ml predicted plasma levels), with the increases in activation being primarily hypothesised as a result of increased glutamate release and its effects at non-NMDA glutamatergic receptors ((R. A. Honey et al., 2004); see Chapter 1 Section 1.1.2).

These studies support the notion that the glutamatergic system is involved in WM function, and suggest that the N-Back task is suitable to detect glutamate-induced disruption in WM brain networks. Nonetheless it is important to substantiate previous findings with the use of ketamine at a dose which minimises both psychotomimetic symptoms and overt performance deficits in order to be able to identify changes in brain activation due to drug effects in the cognitive task network of interest, that are not the result of drug-induced side effects and performance changes. To satisfy this aim, healthy human volunteers were administered ketamine whilst performing a WM N-Back task (with a maximum load of 3-back), at a dose shown to produce robust brain activation but minimal subjective effects (see Chapter 4). Specifically, in order to test predictions with regards to the effects of ketamine on WM in a principled manner, an ROI-based approach was taken with a focus on bilateral DLPFC, cingulate, caudate, thalamus and hippocampus. These ROIs were chosen on the basis of their acknowledged (1) role in WM function, (2) presence in the N-Back task brain network and (3) high concentration of NMDARs and sensitivity to the effects of ketamine determined by previous studies (see Chapter 1, Section 1.1.1.3) and the ketamine phMRI brain response described in Chapter 4 of this thesis. The DLPFC also plays an important role in the manipulation component of WM, a process found to be particularly affected by ketamine.

Thus in consideration of the findings summarised above it was hypothesised that:

(1) Under placebo conditions, N-Back performance (in terms of correct responses) should decrease as a function of WM load. On the other hand, activation within the chosen ROIs should increase linearly as a function of WM load due to the fact that WM capacity should not be exceeded at 3-back in these highly performing healthy volunteers. This hypothesis is more speculative with regards to the hippocampus as its response to load has not previously been investigated.

(2) Under ketamine administration, no performance deficits should be seen due to the low dose used. In terms of activation (a) increases in activation when compared to placebo should occur (at all memory loads) or (b) even in the absence of overt WM capacity deficits, the greater sensitivity of fMRI to drug-

induced effects (e.g. Honey et al., (2005)) may detect subtle changes in cortical efficiency, evidenced by altered load-response profiles.

6.1.5 The Effects of Risperidone and Lamotrigine on the Glutamate System and Working Memory

In order to further investigate the contribution of the glutamatergic system to the neurobiology of WM, two medications expected to indirectly or directly mediate this neurotransmitter system, risperidone and lamotrigine, were given alone and in conjunction with ketamine to determine their modulatory effects in the context of the WM N-back task.

Lamotrigine has been shown to reverse NMDAR antagonist-induced deficits in measures of sensory-motor gating (ketamine - (Brody et al., 2003)) and reversal learning (PCP - (Idris et al., 2005)). In healthy humans, the administration of lamotrigine alone has been shown to either have no effect on baseline cognitive functioning (Anand et al., 2000) or to improve performance in simple reaction time tasks (Aldenkamp et al., 2002). On the other hand, when administered in conjunction with ketamine, it has been shown to reverse ketamine's detrimental effects on behavioural and cognitive measures (Anand et al., 2000), and attenuate ketamine-induced increases in resting state BOLD fMRI signal (Deakin et al., 2008). These effects have been hypothesised to be due the inhibition of ketamine-induced increases in glutamate release (Large et al., 2005). Finally, combining lamotrigine with atypical antipsychotics, has been shown to be beneficial in the treatment of psychosis in schizophrenic patients (Dursun & Deakin, 2001; Dursun et al., 1999; Kremer et al., 2004; Tiihonen et al., 2003).

In parallel, 5HT2A selective antagonists and risperidone itself have been found to block NMDAR antagonist induced deficits in locomotor function (H. Y. Meltzer et al., 2011), and cognitive function (Didriksen et al., 2007; Mirjana, Baviera, Invernizzi, & Balducci, 2004; Varty, Bakshi, & Geyer, 1999), deficits thought to be the result of a frontal hyperglutamatergic state (see Chapter 1 for details). Risperidone has also been found to treat WM deficits in schizophrenia, a disorder, as we have seen, with potential underlying glutamatergic dysfunction (H. Y. Meltzer & McGurk, 1999). Furthermore, risperidone has been found to 'normalise' WM networks in schizophrenic patients compared to haloperidol (G. D. Honey et al., 1999; Surguladze et al., 2007). Nonetheless, in healthy humans, risperidone has been shown to increase the numbers of errors and slow reaction times in the context of an N-Back task (Koychev et al., 2012). In addition, while

risperidone has been found to modulate the glutamatergic system potentially through its actions at 5HT2A receptors, it has also been known to affect the dopamine system (Breese, Knapp, & Moy, 2002). Together with this fact and the role of dopamine D2 receptors in WM itself (Cools & D'Esposito, 2011; Mehta, Montgomery, Kitamura, & Grasby, 2008; Mehta, Sahakian, McKenna, & Robbins, 1999), the effects of risperidone on NMDAR antagonist-induced deficits in WM may be of a more complex nature than a simple inhibitory action on the release of glutamate.

Although the brain response to lamotrigine or risperidone in the context of WM fMRI, alone or in conjunction with ketamine, has not been previously assessed in healthy volunteers the studies above allow certain predictions to be made:

(1) When administered alone, lamotrigine will either show no effect on load-related performance or a trend towards an improvement, specifically in reaction time measures. With regards to changes in the ROIs, lamotrigine will have no significant effect when compared to the placebo condition due to its pharmacological effects being primarily use dependent (Messenheimer, 1995).

(2) When combined with ketamine, and assuming that ketamine will have had no detrimental effects on performance, no additional modulatory effects of lamotrigine pre-treatment will be seen on performance measures. In terms of brain activation in response to the N-Back task, lamotrigine will attenuate the effects of ketamine, i.e. blunt the increase in activation seen with ketamine.

The predictions with regards to risperidone are more tentative, due to the relative lack of studies investigating its effects in healthy volunteers and its complex pharmacological mechanisms.

(3) When administered alone, risperidone will decrease the number of correct responses and increase reaction times. This performance effect may still be evident when combined with ketamine. With regards to activation, specific predictions are harder to make due to risperidone's actions at both 5HT2A and D2 receptors. If one was to base its hypothesised effects on 5HT2A antagonism alone the drug may attenuate the effects of ketamine. In contrast, as seen in patients, it may itself cause an increase in activation, an effect suggested to be due to a D2-mediated increase in dopamine in cortical areas (G. D. Honey et al., 1999).

The predictions with regards to risperidone and lamotrigine were also tested within the ROIs specified above.

Within this chapter the reliability of N-Back performance and its associated network is presented first in order to confirm its ability to reliably assess function and elicit WM

networks in healthy volunteers. This is followed by the ROI results and a whole brain analysis which was performed in order to explore any treatment effects outside of the chosen ROIs. Assessment of performance in each treatment condition was conducted.

6.2 METHODS

Please refer to the relevant sections in Chapter 3 for details on the participants, experimental design, infusion protocol and image acquisition.

6.2.1 N-Back Working Memory Task

This task uses a parametric block design to assess WM function across three levels of cognitive (WM) load (see Figure 6-1). During the task, letters appeared one at a time in the centre of a projected computer screen viewed through periscopic mirrors. The participant was required to monitor this series of letters in order to respond to a particular letter which was the same as the one presented n trials previously, where n is a previously specified number, in this case 0, 1, 2 or 3 (Gevins, 1990; Owen et al., 2005). Zero-back is viewed as the control condition as the participant is merely required to respond to a pre-specified single letter, in this case an 'X' when it appears on the screen. The load on WM is varied by using the 1-back, 2-back and 3-back conditions. In this specific task, 3 blocks of each condition (control, 1-back, 2-back and 3-back) were used, for a total of 12 blocks. There were 21 letters presented per block with 4 of these letters being the targets (each block lasting 42 seconds). Stimulus trials were displayed for 1 second with an interstimulus interval of 2 seconds. In between blocks instructions were displayed on the screen (for 2 seconds; inter-trial interval between instruction and stimulus trials was 3 seconds) in order to warn the participant what condition was to follow ('Is it X'/'1-back'/'2-back'/'3-back'). The task lasted 9 minutes and 9 seconds.

In total, nine different task playlists were constructed, one of which was used for training the participants during their screening visit. All eight remaining playlists were administered to each participant, in a pseudo-randomised order and counterbalanced across participants (i.e. each participant performed the task eight times). Only two of these playlists were used in the reliability study. Each playlist consisted of twelve pseudo-randomised blocks.
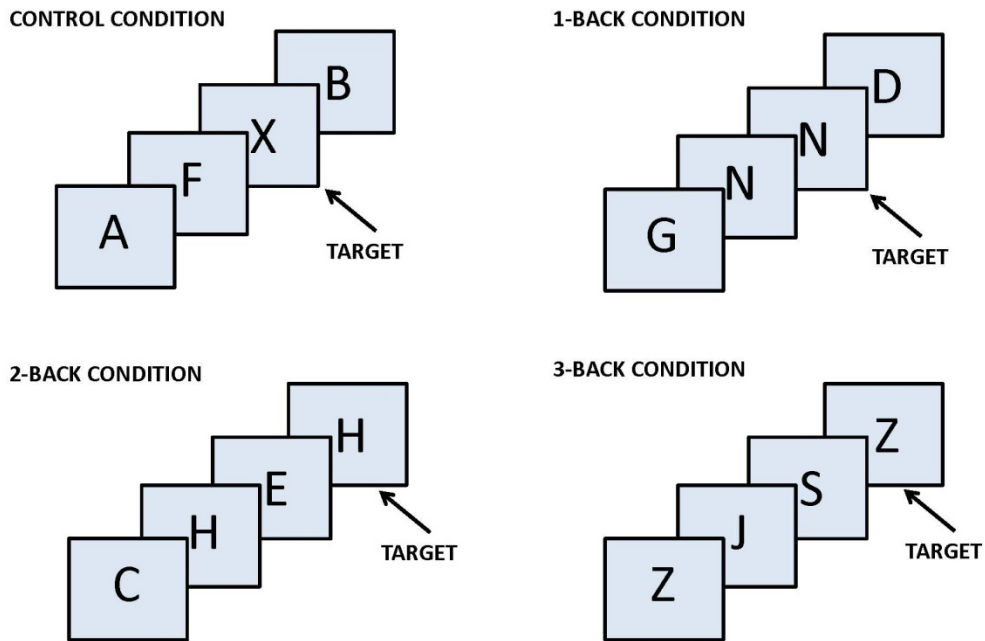
Figure 6-1 The N-Back task

Adapted from Braver et al., 1997. Description of the control conditions and three WM load conditions in the N-BACK task.

6.2.2 N-BACK Performance Analysis

All performance data analysis was conducted in SPSS 20.0 (www.ibm.com/spss). Initial data screening was performed in order to assess whether assumptions for parametric analysis were met. Accordingly, exploratory analysis and Shapiro-Wilk tests were completed. In both the reliability and modulation studies, accuracy data (% correct responses and % false positives) were found to violate the assumptions, thus non-parametric tests were applied to the data. Reaction time (RT) data were found to be normally distributed, thus parametric tests were used.

6.2.2.1 Reliability Study

Data were acquired from 11 healthy male participants on two separate occasions, at least one week apart. Wilcoxon signed-rank matched pairs tests were used to test whether there was an effect of session on accuracy data. Friedman's ANOVA was used to test whether an effect of load was present in each session for the accuracy data. A multifactorial repeated measures ANOVA was used to test for an effect of load and session in the RT data. A paired t-test was used to test whether there was an effect of session in the reactions times for the control condition.

Reliability analyses for the accuracy and RT data were calculated using the ICC (3,1) corresponding to a two-way, mixed-effects model with session as a fixed effect and subject as a random effect (Shrout & Fleiss, 1979) (see Chapter 3 for more details).

6.2.2.2   Modulation Study

Data were acquired from 16 healthy male participants on four separate occasions (both pre- and post-infusion), at least ten days apart. Please refer to Figure 3-4 (Chapter 3) for how the different drug conditions are referred to for analysis purposes.

6.2.2.3   Performance by Session

These analyses were performed in order to detect any session (practice) effects in the study. Friedman's ANOVA was applied to the accuracy data to test whether there was an effect of session (Session1, Session2, Session3, Session4) for the control and load conditions. This was done for both the pre- and post-infusion performance data.

RT data were analysed using (1) a multifactorial repeated measures ANOVA with session and load as within-subject factors for both pre- and post-infusion conditions separately, and (2) a single-factor repeated measures ANOVA with only session as a factor to investigate session effects in the control condition.

6.2.2.4   Performance by Treatment

6.2.2.4.1   Effect of Treatment and Load

Friedman's ANOVA was applied to the accuracy data to test whether there was an effect of load (1-back, 2-back and 3-back) within each treatment condition and to test for an effect of treatment between pre-infusion conditions (Placebo_S, Placebo_K, Risperidone_K, Lamotrigine_K) and between post-infusion conditions (P_Saline, P_Ketamine, R_Ketamine, L_Ketamine). Post-hoc Bonferroni corrected (p-value 0.002) Wilcoxon signed-rank matched pairs tests were then used to investigate any significant main effects of treatment in the control condition ('Is it X') and at each WM load condition (1-back, 2-back and 3-back).

RT data were analysed using (1) a 4x3 multifactorial repeated measures ANOVA with treatment (as above), and load (as above), as within-subject factors for pre- and post-infusion conditions separately and (2) a single-factor repeated measures ANOVA with only treatment as a factor to investigate treatment effects in the control condition as this

is not considered a measure of WM load. Significant main effects and interactions were interpreted using Bonferroni corrected pair-wise comparisons.

6.2.2.4.2   The Influence of Pre-infusion Conditions on Post-infusion Modulation Effects

The analyses described below were conducted in order to assess the post-infusion ketamine modulation effects while taking into account the pre-infusion effects of the oral drugs.

For accuracy data Wilcoxon tests were used to test for any effect of time (pre- versus post-infusion) at each load (Bonferroni corrected p-value is 0.003). Interaction effects cannot be directly calculated with non-parametric tests, thus this was not formally assessed.

For reaction time data a 2x4x3 multifactorial repeated measures ANOVA with time (pre- and post-infusion conditions) treatment arm and load as within-subject factors. Three separate 2x2x3 ANOVA models were conducted in order to investigate (1) any significant interaction effects found in the 2x4x3 model and (2) whether any significant interactions were present when the treatment comparisons of interest were isolated. A separate 2x4 ANOVA model (with time and treatment arm as factors) was conducted for the control condition. Significant interactions were interpreted using pair-wise comparisons, Bonferroni corrected for multiple comparisons. The three 2x2x3 models included:

(1) The placebo and ketamine treatment arms;

(2) The ketamine and risperidone treatment arms;

(3) The ketamine and lamotrigine treatment arms.

6.2.3   N-BACK fMRI analysis

6.2.3.1   Image Analysis

Preprocessing Procedures were identical to that performed on the phMRI data (please see Chapter 4, Section 4.2.1.2.1). However analyses were performed using SPM8 (version 8-update 4010: Wellcome Department of Cognitive Neurology, London, UK) and applied to the NBACK timeseries of 270 images. The SPM software underlying the analysis implemented in this thesis does not differ between SPM 5 and SPM 8.

First Level Model Specification

With the use of a GLM, statistical parametric maps were calculated for each participant, each condition (session or treatment) with the use of the same first level model (see Figure 6-2). The first level model used for both the reliability and modulation studies included explanatory variables representing the control, 1-back, 2-back and 3-back conditions separately. Two variables representing the instructions and button presses (which included the correct responses and false positives) were also included. Nuisance variables consisting of the six motion parameters derived from the realignment preprocessing stage were added to the model. The onsets for each block within each explanatory variable were defined by the time at which the first stimulus of the block was presented. Durations of each block were defined by the length of time between the first stimulus presentation and when the last stimulus disappeared off the computer screen. Onsets for the button press events were defined using the time at which the stimulus to which the participant was responding (including correct responses and false positives) was presented. The durations of these events were defined using the reaction time. All explanatory variables were convolved with a canonical HRF generating a design matrix of the predicted BOLD response (see Chapter 3, Section 3.5.2 for more detail).

After model specification, beta (β) parameters were then estimated for each variable with the use of the restricted maximum likelihood method (see Chapter 3, Section 3.5.2.). T-contrast images were then created; 1-back greater than control, 2-back greater than control, 3-back greater than control and N-back (average response across the memory loads) greater than control. These unthresholded contrasts were then taken through to separate second level analyses.
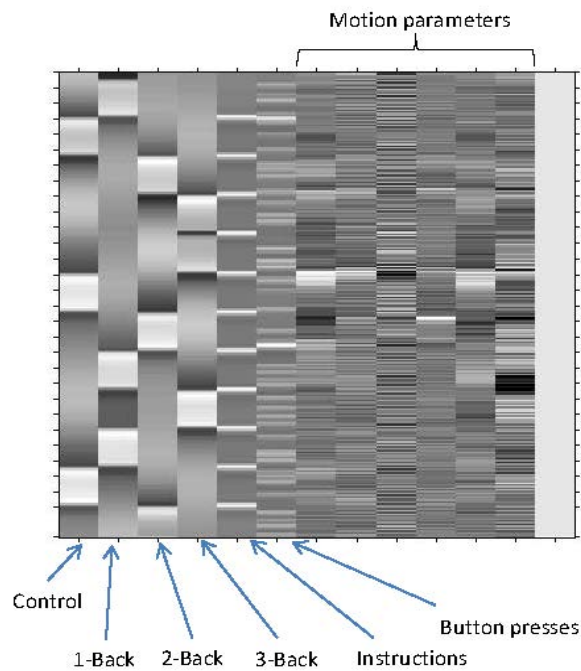
Figure 6-2 Design matrix displaying all variables included in the first level N-Back models

6.2.4 Reliability study

6.2.4.1 Reliability Analysis

Individual T-contrasts were used in a random effects flexible factorial model with subject, session and load as within-subject factors to detect any session effects. Four one-sample t-tests for each WM load contrast (for Session 1 only) were conducted to derive the response networks used in the reliability analysis. Voxelwise ICC values were then calculated using the ICC Matlab toolbox ((Caceres et al., 2009); see Chapter 3, Section 3.6 for details). ICC values represent the median (with 99% confidence intervals) of the ICC distribution across voxels within the brain networks significantly responding (in terms of activation or deactivation) to the N-Back task at the group level. Reliability of N-Back task network ROIs (DLPFC, cingulate and thalamus-defined below) was also calculated. This was performed for each memory load contrast separately and also across loads to maintain consistency across behavioural and imaging results.

6.2.5 Modulation Study

6.2.5.1 Region of Interest Definitions and Analysis

Based on the phMRI response to ketamine reported in Chapter 4 on a separate cohort of participants, a set of ROIs were defined *a priori* for the assessment of the predictions

outlined in the introduction to this chapter. These comprised ten coordinate-based ROIs, consisting of 5mm radius spheres centered on the Montreal Neurological Institute coordinates corresponding to areas most commonly associated with the N-Back network; the DLPFC (right: [50,20,34]; left [-26,44,40]), anterior/mid-cingulate (right: [2,14,42]; left [-8,26,32]), and thalamus (right: [6,-30,6]; left [-6,-16,-2]), and areas involved more generally in WM with a potential sensitivity to the compounds used in this thesis; the caudate(right: [12,-6,16]; left [-18,-18,20]), and hippocampus (right: [34,-18,-8]; left [-32,-12,-12]). For each region, treatment condition and WM load (including the control condition), mean parameter estimates (beta values) were extracted using the SPM8 toolbox MarsBar. Contrast estimates were then calculated in order to ascertain the differences in betas between each WM load condition and the control condition.

All ROI data analysis was conducted in SPSS 20.0 (www.ibm.com/spss). Initial data screening was performed in order to assess whether assumptions for parametric analysis were met. Accordingly, exploratory analysis and Shapiro-Wilk tests were completed. Contrast estimate data were found to be normally distributed, thus parametric tests were used. As with the behavioural data, contrast estimates were analysed using two 4x3 multifactorial repeated measures ANOVAs with treatment and load, as within-subject factors were conducted to investigate pre-ketamine and post-ketamine infusion conditions separately. In order to assess the post-infusion ketamine modulation effects while taking into account the pre-infusion effects of the oral drugs, a 2x4x3 multifactorial repeated measures ANOVA with time (pre- and post-infusion conditions), treatment arm and load as within-subject factors was conducted. Furthermore, three 2x2x3 repeated measures ANOVAs were performed in order to investigate (1) any significant interaction effects found in the 2x4x3 model and (2) whether any significant interactions were present when the treatment comparisons of interest were isolated. This addresses the question of whether the pre-infusion effects of placebo, risperidone and lamotrigine affect the interpretation of any treatment effects seen with ketamine. Significant main effects and interactions were interpreted using pair-wise comparisons, Bonferroni corrected for multiple comparisons.

6.2.5.2   Whole Brain (voxelwise) Analysis

For second level group analyses, individual T-contrasts (from the first level models) for each WM load were used in three random effects analyses (see Figure 6-3). A multifactorial repeated measures ANOVA (flexible factorial model in SPM) was

conducted with subject as a random effect between-subjects factor, and treatment and load as within-subject factors, for pre-ketamine infusion and post-ketamine infusion conditions separately. To investigate the post-infusion ketamine modulation effects while taking into account the pre-infusion effects of the oral drugs, a full factorial model with time, treatment and load as factors was conducted. Group maps were thresholded at $p < 0.001$ uncorrected at the voxel level and cluster corrected at $p < 0.05$.

FLEXIBLE FACTORIAL   **FULL FACTORIAL**
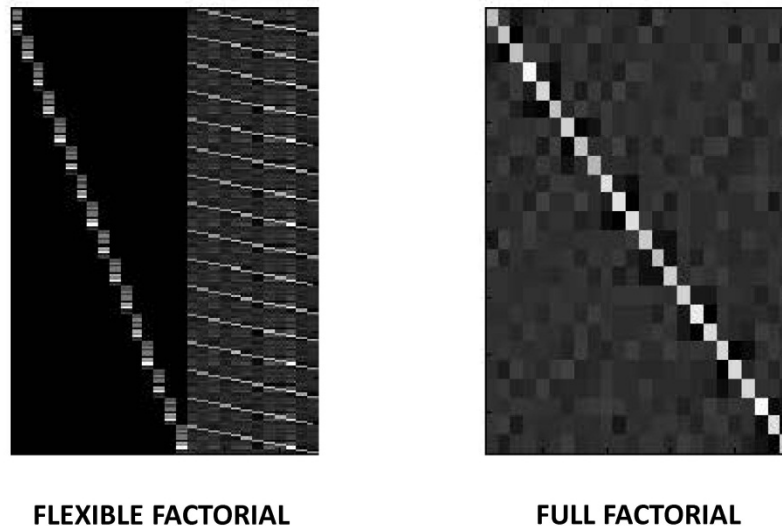
Figure 6-3 Second level design matrices for the N-Back task

6.3   RESULTS

6.3.1   N-BACK Performance

6.3.1.1   Reliability Study

6.3.1.1.1   Accuracy Data

No session effects were found for correct responses (Wilcoxon tests: control ($z = 0.00$, $p = 1.00$); 1-back ($z = -0.447$, $p = 0.655$); 2-back ($z = -0.172$, $p = 0.863$); 3-back ($z = -0.867$, $p = 0.386$)) or false positives (Wilcoxon tests: control ($z = 0.00$, $p = 1.00$); 2-back ($z = -1.342$, $p = 0.18$); 3-back ($z = -0.845$, $p = 0.398$). However, an effect of session was found for the false positives in the 1-back condition, with a decrease in session 2 ($z = -2.236$, $p < 0.05$).

Friedman's ANOVA showed, as expected, a main effect of load on the correct responses (Session 1 and 2 ($x^2(2) = 14$, $p < 0.01$) and false positives (Session 1 ($x^2(2) = 12.722$, $p < 0.01$; Session 2 ($x^2(2) = 10.129$, $p < 0.01$)).

6.3.1.1.2 Reaction Time Data

A repeated measures ANOVA found a significant effect of load ($F_{(2,20)} = 22.256$, $p < 0.01$). No effect of session ($F_{(1,10)} = 0.132$, $p = 0.724$) or session by load interaction was found ($F_{(2,20)} = 0.008$, $p = 0.992$). No effect of session was found for the control condition ($t_{(10)} = 0.699$, $p = 0.501$).

6.3.1.1.3 Reliability Data

Please see Table 6-1.

Table 6-1 Performance data and reliability of responses

	Load	Session 1	Session 2	ICC (3,1) +/- SE
Correct Responses (% +/-SE)	**X**	100	100	N/A[1]
	1B	97.73 (1.20)	97.73 (1.62)	0.69 (0.16)
	2B	93.94 (1.98)	93.94 (2.99)	0.41 (0.23)
	3B	71.48 (8.51)	79.54 (3.95)	0.19 (0.25)
Reaction Time (ms +/-SE)	*X*	*551.67 (27.15)*	*541.79 (26.96)*	*0.86 (0.09)*
	1B	*592.70 (27.58)*	*597.89 (30.39)*	*0.85 (0.09)*
	2B	*661.28 (38.49)*	*668.78 (35.90)*	*0.65 (0.17)*
	3B	*774.18 (50.73)*	*784.43 (54.19)*	*0.69 (0.16)*
False Positives (% +/-SE)	**X**	0	0	N/A[1]
	1B	0.89 (0.31)	0	0 (0.26)
	2B	0.36 (0.24)	0.89 (0.31)	0.04 (0.26)
	3B	3.92 (0.79)	2.85 (0.81)	0.05 (0.26)
Reaction Time (ms +/-SE)	*X*	-	-	*N/A[2]*
	1B	*804.6 (101.87)*	-	*N/A[2]*
	2B	*691.5 (18.24)*	*891.6 (113.81)*	*N/A[2]*
	3B	*871.47 (48.05)*	*820.79 (51.72)*	*N/A[2]*

[1] **Zero variance. ICC cannot be computed.** [2] **Not all subjects gave false positive responses and ICC cannot be computed for the group.**

6.3.1.2   Modulation Study

Please see Section 6.3.1.3 for a summary of the following results.

6.3.1.2.1 Accuracy

Pre-infusion As shown in Figure 6-4, a main effect of load on the correct responses was found for all treatment conditions, whereby these decreased linearly as a function of load (Placebo_S ($x^2(2)$ = 17.08); Placebo_K ($x^2(2)$ = 11.18); Risperidone_K ($x^2(2)$ = 14.68); Lamotrigine_K ($x^2(2)$ = 18.04)), all significant at $p<0.01$.

A trend towards a main effect of treatment was found for the 3-back condition ($x^2(3)$ = 6.544, p = 0.088) driven by lower accuracy in the Risperidone_K condition when compared to both placebo conditions (Placebo_S (z = -2.175, $p=0.03$); Placebo_K (z = -1.966, $p=0.049$). No other treatment effects were found (control ($x^2(3)$ = 4.176, p = 0.243) 1-back ($x^2(3)$ = 5.609, p = 0.132), 2-back ($x^2(3)$ = 3.222, p = 0.359)).

A main effect of load on the false positives was found for all treatment conditions, whereby these increased linearly as a function of load (Placebo_S ($x^2(2)$ = 19, p <0.01); Risperidone_K ($x^2(2)$ = 16.478, p <0.01); Lamotrigine_K ($x^2(2)$ = 8.974, p <0.05) except the Placebo_K condition ($x^2(2)$ = 3.95, p = 0.139). No main effect of treatment was found (Control ($x^2(3)$ = 3.667, p = 0.30); 1-back ($x^2(3)$ = 3.511, p = 0.319); 2-back ($x^2(3)$ = 4.303, p = 0.231); 3-back ($x^2(3)$ = 0.699, p = 0.873).

No main effect of session was found for correct responses or false positives in any task condition (control ($x^2(3)$ = 4.176, p = 0.243; $x^2(3)$ = 3.667, p = 0.30), 1-back ($x^2(3)$ = 4.467, p = 0.215; $x^2(3)$ = 0.702, p = 0.873), 2-back ($x^2(3)$ = 0.2, p = 0.978; $x^2(3)$ = 4.303, p = 0.231) or 3-back ($x^2(3)$ = 0.203, p = 0.977)).

Pre-Infusion
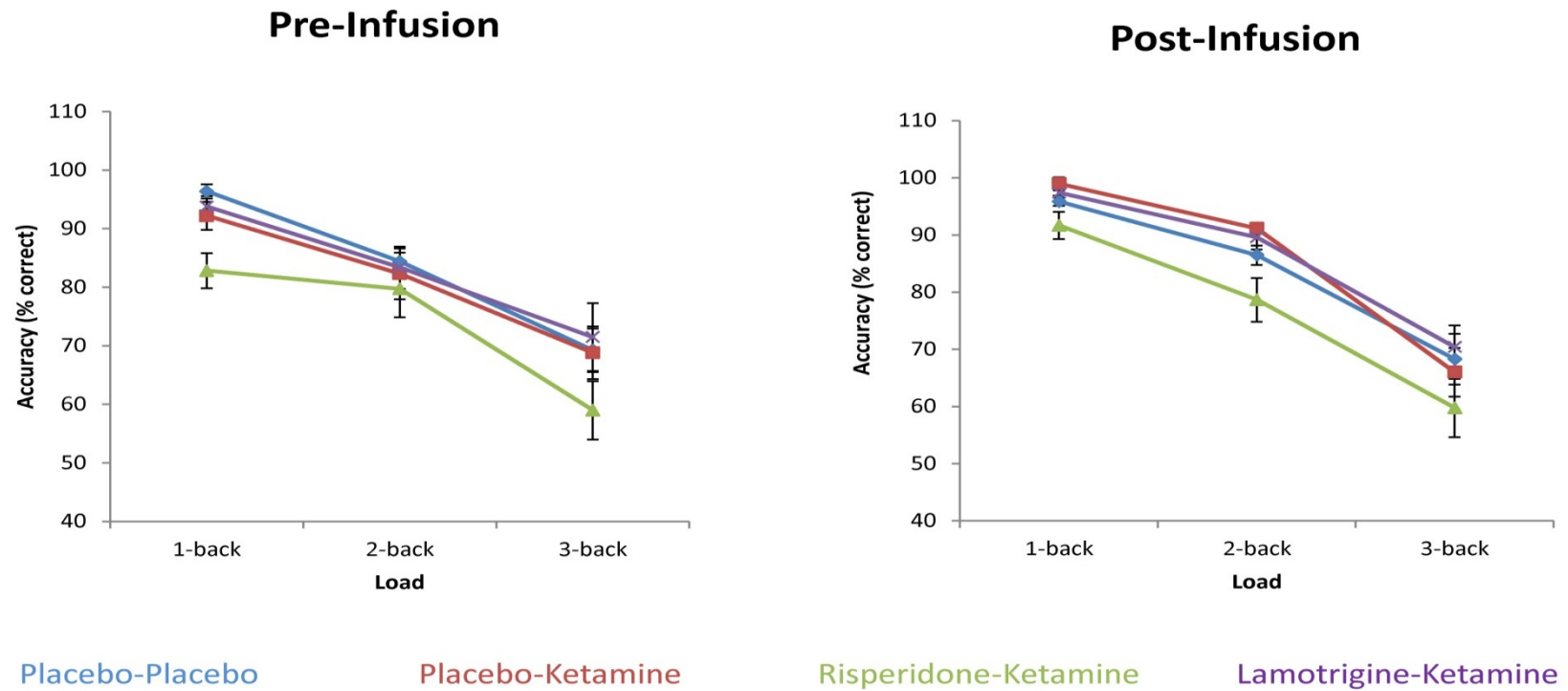
Post-Infusion

Figure 6-4 N-Back performance - Accuracy

Accuracy (percent correct responses) for all treatment conditions (pre- and post-infusion) across all WM loads. Placebo-Saline treatment arm (blue); Placebo-Ketamine treatment arm (red); Risperidone-Ketamine treatment arm (green); Lamotrigine-Ketamine treatment arm (purple). Error bars represent the standard error of the mean.

Post-infusion As shown in Figure 6-4, a main effect of load on the correct responses was found for all treatment conditions (P_Saline ($x^2(2) = 16.04$); P_Ketamine ($x^2(2) = 22.39$); R_Ketamine ($x^2(2) = 12.45$); L_Ketamine ($x^2(2) = 24.12$)), all significant at $p<0.01$.

A main effect of treatment was found in the 2-back condition ($x^2(3) = 8.098$, $p <0.05$), driven by lower accuracy in the R_Ketamine condition ($z = -2.033$, $p=0.042$) when compared to P_Ketamine. No other treatment effects were found (control ($x^2(3) = 4.000$, $p = 0.261$), 1-back ($x^2(3) = 3.868$, $p = 0.276$) or 3-back ($x^2(3) = 3.166$, $p = 0.367$).

A main effect of load on the false positives was found for all treatment conditions (P_Saline ($x^2(2) = 12.235$, $p <0.01$); P_Ketamine ($x^2(2) = 14.368$, $p <0.01$); R_Ketamine ($x^2(2) = 9.116$, $p = 0.01$); L_Ketamine ($x^2(2) = 22.205$, $p <0.01$). No main effect of treatment was found (Control ($x^2(3) = 3.667$, $p = 0.30$); 1-back ($x^2(3) = 4.22$, $p = 0.239$); 2-back ($x^2(3) = 1.172$, $p = 0.760$); 3-back ($x^2(3) = 2.488$, $p = 0.477$).

No main effect of session was found for correct responses or false positives in any task condition (control ($x^2(3) = 3.222$, $p = 0.359$; $x^2(3) = 3.667$, $p = 0.30$), 1-back ($x^2(3) = 1.539$, $p = 0.673$; $x^2(3) = 0.966$, $p = 0.809$), 2-back ($x^2(3) = 0.88$, $p = 0.83$; $x^2(3) = 2.109$, $p = 0.550$) or 3-back ($x^2(3) = 0.931$, $p = 0.818$; $x^2(3) = 0.233$, $p = 0.972$).

The Influence of Pre-infusion Conditions on Post-infusion Modulation Effects

The effect of the oral drugs on ketamine were evaluated; at 2-back, a trend (based on bonferroni corrected significance levels) towards increased correct responses were found in the P_Ketamine condition when compared to Placebo_K (Wilcoxon: $z = -2.572$, $p=0.01$) and at 3-back, increased false positives were found in the R_Ketamine condition when compared to Risperidone_K (Wilcoxon: $z = -1.974$, $p=0.048$). No other differences were found between pre- and post-infusion conditions.

6.3.1.2.2   Reaction Time

Effects of Treatment and Load

Pre-infusion As Figure 6-5 shows, a significant main effect of load was found in all treatment conditions ($F(2,30) = 19.20$, $p<0.01$) and is driven by the significant increase in reaction time between the 1-back and 3-back loads.

No main effect of treatment was found (control ($F(3,45) = 1.44$, $p = 0.244$); load conditions ($F(3,45) = 1.853$, $p = 0.151$)). No interaction between treatment and load was found ($F(6,90) = 0.775$, $p = 0.591$).

No main effect of session was found (control ($F(3,45) = 2.289$, $p = 0.091$); load conditions ($F(3,45) = 1.394$, $p = 0.257$).
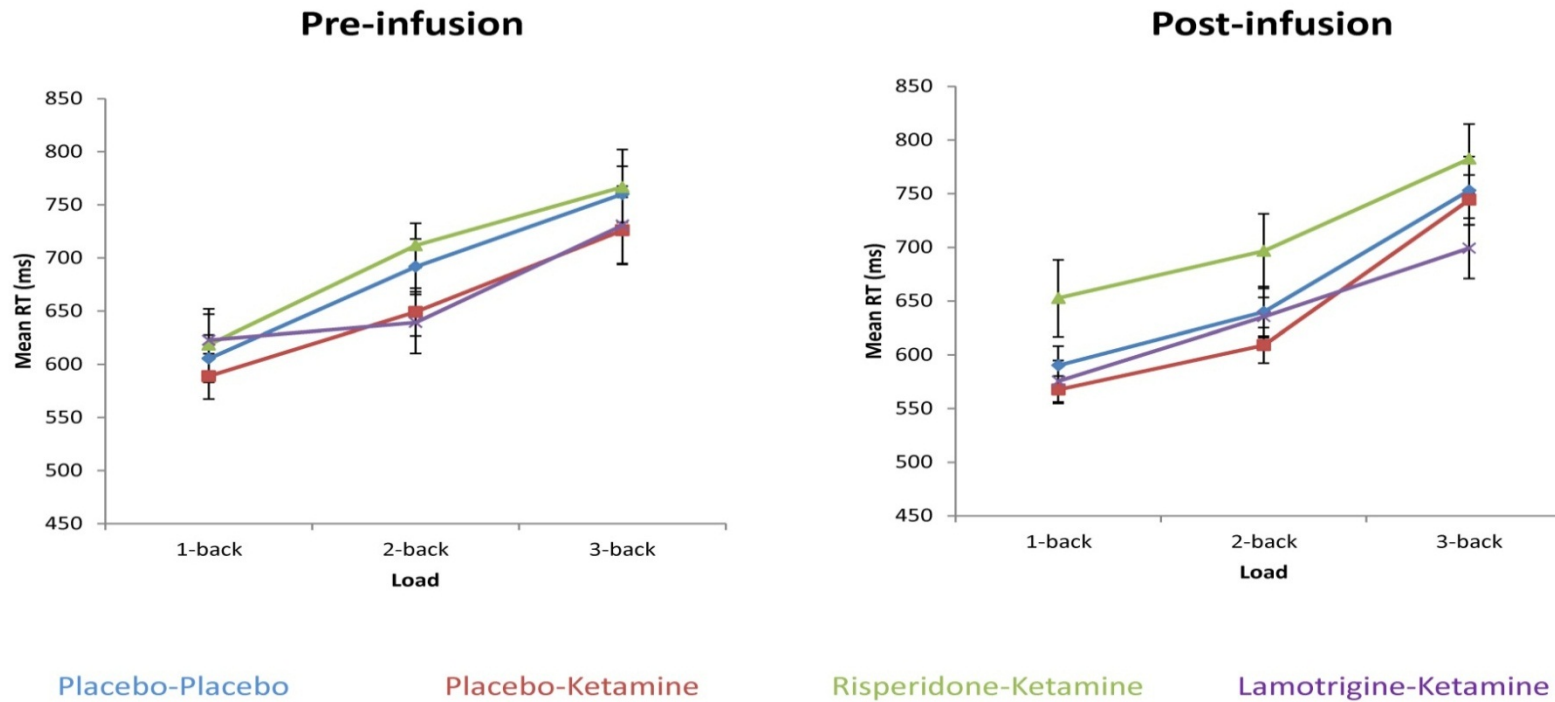
Figure 6-5 N-Back performance - Reaction Times

Reaction times for all treatment conditions (pre- and post-infusion) across all WM loads. Placebo-Saline treatment arm (blue); Placebo-Ketamine treatment arm (red); Risperidone-Ketamine treatment arm (green); Lamotrigine-Ketamine treatment arm (purple). Error bars represent the standard error of the mean.

Post-infusion A significant main effect of load was found ($F_{(2,30)}$ = 13.44, p<0.01) and is driven by a significant increase in reaction time between the 1-back and 3-back conditions, although this is not significant in the R_Ketamine condition.

A significant main effect of treatment was found ($F_{(3,45)}$ = 5.49, p<0.01) and is due to a significant increase in reaction times at 2-back in the R_Ketamine condition. This increase in reaction times was also found in the R_Ketamine control condition ($F_{(3,45)}$ = 3.455, p<0.05), although this is not significant in comparison to P_Saline.

No significant interaction between treatment and load was found ($F_{(6,90)}$ = 0.718, p = 0.636).

No main effect of session was found (control ($F_{(3,45)}$ = 1.796, p = 0.161); load conditions ($F_{(3,45)}$ = 0.276, p = 0.842).

False positive reaction time analysis could not be computed due to too few data points.

THE INFLUENCE OF PRE-INFUSION CONDITIONS ON POST-INFUSION MODULATION EFFECTS

No significant effects were found in the 2x4x3 repeated-measures ANOVA including all treatment arms or the more specific 2x2x3 models including either the placebo and ketamine treatment arms or the ketamine and risperidone treatment arms (Full model: Time ($F_{(1,15)}$ = 2.187, p = 0.16), time by treatment arm interaction ($F_{(3,45)}$=0.937, p=0.431); time by treatment arm by load interaction ($F_{(6,90)}$=0.997, p=0.432); placebo and ketamine treatment arms: Effect of time ($F_{(1,15)}$ = 2.166, p = 0.162), time by treatment arm interaction ($F_{(1,15)}$=0.215, p=0.65), time by treatment arm by load interaction ($F_{(2,30)}$=0.239, p=0.789); ketamine and risperidone treatment arms (Effect of time ($F_{(1,15)}$ = 0.016, p = 0.9), time by treatment arm interaction ($F_{(1,15)}$=0.736, p=0.404), time by treatment arm by load interaction ($F_{(2,30)}$=0.847, p=0.439)).

In the model including the ketamine and lamotrigine treatment arms a trend towards a significant effect of time was found ($F_{(1,15)}$ = 3.345, p=0.087). This is due to faster reaction times overall in the L_Ketamine condition when compared to the Lamotrigine_K condition. No interactions were found in this model (time by treatment $F_{(1,15)}$=0.211; time by treatment by load $F_{(2,30)}$=1.321, p=0.282).

A significant effect of time was also found on the control condition ($F_{(1,15)}$=6.445, p<0.05). This is due to significantly faster reactions time in the post-infusion P_Ketamine

and L_Ketamine conditions compared to the corresponding pre-infusion conditions. No time by treatment interaction was found ($F_{(3,45)}=0.767$, $p=0.518$).

6.3.1.3   Summary of Drug Effects on N-Back Performance

Ketamine

When compared to saline infusion no ketamine induced effects were found. However, when compared to pre-infusion placebo, ketamine speeded reaction times and improved accuracy in terms of correct responses (although the effect on accuracy was not significant when corrected for multiple comparisons).

Risperidone

When compared to placebo, risperidone decreased accuracy in terms of correct responses in the 3-back condition, although this was not significant when corrected for multiple comparisons. A trend was also present at 2-back when risperidone-ketamine was compared to ketamine alone, in addition to slowed reaction times and a blunted load-response. An increase in false positives was also found in the risperidone-ketamine condition when compared to risperidone alone.

Lamotrigine

When compared to placebo no lamotrigine induced effects were found. When compared to ketamine alone, no effect of lamotrigine pre-treatment was found on ketamine administration. However, significantly faster reaction times were found in the lamotrigine-ketamine condition across WM load and control conditions when compared to lamotrigine alone.

6.3.2   Imaging Results

6.3.2.1   Reliability Study

6.3.2.1.1   Reliability of the N-Back Task Network

No main effect of session was found between the brain response to the N-Back on session 1 compared to session 2, indicating stability of the N-Back network across time at a group level. It appears both the activation and deactivation networks are most stable in the 3-back condition, although there is greater discrepancy between loads for the deactivation network. The weaker reliability of the deactivation network confirms what previous studies have found (Caceres et al., 2009). Although reliability of the overall task network appears slightly lower than that seen in the Caceres et al study, reliability of the

individual N-Back network ROIs is good, with the DLPFC demonstrating the highest reliability in the context of the 3-back. These results may have implications for the use of the N-Back task in future repeated-measures studies and on the strength of the modulation study outcomes.

Table 6-2 Reliability of the N-Back network at each WM load

	ICC (3,1) +/- SE			
	1-back	**2-back**	**3-back**	**N-Back**
Activation Network	0.25 (0.011)	0.27 (0.006)	0.33 (0.007)	0.33 (0.007)
Deactivation Network	0.01 (0.052)	0.12 (0.004)	0.21 (0.008)	0.19 (0.006)
R DLPFC	0.16 (0.044)	0.26 (0.045)	0.57 (0.035)	0.51 (0.043)
L DLPFC	0.02 (0.018)	0.39 (0.03)	0.71 (0.012)	0.51 (0.014)
R Cingulate	-0.01 (0.009)	0.023 (0.014)	0.41 (0.013)	0.23 (0.01)
L Cingulate	0.30 (0.018)	0.002 (0.024)	0.44 (0.013)	0.32 (0.017)
R Thalamus	-0.45 (0.022)	-0.50 (0.016)	0.24 (0.016)	0.06 (0.013)
L Thalamus	-0.24 (0.009)	-0.15 (0.018)	0.17 (0.01)	0.11 (0.011)
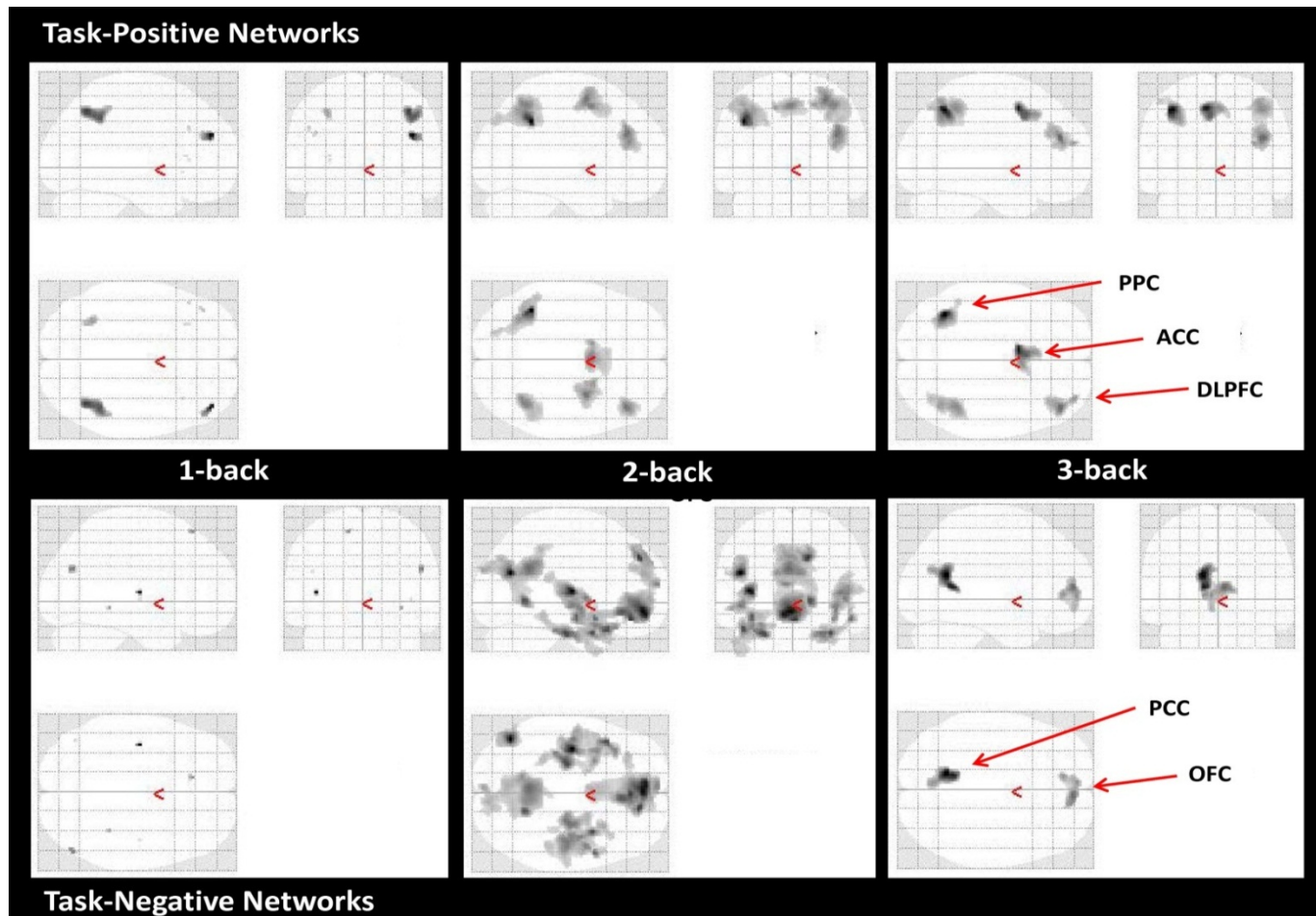
Session 1 N-back networks displayed on glass brains for the 1-back (p<0.001 uncorrected), 2-back and 3-back conditions (p<0.001 uncorrected, cluster corrected at 0.05).

PPC (Posterior Parietal Cortex); DLPFC (Dorsolateral Prefrontal Cortex); ACC (Anterior Cingulate Cortex); PCC (Posterior Cingulate Cortex); OFC (Orbitofrontal Cortex).

Figure 6-6 N-Back brain network for the reliability study (Session 1)

6.3.2.2   Modulation Study

Please see Section 6.3.2.3 for a summary of the following results.

6.3.2.2.1   N-Back Working Memory Network

As expected under placebo conditions, the brain network recruited with the NBACK when comparing the WM loads (as one WM condition) to the control condition (see Figure 6.7.; task network) included activation in bilateral DLPFC, VLPFC, parietal and cingulate cortices, and insular cortex at an uncorrected threshold of $p<0.001$, family-wise corrected for multiple comparisons at $p<0.05$ (task-positive network) on the basis of cluster extent. Bilateral caudate was also present at the lower uncorrected threshold of $p<0.01$. The deactivation network (task-negative network) included the posterior cingulate and the orbitofrontal cortices, areas established as part of the default mode network whose suppression has been found to be beneficial for cognitive task performance (see Figure 6.7; (Whitfield-Gabrieli & Ford, 2012)). When looking for regions that respond differentially to WM load, similar task network areas are present, with the additional recruitment of the thalamus and cerebellum (see Figure 6.7; load network). Both the task and load N-Back networks were present in all treatment conditions. Treatment related differences in the extent to which these networks were recruited at each load were assessed using pre-defined ROIs. For the results of this analysis please see below.
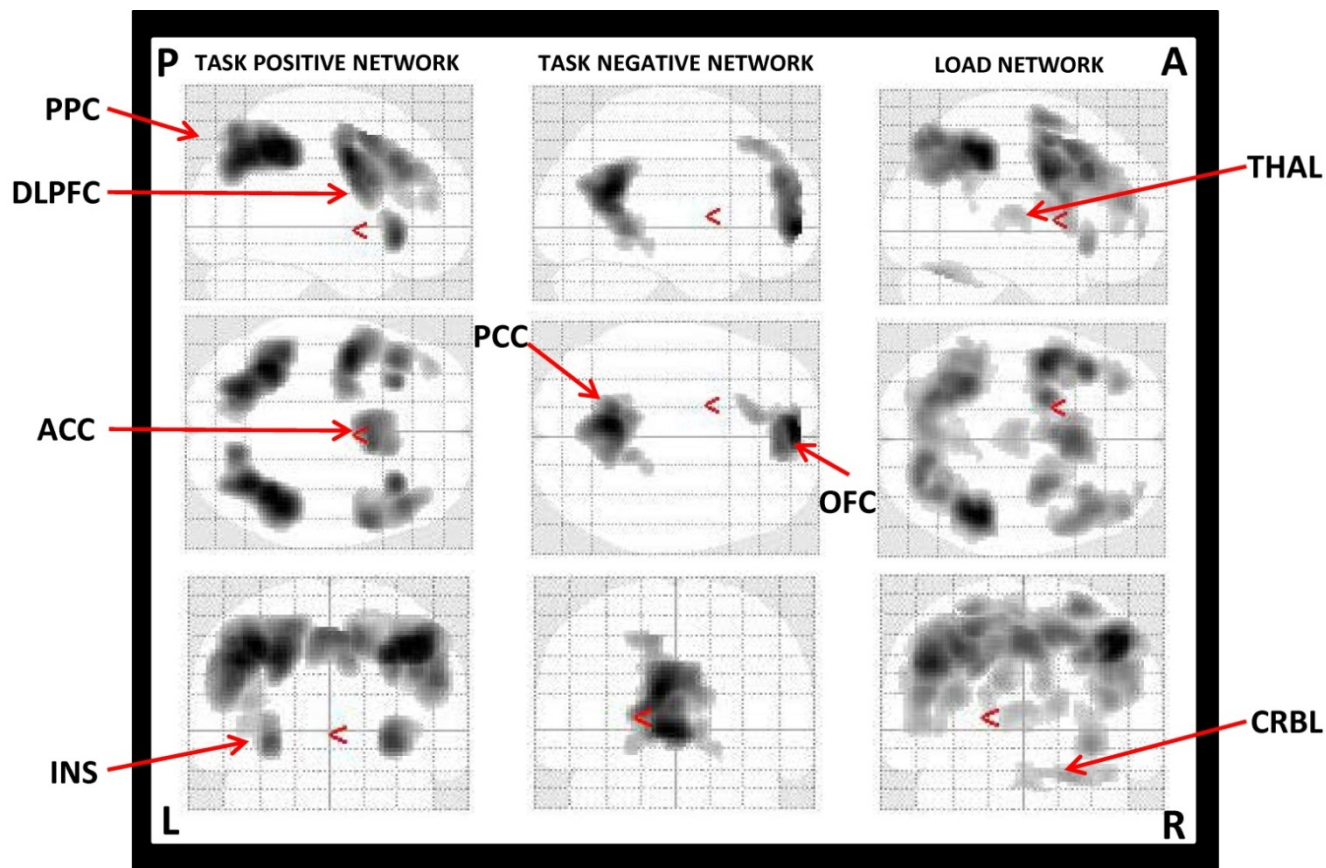
Figure 6-7 N-Nack task networks from the modulation study displayed on glass brains

All networks were defined using the full factorial model. No significant differences were found between the Placebo_S and P_saline networks thus the Placebo_S network was used for display purposes.

Task positive and negative networks are defined using the N-Back (across loads) versus control condition T-contrast (presented at p<0.001 uncorrected, cluster corrected at p<0.05). The load network was defined using the F-contrast for main effect of load.

PPC (Posterior Parietal Cortex); DLPFC (Dorsolateral Prefrontal Cortex); ACC (Anterior Cingulate Cortex); INS (Insula); PCC (Posterior Cingulate Cortex); OFC (Orbitofrontal Cortex); THAL (Thalamus); CRBL (Cerebellum).

6.3.2.2.2   Region of Interest (ROI) Analysis
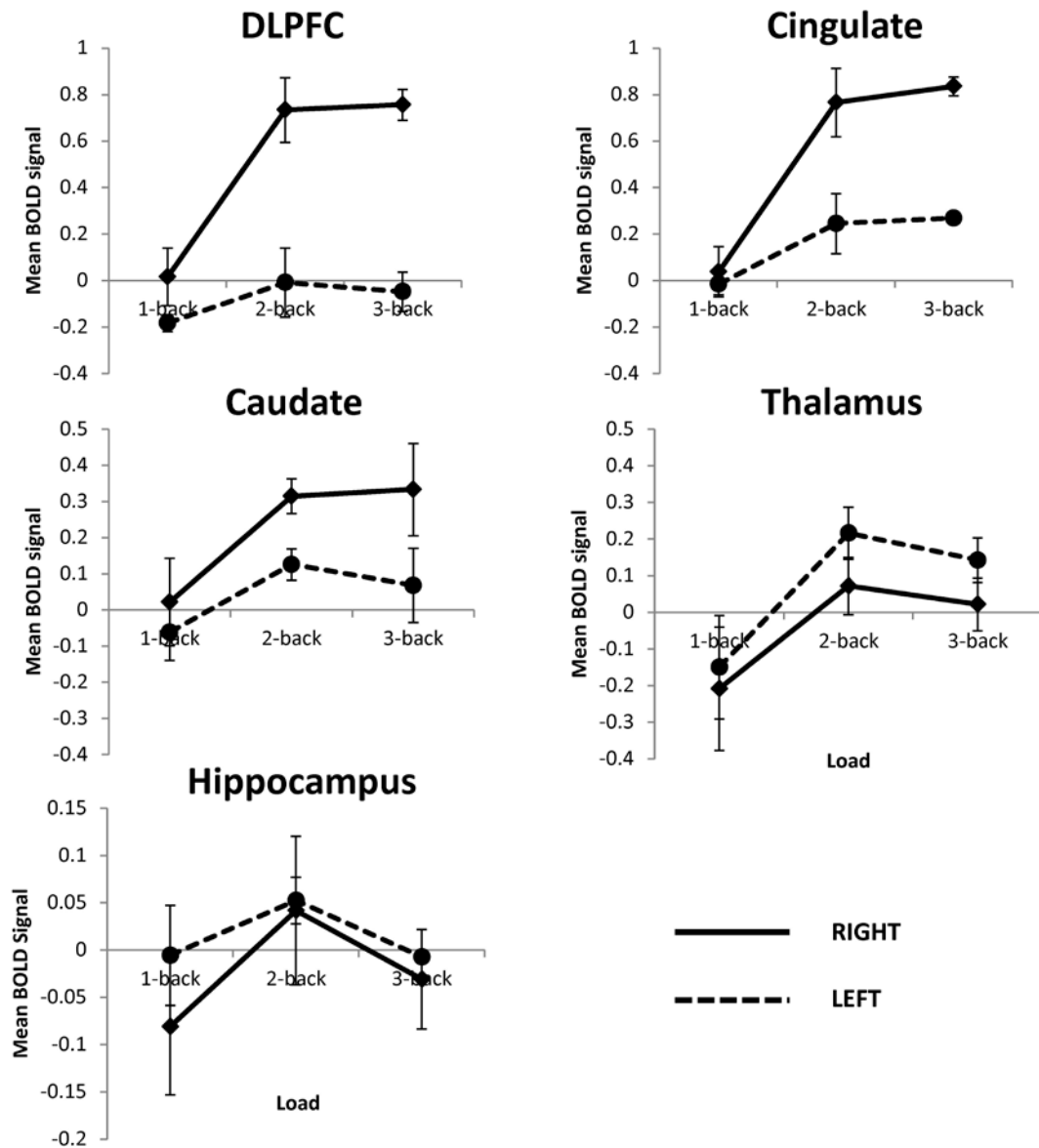
Figure 6-8 The ROI load response to the N-Back task on placebo

The change in BOLD signal as a function of load for each individual ROI across the placebo conditions (Placebo_S, Placebo_K and P_Saline). Error bars represent the standard error of the mean (i.e. variance in response across placebo conditions).

Pre-infusion Please refer to Table 6.3. for all relevant statistics. A significant effect of load across treatment conditions on brain activation within the right DLPFC, cingulate and caudate and left cingulate, caudate and thalamus was found. The left DLPFC, right thalamus and right and left hippocampus ROIs do not show a significant main effect of load (please refer to Figure 6-8 for the load response on placebo).

Post-hoc pairwise comparisons, indicate that the significant effect of load is driven by the increase in activation between the 1-back and 3-back conditions. Overall, this pattern is seen in the placebo and lamotrigine conditions, whereas the effect of load is not significant in the risperidone condition; these findings are consistent across ROIs demonstrating a significant effect of load apart from the left caudate. This ROI demonstrates a blunted effect of load with lamotrigine but a significant effect of load with risperidone; however this is characterised by a *decrease* rather than an increase in activation between the 1-back and 3-back conditions.

The left caudate also demonstrates a significant treatment by load interaction ($p<0.01$) which is driven by increased activation at 1-back on risperidone when compared to placebo (Placebo_S and Placebo_K). The reverse occurs in the 3-back condition whereby risperidone demonstrates lower activation compared to placebo (Placebo_K), although this comparison is not significant (see Appendix C). A similar effect occurs in the right caudate which demonstrates a trend towards a treatment by load interaction ($p=0.059$), although this is in comparison to Placebo_S. However, one must be cautious in interpreting these findings due to the variability in response between the two placebo sessions (see Appendix C).

The right DLPFC also demonstrates a significant treatment by load interaction ($p<0.05$). Similar to the caudate, pairwise comparisons indicate that this is due to increased activation on risperidone at 1-back when compared to placebo (Placebo_S) and reversal of this difference in the 3-back condition (see Appendix C).

The treatment by load interaction in the right thalamus ($p<0.01$) and left thalamus ($p = 0.063$), is also driven by significantly increased activation on risperidone at 1-back when compared to placebo (see Appendix C). A trend risperidone-induced interaction effect is also present in the right cingulate ($p=0.051$), right ($p=0.057$) and left ($p=0.068$) hippocampus, although this does not survive correction for multiple comparisons.

In order to assess whether any order effects were present a repeated-measures ANOVA was employed with treatment, load and ROI as within-subjects factors and order as a between-subjects factor. No main effect of order was found ($F_{(3,12)}=0.750$, $p=0.543$). No significant interaction was found between treatment and order ($F_{(9,36)}=0.967$, $p=0.482$), treatment, ROI and order ($F_{(81,324)}=0.842$, $p=0.823$), or treatment, load, ROI and order ($F_{(162,648)}=0.985$, $p=0.539$).

Post-infusion Please refer to Table 6.3. for all relevant statistics. A significant effect of load on brain activation within the right DLPFC, cingulate and caudate and left cingulate, caudate and thalamus was found, the same ROIs demonstrating this effect in the pre-infusion conditions. As before, pairwise comparisons demonstrate that this effect is due to an increase in activation between the 1-back and 3-back loads in the placebo (P_Saline) and lamotrigine-ketamine (L_ketamine) conditions. This difference between the 1-back and 3-back conditions is attenuated in the ketamine (P_Ketamine) and risperidone-ketamine (R_Ketamine) conditions, which demonstrate a more inverted-U shaped response (see figures 6.9 & 6.10). A significant effect of load is also seen in the left caudate, however the increase in activation between the 1-back and 3-back conditions is attenuated in *all* treatment conditions. Instead, in this ROI the significant effect of load reflects an inverted-U shaped pattern of response.

A trend-level effect of treatment was found in the left cingulate ($p = 0.089$) and left thalamus ($p=0.064$). Pairwise comparisons, although not significant, indicate that this is due to an increase in activation in the L_Ketamine condition compared to P_Ketamine and P_Saline.

The right DLPFC demonstrates a significant treatment by load interaction ($p<0.05$). Pairwise comparisons, although not significant when corrected for multiple comparisons, indicate that this is due to increased activation in all ketamine conditions at 1-back when compared to P_Saline and decreased activation in the P_Ketamine and R_Ketamine conditions at 3-back when compared to P_Saline and L_Ketamine (see Appendix C). Separate 2x2 ANOVAs indicate that this effect is driven by a significant interaction between P_Saline and P_Ketamine ($p<0.01$; see Figure 6-9 for the effect of ketamine in the right DLPFC). A significant interaction is also seen between the P_Saline and R_Ketamine conditions ($p<0.05$) whereas there is a trend towards an interaction between P_Ketamine and L_Ketamine ($p=0.089$).

In order to assess whether any order effects were present a repeated-measures ANOVA was employed with treatment, load and ROI as within-subjects factors and order as a between-subjects factor. No main effect of order was found ($F_{(3,12)}=2.951$, $p=0.076$). No significant interaction was found between treatment and order ($F_{(9,36)}=0.997$, $p=0.460$), or treatment, ROI and order ($F_{(81,324)}=0.1.088$, $p=0.302$). A significant interaction between treatment, load, ROI and order ($F_{(162,648)}=1.324$, $p<0.01$) was found.

Bonferroni corrected pairwise comparisons indicate that this interaction is driven by the following results. The summary below describes the order effects which occur in a particular ROI, at a particular working memory load in all treatment arms (no order effects were detected for the placebo treatment arm).

Treatment Arm	ROI	Load	Order Effect
Ketamine	rDLPFC	2	The brain response is *increased* when ketamine is administered on the second visit compared to when it is administered on the first or third visit.
	rCAU	3	The brain response is *decreased* when ketamine is administered on the second visit compared to when it is administered on the third or fourth visit.
	rCING	1	The brain response is *increased* when ketamine is administered on the second visit compared to when it is administered on the third visit.
Risperidone	rTHAL	1	The brain response is *decreased* when risperidone pre-treatment is administered on the second visit compared to when it is administered on the fourth
Lamotrigine	rCAU	1	The brain response is *decreased* when lamotrigine pre-treatment is administered on the second visit compared to when it is administered on the fourth

Nevertheless, these results do not affect the outcome of the NBACK chapter as order effects are not present in any of the ROIs demonstrating a significant modulatory effect of treatment.

Table 6-3 Repeated-measure ANOVA describing treatment and load effects in pre-infusion and post-infusion conditions separately

ROIs	Pre-Infusion 4x3 ANOVA						Post-Infusion 4x3 ANOVA					
	Main Effect of Treatment		Main Effect of Load		Treatment x Load Interaction		Main Effect of Treatment		Main Effect of Load		Treatment x Load Interaction	
	F[3,45]	P	F[2,30]	P	F[6,90]	P	F[3,45]	P	F[2,30]	P	F[6,90]	P
rDLPFC	0.359	P=0.783	14.702	p<0.01	2.520	p<0.05	0.717	P=0.547	9.721	p<0.01	2.467	p<0.05
lDLPFC	1.364	P=0.266	0.185	P=0.832	0.959	P=0.457	0.536	P=0.660	0.021	P=0.979	0.892	P=0.504
rCING	0.229	P=0.875	12.982	p<0.01	2.191	P=0.051	0.678	P=0.570	12.701	p<0.01	0.826	P=0.553
lCING	0.119	P=0.948	10.180	p<0.01	1.305	P=0.263	2.390	p=0.081	5.671	p<0.01	1.398	P=0.224
rCAU	1.411	P=0.252	10.870	p<0.01	2.115	P=0.059	0.330	P=0.804	4.720	p<0.05	1.058	P=0.394
lCAU	1.309	P=0.283	4.411	P<0.05	4.524	p<0.01	0.713	P=0.55	7.476	p<0.01	0.534	P=0.781
rTHAL	1.441	P=0.243	1.457	P=0.249	3.120	p<0.01	1.216	P=0.315	0.511	P=0.605	0.358	P=0.903
lTHAL	1.445	P=0.242	5.851	p<0.01	2.082	P=0.063	2.590	P=0.064	4.520	p<0.05	0.757	P=0.605
rHIPP	2.175	P=0.104	0.889	P=0.422	2.134	P=0.057	0.988	P=0.407	0.684	P=0.512	0.230	P=0.966
lHIPP	1.204	P=0.319	1.727	P=0.195	2.046	P=0.068	1.061	P=0.375	0.400	P=0.674	0.054	P=0.999

A 2x4x3 repeated measures ANOVA was conducted to describe the effects of time and the interaction between the pre-infusion conditions and the post-infusion conditions (see Appendix C; Table C-5). A significant main effect of time was found in bilateral caudate ($F_{(1,15)} = 10.777$, $p<0.01$; $F_{(1,15)} = 17.962$, $p<0.01$), thalamus ($F_{(1,15)} = 6.836$, $p<0.05$; $F_{(1,15)} = 6.339$, $p<0.05$) and hippocampus $F_{(1,15)} = 6.492$, $p<0.05$) with a trend towards significance in the right DLPFC ($F_{(1,15)} = 3.704$, $p=0.073$). This effect of time was found to be driven by a decrease in activation in post-infusion conditions, P_Ketamine and R_Ketamine, when compared to Placebo_K and Risperidone_K conditions respectively. Additionally, significant time by treatment arm by load interactions were found in the left caudate ($F_{(6,90)} = 2.344$, $p<0.05$) and right thalamus ($F_{(6,90)} = 2.569$, $p<0.05$). Three separate 2x2x3 ANOVA models were conducted in order to investigate the significant interaction effects found in the 2x4x3 ANOVA by isolating the treatment comparisons of interest. Furthermore, due to the number of participants in the modulation study it is possible that the high number of factors in the larger ANOVA may reduce the power to detect drug effects which will be present in the more specific models.

KETAMINE

The first ANOVA included the placebo and ketamine treatment arms in order to isolate any effects of ketamine in comparison to both pre-infusion and post-infusion placebo conditions. Please refer to Figure 6-9 to visualise specific results and Table 6-4 for all relevant statistics.
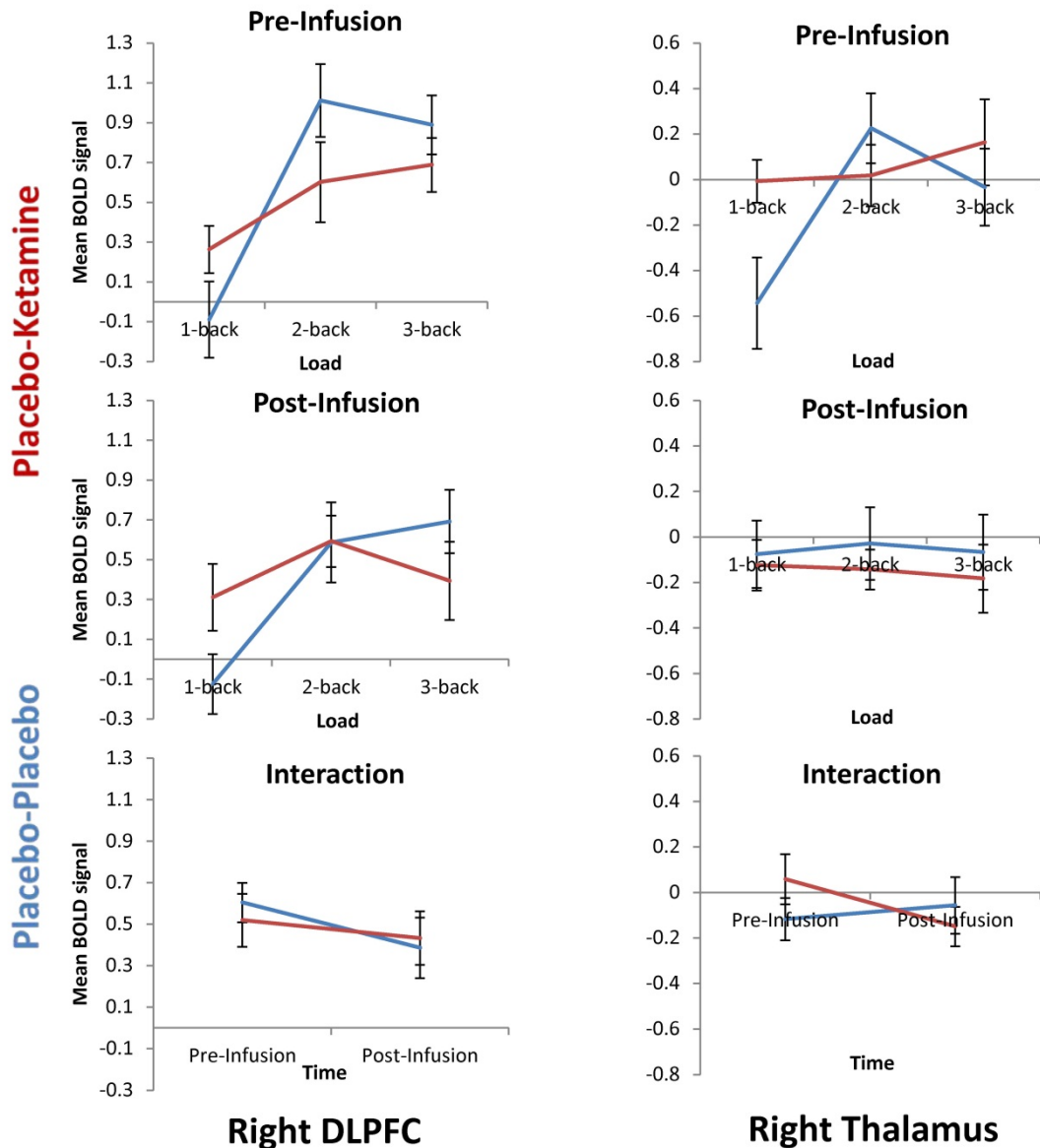
Figure 6-9 The effect of the placebo and ketamine treatment arms in the right DLPFC and thalamus

The DLPFC was found to exhibit a significant treatment by load interaction at post-infusion whereas the thalamus exhibited a significant time by treatment by load interaction between the pre-infusion and post-infusion conditions. However, it is important to note that (apart from the post-infusion DLPFC graph) none of the comparisons shown above are significant; the graphs are for visualisation purposes alone. The interaction graphs illustrate the time by treatment effect. Error bars represent the standard error of the mean. Pre-infusion placebo arm = placebo; Post-infusion placebo arm = saline; Pre-infusion of ketamine arm = placebo; Post-infusion of ketamine arm = ketamine.

As Figure 6-9 shows, a significant time by treatment arm by load interaction is seen in the right thalamus (p<0.01). The three-way interaction in this ROI is driven by the fact that at pre-infusion activation on Placebo_K is greater than Placebo_S at 1-back and 3-back together with the reversal of this effect at post-infusion whereby activation on P_Ketamine is decreased compared to P_Saline. However, even though variability is present between placebo conditions the overall effects of time in each treatment arm can help to clarify what this three-way interaction demonstrates. In the placebo treatment arm activation increases over time (this is significant at 1-back; see Appendix C), in contrast to a decrease in activation in the ketamine treatment arm. Thus it appears that the overall effect of ketamine is in opposition to what occurs over time in placebo conditions (see Appendix C).

The right (p=0.063) and left cingulate (p<0.05) also demonstrate a time by treatment arm by load interaction. These interactions are driven by the fact that the relationship between treatment arms is different depending on whether one compares the pre-infusion or the post-infusion conditions. In the right cingulate, the interaction is driven by the fact that at pre-infusion 2-back, activation in the Placebo_K condition is decreased compared to Placebo_S, whereas at post-infusion, activation in P_Ketamine is increased compared to P_Saline. However none of these comparisons are significant. In the left cingulate, the interaction is driven by a trend-level decrease in activation in the P_Ketamine compared to P_Saline condition at 3-back and a non-significant decrease in the P_Ketamine condition at 1-back together with the reversal of this effect at pre-infusion whereby activation at 1-back is greater in Placebo_K than in Placebo_S. However, no significant differences are evident between placebo conditions indicating that the overall effect of a placebo-ketamine comparison is that of a decrease in activation with ketamine (see Appendix C).

The right caudate demonstrates a trend towards a significant time by treatment interaction (p=0.094). Pairwise comparisons indicate that this interaction is due to the fact that there is a significant decrease in activation over time in the ketamine treatment arm (i.e. between pre-infusion Placebo_K and post-infusion P_Ketamine conditions), whereas this effect is attenuated in the placebo treatment arm. Thus, although variability is seen between the pre-infusion placebo conditions, ketamine appears to decrease activation to a greater extent than is seen over time in placebo conditions.

Table 6-4 Repeated-measures ANOVA including the placebo and ketamine treatment arms

ROIs	2x4x3 ANOVA (placebo-ketamine)					
	Main Effect of Time		**Time x Treatment Interaction**		**Time x Treatment x Load Interaction**	
	F[1,15]	P	F[1,15]	P	F[2,30]	P
rDLPFC	2.163	p=0.162	0.272	p=0.610	2.366	p=0.111
lDLPFC	0.048	p=0.830	0.001	P=0.982	0.250	p=0.780
rCING	1.006	p=0.332	0.121	p=0.732	3.033	p=0.063
lCING	1.628	p=0.221	0.122	p=0.731	5.150	p<0.05
rCAU	4.730	p<0.05	3.202	p=0.094	1.676	p=0.204
lCAU	6.803	p<0.05	0.590	p=0.454	1.966	p=0.158
rTHAL	0.632	p=0.439	1.823	p=0.197	6.066	p<0.01
lTHAL	3.431	p=0.084	0.750	p=0.400	0.931	p=0.405
rHIPP	9.829	p<0.01	1.204	p=0.290	1.115	p=0.341
lHIPP	1.446	p=0.248	1.471	p=0.244	1.208	p=0.313

RISPERIDONE

The second ANOVA included the ketamine and risperidone treatment arms in order to isolate any effects of risperidone on ketamine with respect to its pre-infusion activation. Please refer to Figure 6-10 to visualise specific results and Table 6-5 for all relevant statistics.
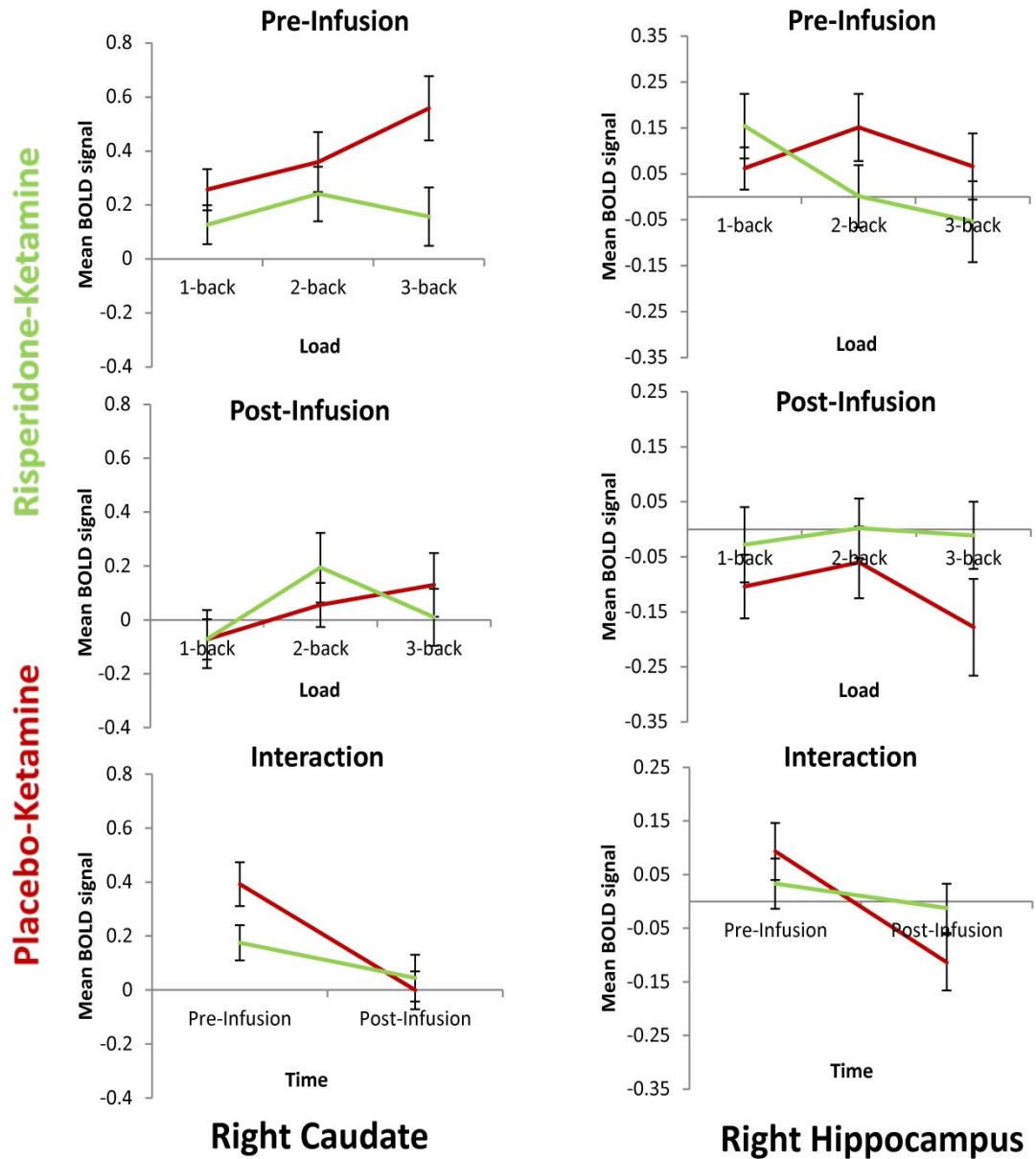
Figure 6-10 The effect of the ketamine and risperidone treatment arms in the right caudate and hippocampus

These two ROIs were found to exhibit a trend-level and significant time by treatment interaction respectively between the pre-infusion and post-infusion conditions of these treatment arms. The interaction graphs illustrate the time by treatment effect. It is important to note that the pre-infusion and post-infusion comparisons are not significant; these graphs are for visualisation purposes alone. Error bars represent the standard error of the mean. **Pre-infusion ketamine arm = placebo; Post-infusion ketamine arm = ketamine; Pre-infusion risperidone arm = risperidone; Post-infusion of risperidone arm = risperidone-ketamine.**

As Figure 6-10 shows, the right caudate (p=0.081) and right hippocampus (p<0.05) demonstrate a time by treatment interaction effect. These interactions are driven by the fact that the effect of 'time' differs between treatment arms. In the ketamine treatment arm activation significantly decreases between pre-infusion (Placebo_K) and post-infusion (P_Ketamine) conditions, whereas in the risperidone treatment arm this decrease is not significant (Appendix C).

The left caudate demonstrates a trend-level time by treatment by load interaction (p=0.08). The interaction is driven by the fact that the relationship between the ketamine and risperidone treatments arms is reversed between pre- and post-infusion conditions. At pre-infusion, risperidone demonstrates increased activation at 1-back compared to placebo but significantly decreased activation at 3-back, whereas activation does not differ in the 2-back condition between these two treatments. On the other hand, at post-infusion, no difference in activation is found between P_ketamine and R_Ketamine at 1-back or 3-back, whereas R_Ketamine shows an increase compared to P_Ketamine at 2-back. The overall effects of 'time' demonstrate that although both post-infusion conditions (P_Ketamine and R_Ketamine) demonstrate an overall decrease in activation compared to their pre-infusion conditions, it appears that there is a lesser decrease between Risperidone_K and the R_Ketamine conditions than there is between the Placebo_K and P_Ketamine conditions.

Table 6-5 Repeated-measures ANOVA including the ketamine and risperidone treatment arms

ROIs	2x2x3 ANOVA (ketamine-risperidone)					
	Main Effect of Time		**Time x Treatment Arm Interaction**		**Time x Treatment Arm x Load Interaction**	
	F[1,15]	P	F[1,15]	P	F[2,30]	P
rDLPFC	1.99	p=0.179	0.39	p=0.542	0.58	p=0.566
lDLPFC	1.221	p=0.287	0.581	p=0.458	2.173	P=0.131
rCING	1.43	p=0.25	0.836	p=0.375	0.124	p=0.884
lCING	3.137	p=0.097	0.004	p=0.953	0.251	p=0.78
rCAU	12.103	p<0.01	3.494	p=0.081	0.84	p=0.442
lCAU	8.29	p<0.05	0.575	p=0.46	2.744	p=0.08
rTHAL	8.228	p<0.05	0.451	p=0.512	0.848	p=0.438
lTHAL	7.825	p<0.05	0.105	p=0.75	0.461	p=0.635
rHIPP	3.699	p=0.074	5.924	p<0.05	1.668	p=0.206
lHIPP	3.633	p=0.076	0.783	p=0.39	0.976	p=0.388

LAMOTRIGINE

The third ANOVA included the Placebo_K – P_Ketamine and Lamotrigine_K – L_Ketamine treatment arms in order to isolate any effects of risperidone on ketamine with respect to its pre-infusion baseline activation. Please refer to Figure 6-11 to visualise specific results and Table 6-6 for all relevant statistics.
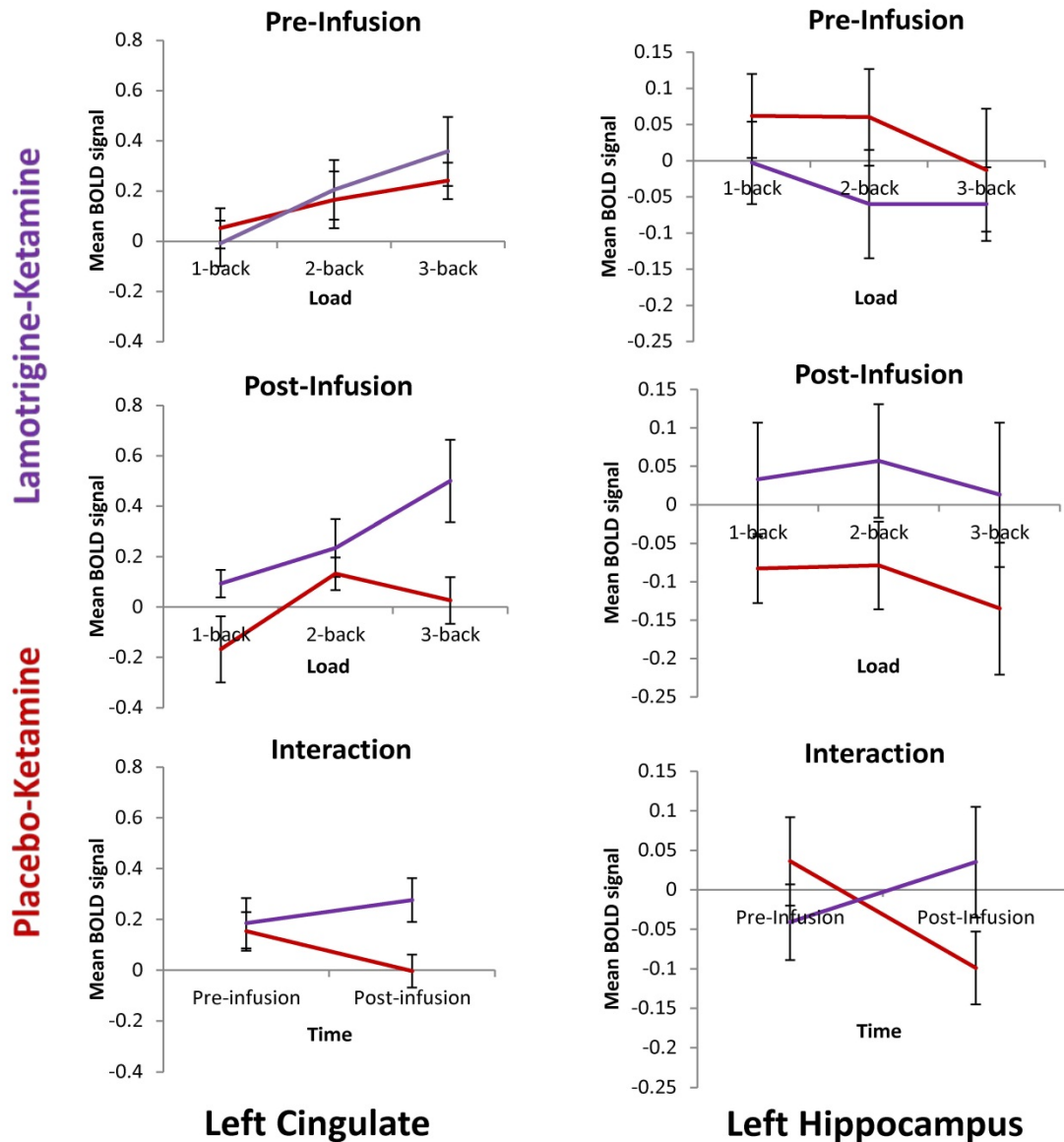
Figure 6-11 The effect of the ketamine and lamotrigine treatment arms in the left cingulate and hippocampus

The left cingulate was found to demonstrate a trend-level effect of treatment between the post-infusion conditions whereas the left hippocampus demonstrated a trend-level time by treatment interaction between the pre-infusion and post-infusion conditions. The interaction graphs illustrate the time by treatment effect. It is important to note that the left cingulate interaction and the pre-infusion and post-infusion comparisons for the left hippocampus are not significant; the graphs are for visualisation purposes alone. Error bars represent the standard error of the mean. **Pre-infusion ketamine arm = placebo; Post-infusion ketamine arm = ketamine; Pre-infusion lamotrigine arm = lamotrigine; Post-infusion of lamotrigine arm = lamotrigine-ketamine.**

As Figure 6-11 shows, the left hippocampus demonstrated a trend-level time by treatment interaction (p=0.084). This interaction is driven by (1) a trend-level (p=0.068) decrease in activation in the P_Ketamine condition compared to Placebo_K and (2) a trend level-level (p=0.089) increase in activation in the L_Ketamine condition compared to P_Ketamine. No effect of Lamotrigine_K is seen in comparison to Placebo_K, thus the effect is due to a decrease in activation with ketamine and a subtle reversal of this effect with lamotrigine. No other interactions were present in any other ROIs.

Table 6-6 Repeated-measures ANOVA including the ketamine and lamotrigine treatment arms

ROIs	2x2x3 ANOVA (ketamine-lamotrigine)					
	Main Effect of Time		**Time x Treatment Arm Interaction**		**Time x Treatment Arm x Load Interaction**	
	F[1,15]	P	F[1,15]	P	F[2,30]	P
rDLPFC	0.464	p=0.506	0.001	p=0.975	1.469	p=0.63
lDLPFC	0.003	p=0.956	0.078	p=0.784	1.379	p=0.267
rCING	0.103	p=0.753	0.568	p=0.463	0.767	p=0.473
lCING	0.212	p=0.652	3.044	p=0.101	1.532	p=0.233
rCAU	16.36	p<0.01	2.209	p=0.158	0.629	p=0.54
lCAU	10.05	p<0.01	0.955	p=0.344	1.38	p=0.267
rTHAL	2.872	p=0.111	0.56	p=0.466	0.673	p=0.518
lTHAL	1.882	p=0.19	2.169	p=0.162	0.351	p=0.707
rHIPP	4.264	p=0.057	2.649	p=0.124	0.346	p=0.710
lHIPP	0.382	p=0.546	3.42	p=0.084	0.149	p=0.862

6.3.2.3   Summary of Imaging Results: by Treatment

Placebo

As can be seen in Figure 6-8, a degree of variation exists between ROIs in their response to WM load. The right DLPFC, cingulate and caudate appear to respond with an increase in activation mainly between the 1-back and 2-back conditions and plateau between the two-back and three-back. The left DLPFC, cingulate and caudate appear to be responding to a lesser extent to load, with the caudate, together with the thalamus and hippocampus demonstrating a more 'inverted U-shaped' response.

Ketamine

When compared to saline infusion, ketamine appears to blunt the response to load seen in the right DLPFC and bilateral cingulate, with a decrease in activation in the 3-back condition. This effect is most clearly apparent in the right DLPFC which demonstrates a significant treatment by load interaction, driven by a decrease in activation at 3-back in the ketamine condition when compared to the placebo (see Figure 6-9).

However, when pre-infusion conditions are taken into account when interpreting the effect of ketamine, a pattern of subtle decreases in activation emerges. Significant interactions seen in the right thalamus and left cingulate indicate that although variability is seen between pre-infusion placebo conditions, the ketamine-induced decrease in activation is in opposition to what occurs over time in placebo conditions. The right caudate also demonstrates a significant decrease across loads when the ketamine condition is compared to its baseline placebo.

Risperidone

When compared to placebo, risperidone appears to induce a widespread load-related effect. Significant risperidone-induced treatment by load interactions characterised by increases in activation at 1-back and decreases in activation at 3-back are present in the right DLPFC and left caudate. The left caudate nucleus, right cingulate, bilateral thalamus and hippocampus also appear sensitive to this effect. This pattern of activation also appears present when risperidone is combined with ketamine.

However, when pre-infusion conditions are taken into account when interpreting the effects of risperidone pre-treatment on ketamine, it appears that risperidone may in fact be having a subtle modulatory effect on ketamine. For example, the right hippocampus demonstrates a significant interaction due to risperidone reducing activation at baseline.

In addition, the change in activation between risperidone and risperidone-ketamine is less than the change between placebo and ketamine, suggesting that risperidone may be blocking the decreases seen with ketamine alone. Bilateral caudate nucleus also appears sensitive to this effect.

Lamotrigine

When compared to placebo, no lamotrigine-induced effects were found. No significant effects of lamotrigine pre-treatment were found on the ketamine response. However, the left cingulate, thalamus and hippocampus appear to demonstrate a trend towards lamotrigine-induced reversal of ketamine's effects.

6.3.2.4   Whole Brain Analysis

Whole brain analyses of the NBACK task were conducted for exploratory purposes, in order to detect any treatment effects outside of the selected pre-defined regions of interest. Treatment effects or treatment by load interaction effects were investigated with the use of two separate repeated measures ANOVAs (flexible factorials) for the pre-infusion and post-infusion conditions separately, to examine the main effects of the oral drugs and then their effects on the ketamine response. This was followed by a full-factorial analysis combining the pre- and post-infusion conditions in order to investigate ketamine modulation effects while taking into account the effects of the oral drugs.

At a voxelwise significance threshold of $p<0.001$ uncorrected, no main effect of treatment was found between pre-infusion conditions. At post-infusion, the posterior cingulate cortex (PCC) was present at $p<0.001$ uncorrected, however this effect of treatment was not significant at a corrected threshold (see Figure 6.12; dark blue bars for the post-infusion response).

However, the full factorial analysis demonstrates a significant time by treatment interaction in this region (family-wise corrected for multiple comparisons at $p<0.05$ on the basis of peak amplitude) (see Figure 6.12). As expected, deactivation of the PCC occurs under placebo conditions, although this appears to lessen over time. In comparison, the administration of ketamine appears to increase this deactivation in comparison to its baseline placebo. Risperidone alone appears to inhibit the required deactivation in comparison to placebo, whereas when it is combined with ketamine it increases the deactivation to a greater extent than is seen with either placebo or ketamine. On the other hand, the deactivation seen with Lamotrigine alone does not appear to differ from placebo conditions. However, when combined with ketamine it constrains the

deactivation seen with ketamine alone. The interaction effect thus appears to be due to the opposing effects of the risperidone and ketamine (decrease in activation at post-infusion) compared to the placebo and lamotrigine (increase in activation at pre-infusion) treatment arms. Nonetheless, the pattern of activation described above is merely descriptive; only one 2x2 interaction (between the placebo and risperidone treatment arms) performed within this full-factorial and masked within a PCC-precuneus ROI demonstrate was found to be significant (FWE corrected for small volumes at $p < 0.05$).
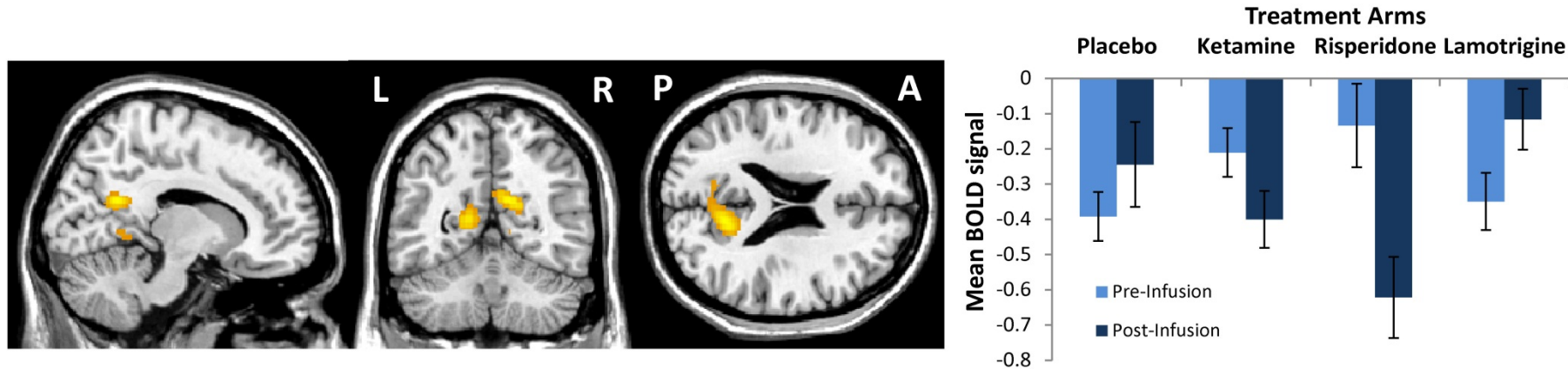
Figure 6-12 A time by treatment arm interaction in the posterior cingulate cortex

Figure 6.13. The posterior cingulate cortex showing a significant time (pre- versus post-infusion) by treatment arm (placebo, ketamine, risperidone and lamotrigine) interaction effect. The F-test is displayed at an uncorrected threshold ($p<0.001$) but is significant at a family-wise error ($p<0.05$) corrected threshold on the basis of peak amplitude. The adjacent plot displays the mean BOLD signal (allback versus control) for both pre-infusion (light blue) and post-infusion (dark blue) treatment conditions (extracted from a sphere centred on the MNI coordinate (12, -56, 22), representing the peak signal of the PCC). Error bars represent the standard error of the mean.

6.3.2.5 Individual Differences

As seen in Figure 6-9, a significant treatment by load interaction is seen between placebo and ketamine conditions in the right DLPFC, whereby ketamine induces a decrease in activation at 3-back. Based on the literature, this decrease in activation could suggest that either (1) WM memory capacity is disrupted by ketamine; an effect characterised by disengagement of the DLPFC at higher memory loads or (2) cortical efficiency is increased (Callicott et al, 2000). Given that individual variability in performance impacts on prefrontal cortical recruitment in a non-linear fashion (e.g. impaired performance can be coupled to either an increase or decrease in brain activation representing increased effort or disengagement respectively) (Rypma & D'Esposito, 1999; Callicott et al, 2000; Rypma et al., 2002; Cools & D'Esposito, 2011) an analysis of the individual responses to ketamine in the DLPFC may clarify the relationship between performance and the observed decrease in activation presented in this chapter.
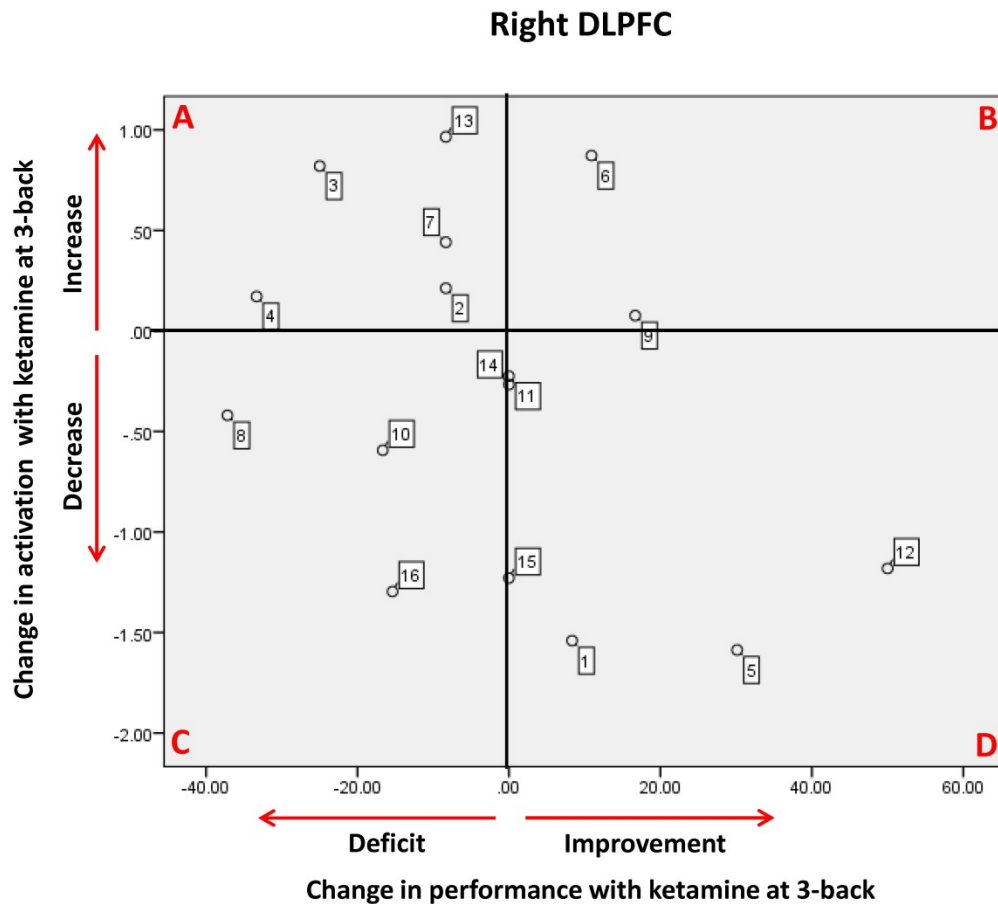
Right DLPFC

Figure 6-13 Individual Differences in the Ketamine Response

Relationship between ketamine-induced changes in activation in the right DLPFC and ketamine-induced performance changes (% correct) at 3-back. Numbers 1-16 represent all sixteen participants in the modulation study.

In order to see whether there was an overall group effect with regards to the relationship between performance and activation in the right DLPFC, a two-tailed Pearson's correlation was performed between the change (from placebo) in performance with ketamine and the change in activation with ketamine at 3-back. This correlation was not found to be significant ($p=0.138$), indicating that no relationship exists, possibly due to a heterogeneous response across individuals. As Figure 6-13 shows, some individuals demonstrated poor performance (Quadrant A and C), with those in Quadrant A demonstrating an increase in activation, a pattern suggestive of cortical inefficiency. On the other hand, Quadrant C individuals demonstrated a decrease in activation, suggestive of a potential disengagement from the task (or exceeded WM capacity). In contrast, the remaining individuals demonstrate improved performance with either increased activity, suggesting normal engagement with the task (Quadrant B) or decreased activity, indicative

of increased cortical efficiency (Quadrant D). Those on the border (e.g. participants 11, 14 and 15) demonstrate no change in performance but a decrease in activity, also suggestive of improved efficiency. Thus, it is apparent that the brain response to ketamine and what it represents depends very much on the individual.

6.4   DISCUSSION

6.4.1   Summary of Main Results

This chapter shows that administration of low dose ketamine demonstrates subtle localised modulation of N-Back task-dependent activation. No increases in activation were seen in contrast to previous studies (G. D. Honey et al., 2008; R. A. Honey et al., 2004). However, a ketamine-related altered load-response was found within the DLPFC, a region with an established role in monitoring and manipulation WM processes. Furthermore, when pre-infusion conditions were considered, subtle decreases in activation were seen with ketamine in the right thalamus and caudate nucleus, and left cingulate; an effect which opposes that which is seen over time in placebo conditions. Ketamine did not induce any performance deficits as predicted; instead it was seen to improve performance when pre-infusion placebo conditions were taken into account.

As expected, risperidone did produce a trend towards performance deficits in the N-Back task. Risperidone was also found to induce a widespread pattern of activation characterised by increased activation at lower loads and decreased activation at higher loads, an effect most evident in the right DLPFC and left caudate although other regions were also sensitive to this effect. When combined with ketamine, this load-related pattern was still evident. On the other hand, modulatory effects of risperidone were only apparent if pre-infusion conditions were taken into account, with a 'reversal' of ketamine-induced decreases in the right hippocampus. The posterior cingulate cortex also demonstrated a risperidone-induced effect, with reduced deactivation at pre-infusion compared to enhanced deactivation when combined with ketamine.

Lamotrigine had no effect on N-Back performance or brain response. When combined with ketamine certain regions of interest demonstrated a potential reversal of the effects of ketamine, such as the left cingulate and thalamus and right DLPFC and hippocampus, however these did not reach significance.

These findings are discussed further below.

6.4.2   Reliability of the N-Back Task: Performance and Brain Network

Reliability analyses in terms of performance demonstrate that accuracy data is more reliable at lower loads, with performance on the 3-back being less consistent across time within the same individual, although group-level consistency is present (i.e. no session effects). On the other hand reaction time measures demonstrate high reliability across all memory loads, suggesting that this performance measure may be more sensitive in terms of assessing WM function in repeated measures studies.

In terms of the cognitive brain network elicited by the N-Back task, the highest reliability was found for the 3-back load in the activation network. The deactivation network appears less reliable overall although this was also the highest at 3-back. The reliability of the task network may appear lower than expected; this may be due to the relatively small sample size used and thus a poorer representation of the true task network. However, ROI analysis confirms the load-related finding and demonstrates that regions within the N-Back task network exhibit reasonable reliability estimates. These results may influence the confidence with which one can interpret the results from the modulation study; it suggests that results with respect to the 3-back may be the most robust in terms of reaction times and brain activation, specifically within the DLPFC.

6.4.3   The Effects of Ketamine on the N-Back Task Network: Increases or Decreases?

The results from the modulation study with regards to ketamine, suggest that acute low-dose ketamine administration to healthy volunteers does not have a generalised effect on WM as assessed by an fMRI N-Back task. Instead, ketamine's effects appear restricted to only certain brain regions and furthermore, may only be detected when the load-related response is investigated.

The hypothesised brain response to ketamine, which has been found in previous WM imaging studies, was that of an increase in activation across the WM network (G. D. Honey et al., 2008; R. A. Honey et al., 2004). However, additional studies have demonstrated that ketamine given at higher doses can also induce task-related reductions in BOLD signal (Abel, Allin, Kucharska-Pietura, et al., 2003; Northoff et al., 2005) (Scheidegger, Boeker, et al., 2011; Scheidegger, Henning, et al., 2011), a finding more in line with the results of this chapter, whereby ketamine induced decreases are apparent in the cingulate, caudate and thalamus, nodes of the WM cortico-striato-thalamic circuit shown to be extensively affected by NMDAR antagonism ((Moghaddam et al., 1997; C. J.

Morgan, Mofeez, et al., 2004a; Olney & Farber, 1995a; Santana, Troyano-Rodriguez, Mengod, Celada, & Artigas, 2011); Chapter 1 – Section 1.3.2).

The ketamine-induced increases in BOLD signal have been hypothesised to be due the excessive release of glutamate, and thus increased neuronal activity (Krystal, Anand, & Moghaddam, 2002). However, studies have shown that the effects of NMDAR antagonists are more complex. For example, in the context of a WM task in animals, Jackson et al., (2004) demonstrated that although the NMDAR antagonist, MK-801, increased irregular neuronal spike activity, it also reduced neuronal burst activity which is "more effective [] at enhancing neurotransmitter release and information processing" (p.8471). Consequently the efficiency of signal transmission in brain areas such as the PFC or hippocampus is disrupted, potentially leading to decreased BOLD signal. Furthermore, the decreases in activation seen with ketamine is also in agreement with the putative role of the NMDAR in WM described by Wang and colleagues (Compte et al., 2000; Lisman et al., 1998; X. J. Wang, 1999), whereby unsettling "NMDAR-mediated transmission at recurrent synapses should lead to a decrease in memory-associated firing" ((Lisman et al., 1998), p.275) and thus affect the ability to maintain information 'online'. However, this physiological effect may only impact upon performance at higher doses (Adler et al., 1998; C. J. Morgan, Mofeez, et al., 2004a).

In methodological terms, it is possible that the results in this chapter differ from previous studies due to the lower dose of ketamine used, for example in comparison to Honey et al., (2008) who used targeted plasma levels of 100ng/ml. Furthermore, in this study, the N-Back task was performed at least 20 minutes post-infusion, and thus under steady-state conditions, whereas previous studies administered the task much closer to the initial bolus infusion (R. A. Honey et al., 2004) whereby the initial reactions to ketamine administration may obscure the more specific and discreet cognitive effects of this drug. It may thus be important to confirm the findings of this chapter with the use of a higher dose of ketamine still within a steady state context.

6.4.4 The Effects of Ketamine on Working Memory Capacity

As described in the introduction, a decrease in regional activation during a WM task has been hypothesised to reflect the capacity limitations of the memory system (Callicott et al., 1999). Thus ketamine-induced hypoactivity at higher memory loads could suggest that the drug may be impacting upon WM capacity, whereby the system reaches capacity at lower memory loads. In fact, when compared to the placebo response, the right DLPFC

demonstrates a ketamine-induced pattern of activation suggestive of a shift in the WM 'load-activation curve', with increased activation at low memory loads and decreased activation at high memory loads. This shift in response is reminiscent of that hypothesised to explain the hypo- and hyperactivation seen during WM tasks in schizophrenic patients when compared to healthy volunteers (Callicott et al., 2003). This particular effect in the DLPFC is also of interest due to the fact that this area has been hypothesised to be primarily involved in WM manipulation processes (Owen, 2000; Petrides, 2000), processes that have also been found to be specifically affected by ketamine (R. A. Honey et al., 2004; R. A. Honey et al., 2003).

In addition, ketamine was not found to cause performance deficits but instead was found to improve performance overall. This suggests that the decrease in activation seen with ketamine at higher memory loads within the right DLPFC may instead be indicative of increased cortical efficiency and improved information processing (Callicott et al., 2000), not impaired WM capacity. In support of this, beneficial effects of the NMDAR antagonist memantine have been found on cognition (Parsons, Stoffler, & Danysz, 2007), however one must be cautious in generalising across different NMDAR antagonists as their pharmacological profiles still differ to a great extent (Dix et al., 2010). Furthermore, this idea is refuted by the fact that risperidone demonstrates a similar activation load-response to that seen with ketamine, whilst performance is impaired. However, investigation into individual differences demonstrates that the answer to this question is not a simple one, as it is apparent that there is great heterogeneity between individuals, with regards to their relationship between ketamine-induced performance changes and accompanying brain response. Nonetheless, whether low-dose ketamine is detrimental or beneficial to WM, these results support the involvement of the glutamatergic system, and specifically NMDAR-mediated transmission, in WM function.

6.4.5 Reversal of the Ketamine-induced Brain Response: A Role of the Glutamatergic System in Working Memory?

Contrary to the hypothesis stated in the introduction to this chapter and previous studies that demonstrate an inhibitory action of lamotrigine on the effects of ketamine (Anand et al., 2000; Deakin et al., 2008), lamotrigine was not found to significantly reverse the effects of ketamine on brain activation in the context of a WM task. However, the direction of the trend effects within regions such as the DLPFC, cingulate, thalamus or hippocampus does lend support to the notion that ketamine exerts its effects through the

excessive release of glutamate, with the subtlety of lamotrigine's 'reversal' merely due to the similarly subtle ketamine induced changes in brain activation.

With respect to the effects of risperidone, a modulation of the effects of ketamine was found in the right hippocampus with a trend towards a similar effect in the caudate nucleus. As hypothesised in the introduction, this modulation may be due to the effects of risperidone at 5HT2A receptors, although it cannot be excluded that this may be due to the effects of risperidone at dopamine D2 receptors, given the high density of such receptors in striatal areas (Camps, Cortes, Gueye, Probst, & Palacios, 1989). In fact, Krystal et al., (1999) demonstrated that haloperidol pre-treatment, an antipsychotic highly selective for the D2 receptor, was able to attenuate ketamine-induced impairments on the Wisconsin Card Sorting Test (WCST), a task with an important WM component. Furthermore, in this study, no risperidone-ketamine interaction effects were observed in cortical regions where 5HT2A-mediated mechanisms may be more pronounced, supporting the idea that, in the context of a WM task, the dopaminergic effects of the drug may dominate. This is also in keeping with animal studies that demonstrate that 5HT2A antagonism is 'necessary but not sufficient' to reverse NMDAR antagonist induced cognitive deficits (H. Y. Meltzer et al., 2011). However, due to the non-specific nature of fMRI results in terms of neurotransmitters, further research is needed in order to elucidate which neurochemical actions underlie the interaction between risperidone and ketamine.

In contrast, it is important to acknowledge that risperidone appears to be detrimental to WM performance, alone and in conjunction with ketamine. This finding is in keeping with previous studies that have investigated the role of D2 receptors in WM and have found that their antagonism induces WM-related impairments (Mehta et al., 2008; Mehta et al., 1999), although the possibility that the deficits are not related to WM per se but instead to non-specific sedatory effects cannot be ruled out (D. D. Miller, 2004). In terms of brain response, the load-activation response to risperidone is similar to that seen with ketamine in the DLPFC; furthermore it is still present when the two drugs are combined. As described in the introduction, prefrontal cortical regulation of dopamine levels is essential to WM function (Cools & D'Esposito, 2011) and, in the highly performing healthy volunteers included in this study one could assume that these levels are optimal on average. Thus a drug-induced increase in dopamine release and resulting excessive D1 stimulation could lead to a deficit in WM (Castner & Williams, 2007). In fact, risperidone, through D2 blockade, has been found to increase dopamine release in the PFC (Ichikawa

& Meltzer, 1999), a mechanism which has been proposed to underlie the effects of risperidone on the brain response to a WM task in schizophrenic patients (G. D. Honey et al., 1999). The different pharmacological profiles of ketamine and risperidone suggest that their effects on the load-activation curve, although similar in the DLPFC, are due to distinct mechanisms, one glutamatergic and the other dopaminergic. However it is possible that these effects may be the result of a common pathway, as ketamine has also been found to release dopamine in cortical areas (Lindefors et al., 1997; Verma & Moghaddam, 1996). Nonetheless, in task-positive cortical regions the combination of these two compounds does not appear to be additive in terms of brain response.

6.4.6 The Effects of Ketamine on the Posterior Cingulate Cortex

The posterior cingulate cortex is part of the default mode network (DMN); a task negative network whose activation is suppressed during the performance of cognitive tasks (Raichle et al., 2001). The effects of ketamine on the PCC are minimal and load-independent, although the results do suggest that they oppose the effects of placebo across time, with a decrease in activation compared to an increase. These findings may be relevant for schizophrenia, where enhanced deactivation of the DMN has in fact been shown (Garrity et al., 2007). This trend is also in agreement with a study by Scheidegger and colleagues (2011) that demonstrated a ketamine-enhanced deactivation in the pregenual cingulate cortex (a region of the DMN), an effect which was also found to be correlated with the glutamine-glutamate ratio, a marker of glutamatergic neurotransmission. Similar findings were reported by Northoff and colleagues who demonstrated ketamine-induced decreases in activation in the PCC during an episodic memory task (Northoff et al., 2005) and, in a separate study, that glutamate levels in a region of the DMN (perigenual cingulate cortex) predicted the level of activation in the anterior cingulate cortex (ACC), a task-positive region (N. W. Duncan, Enzi, Wiebking, & Northoff, 2011). In addition, this group found a degree of functional connectivity between these task-positive and task-negative regions, however they did not directly assess whether the levels of glutamate modulated this connectivity. It would be interesting to see whether, with the use of ketamine, modulation of the glutamatergic system alters this relationship within the context of a WM task, so as to have further insight into the role the glutamatergic system (or more specifically NMDAR hypofunction) in aspects of brain function important for cognition. Furthermore, this would strengthen the findings of this chapter which demonstrate only subtle effects of ketamine on univariate analyses at the dose used.

Lamotrigine is seen to counteract the ketamine-induced 'deactivation', supporting the idea that NMDAR antagonism leads to an increase in glutamate release in cortical areas. On the other hand, risperidone appears to increase the deactivation seen with ketamine. The DMN network has previously been shown to be modulated by the dopaminergic system in humans, with potent dopamine-enhancing compounds enhancing the deactivation characteristic of the PCC in a WM task (Marquand et al., 2011; Tomasi et al., 2011). In addition, the administration of ketamine has been shown to increase dopamine release in the PCC (Aalto et al., 2005), although this was at a much higher dose (200ng/ml) compared to that used in this study. Thus, together with the fact that risperidone induces dopamine release in cortical areas (Ichikawa & Meltzer, 1999), it is possible that when risperidone and ketamine are combined the dopaminergic release supersedes the glutamatergic release in this task-negative region, at least in a WM framework. It is also possible however that risperidone potentiates the effects of ketamine through other systems, for example both ketamine and risperidone increase the release of serotonin in cortical areas (Aghajanian & Marek, 2000; Ichikawa & Meltzer, 1999). Fittingly, risperidone has also been found to promote DMN deactivation in schizophrenic patients, although this was not significantly different from the deactivation seen in healthy controls (Surguladze et al., 2007).

6.4.7 Caveats and Limitations

As described in the introduction to this chapter, the successful completion of an N-Back task requires the use of a multitude of processes within WM. Although this confers many advantages if one wishes to assess pharmacological modulation of WM as a single construct, it does not allow the assessment of drug-induced effects on these individual processes. Thus, it may be more informative in terms of the effects of ketamine on WM, to use a task that enables the separate study of processes such as manipulation and maintenance, as was done by Honey et al (2004). Nevertheless, from the results of this chapter it is apparent that the parametric modulation of load within a WM task is a significant advantage, as this method appears suitable for detecting specific effects of the drug being administered.

As well as affecting manipulation processes (see Chapter 1, Table 1-1), ketamine has also been shown to specifically affect encoding (versus retrieval) processes in cognition ((G. D. Honey, Honey, Sharar, et al., 2005); see Chapter 7 for more details). Thus, a task that includes an encoding, manipulation and load component but maintains separation

between these different conditions could potentially tease apart the effects of ketamine, and thus the role of NMDA hypofunction in WM processes. For example, one could incorporate a manipulation component to a variant of the WM delayed-match-to-sample task (DMS) as used by Habeck et al., (Habeck et al., 2005), which characteristically includes three different phases; encoding, maintenance and retrieval within different load conditions.

6.4.8   Conclusions

This chapter demonstrates that acute administration of low-dose ketamine has a subtle and region-specific effect on the brain response to a WM N-Back task supporting the notion that NMDAR-mediated transmission plays a role in WM function and that fMRI is able to detect this. Ketamine has been hypothesised to exert its effects through a hyperglutamatergic state; however the subtle modulatory effects of lamotrigine do not strongly support this claim. Furthermore, the modulatory effects of risperidone suggest that under certain circumstances other neurotransmitter systems, including the dopamine system, may override the pure glutamatergic effects of ketamine. Whether this is specific to WM function alone is a question that the following chapter may shed light on (Chapter 7). Although these findings have implications for the role of the glutamatergic system in WM, further research into the more specific effects of ketamine is required, in order to identify the exact cognitive targets of NMDAR hypofunction.

Chapter 7 The Effect of Ketamine on an Associative Memory and Learning Task in Healthy Volunteers: Modulation with Risperidone and Lamotrigine

As described in Chapter 1, the role of glutamatergic signalling in cognitive function, and specifically learning and memory, has gained prominence. This chapter describes how ketamine, a probe of the glutamatergic system in healthy volunteers, affects associative learning function and its neural correlates in the context of an fMRI PAL task. In addition, the modulatory effects of lamotrigine and risperidone, compounds expected to counteract the effects of ketamine, are also described. Overall the PAL task was not shown to be sensitive to the effects of ketamine either in terms of performance or cognitive brain networks. No significant modulatory effects of risperidone were found. The interactions between ketamine and lamotrigine were more pronounced and were apparent in the DLPFC, VLPFC and inferior temporal lobe (ITL). These findings have implications on the role of NMDAR hypofunction in associative learning and the utility of fMRI, and specifically the PAL task, in detecting the effects of low-dose ketamine.

7.1 INTRODUCTION

7.1.1 The Neural Basis of Associative Memory and Learning

Associative learning has been described as the "ability to link arbitrary stimuli or actions together in memory" ((Suzuki, 2007a), p.842). In addition, the acquisition and expression of learning relies on the integrity of encoding and consolidation processes and the retrieval of such associations from memory (Banyai et al., 2011).

The underlying neural correlates of encoding and retrieval processes in the context of human associative learning have been extensively investigated with the use of fMRI. Studies using paired associate paradigms, whereby participants are required to learn word-word or object-location associations, have demonstrated the central role of both the prefrontal cortex and temporal lobe structures, such as the parahippocampal gyrus and the hippocampus itself, in both encoding and retrieval (Bunge et al., 2004; Hales, Israel, Swann, & Brewer, 2009; J. A. Meltzer & Constable, 2005; Mottaghy et al., 1999; Ranganath, Cohen, Dam, & D'Esposito, 2004). However, it has been shown that the brain networks involved in associative encoding and retrieval functions can sometimes be dissociated. For example, successful encoding has been found to be accompanied by greater activity in the right anterior hippocampus and ventrolateral prefrontal cortex

(VLPFC), whereas successful retrieval elicited greater activity in the right posterior hippocampus and dorsolateral prefrontal cortex (DLPFC) (Chua, Schacter, Rand-Giovannetti, & Sperling, 2007; Prince, Daselaar, & Cabeza, 2005). Furthermore, successful retrieval has been found to require activation in the precuneus and posterior cingulate cortex, areas considered to be part of the default mode network (DMN), whereas successful encoding, as is customary during the performance of a cognitive task, requires these areas to be deactivated (Daselaar et al., 2009; Huijbers, Pennartz, Cabeza, & Daselaar, 2011; H. Kim, Daselaar, & Cabeza, 2010).

The studies described above have focused on the cognitive brain networks recruited during associative *memory* processes but have not directly assessed associative *learning*, i.e. how the brain responds to the repeated presentation of the same paired associate stimuli over time. In this context, electrophysiological studies have investigated the neuronal response in non-human primates, primarily with the use of visuomotor associative learning tasks. As described in Chapter 1 (section 1.2.1.2.) Suzuki and colleagues (Suzuki, 2007a, 2007b, 2008) demonstrated that prefrontal, hippocampal and striatal neurons displayed varying kinds of learning-related signals; increases, decreases or transient activity were the most common signals detected. The decrease in activity with time or 'repetition suppression' in such brain areas has been found in electrophysiological studies (E. K. Miller, Li, & Desimone, 1991), and in PET and fMRI imaging studies (Buchel, Coull, & Friston, 1999; P. Fletcher, Buchel, Josephs, Friston, & Dolan, 1999; P. C. Fletcher & Henson, 2001; Molchan, Sunderland, McIntosh, Herscovitch, & Schreurs, 1994; Squire et al., 1992) suggesting an adaptation or increased efficiency of the neural response with increasing familiarity (Molchan et al., 1994). Nonetheless, increases in fMRI BOLD signal or CBF have also been found in associative learning paradigms (Law et al., 2005; Passingham, Toni, & Rushworth, 2000; Toni et al., 2001). Differences found in the temporal dynamics of the response may be due to dissimilarities in the experimental tasks used or perhaps the stage of learning at which the signal was captured. Findings from associative learning studies demonstrate that the brain areas recruited during associative memory, also respond as a function of associative learning, again with a principal role for the prefrontal-temporal circuit (Toni et al., 2001). Furthermore, the functional connection between prefrontal and temporal areas appears crucial to associative learning with animal lesion studies demonstrating that a disconnection between these areas disrupts the acquisition of associations (Passingham et al., 2000).

7.1.2 The Paired Associative Learning Task

More recently, a task that combines the ability to explicitly assess associative encoding and retrieval processes separately with the ability to assess changes in these processes over time with learning has been developed. The paired associative learning (PAL) task used in this study requires learning of stimulus-location associations and was adapted from a task included in the Cambridge Neuropsychological Test Automated Battery (CANTAB), which was initially developed for the early detection of Alzheimer's disease (Fowler et al., 2002; Swainson et al., 2001). This task has proved sensitive to the performance deficits arising from hippocampal pathology as is seen in Alzheimer's (Blackwell et al., 2004; Fowler et al., 2002; O'Connell et al., 2004), and to changes in hippocampal activation seen with this disease (de Rover et al., 2011). This particular task and similar paired associative learning tasks have been shown to recruit a brain network in healthy volunteers including prefrontal (specifically DLPFC and VLPFC), anterior and posterior cingulate, temporal, parietal and occipital cortices (de Rover et al., 2011; Dolan & Fletcher, 1997; Hales et al., 2009), together with the cuneus, precuneus, caudate, cerebellum and importantly, the hippocampus (de Rover et al., 2011; Gould et al., 2005; Kirwan & Stark, 2004; Small et al., 2001; Sperling, 2007; Zeineh, Engel, Thompson, & Bookheimer, 2003). In addition, the CANTAB PAL task has been shown to be sensitive to pharmacological modulation, although this has yet to be corroborated in terms of fMRI imaging (Jakala et al., 1999; Morein-Zamir et al., 2010). This task is thus well-suited to investigate associative memory and learning processes that are (1) coordinated by prefrontal and temporal cortices and (2) sensitive to pharmacological agents.

7.1.3 The Role of the Glutamate System in Associative Memory and Learning

As described in Chapter 1, the neurobiological basis of associative learning and memory has been hypothesised to involve long-term potentiation (LTP), a process whereby activity-dependent increases in strength or efficacy of synaptic connections occurs (Hoehn-Saric, McLeod, & Glowa, 1991; Tsien, 2000). This form of synaptic modification is thought to arise through the coincident detection of both pre- and post-synaptic activity, an ability characteristic of the glutamatergic NMDA receptor (Fanselow & Poulos, 2005; G. Riedel et al., 2003). Furthermore, the highest levels of NMDARs within the brain are found in the cortex, thalamus and hippocampus (G. Riedel et al., 2003), suggesting that this circuit may be fundamental to learning and memory, a suggestion supported by the fMRI studies described above.

In addition, studies that have utilised NMDAR antagonists have found that administration of such compounds result in profound impairments in learning and memory-related processes in both animals (Hoehn-Saric et al., 1991) and healthy humans (Rockstroh, Emre, Tarral, & Pokorny, 1996; Schugens et al., 1997). However such learning and memory impairments appear to be due to disruption only to certain phases of the associative process (Rezvani, 2006; G. Riedel et al., 2003). For example, with the use of paired associate tasks numerous animal studies have shown that NMDAR function is only required during encoding (Barker & Warburton, 2008; Bethus, Tse, & Morris, 2010; Day, Langston, & Morris, 2003) and potentially consolidation (Winters & Bussey, 2005), but not during retrieval, a process seemingly more reliant on AMPARs (Bast, da Silva, & Morris, 2005). Furthermore, concomitant activation of NMDARs within the prefrontal cortex and perirhinal (temporal) cortex is essential for the long-term encoding of object-location associations (Barker & Warburton, 2008).

The role of the NMDAR in learning and memory processes has also gained interest due to the fact that patients suffering from schizophrenia, a disorder which has been proposed to involve NMDAR hypofunction (see Chapter 1), also demonstrate deficits in such cognitive functions. Deficits have been found specifically in the PAL task, with impairments in remembering object-location associations and subtle reductions in learning rates (Barnett et al., 2005; Brambilla et al., 2011; Diwadkar et al., 2008; Wood et al., 2002).   Thus the use of NMDAR antagonists in healthy humans may not only shed light on the role of glutamatergic signalling in learning and memory but also the neurobiological mechanisms underlying schizophrenia-related cognitive dysfunction.

A role for the neurotransmitter dopamine has also been found for associative learning (E. K. Miller & Cohen, 2001). Dopamine signalling in the striatum has been hypothesised to underlie 'prediction error', or the "mismatch between expectancy and experience", a process which affects how and how well associations are learnt ((Corlett, Murray, et al., 2007), p. 2387). Abnormal dopamine neuron firing has in fact been used to explain the mechanism behind delusional belief formation in psychoses whereby aberrant prediction error signalling allocates salience to irrelevant stimuli, leading to misplaced associations (Corlett, Honey, et al., 2007; Corlett, Murray, et al., 2007; J. A. Gray, Feldon, Rawlins, Hemsley, & Smith, 1991; Kapur, 2003). However, a more recent theoretical perspective attributes prediction error per se to NMDAR- and AMPAR-mediated glutamatergic transmission, whereas dopamine serves a more modulatory role, coding the degree of certainty one has in such errors (Corlett et al., 2011). Dopaminergic projections to the

PFC are also thought to act as a 'gating' mechanism, whereby dopaminergic signalling exerts control over what information is to be maintained in memory and what should be discarded (Braver, Barch, & Cohen, 1999; Braver & Cohen, 1999). This function is important for associative learning as it influences which experiences are held together in memory and thus associated (E. K. Miller & Cohen, 2001). Nonetheless, a role for a fronto-striatal dopaminergic circuit may be more relevant for studies directly assessing reward prediction error, in contrast to the PAL task which, in the context of this thesis, focusses on successful learning.

7.1.4   The Effects of Ketamine on Associative Memory and Learning

As with other NMDAR antagonists, the administration of ketamine has been shown to impair learning and memory in animals (Pallares, Nadal, Silvestre, & Ferre, 1995) and humans, specifically in the encoding phase (Hetem et al., 2000; G. D. Honey, Honey, Sharar, et al., 2005; C. J. Morgan, Mofeez, et al., 2004b; Oye et al., 1992; Rowland, Astur, et al., 2005). Such studies demonstrated that retrieval of information from memory was only impaired if encoding occurred during drug administration, whereas if encoding was performed beforehand, retrieval performance was unaffected. In terms of fMRI BOLD imaging, ketamine has been found to induce task-related changes in activation during both encoding and retrieval phases. For example, ketamine-induced increases in activation during encoding and retrieval in the prefrontal cortex and hippocampus respectively and decreases in activation during retrieval in areas such as the prefrontal cortex, anterior and posterior cingulate have been found (G. D. Honey, Honey, O'Loughlin, et al., 2005; Northoff et al., 2005).

Studies that have focused on the effect of ketamine on explicit learning, have most commonly used the Hopkins Verbal Learning Task (HVLT) which includes three learning trials (i.e. three presentations of the word list each followed by a free recall trial), a delayed recall trial and a yes/no delayed recognition trial (Benedict, Schretlen, Groninger, & Brandt, 1998). Many studies have demonstrated a ketamine-induced impairment on this task in terms of number of words remembered compared to placebo (Anand et al., 2000; Krystal, Abi-Saab, et al., 2005; Krystal, Perry, et al., 2005; Parwani et al., 2005), however only Anand and colleagues (2000) demonstrated that ketamine blunted learning across trials.

Few studies have investigated the effects of ketamine on *associative* learning and memory and none, to my knowledge, have used the visuo-spatial task to do this. Harborne et al.,

(1996) showed that ketamine impaired an individuals' ability to recall words associated with faces, whereas Freeman et al., (2009) demonstrated that acute ketamine had no effect on superstitious conditioning (a classical form of associative learning), although this study used a between-subjects design. Only one study has investigated the effects of ketamine on associative learning with the use of fMRI; demonstrating an effect on the prefrontal cortex. However, although informative, the focus of this study was on the subject of prediction error (or trial and error learning) and not specifically on what occurs to encoding and retrieval processes over time, a question more relevant to this chapter (Corlett et al., 2006).

Thus in order to be able to investigate the role of NMDAR hypofunction in associative learning and memory processes, with the added advantage of separating encoding and retrieval phases, ketamine was administered to healthy volunteers whilst performing an fMRI version of the CANTAB PAL task. This will allow us to investigate whether NMDAR antagonism alters the temporal dynamics of the BOLD response to repeated presentations of the same pairs of stimuli and further examine the role of the glutamatergic system in human associative learning. Specifically, in order to test predictions in a principled manner, an ROI-based approach was taken with a focus on the DLPFC, VLPFC, posterior cingulate cortex (PCC), parahippocampal gyrus, and anterior hippocampus. These ROIs were chosen on the basis of their acknowledged (1) role in associative memory and learning function, (2) presence in the PAL task brain network and (3) high concentration of NMDARs and sensitivity to the effects of ketamine determined by previous studies (see Chapter 1, Section 1.1.1.3) and the ketamine phMRI brain response described in Chapter 4 of this thesis. The anterior hippocampus was specifically chosen because it has been found to be particularly associated with encoding, a process found to be affected by ketamine.

On the basis of the findings summarised above it was hypothesised that:

(1) Under placebo conditions, PAL performance (in terms of correct responses) should increase as a function of learning phase. On the other hand, activation within the chosen ROIs should either (a) decrease between the initial presentation of the stimuli and the final learning phase in both encoding and retrieval conditions due to stimulus familiarity, predictability or reduced response demands (i.e. the 'repetition suppression' phenomenon) or (b) increase in both encoding and retrieval conditions as a function of learning phase if a degree of automaticity of response has not been achieved. No study has previously investigated the effects of learning phase during separate encoding and retrieval phases thus the predictions with regards to the pattern of response remain tentative.

(2) Under ketamine administration, no performance deficits should be seen due to the low dose used. However, due to the potentially increased sensitivity of fMRI to the subtle effects of ketamine (Honey et al., 2005), the pattern of learning-related changes in activity seen on placebo may be disrupted by ketamine (e.g. no activation change between learning phases) in both encoding and retrieval conditions due to the fact that both of these are performed during ketamine administration.

7.1.5 The Effects of Risperidone and Lamotrigine on Associative Memory and Learning

In order to further investigate the contribution of the glutamatergic system to the neurobiology of associative learning and memory, two medications expected to indirectly or directly modulate this neurotransmitter system, risperidone and lamotrigine, were given in conjunction with ketamine to determine their modulatory effects in the context of the PAL task.

As described in chapter 1 and 6, lamotrigine has been found to reverse the effects of NMDAR antagonists in learning contexts; for example, in reversal learning (PCP - (Idris et al., 2005)) and HVLT paradigms (ketamine - (Anand et al., 2000)). This has been hypothesised to be due to the inhibition of NMDAR antagonist-induced glutamate release, through lamotrigine's actions at sodium and calcium channels (Large et al., 2005). Reversal of the effects of ketamine by this compound has also been confirmed in the study by Deakin et al., (2008) on resting state BOLD phMRI. Lamotrigine alone has been found to have minimal effects in healthy volunteers (Aldenkamp et al., 2002; Anand et al., 2000). In consideration of these findings one can make certain predictions about the effect of lamotrigine on the fMRI PAL task alone or in conjunction with ketamine:

(1) When administered alone, Lamotrigine will either show no effect on learning-related performance or a trend towards an improvement, specifically in reaction time measures. With regards to changes in the ROIs, Lamotrigine will have no significant effect when compared to the placebo condition due to its pharmacological effects being primarily use dependent (Messenheimer, 1995).

(2) When combined with ketamine, and assuming that ketamine will have had no detrimental effects on performance, no additional modulatory effects of lamotrigine pre-treatment will be seen on performance measures. In terms of brain activation in response to the PAL task, lamotrigine will attenuate the effects of ketamine, i.e. maintain the pattern of activation across learning phases seen in the placebo condition.

The atypical antipsychotic risperidone has also been found to reverse the effects of NMDAR-antagonist induced deficits in preclinical learning paradigms; for example in the Morris water maze task (Celikyurt, Akar, Ulak, Mutlu, & Erden, 2011; Didriksen et al., 2007), in reversal learning (McLean et al., 2010) or in place avoidance, a test of associative conditioning (Bubenikova-Valesova, Stuchlik, Svoboda, Bures, & Vales, 2008; Ishiyama et al., 2007). This has been hypothesised to be due to its actions at the serotonergic 5HT2A receptor and its subsequent inhibition of glutamate release (Aghajanian & Marek, 2000; Large, 2007). Furthermore, beneficial effects of risperidone have been found in schizophrenia, with patients demonstrating improvements in learning and memory tasks, although this is usually consequent to long-term treatment (Harvey, Green, McGurk, & Meltzer, 2003; Kern et al., 1999).

Nonetheless, while risperidone has been found to attenuate NMDAR antagonist-induced learning deficits, selective 5HT2A antagonists when administered alone have not consistently been found to do so, suggesting that risperidone's effects at dopamine D2 receptors are also important in its mechanism of action (Snigdha et al., 2010). In fact, dopamine D2 antagonism has been shown to promote learning in both animals and humans (Eyny & Horvitz, 2003; Mehta, Hinton, Montgomery, Bantick, & Grasby, 2005). However, as described in Chapter 6, risperidone has also been found to have detrimental effects on cognitive performance measures in healthy humans (Koychev et al., 2012). Together with the role of dopamine in prediction error and subsequent associative learning processes (Corlett et al., 2011), such findings suggest that the effects of risperidone on NMDAR antagonist-induced deficits may be of a more complex nature than a simple inhibitory action on the release of glutamate.

In comparison to lamotrigine the predictions with regards to risperidone are more cautious, due to the relative lack of studies investigating its effects in healthy volunteers and its complex pharmacological mechanisms.

(3) When administered alone, risperidone will decrease the number of correct responses and increase reaction times. This performance effect may still be evident when combined with ketamine. With regards to activation, specific predictions are harder to make due to risperidone's actions at both 5HT2A and D2 receptors. If one was to base its hypothesised effects on 5HT2A antagonism alone the drug may attenuate the effects of ketamine.

The predictions with regards to risperidone and lamotrigine were also tested within the ROIs specified above.

Given the role of NMDARs in neuroplasticity, learning networks and contrasts representing learning-induced changes in response amplitude were specifically chosen for analysis purposes rather than contrasts representing simple task-related activation. Within this chapter the reliability of PAL performance and its associated learning network is presented in order to confirm its ability to reliably assess function and elicit associative learning networks in healthy volunteers. This is followed by an assessment of performance in each treatment condition, the ROI results and a whole brain analysis performed in order to explore any treatment effects outside of the chosen ROIs. Assessment of performance in each treatment condition was conducted. None of the compounds included in this study has previously been administered in conjunction with the fMRI PAL task in healthy humans, thus the results in this chapter are described from an exploratory perspective.

7.2   METHODS

Please refer to the relevant sections in Chapter 3 for details on the participants, experimental design, infusion protocol and image acquisition. All treatment conditions will be referred to as described in Chapter 3 (Figure 3-4), in the remainder of the text.

7.2.1   Visuo-spatial Paired Associative Memory and Learning (PAL) Task

Initially, six distinct stimuli appeared on a computer screen, one by one in a pseudorandom order. Each stimulus appeared in a different location and remained there for one second (six second encoding stage). One second after the last stimulus was revealed, the six stimuli were then shown again one by one in the centre of the screen for three seconds each. Participants responded to each stimulus by moving the joystick towards the position they believed to be the original location of the stimulus (eighteen second retrieval stage). This cycle was presented twice more with the same stimuli in the same locations but presented in a different order. In between cycles the participants were presented, irrespective of performance, with the prompt 'Try again'. Thus in one block of the task, participants had three opportunities (learning phase 1, 2 and 3) to learn the locations of the six stimuli. Overall, the task consisted of six blocks, each time with a new stimulus set. The prompt 'New shapes' was shown at the start of each block. The control condition involved viewing a single stimulus appearing in each of the six locations. This

was followed by the same stimulus appearing in the centre of the screen accompanied by a cue (grey circle) highlighting the direction in which the joystick should be moved. Participants were prompted to 'Follow the grey circle' at the start of each control phase. The control condition was also presented six times, controlling for the visual and motor requirements within the same framework as the learning conditions. The presentation of the verbal instructions always lasted four seconds. The total task length was 11 minutes and 12 seconds.

Thus, in total, within the object-location learning conditions there were eighteen encoding phases and eighteen retrieval phases. The control condition included six encoding and retrieval phases. For this task, nine different task playlists were constructed, one of which was used for training the participants during their screening visit. All eight remaining playlists were administered to each participant, in a pseudo-randomised order and counterbalanced across participants (i.e. each participant performed the task eight times). Only two of these playlists were used in the reliability study. Each playlist consisted of six pseudo-randomised blocks.
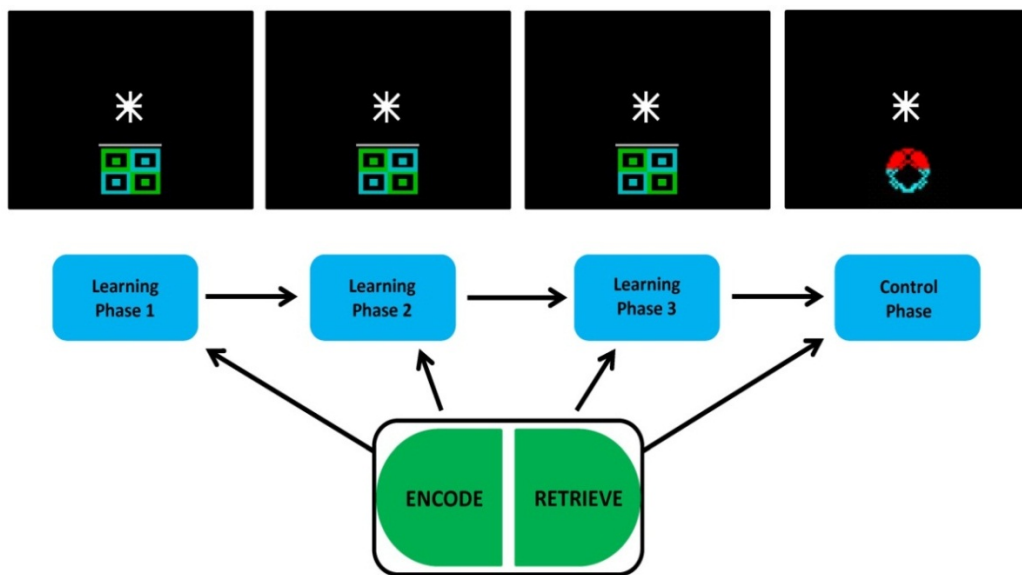
Figure 7-1 Example screen display of the PAL task in the 3 learning phases and control condition

Each presentation is separated into encoding (viewing) of six stimuli followed by retrieval of the previous locations of the same six stimuli. A separate condition is used to control for the visuo-motor components of the task and the entire procedure is repeated a total of six times with new stimuli to maximise power to detect drug-induced differences in behaviour and brain activation patterns.

7.2.2 PAL Performance Analysis

All performance data analysis was conducted in SPSS 20.0 (www.ibm.com/spss). Initial data screening was performed in order to assess whether assumptions for parametric analysis were met. Accordingly, exploratory analysis and Shapiro-Wilk tests were completed. In both the reliability and modulation studies, accuracy data (% correct responses, % misses) were found to violate the assumptions, thus non-parametric tests were applied to the data. Reaction time (RT) data were found to be normally distributed, thus parametric tests were used.

7.2.2.1 Reliability Study

Data were acquired from 11 healthy male participants on two separate occasions, at least one week apart. Wilcoxon signed-rank matched pairs tests were used to test whether there was an effect of session on accuracy data. Friedman's ANOVA was used to test whether an effect of learning was present in each session for the accuracy data. A multifactorial repeated measures ANOVA was used to test for an effect of learning phase and session in the RT data. A paired t-test was used to test whether there was an effect of session in the reactions times for the control condition.

Reliability analyses for the accuracy and RT data were calculated using the ICC (3,1) corresponding to a two-way, mixed-effects model with session as a fixed effect and subject as a random effect (Shrout & Fleiss, 1979) (see Chapter 3 for more details).

7.2.2.2 Modulation Study

Data were acquired from 16 healthy male participants on four separate occasions (both pre- and post-infusion), at least ten days apart.

7.2.2.2.1 Performance by Session

These analyses were performed in order to detect any session (practice) effects in the study. Friedman's ANOVA was applied to the accuracy data to test whether there was an effect of session (Session1, Session2, Session3, Session4) for the control and learning conditions. This was done for both the pre- and post-infusion performance data.

RT data were analysed using (1) a multifactorial repeated measures ANOVA with session and learning phase as within-subject factors for both pre- and post-infusion conditions separately, and (2) a single-factor repeated measures ANOVA with only session as a factor to investigate session effects in the control condition.

7.2.2.2.2 Performance by Treatment

Friedman's ANOVA was applied to the accuracy data to test whether there was an effect of learning (phase 1, phase 2, phase 3) within each treatment condition and to test for an effect of treatment between pre-infusion conditions (Placebo_S, Placebo_K, Risperidone_K, Lamotrigine_K) and between post-infusion conditions (P_Saline, P_Ketamine, R_Ketamine, L_Ketamine). Bonferroni corrected post-hoc Wilcoxon signed-rank matched pairs tests were then used to investigate any significant main effects of treatment at each learning phase and in the control condition (Bonferroni corrected p-value is 0.002).

RT data were analysed using (1) a 4x3 multifactorial repeated measures ANOVA with treatment (as above), and learning phase (as above), as within-subject factors for pre- and post-infusion conditions separately and (2) a single-factor repeated measures ANOVA with only treatment as a factor to investigate treatment effects in the control condition as this is not considered a measure of associative memory. Significant main effects and interactions were interpreted using Bonferroni corrected pair-wise comparisons.

THE INFLUENCE OF PRE-INFUSION CONDITIONS ON POST-INFUSION MODULATION EFFECTS

The analyses described below were conducted in order to assess the post-infusion ketamine modulation effects while taking into account the pre-infusion effects of the oral drugs.

For accuracy data Wilcoxon tests were used to test for any effect of time (pre- versus post-infusion) at each learning phase (Bonferroni corrected p-value is 0.003). Interaction effects cannot be directly calculated with non-parametric tests, thus this was not formally assessed.

For reaction time data a 2x4x3 multifactorial repeated measures ANOVA with time (pre- and post-infusion conditions) treatment arm and learning as within-subject factors. Three separate 2x2x3 ANOVA models were conducted in order to investigate (1) any significant interaction effects found in the 2x4x3 model and (2) whether any significant interactions were present when the treatment comparisons of interest were isolated. A separate 2x4 ANOVA model (with time and treatment arm as factors) was conducted for the control

condition. Significant interactions were interpreted using pair-wise comparisons, Bonferroni corrected for multiple comparisons. The three 2x2x3 models included:

(1) The placebo and ketamine treatment arms;

(2) The ketamine and risperidone treatment arms;

(3) The ketamine and lamotrigine treatment arms.

7.2.3 PAL fMRI Analysis

7.2.3.1 Image Analysis

Preprocessing Procedures were identical to that performed on the phMRI data (please see Chapter 4, Section 4.2.1.2.1). However analyses were performed using SPM8 (version 8-update 4010: Wellcome Department of Cognitive Neurology, London, UK) and applied to the PAL timeseries of 336 images. The SPM software underlying the analysis implemented in this thesis does not differ between SPM5 and SPM 8.

First Level Model Specification

With the use of a GLM, statistical parametric maps were calculated for each participant, each condition (session or treatment) with the use of the same first level model (see Figure 7-2). The first level model used for both the reliability and modulation studies included explanatory variables representing the control, learning phase 1, learning phase 2 and learning phase 3 for encoding and retrieval conditions separately. Two variables representing the instructions and errors (which included the incorrect responses and misses) were also included. Nuisance variables consisting of the six motion parameters derived from the realignment preprocessing stage were added to the model. The onsets for each block within each explanatory variable were defined by the time at which the first stimulus of the block was presented. Durations of each block were defined by the length of time between the first stimulus presentation and when the last stimulus disappeared off the computer screen (encoding blocks = 6 seconds, retrieval blocks = 18 seconds). Onsets for the error events were defined using the time at which the stimulus to which the participant was responding was presented. The durations of these events were defined using the amount of time that was available to the participant to respond to the stimulus (3 seconds). All explanatory variables were convolved with a canonical HRF generating a design matrix of the predicted BOLD response (see Chapter 3, Section 3.5.2 for more detail).

After model specification, beta (β) parameters were then estimated for each variable with the use of the restricted maximum likelihood method (see Chapter 3, Section 3.5.2.). T-contrast images were then created and then taken through to separate second level analyses (see whole brain analysis section). In order to assess what occurs to the encoding and retrieval networks across learning phases two additional t-contrasts were created; encode3 (learning phase 3) greater than encode1 (learning phase 1) and retrieve3 (learning phase 3) greater than retrieve1 (learning phase 1). These two contrasts (from now on referred to as the 'encode-learn' and 'retrieve-learn' contrasts) were used in the second level analyses used to investigate treatment effects.
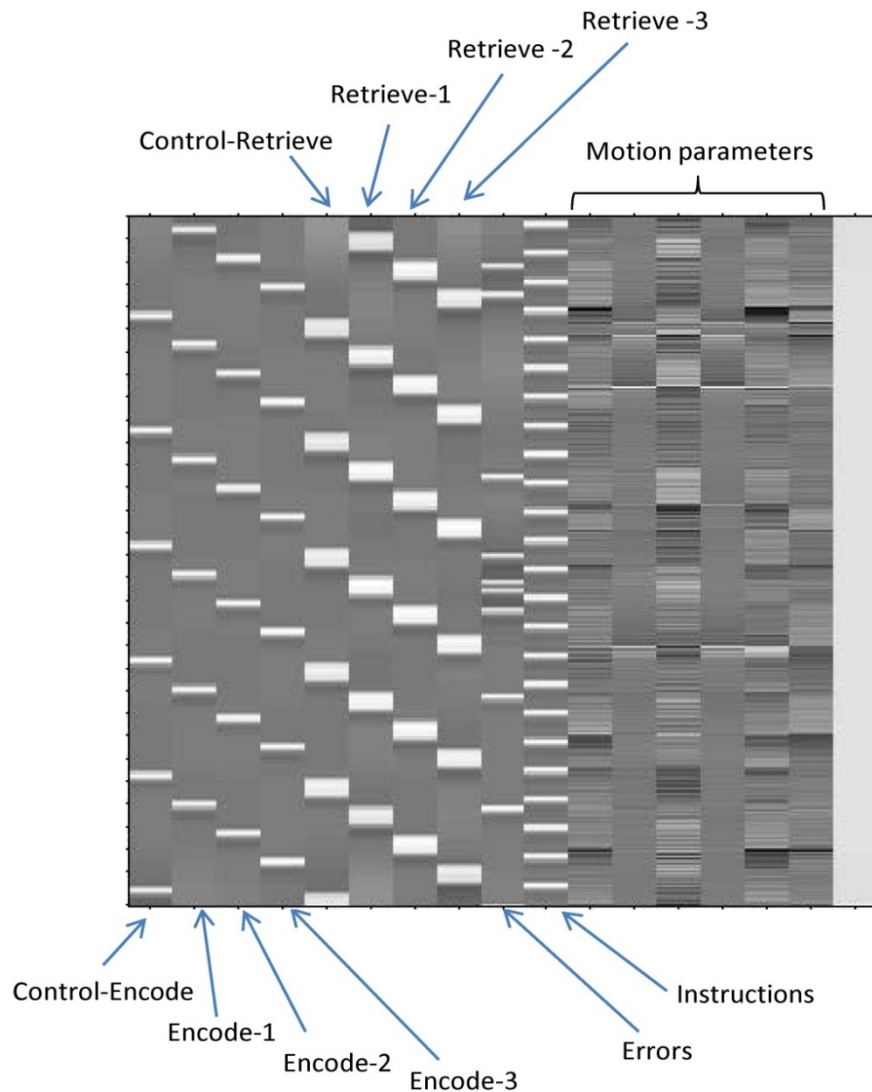
Figure 7-2 Design matrix displaying all variables included in the PAL first level models

Numbers 1, 2 and 3 represent learning phases 1, 2 and 3.

7.2.4   Reliability Study

7.2.4.1   Reliability Analysis

Individual T-contrasts (encode-learn and retrieve-learn) were used in two random effects flexible factorial models with subject as a between subject factor and session as a within-subject factor to detect any session effects for encoding and retrieval conditions separately. Two one-sample t-tests including the same contrasts (for Session 1 only) were conducted to derive the response networks used in the reliability analysis. Voxelwise ICC values were then calculated using the ICC Matlab toolbox (Caceres et al., 2009; see Chapter 3, Section 3.6 for details). ICC values represent the median (with 99% confidence

intervals) of the ICC distribution across voxels within the brain networks significantly responding to the PAL task learning network at the group level. Reliability of PAL task network ROIs (DLPFC, VLPFC, PCC, parahippocampal gyrus and hippocampus-defined below) was also calculated. This was performed for the encode-learn and retrieve-learn contrasts separately.

7.2.5 Modulation Study

7.2.5.1 Region of Interest Definitions and Analysis

Based on the phMRI response to ketamine reported in Chapter 4 on a separate cohort of participants, a set of ROIs were defined *a priori* for the assessment of the predictions outlined in the introduction to this chapter. These comprised ten coordinate-based ROIs, consisting of 5mm radius spheres centered on the Montreal Neurological Institute coordinates corresponding to the DLPFC (right: [50,20,34]; left [-26,44,40]), VLPFC (right: [42, 26, -8]; left [-38, 18, -16]), PCC (right: [6, -42, 6]; left [-4, -42, 32]), the parahippocampal gyrus (right: [22, -36, -14]; left [-28, -32, -18]), and anterior hippocampus (right: [20, -20, -10]; left [-36, -10, -20]). For each region, treatment condition and learning phase mean parameter estimates (beta values) were extracted using the SPM8 toolbox MarsBar. Contrast estimates were then calculated in order to ascertain the differences in betas between learning phase 3 and learning phase 1 for both encoding and retrieval conditions (using the encode-learn and retrieve-learn contrasts specified above). A positive beta value in these conditions thus indicates that activity in learning phase 3 is greater than in learning phase 1 whereas a negative beta value indicates activity in learning phase 1 is greater than in learning phase 3.

All ROI data analysis was conducted in SPSS 20.0 (www.ibm.com/spss). Initial data screening was performed in order to assess whether assumptions for parametric analysis were met. Accordingly, exploratory analysis and Shapiro-Wilk tests were completed. Contrast estimate data were found to be normally distributed, thus parametric tests were used. Analyses for the encoding and retrieval contrast estimates were performed separately to adequately assess the drug-induced effects on these different cognitive processes. Each was analysed with two one-way repeated measures ANOVAs in order to assess the treatment effect pre- and post-ketamine infusion. In addition, in order to assess the post-infusion ketamine modulation effects while taking into account the pre-infusion effects of the oral drugs, a 2x4 multifactorial repeated measures ANOVA with time (pre- and post-infusion conditions), treatment arm as within-subject factors was conducted.

Furthermore, three 2x2 repeated measures ANOVAs were performed in order to investigate (1) any significant interaction effects found in the 2x4 model and (2) whether any significant interactions were present when the treatment comparisons of interest were isolated. This addresses the question of whether the pre-infusion effects of placebo, risperidone and lamotrigine affect the interpretation of any treatment effects seen with ketamine. Significant main effects and interactions were interpreted using pair-wise comparisons, Bonferroni corrected for multiple comparisons.

7.2.5.2   Whole Brain (voxelwise) Analysis

For second level group analyses, individual T-contrasts (from the first level models) representing learning within the encoding and retrieval conditions were used in random effects analyses (see Figure 7-3). As with the ROI data, encoding and retrieval contrast estimates were analysed separately. Each contrast was taken through to three multifactorial repeated measures ANOVAs (flexible factorial model in SPM). Two were conducted with subject as a random effect between-subjects factor and treatment as a within-subject factor to assess any treatment effects pre- and post-ketamine infusion separately. To investigate the post-infusion ketamine modulation effects while taking into account the pre-infusion effects of the oral drugs, an additional flexible factorial model with subject as a random effect between-subjects factor and time and treatment arm as within-subject factors was conducted. Group maps were thresholded at $p<0.001$ uncorrected at the voxel level and cluster corrected at $p<0.05$.

FLEXIBLE FACTORIALS

Figure 7-3 Second level design matrices for the PAL task

Second level design matrices for (a) the pre- and post-infusion flexible factorials with subject and treatment as factors and (b) the flexible factorial with subject, time and treatment arm as factors.

7.3 RESULTS

7.3.1 PAL Performance Analysis

7.3.1.1 Reliability Study

7.3.1.1.1 Accuracy Data

No effect of session was found for correct responses in learning phase 3 ($z = -0.816$, $p = 0.414$) or the control condition ($z = 0.00$, $p = 1.00$). However, a session effect was found for learning phase 1 ($z = -2.407$, $p < 0.05$) and learning phase 2 ($z = -2.524$, $p < 0.05$).

No main effect of session was found for the misses in the control condition ($z = 0.00$, $p = 1.00$); learning phase 1 ($z = -1.289$, $p = 0.197$) or learning phase 3 ($z = -1.342$, $p = 0.18$). However, a trend towards a session effect in learning phase 2 ($z = -1.89$, $p = 0.059$) was found.

A main effect of learning was found on the correct responses (Session 1 ($x^2(2) = 20.182$, $p < 0.01$) and Session 2 ($x^2(2) = 13.610$, $p < 0.01$)) but not on the misses (Session 1 ($x^2(2) = 0.4$, $p = 0.819$; Session 2 ($x^2(2) = 2$, $p = 0.386$)).

7.3.1.1.2   Reaction Time Data

A trend towards a learning effect ($F_{(2,20)}$ = 3.391, p = 0.054) and no significant effect of session ($F_{(1,10)}$ = 2.276, p = 0.162) was found. A significant session by learning phase interaction was found ($F_{(2,20)}$ = 3.518, p <0.05) driven by the fact that the learning effect is lower in Session 1 compared to Session 2 where there is a decrease in reaction time as a function of learning phase.

7.3.1.1.3   Reliability Data

As Table 7-1 shows, reliability of the various performance measures ranges from poor to excellent (-0.07 – 0.79), with highest reliability estimates for the reaction times.

Table 7-1 Performance data and reliability of responses for the PAL task

	Learning Phase	Session 1	Session 2	ICC (3,1) +/- SE
Correct Responses (% +/-SE)	**1**	52.78 (5.11)	69.19 (5.61)	0.54 (0.20)
	2	75.51 (5.023)	86.11 (3.98)	0.71 (0.15)
	3	87.63 (4.37)	91.67 (2.31)	0.36 (0.24)
	Control	93.94 (2.82)	95.96 (1.42)	-0.07 (0.26)
Reaction Time (ms +/-SE)	*1*	*908.45 (40.45)*	*934.17 (51.19)*	*0.52 (0.21)*
	2	*928.50 (52.79)*	*863.91 (50.05)*	*0.69 (0.16)*
	3	*889.48 (58.28)*	*792.30 (41.49)*	*0.74 (0.14)*
	Control	*614.96 (28.79)*	*578.34 (21.54)*	*0.79 (0.12)*
Misses (% +/- SE)	**1**	1.77 (1.01)	0.51 (0.34)	0.21 (0.25)
	2	1.26 (0.58)	0.25 (0.25)	0.55 (0.20)
	3	1.26 (0.87)	0.00 (0.00)	0 (0.26)
	Control	0.00 (0.00)	0.00 (0.00)	-

7.3.1.2   Modulation Study

7.3.1.2.1   Accuracy

<div align="center">EFFECT OF TREATMENT AND LEARNING</div>

Pre-infusion As shown in Figure 7-4, a main effect of learning (p<0.01) on the correct responses was found for all treatment conditions, whereby these increased as a function of learning phase ((Placebo_S (x^2(2) = 18.533); Placebo_K (x^2(2) = 23.831); Risperidone_K (x^2(2) = 16.645); Lamotrigine_K (x^2(2) = 28.222)).

A significant main effect of treatment was found for learning phase 3 (x^2(3) = 9.148, p <0.05), driven by increased accuracy in the Lamotrigine_K condition when compared to Risperidone_K (z = -2.707, p=0.007) and Placebo_S (z = -1.773, p = 0.076). No other treatment effects were found (control (x^2(3) = 3.35, p = 0.341), learning phase 1 (x^2(3) = 2.404, p = 0.493), learning phase 2 (x^2(3) = 3.313, p = 0.346)).

A trend towards a learning effect on misses was found in the Placebo_K condition with a higher percentage of misses in learning phase 2 (x^2(2) = 4.667, p = 0.097). No other learning effects were found (Placebo_S (x^2(2) = 1.52, p = 0.468); Risperidone_K (x^2(2) = 0.5, p = 0.779); Lamotrigine_K (x^2(2) = 2, p = 0.368)).

A trend towards a main effect of treatment in misses was found in learning phase 1 and learning phase 3 (x^2(3) = 6.83, p = 0.078; x^2(3) = 0.7.408, p = 0.06). The trend in learning phase 1 was driven by less misses in the Placebo_K (z = -2.032, p=0.042) and Lamotrigine_K (z = -1.802, p = 0.072) conditions when compared to Placebo_S. The trend in learning phase 3 was also driven by less misses in the Lamotrigine_K (z = -2.06, p=0.039) condition when compared to Placebo_S. No other treatment effects were found (control (x^2(3) = 1.214, p = 0.75); learning phase 2 conditions (x^2(3) = 4.5, p = 0.212)).

No main effect of session was found for correct responses (control (x^2(3) = 3.219, p = 0.359), learning phase 1 (x^2(3) = 2.444, p = 0.486), learning phase 2 (x^2(3) = 4.021, p = 0.259), learning phase 3 (x^2(3) = 6.613, p = 0.085)). A significant main effect of session was found for the misses in the control (x^2(3) = 10.214, p <0.05) and learning phase 3 (x^2(3) = 13.041, p <0.01) conditions, an effect driven by a lower number of misses on session 2. No other session effects were found (learning phase 1 (x^2(3) = 5.809, p = 0.121); learning phase 2 (x^2(3) = 4.833, p = 0.184).
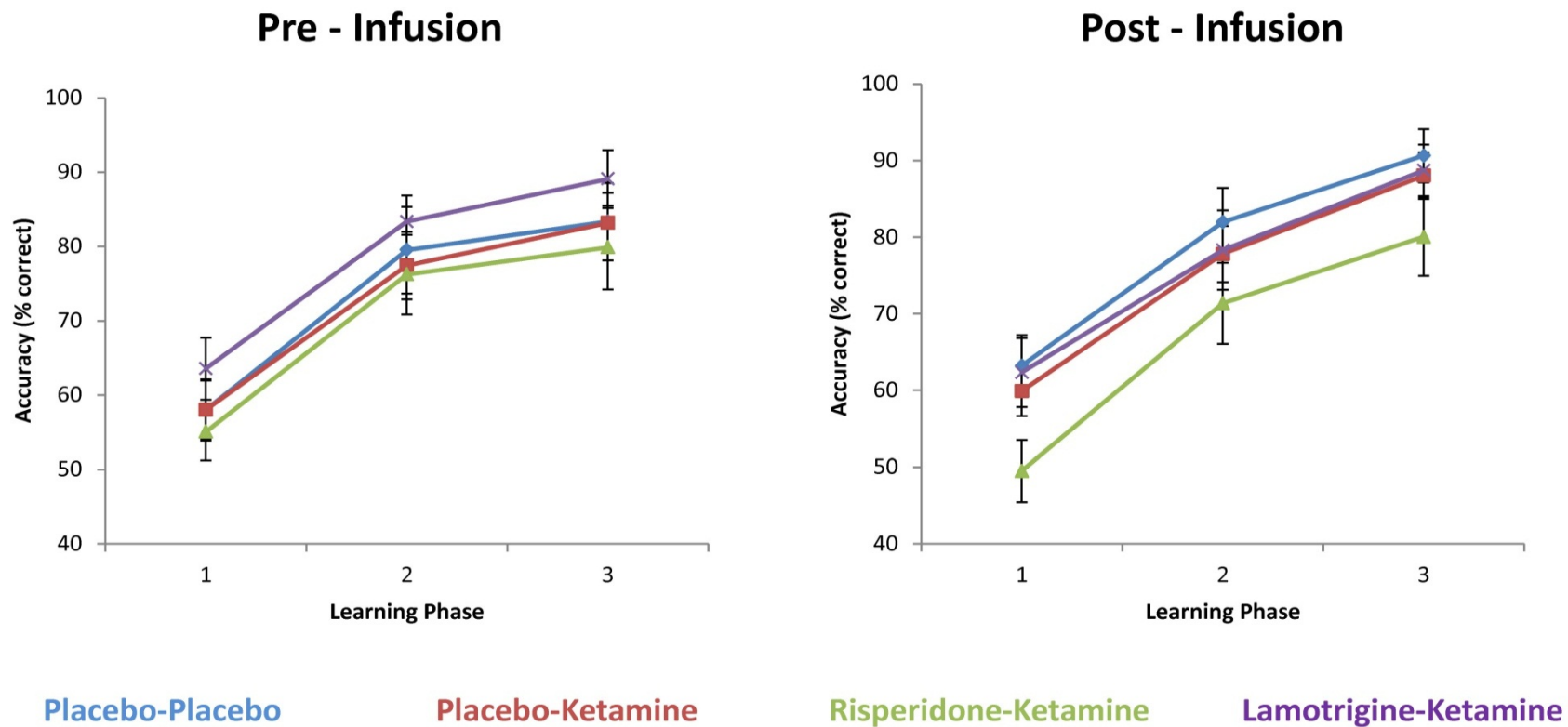
Figure 7-4 PAL performance - Accuracy

Accuracy (percent correct responses) for all treatment conditions (pre- and post-infusion) across all learning phases. Placebo-Placebo treatment arm (blue); Placebo-Ketamine treatment arm (red); Risperidone-Ketamine treatment arm (green); Lamotrigine-Ketamine treatment arm (purple). Error bars represent the standard error of the mean.

Post-infusion A main effect of learning (p<0.01) on correct responses was found for all treatment conditions ((P_Saline (x^2(2) = 29.129); P_Ketamine (x^2(2) = 29.032); R_Ketamine (x^2(2) = 29.746); L_Ketamine (x^2(2) = 18.677)).

A main effect of treatment was found for learning phase 3 (x^2(3) = 12.263, p <0.01) and a trend was found for learning phase 2 (x^2(3) = 7.262, p = 0.064). These effects were driven by a lower number of correct responses in the R_Ketamine condition when compared to P_Saline in learning phase 2 (z = -2.234, p=0.026) and when compared to P_Saline (z = -2.985, p=0.003), P_Ketamine (z = -2.454, p=0.014) and L_Ketamine (z = -2.674, p=0.008) in learning phase 3, although these were not significant when corrected for multiple comparisons. No other treatment effects were found (control (x^2(3) = 5.84, p = 0.12); learning phase 1 (x^2(3) = 6.000, p = 0.112)).

No main effect of learning on misses was found for any treatment condition ((P_Saline (x^2(2) = 2, p = 0.368); P_Ketamine (x^2(2) = 0.8, p = 0.67); R_Ketamine (x^2(2) = 1.182, p = 0.554); L_Ketamine (x^2(2) = 0.5, p = 0.779)).

A trend towards an effect of treatment was found in learning phase 1 (x^2(3) = 7.435, p = 0.059), driven by an increase in misses in the R_Ketamine condition when compared to P_Ketamine (z = -2.02, p=0.043) and L_Ketamine (z = -2.058, p=0.04). No other treatment effects were found (control (x^2(3) = 3, p = 0.392); learning phase 2 (x^2(3) = 2.7, p = 0.44) or learning phase 3 (x^2(3) = 4.778, p = 0.189)).

No main effect of session was found for correct responses or misses in any condition (control (x^2(3) = 5.84, p = 0.12; x^2(3) = 0.474, p = 0.925), learning phase 1 (x^2(3) = 1.745, p = 0.627; x^2(3) = 3.261, p = 0.353), learning phase 2 (x^2(3) = 0.476, p = 0.924; x^2(3) = 1.5, p = 0.682) or learning phase 3 (x^2(3) = 0.526, p = 0.913; x^2(3) = 3, p = 0.392)).

THE INFLUENCE OF PRE-INFUSION CONDITIONS ON POST-INFUSION MODULATION EFFECTS

No significant differences between pre-infusion and post-infusion conditions were found in correct responses or misses when corrected for multiple comparisons.

7.3.1.2.2 Reaction Time

EFFECT OF TREATMENT AND LEARNING

Pre-infusion As shown in Figure 7-5, a significant main effect of learning was found for all treatment conditions (F(2,30) = 13.089, p<0.01) and is driven by the decrease in reaction time between learning phase 1 and learning phases 2 and 3.

A significant main effect of treatment was found ($F_{(3,45)} = 4.072$, $p < 0.05$) and is due to significantly longer reaction times in the Risperidone_K condition in comparison to Lamotrigine_K. No other treatment effects were found (control ($F_{(3,45)} = 1.65$, $p = 0.191$).No significant interaction between treatment and learning was found ($F_{(6,90)} = 0.733$, $p = 0.625$).

No main effect of session was found learning conditions (control ($F_{(3,45)} = 1.214$, $p = 0.316$); learning conditions ($F_{(3,45)} = 1.887$, $p = 0.145$).
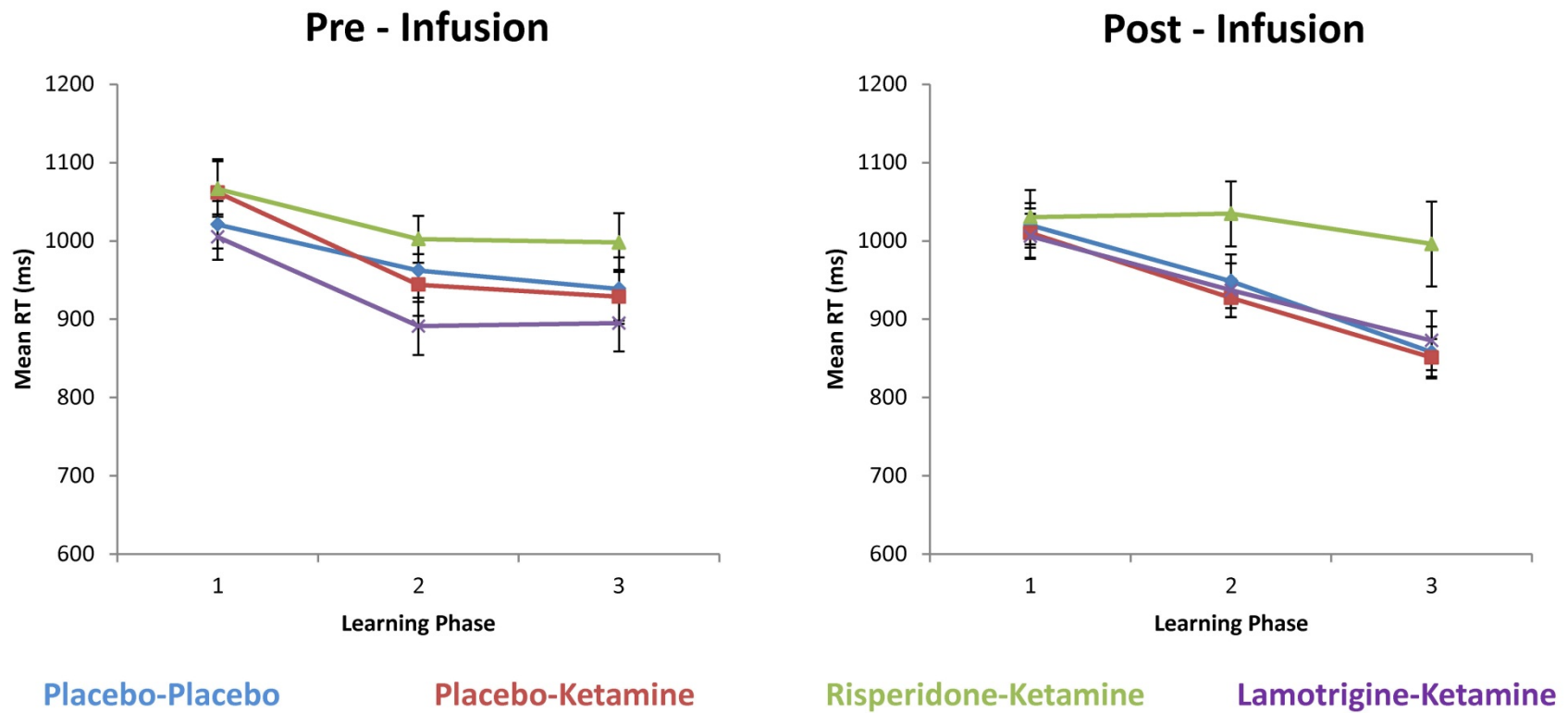
Figure 7-5 PAL performance - Reaction Times

Reaction times for all treatment conditions (pre- and post-infusion) across all learning phases. Placebo-Placebo treatment arm (blue); Placebo-Ketamine treatment arm (red); Risperidone-Ketamine treatment arm (green); Lamotrigine-Ketamine treatment arm (purple). Error bars represent the standard error of the mean.

Post-infusion As Figure 7-5 shows, a significant main effect of learning was found ($F_{(2,30)}$ = 14.92, p<0.01), although this is absent in the R_Ketamine condition.

A significant main effect of treatment was found ($F_{(3,45)}$ = 5.733, p <0.01), driven by significantly longer reaction times in the R_Ketamine condition when compared to P_Ketamine. A trend towards a significant interaction between treatment and learning was found ($F_{(6,90)}$ = 1.925, p = 0.085). This is due to the absence of a learning effect on reaction time in the R_Ketamine condition, whereas reaction times in all other treatment conditions decrease linearly as a function of learning. A significant main effect of treatment was also found in the control condition ($F_{(3,45)}$ = 6.482, p <0.01) due to significantly increased reaction times in the R_Ketamine condition in comparison to both P_Saline and P_Ketamine.

No main effect of session was found (control ($F_{(3,45)}$ = 0.575, p = 0.634); load conditions ($F_{(3,45)}$ = 0.599, p = 0.619)).

THE INFLUENCE OF PRE-INFUSION CONDITIONS ON POST-INFUSION MODULATION EFFECTS

No effects were found in the 2x4x3 ANOVA including all treatment arms or in the 2x2x3 model including the ketamine and risperidone treatment arms (Full model: effect of time ($F_{(1,15)}$ = 1.667, p = 0.216); time by treatment arm interaction ($F_{(3,45)}$=1.671, p=0.187); time by treatment arm by learning interaction ($F_{(6,90)}$=0.547, p=0.771); ketamine and risperidone: effect of time ($F_{(1,15)}$=2.076, p=0.17); time by treatment arm ($F_{(1,15)}$=1.911, p=0.187); time by treatment arm by learning interaction ($F_{(2,30)}$=0.26, p=0.773)).

The 2x2x3 model including the placebo and ketamine treatment arms found a significant effect of time ($F_{(1,15)}$ = 4.735, p <0.05), driven by a decrease in reaction times in the post-infusion conditions, although pairwise comparisons indicate that this was significant only for the P_Ketamine condition. No significant time by treatment arm interaction ($F_{(1,15)}$=0.463, p=0.507) or time by treatment arm by learning interaction ($F_{(2,30)}$=0.842, p=0.441)was found. A trend towards a significant time by treatment interaction ($F_{(1,15)}$=4.511, p=0.051) was found in the model including the ketamine and lamotrigine treatment arms. This appears to be due to a slight increase in reaction time between the Lamotrigine_K and L_Ketamine conditions, whereas there is a significant decrease in reaction times between the Placebo_K and P_Ketamine conditions. No

significant effect of time (F(1,15)=1.481, p=0.242) or time by treatment by learning interaction (F(2,30)=0.031, p=0.969) was found.

A separate 2x4 ANOVA repeated measures model demonstrated that there was a significant effect of time on the control condition (F(1,15)=16.694, p<0.01) and a trend towards a time by treatment interaction (F(3,45)=2.68, p=0.058). This is due to a significant decrease in reaction times in the P_Saline and P_Ketamine conditions when compared to pre-infusion conditions and the fact that this change over time does not occur in the risperidone or lamotrigine treatment arms.

7.3.1.3 Summary of Drug Effects on PAL Performance

Ketamine

When compared to saline no ketamine induced effects were found. However, when compared to pre-infusion placebo, ketamine speeded reaction times in both learning and control conditions.

Risperidone

When compared to placebo, no risperidone induced effects were found. When given in conjunction with ketamine, a trend towards reduced correct responses and increased misses was found when compared to when ketamine was administered alone. Reactions times were also found to be significantly longer when compared to ketamine alone. Furthermore the learning effect was absent in this condition.

Lamotrigine

When compared to placebo, no lamotrigine induced effects were found. No significant effect of lamotrigine pre-treatment was found on the response to ketamine in terms of either accuracy or reaction times.

7.3.2 Imaging Results

7.3.2.1 Reliability Study

7.3.2.1.1 Reliability of the PAL Task Learning Network

No main effect of session was found for the encoding or retrieval learning networks, indicating stability/reproducibility of the PAL learning network across time at a group level. In terms of reliability, it appears that an increase in activation across learning phase is the most reliable effect in the encoding network whereas, for the retrieval network, the

opposite is true (see Table 7-2). The ROIs demonstrating highest reliability are the right DLPFC and VLPFC, left PCC and anterior hippocampus. Overall, within these ROIs it is the retrieval learning network which appears more reliable compared to the encoding learning network. These results may have implications for the use of the PAL task in future repeated-measures studies and on the strength of the modulation study outcomes.

Table 7-2 Reliability of the PAL learning network for both encoding and retrieval conditions

	ICC (3,1) +/- SE	
	ENCODING	**RETRIEVAL**
Learning Network (increase in activation across learning phase)	0.26 (0.007)	0.04 (0.054)
Learning Network (decrease in activation across learning phase)	0.14 (0.025)	0.24 (0.018)
R DLPFC	0.28 (0.019)	0.54 (0.010)
L DLPFC	-0.15 (0.016)	0.21 (0.024)
R VLPFC	0.29 (0.041)	0.55 (0.017)
L VLPFC	0.26 (0.010)	0.21 (0.012)
R PCC	-0.23 (0.018)	0.14 (0.017)
L PCC	0.34 (0.013)	0.27 (0.007)
R Parahippocampal G	0.18 (0.022)	0.15 (0.014)
L Parahippocampal G	0.21 (0.014)	0.27 (0.013)
RA Hippocampus	0.26 (0.010)	0.05 (0.008)
LA Hippocampus	0.37 (0.017)	0.47 (0.012)

ENCODING

RETRIEVAL

Increases in activation over learning phase

Decreases in activation over learning phase
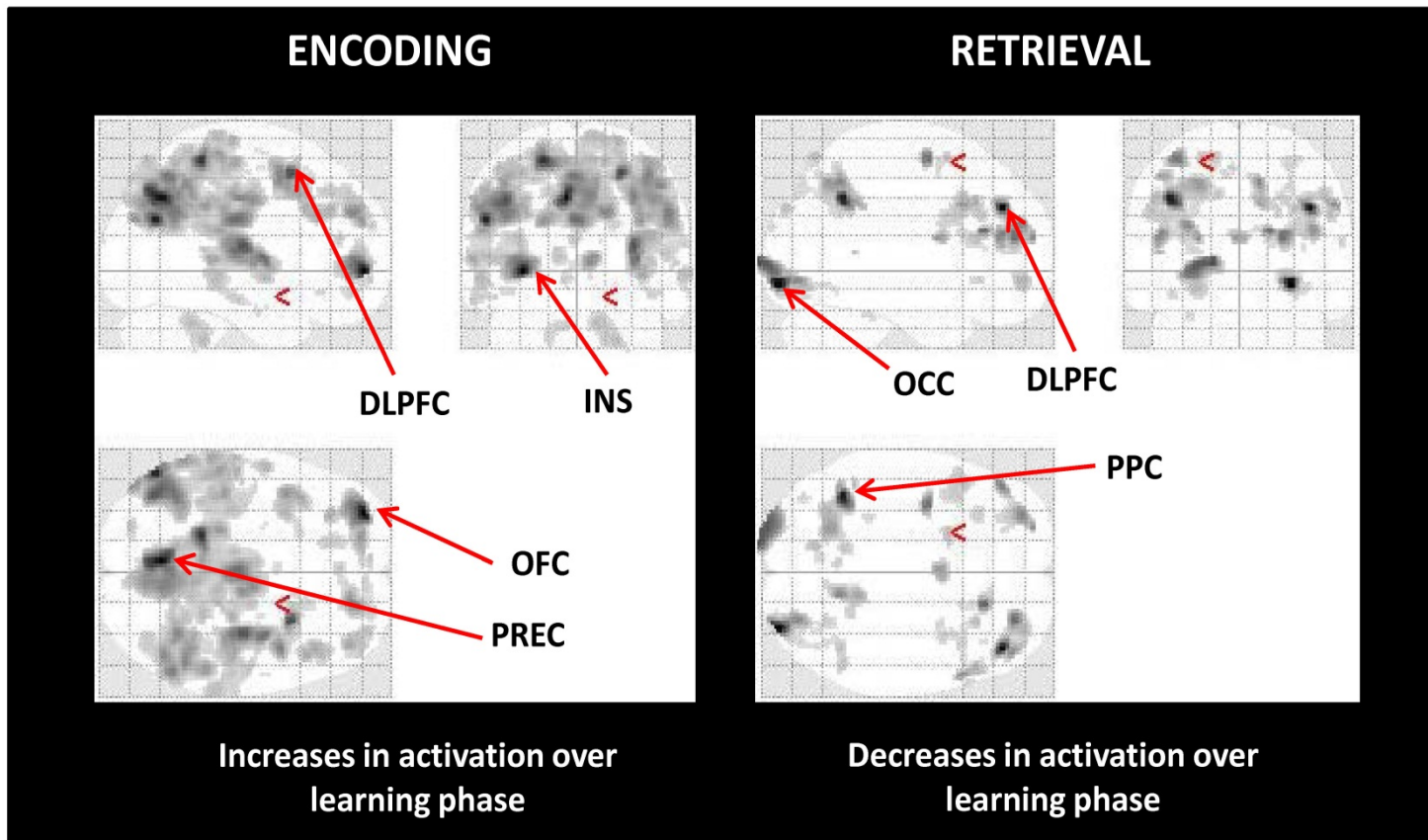
Learning networks displayed on glass brains for the encoding and retrieval (p<0.01 uncorrected). These networks were chosen for display as they demonstrate the more reliable learning effect (see Table 7-2).

PPC (Posterior Parietal Cortex); DLPFC (Dorsolateral Prefrontal Cortex); OCC (Orbitofrontal Cortex); PREC (Precuneus); OCC (Occipital Cortex).

Figure 7-6 Session 1 PAL learning networks

7.3.2.2   Modulation Study

7.3.2.2.1   The PAL Task Network

This study is the first to investigate the effects of learning on the BOLD signal in both encoding and retrieval conditions. Encoding and retrieval learning networks were defined using the flexible factorial model with subject, time and treatment arm as factors although the subject columns were not included in the design matrix in order to retain between-subject variance within the model. As Figure 7-7 shows, the most robust results are increases in activation across learning phase during both encoding and retrieval (significant at an uncorrected threshold of $p<0.001$, family-wise corrected (FWE) for multiple comparisons at $p<0.05$ on the basis of cluster extent). For encoding this includes increases in activation in the DLPFC, regions of the temporal lobe and precuneus-posterior cingulate cortices. For retrieval, increases are apparent in orbitofrontal areas together with the mid-posterior cingulate and precuneus. At an uncorrected threshold ($p<0.001$), decreases are also seen during retrieval in the DLPFC. Treatment related differences in learning-related activation were assessed using pre-defined ROIs. For the results of this analysis please see below.

P ENCODING INCREASES | RETRIEVAL INCREASES | RETRIEVAL DECREASES **A**

CUNEUS/ PREC/PCC

TEMP

DLPFC

ANG   OFC   DLPFC

L   **R**

No significant differences were found between the Placebo_S and P_saline networks thus the Placebo_S network was used for display purposes.

The T-maps displayed represent increases and decreases in activation (across learning phase) in both encoding and retrieval conditions at group level. The increases are displayed at an uncorrected threshold of p<0.001, FWE corrected for multiple comparisons at p<0.05 on the basis of cluster extent. Decreases are displayed at an uncorrected threshold (p<0.001).

DLPFC (Dorsolateral Prefrontal Cortex); PCC (Posterior Cingulate Cortex); PREC (Precuneus); ANG (Angular Gyrus); TEMP (Medial Temporal Lobe); OFC (Orbitofrontal Cortex); A (Anterior); P (Posterior); R (Right); L (Left).

Figure 7-7 PAL task networks from the modulation study displayed on glass brains

7.3.2.2.2   Region of Interest Analysis
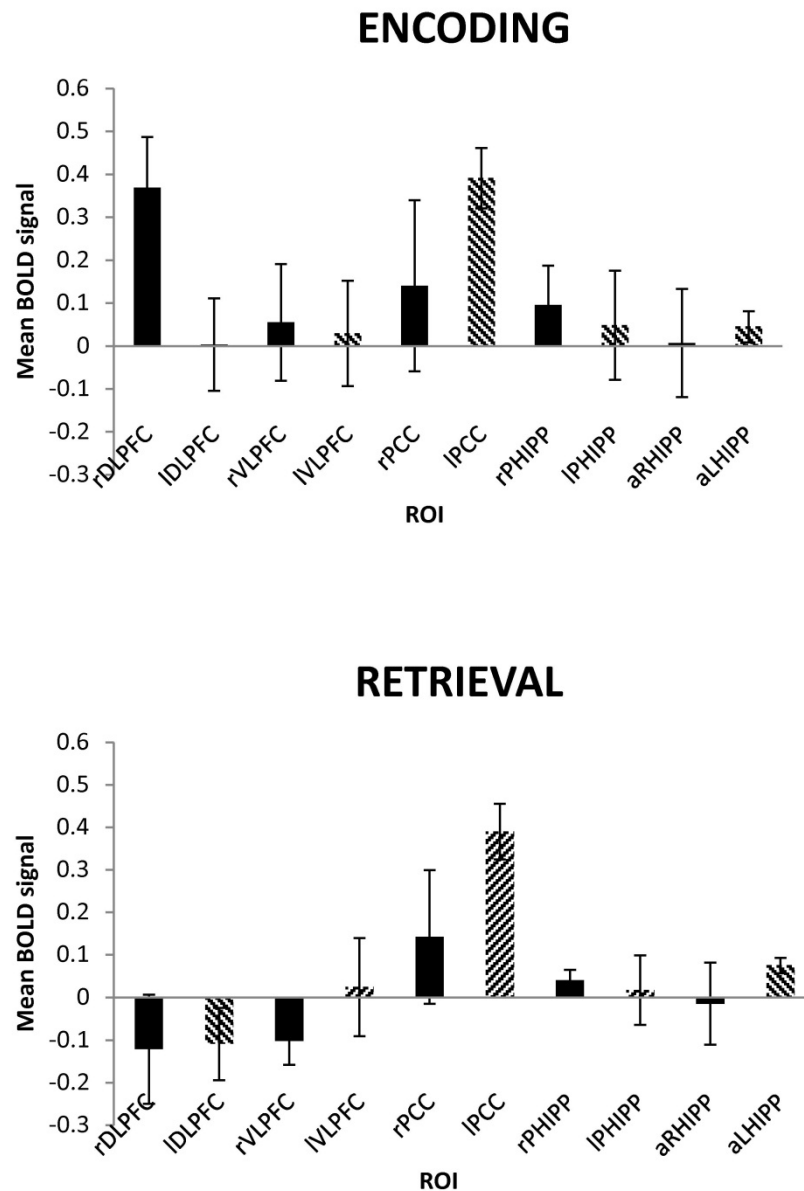
Figure 7-8 The learning response to the PAL task on placebo for each ROI

The learning response to the PAL task on placebo (averaged across Placebo_S, Placebo_K and P_Saline) during both encoding and retrieval conditions. A positive response indicate that activation in learning phase 3 is increased in comparison to learning phase 1, whereas a negative response indicates that activation is decreased in learning phase 3 when compared to learning phase 1. Error bars represent the standard error of the mean (i.e. variance in response across placebo conditions). DLPFC (Dorsolateral Prefrontal Cortex); VLPFC (Ventrolateral Prefrontal Cortex); PCC (Posterior Cingulate Cortex); PHIPP (Parahippcampal Gyrus); HIPP (Hippocampus).

Pre-Infusion Please refer to Table 7-3 for all relevant statistics. No main effect of treatment was found for any of the ROIs except for a trend within the left parahippocampal gyrus (p=0.068). Post-hoc one-sample t-tests indicate that this is due to a significant increase in activation (p<0.05) between learning phase 1 and 3 in the Placebo_S condition (represented by a positive beta value) compared to a decrease in activation between these two learning phases in the Risperidone_K condition (represented by a negative beta value) although this was not found to be significant.

In order to assess whether any order effects were present, a repeated-measures ANOVA was employed with treatment and ROI as within-subjects factors and order as a between-subjects factor. No main effect of order was found (F(3,12)=0.671, p=0.586).No significant interaction was found between treatment and order (F(9,36)=1.626, p=0.145) or treatment, order and ROI (F(81,324)=0.796, p=0.891).

Post-Infusion Please refer to Table 7-3 for all relevant statistics. No main effect of treatment was found for any of the ROIs.

Table 7-3 Repeated Measures One-Way ANOVAs describing treatment effects at encoding in pre-infusion and post-infusion conditions separately

ROIs	ENCODING			
	Pre-Infusion One-Way ANOVA		Post-Infusion One-Way ANOVA	
	Main Effect of Treatment		Main Effect of Treatment	
	F[3,45]	P	F[3,45]	P
rDLPFC	1.455	P=0.239	0.204	P=0.893
lDLPFC	1.300	P=0.286	1.023	P=0.391
rVLPFC	0.916	P=0.441	0.641	P=0.593
lVLPFC	0.816	P=0.492	0.477	P=0.700
rPCC	0.931	P=0.434	0.715	P=0.548
lPCC	0.530	P=0.664	1.000	P=0.402
rPHIPP	1.640	P=0.193	0.284	P=0.837
lPHIPP	2.538	P=0.068	0.346	P=0.793
aRHIPP	0.850	P=0.474	0.370	P=0.775
aLHIPP	0.844	P=0.477	0.426	P=0.736

In order to assess whether any order effects were present, a repeated-measures ANOVA was employed with treatment and ROI as within-subjects factors and order as a between-

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subjects factor. No main effect of order was found ($F_{(3,12)}=1.752$, $p=0.210$). No significant interaction was found between treatment and order ($F_{(9,36)}=1.575$, $p=0.160$). However, a significant interaction was found between treatment, order and ROI ($F_{(81,324)}=1.324$, $p<0.05$).

Following post-hoc Bonferroni corrected pairwise comparisons this interaction appears to be driven by an effect of order in the left parahippocampal gyrus. The response in this region to ketamine infusion (in the ketamine treatment arm of the study), appears to be significantly lower if participants received ketamine on their second visit compared to if they received it on their first visit. Due to this finding, the model describing the interaction between placebo and ketamine treatment arms demonstrating a trend ($p=0.091$; see below) time by treatment arm interaction was repeated including order as a between-subjects factor. The time by treatment arm interaction was no longer found to represent a trend ($F_{(1,12)}=0.428$, $p=0.525$). Nonetheless, this does not change the outcome of the PAL results as this trend interaction is not considered to be a key result and will not be discussed.

ENCODING: THE INFLUENCE OF PRE-INFUSION CONDITIONS ON POST-INFUSION MODULATION EFFECTS

A 2x4 repeated measures ANOVA was conducted to describe the effects of time and the interaction between time (pre- and post-infusion conditions) and treatment arm (see Appendix D; Table D-1 for all relevant statistics). No significant main effect of time was found in any ROI. A trend towards a time by treatment arm interaction was found in the left DLPFC ($p=0.076$) and parahippocampal gyrus ($p=0.07$). Three separate 2x2 ANOVA models were conducted in order to investigate the significant interaction effects found in the 2x4 ANOVA by isolating the treatment comparisons of interest. Furthermore, it is possible that, due to undue influence of unwanted variance in the larger ANOVA, true interaction effects will be masked and will only be identified in the more specific models.

KETAMINE

The first ANOVA included the placebo and ketamine treatment arms in order to isolate any effects of ketamine in comparison to both pre-infusion and post-infusion placebo conditions. Please refer to Figure 7-7 to visualise specific results and Table 7-4 for all relevant statistics.

Left Parahippocampal Gyrus

ENCODING

(y-axis) Mean BOLD signal: 0.5, 0.4, 0.3, 0.2, 0.1, 0, -0.1, -0.2, -0.3, -0.4

Pre-Infusion   Post-Infusion

Time

RETRIEVAL

(y-axis) Mean BOLD signal: 0.5, 0.4, 0.3, 0.2, 0.1, 0, -0.1, -0.2, -0.3, -0.4

Pre-Infusion   Post-Infusion

Time

Placebo-Placebo   Placebo-Ketamine
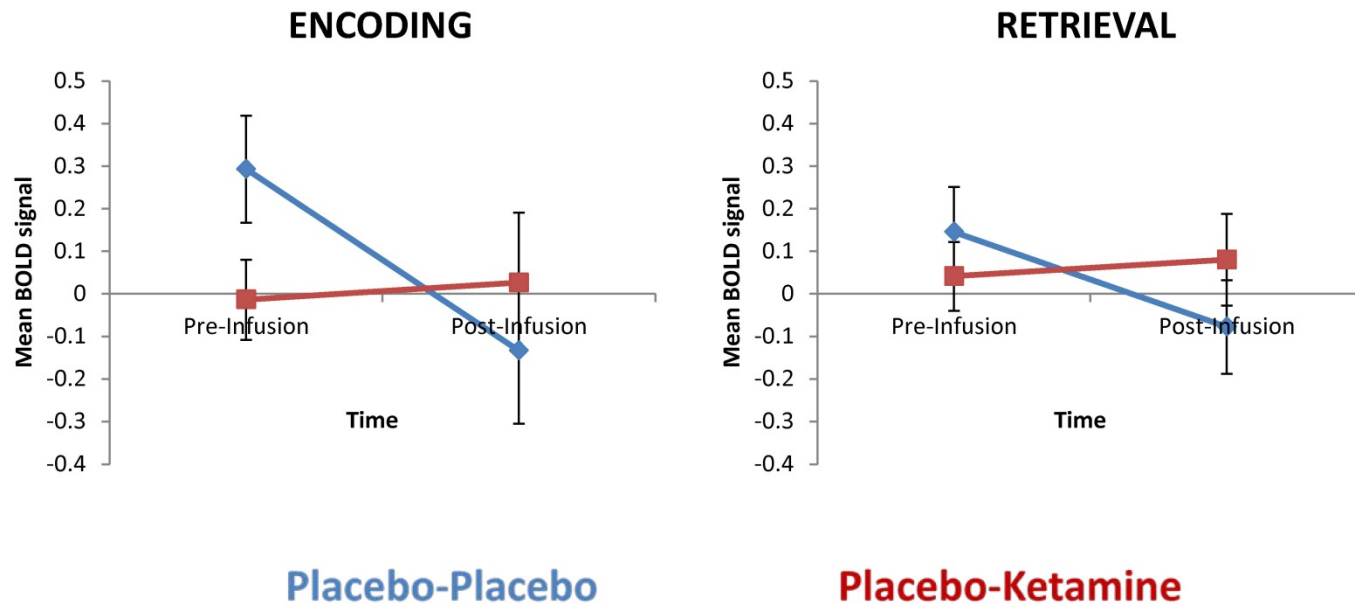
Figure 7-9 Learning-related activity in encoding and retrieval conditions in the left parahippocampal gyrus in both placebo and ketamine treatment arms

Error bars represent the standard error of the mean. Pre-infusion placebo arm = placebo; Post-infusion placebo arm = saline; Pre-infusion of ketamine arm = placebo; Post-infusion of ketamine arm = ketamine.

As Figure 7-9 shows, a trend towards a time by treatment arm interaction (p=0.091) was found in the left parahippocampal gyrus. In the placebo treatment arm there is a change (pairwise comparison: p=0.084) in learning-related activity between pre- and post-infusion conditions; at pre-infusion there is a significant increase in activity between learning phase 1 and 3 whereas at post-infusion there is greater activity in learning phase 1, although this effect is minimal. In contrast, this change in learning-related activity across pre- and post-infusion conditions does not occur in the ketamine treatment arm. Thus it appears that the effect of ketamine is in opposition to what occurs over time in placebo conditions. Nonetheless, variability between the pre-infusion placebo conditions may also be driving this effect.

RISPERIDONE

The second ANOVA included the ketamine and risperidone treatment arms in order to isolate any effects of risperidone pre-treatment on ketamine while taking into account pre-infusion conditions. Please refer to Table 7-4 for all relevant statistics. No significant time by treatment interaction effects were found in any of the ROIs.

LAMOTRIGINE

The third ANOVA included the ketamine and lamotrigine treatment arms in order to isolate any effects of lamotrigine pre-treatment on ketamine while taking into account pre-infusion conditions. Please refer to Table 7-4 for all relevant statistics.

ENCODING
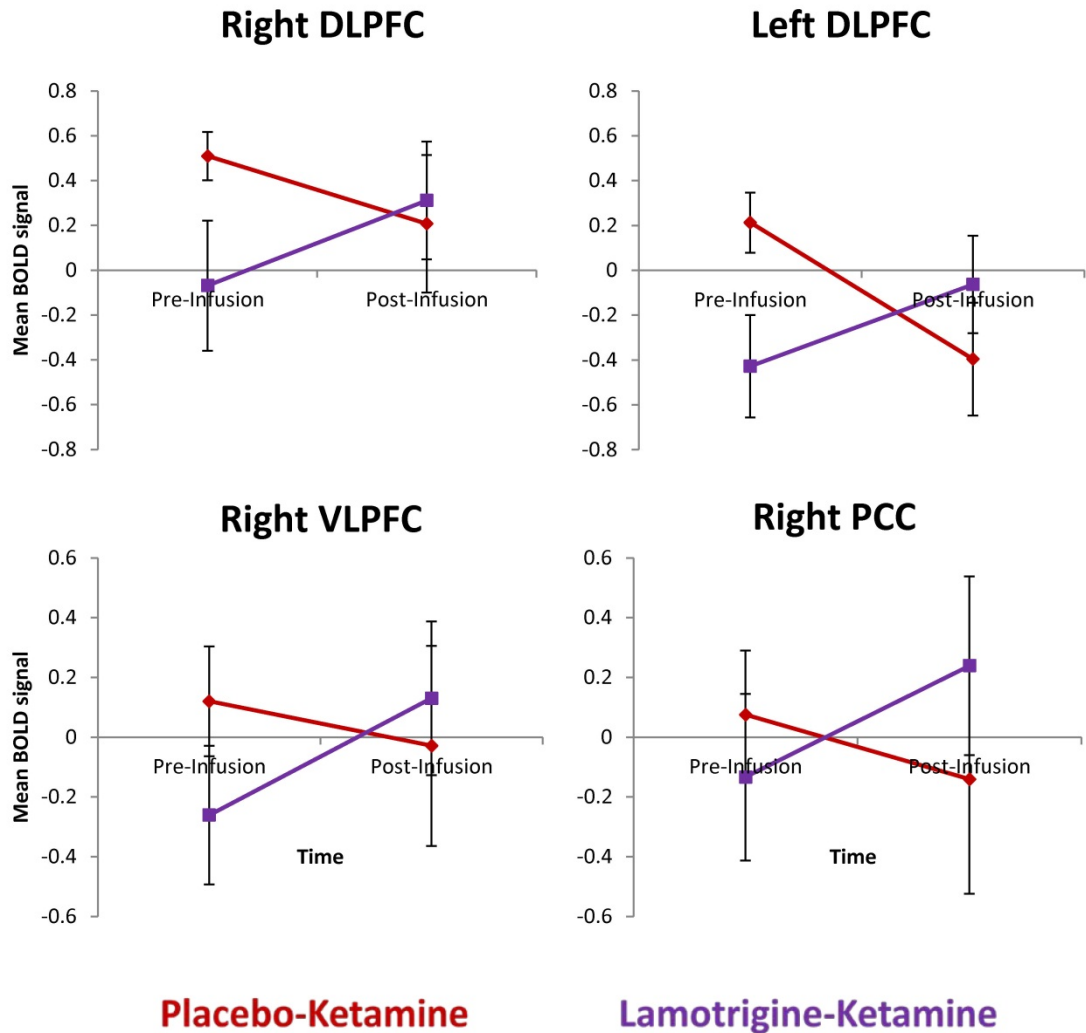
Figure 7-10 Time by treatment interaction effects during encoding between ketamine and lamotrigine treatment arms

Graphs displaying time by treatment interaction effects during encoding between the ketamine (red) and lamotrigine (purple) treatment arms in four regions of interest, the right and left DLPFC and right VLPFC and PCC. The PCC interaction is significant only at trend-level. Error bars represent the standard error of the mean. Pre-infusion ketamine arm = placebo; Post-infusion ketamine arm = ketamine; Pre-infusion lamotrigine arm = lamotrigine; Post-infusion of lamotrigine arm = lamotrigine-ketamine.

As Figure 7-10 shows, significant time by treatment interaction effects were found in the right (p<0.05) and left DLPFC (p<0.01) and right VLPFC (p<0.05). A trend was also found for the right PCC (p=0.097). In the right DLPFC ketamine treatment arm there is a change in learning-related activity between pre- (Placebo_K) and post-infusion (P_Ketamine) conditions characterised by an increase in activity between learning phase 1 and 3 at pre-infusion (positive beta value) and an attenuation of this effect (smaller positive beta value) at post-infusion. The opposite effect occurs in the lamotrigine treatment arm whereby activity decreases with learning phase (negative beta value) at pre-infusion (Lamotrigine_K) but increases with learning phase (positive beta value) at post-infusion (L_Ketamine). However, pairwise comparisons indicate that the changes over time in both treatment arms are not significant (see Appendix D).

In the left DLPFC learning-related activity in the ketamine treatment arm is characterised by greater activity in learning phase 3 at pre-infusion (Placebo_K; positive beta value) in contrast to greater activity in learning phase 1 at post-infusion (P_Ketamine; negative beta value). Pairwise comparisons indicate that this change in activity between pre- and post-infusion conditions is significant (p<0.05; see Appendix D). The interaction within this ROI is thus driven by the fact that in the lamotrigine treatment arm greater activity in learning phase 1 is evident at pre-infusion whereas this effect is attenuated at post-infusion.

The interactions apparent in the right VLPFC and PCC are driven by similar effects. In the ketamine treatment arm, there is an increase in activity with learning phase at pre-infusion (Placebo_K; positive beta value) whereas at post-infusion (P_Ketamine) there is a decrease (negative beta value). The interaction is thus due to the reversal of this effect in the lamotrigine treatment arm; a negative beta value in the Lamotrigine_K pre-infusion condition and positive beta value in the L_Ketamine post-infusion condition. However, pairwise comparisons indicate that the changes over time in both treatment arms are not significant (see Appendix D).

Overall, across these ROIs, it appears that ketamine attenuates the learning-related increase in activity in learning phase 3 seen in pre-infusion placebo conditions. On the other hand, lamotrigine pre-treatment appears to counteract this effect of ketamine.

Table 7-4 Repeated-measures 2x2 ANOVAs describing time by treatment interaction effects during encoding conditions

ROIs	ENCODING					
	PLA-KET 2x2 ANOVA		**KET-RIS 2x2 ANOVA**		**KET-LAM 2x2 ANOVA**	
	Time x Treatment Interaction		Time x Treatment Interaction		Time x Treatment Interaction	
	F[1,15]	P	F[1,15]	P	F[1,15]	P
rDLPFC	0.008	P=0.930	0.126	P=0.728	5.433	P<0.05
lDLPFC	1.787	P=0.201	1.791	P=0.201	15.031	P<0.01
rVLPFC	0.738	P=0.404	0.003	P=0.957	7.552	P<0.05
lVLPFC	1.038	P=0.324	0.056	P=0.816	1.074	P=0.316
rPCC	1.192	P=0.292	0.051	P=0.824	3.136	P=0.097
lPCC	0.002	P=0.964	0.004	P=0.950	1.847	P=0.194
rPHIPP	0.335	P=0.571	0.587	P=0.455	0.223	P=0.644
lPHIPP	3.261	P=0.091	0.446	P=0.514	0.078	P=0.783
aRHIPP	1.620	P=0.222	0.010	P=0.923	0.608	P=0.448
aLHIPP	0.141	P=0.713	0.000	P=0.995	0.148	P=0.705

Pre-Infusion Please refer to Table 7-5. for all relevant statistics. No main effect of treatment was found for any of the ROIs except for a trend within the left anterior hippocampus (p=0.095). Pairwise comparisons indicate that this effect is driven by a difference in learning-related activity between Risperidone_K and Lamotrigine_K conditions, although this effect does not survive multiple comparisons correction.

In order to assess whether any order effects were present, a repeated-measures ANOVA was employed with treatment and ROI as within-subjects factors and order as a between-subjects factor. No main effect of order was found ($F_{(3,12)}$=1.935, p=0.178).No significant interaction was found between treatment and order ($F_{(9,36)}$=0.421, p=0.915) or treatment, order and ROI ($F_{(81,324)}$=0.547, p=0.999).

Post-Infusion Please refer to Table 7-5. for all relevant statistics. No main effect of treatment was found for any of the ROIs.

In order to assess whether any order effects were present within the **post-infusion** data during retrieval, a repeated-measures ANOVA was employed with treatment and ROI as within-subjects factors and order as a between-subjects factor. No main effect of order was found ($F_{(3,12)}$=2.245, p=0.136).No significant interaction was found between treatment and order ($F_{(9,36)}$=0.707, p=0.698) or treatment, order and ROI ($F_{(81,324)}$=0.878, p=0.756).

Table 7-5 Repeated-measures One-Way ANOVAs describing treatment effects at retrieval in pre-infusion and post-infusion conditions separately

	RETRIEVAL			
ROIs	**Pre-Infusion One-Way ANOVA**		**Post-Infusion One-Way ANOVA**	
	Main Effect of Treatment		Main Effect of Treatment	
	F[3,45]	P	F[3,45]	P
rDLPFC	0.335	P=0.800	0.598	P=0.619
lDLPFC	1.246	P=0.304	1.024	P=0.391
rVLPFC	0.055	P=0.983	0.669	P=0.576
lVLPFC	0.658	P=0.582	0.528	P=0.665
rPCC	1.376	P=0.262	0.477	P=0.700
lPCC	1.112	P=0.354	0.296	P=0.828
rPHIPP	0.322	P=0.809	0.288	P=0.834
lPHIPP	0.477	P=0.700	0.678	P=0.570
aRHIPP	1.046	P=0.382	0.315	P=0.815
aLHIPP	2.251	P=0.095	0.130	P=0.942

RETRIEVAL: THE INFLUENCE OF PRE-INFUSION CONDITIONS ON POST-INFUSION MODULATION EFFECTS

A 2x4 repeated measures ANOVA was conducted to describe the effects of time and the interaction between time (pre- and post-infusion conditions) and treatment arm (see Appendix D; Table D-2 for all relevant statistics). No significant main effect of time or time by treatment arm interaction was found in any ROI. Three separate 2x2 ANOVA models were conducted in order to investigate whether, due to undue influence of unwanted variance in the larger ANOVA, true interaction effects will be masked and will only be identified in the more specific models.

KETAMINE

The first ANOVA included the placebo and ketamine treatment arms in order to isolate any effects of ketamine in comparison to both pre-infusion and post-infusion placebo conditions. Please refer to Table 7-6 for all relevant statistics.

As Figure 7-9 shows, a trend towards a time by treatment interaction (p=0.099) was found in the left parahippocampal gyrus. In the placebo treatment arm there is a change (pairwise comparison: p=0.081) in learning-related activity between pre- and post-infusion

conditions; at pre-infusion there is an increase in activity between learning phase 1 and 3 whereas at post-infusion there is greater activity in learning phase 1. In contrast, this change in learning-related activity across pre- and post-infusion conditions does not occur in the ketamine treatment arm with activity being greater at learning phase 3 in both pre-infusion placebo (Placebo_K) and post-infusion ketamine (P_Ketamine) conditions. Thus it appears that the effect of ketamine is in opposition to what occurs over time in placebo conditions.

RISPERIDONE

The second ANOVA included the ketamine and risperidone treatment arms in order to isolate any effects of risperidone pre-treatment on ketamine while taking into account pre-infusion conditions. Please refer to Table 7-6 for all relevant statistics. No significant time by treatment interaction effects were found in any of the ROIs.

LAMOTRIGINE

The third ANOVA included the ketamine and lamotrigine treatment arms in order to isolate any effects of lamotrigine pre-treatment on ketamine while taking into account pre-infusion conditions. Please refer to Table 7-6 for all relevant statistics. No significant time by treatment interaction effects were found in any of the ROIs.

Table 7-6 Repeated-measures 2x2 ANOVAs describing time by treatment interaction effects during retrieval conditions

	RETRIEVAL					
ROIs	**PLA-KET 2x2 ANOVA**		**KET-RIS 2x2 ANOVA**		**KET-LAM 2x2 ANOVA**	
	Time x Treatment Interaction		Time x Treatment Interaction		Time x Treatment Interaction	
	$F[1,15]$	P	$F[1,15]$	P	$F[1,15]$	P
rDLPFC	0.221	P=0.645	0.658	P=0.430	0.354	P=0.561
lDLPFC	0.693	P=0.418	0.000	P=0.997	0.210	P=0.653
rVLPFC	0.001	P=0.980	0.764	P=0.396	0.047	P=0.831
lVLPFC	0.047	P=0.832	0.308	P=0.587	0.158	P=0.697
rPCC	0.190	P=0.669	2.845	P=0.112	2.140	P=0.164
lPCC	0.052	P=0.823	0.381	P=0.547	0.596	P=0.452
rPHIPP	0.064	P=0.804	0.334	P=0.572	0.119	P=0.735
lPHIPP	3.094	P=0.099	0.179	P=0.678	0.054	P=0.819
aRHIPP	0.904	P=0.357	0.652	P=0.432	1.150	P=0.300
aLHIPP	0.450	P=0.512	2.905	P=0.109	0.211	P=0.652

7.3.2.3 Summary of Imaging Results: by Treatment

Placebo

As Figure 7-8 shows, learning-related activity differs depending on the ROI. During encoding, activation in all ROIs appears to be increase with learning phase. On the other hand during retrieval, the PCC, parahippocampal gyrus and hippocampus show this same pattern whereas the DLPFC and VLPFC demonstrate increased activity during learning phase 1. In both encoding and retrieval conditions, the DLPFC and PCC appear to demonstrate the largest learning-related activity changes.

Ketamine

When compared to saline, no ketamine-induced effects were found. However, in the left parahippocampal gyrus, when pre-infusion conditions are taken into account the effect of ketamine appears to be in opposition to what occurs over time in placebo conditions.

Under placebo, activity is greater in learning phase 3 at pre-infusion but greater in learning phase 1 at post-infusion. This effect is not seen in the ketamine treatment arm. The same pattern is apparent in both encoding and retrieval conditions, although it is not significant.

Risperidone

When compared to placebo, only the left parahippocampal gyrus appears sensitive to the effects of risperidone during encoding, although this is not a significant effect. No additional effects of risperidone were evident during retrieval. No modulatory effects of risperidone on ketamine were found.

Lamotrigine

When compared to placebo, no lamotrigine-induced effects were found. No modulatory effects of lamotrigine on ketamine were found when only the post-infusion conditions were investigated. However, when pre-infusion conditions are taken into account when interpreting the effects of lamotrigine pre-treatment on ketamine, it appears that lamotrigine may be having a modulatory effect on the response to ketamine in the DLPFC, right VLPFC and PCC. In these regions, ketamine appears to attenuate the increase in activity evident in learning phase 3 on placebo, whereas when it is combined with lamotrigine this increase is reinstated (to differing degrees). This effect only occurs during encoding.

7.3.2.4   Whole Brain Analysis

Whole brain analyses of the PAL task were conducted for exploratory purposes, in order to detect any treatment effects outside of the pre-selected regions of interest. Treatment effects were investigated with the use of two separate flexible factorials for the pre-infusion and post-infusion conditions separately, to examine the main effects of the oral drugs and their subsequent effects on the ketamine response. This was followed by a larger flexible factorial analysis combining pre- and post-infusion conditions in order to investigate ketamine modulation effects while taking into account the effects of the oral drugs. These three models were performed for both encoding and retrieval contrasts individually.

During encoding, no main effect of treatment was found between pre-infusion conditions even at an uncorrected threshold of $p < 0.001$. At post-infusion, regions in the superior

frontal cortex and precentral gyrus are present at p<0.001 uncorrected, however this effect does not survive at a corrected threshold and thus will not be discussed further.

On the other hand, the flexible factorial analysis including both pre- and post-infusion conditions demonstrates a significant time by treatment interaction in the DLPFC and inferior temporal lobe (family-wise corrected for multiple comparisons at p<0.05 on the basis of peak amplitude) (see Figure 7-11). The directionality of the responses in each treatment condition suggests that this effect could be driven by an interaction between either (1) placebo and ketamine treatment arms, (2) ketamine and risperidone treatments arms or (3) ketamine and lamotrigine treatment arms. These 2x2 interaction F-contrasts within the flexible factorial demonstrate that the significant time by treatment interaction is driven by the ketamine and lamotrigine treatment arms, whereby the DLPFC and inferior temporal lobe are also found to be significant at an FWE corrected threshold (on the basis of peak amplitude) (see Figure 7-11).

Time x Treatment Interaction

Right DLPFC
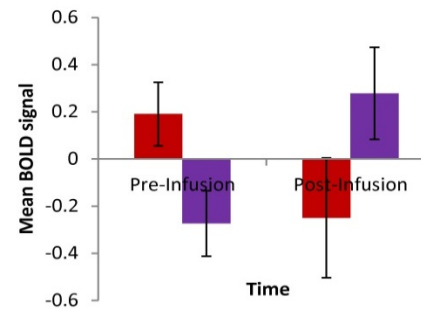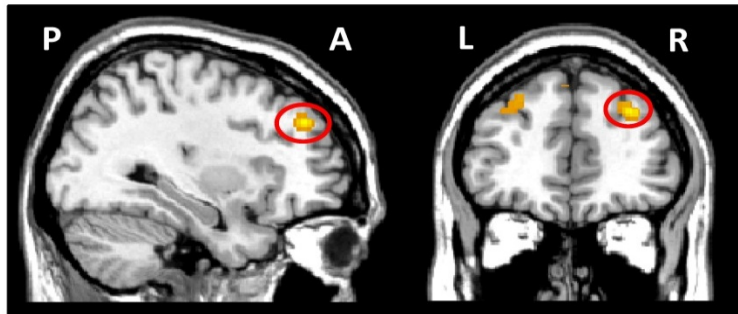
Right Inferior Temporal Lobe
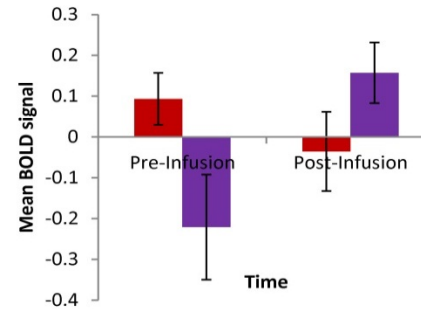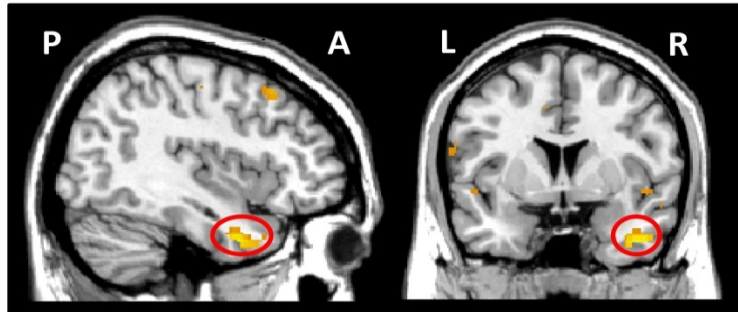
Placebo-Ketamine **Lamotrigine-Ketamine**

The right DLPFC and inferior temporal lobe showing a significant time by treatment arm effect during encoding. The F-test is displayed at an uncorrected threshold (p<0.001) but is significant at an FWE (p<0.05) corrected threshold on the basis of peak amplitude.

The adjacent plots display the mean BOLD signal for the encode-learn contrast in both pre- and post-infusion conditions (extracted from two spheres centred on the MNI coordinates (32,42,34) and (40,4,-36), representing the peak signal of the DLPFC and inferior temporal lobe respectively). Only the ketamine and lamotrigine treatment arms are shown. Error bars represent the standard error of the mean.

Figure 7-11 Whole brain time by treatment interaction in the right DLPFC and ITL

The interactions apparent in the right DLPFC and inferior temporal lobe are driven by the opposing effects of the ketamine and lamotrigine treatment arms. In the ketamine treatment arm, there is greater activity in learning phase 3 compared to learning phase 1 at pre-infusion (Placebo_K; positive beta value) whereas at post-infusion (P_Ketamine) there is greater activity in learning phase 1 (negative beta value). In contrast, the lamotrigine treatment arm demonstrates greater activity at learning phase 1 at pre-infusion (Lamotrigine_K) but greater activity in learning phase 3 at post-infusion (L_Ketamine).

No significant effects were found during retrieval conditions.

7.3.2.4.1 Individual Differences

In order to establish that activity within the DLPFC and inferior temporal lobe during encoding is still evident when individual differences in the degree of learning are taken into account, additional first level models were created together with a second level flexible factorial incorporating subject, time and treatment arm (placebo, ketamine, risperidone and lamotrigine) as factors. This analysis will also demonstrate whether the treatments given impact upon this performance-activity relationship. First level design matrices incorporated explanatory variables representing the encode control condition, an 'encode-all' condition which included all three learning phases and a variable which weighted each encoding learning phase by performance (in terms of correct responses). The same explanatory variables were created for the retrieval condition. As described in the methods, two variables representing the instructions and errors were also included along with the six motion parameters. In this performance related model, a contrast was created for the performance-weighted column. In this context, a positive beta value indicates that activity in a particular brain region is positively coupled to performance, whereas a negative beta indicates that activity is negatively coupled to performance. For example, if performance improves as a function of learning phase (as it does for all treatment conditions) and activity is found to be positively coupled to performance this suggests that an increase in activation occurs across learning phases.

The second level model incorporating the first level T-contrasts representing performance-related activity, demonstrates that a time by treatment interaction, including the ketamine and lamotrigine treatment arms, is apparent within the right inferior temporal lobe. This effect, also found in the whole brain analysis previously described, is

present at 0.01 uncorrected, and approaches significance (p=0.069) when FWE corrected for small volumes at p<0.05 within an inferior temporal anatomical mask.

Right Inferior Temporal Lobe
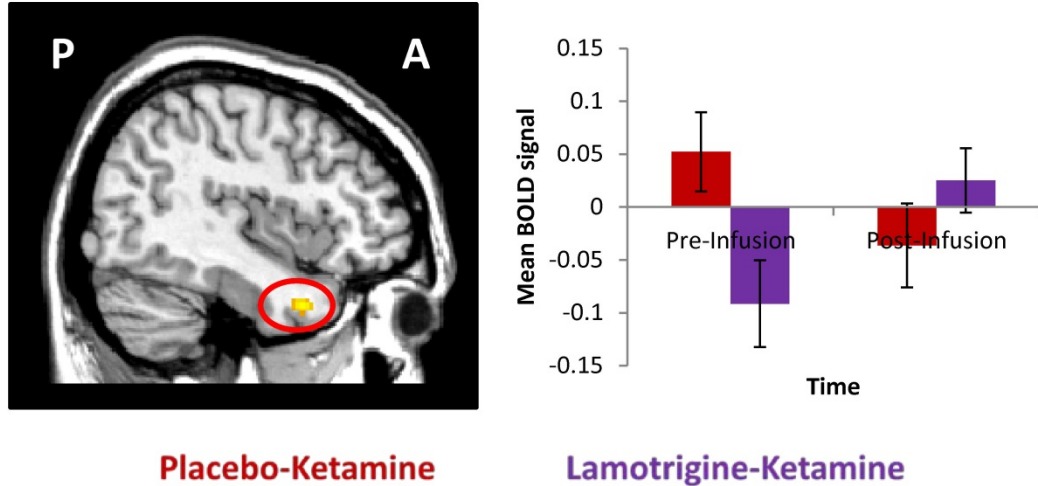
Figure 7-12 Learning-related effect in the right ITL

The inferior temporal lobe showing a significant time by treatment arm (including the ketamine and lamotrigine treatment arms) effect during encoding. The F-test is displayed at an uncorrected threshold p<0.01. The adjacent plot displays the mean BOLD signal representing performance-related activity in both pre- and post-infusion conditions (extracted from the sphere centred on MNI coordinates (42,10,-34)). Error bars represent the standard error of the mean.

Figure 7-12 demonstrates that, under placebo conditions, activity in the inferior temporal lobe is positively coupled to performance. This is also evident when ketamine is administered in conjunction with lamotrigine. On the other hand, when administered alone, both lamotrigine and ketamine disrupt this relationship between performance and activation. This pattern of performance related changes mirrors that of the pure activation changes in the whole brain analysis described above. Thus the performance analysis confirms that activation changes across learning phase during encoding are functionally relevant, giving us an insight into how a particular brain region within an individual responds to task-related changes in performance. Furthermore, it demonstrates how the different treatments impact upon this relationship and may be informative in terms of whether this region is necessary for successful task completion.

7.4   DISCUSSION

7.4.1   Summary of Main Results

This chapter shows that administration of low dose ketamine has minimal effects in the context of a paired associative learning task relative to placebo. As expected, no learning-related performance deficits were found; instead a generalised speeding of reaction times was evident. This is potentially due to a ketamine-induced increase in cortical excitability and resulting motoric stimulation (Di Lazzaro et al., 2003). An indication of a localised effect within the parahippocampal gyrus was found whereby the effects of ketamine opposed that of placebo over time. As hypothesised, this effect was found during both encoding and retrieval conditions, however it only approached significance.

As expected, risperidone induced performance deficits in the PAL task, although only when combined with ketamine. No significant effects of risperidone were evident in the ROI or whole brain analyses, alone or when combined with ketamine. On the other hand, lamotrigine had no effects on performance but, when pre-infusion conditions were taken into account, lamotrigine pre-treatment was found to have a modulatory effect on ketamine in the DLPFC, VLPFC and inferior temporal lobe. Interestingly, this effect is only evident during encoding conditions and, for the inferior temporal lobe, is associated with learning-related performance changes.

These findings will be discussed further below.

7.4.2   Reliability of the PAL Task: Performance and Brain Network

In terms of performance, reliability analyses demonstrate that accuracy is reliable in the early learning phases but less so at the last stage. In addition, reliability is very low in the control phase. Session effects were also found which undermines the group reproducibility of this measure. On the other hand, reaction times demonstrate increasing reliability with learning phase, with the highest value in the control condition and do not show any session effects. These results suggest that drug-induced changes in reaction times may be more indicative of true modulatory effects in repeated measures studies due to their apparent stability over time.

Reliability of the paired associative learning network appears to depend upon the exact process being assessed. Activation in individual ROIs is more reliable during retrieval,

although when the overall network is assessed reliability is similar in both encoding and retrieval networks. It is possible that this difference is a consequence of the task design itself, whereby the time allocated to retrieval processes is triple that given to encoding. Thus it is possible that more power is available in retrieval conditions to adequately assess reliability, simply due to the amount of data analysed. ROIs demonstrating highest reliability (0.34 – 0.55) include the right DLPFC and VLPFC and left PCC and anterior hippocampus, regions which are also the most robustly recruited under placebo in the modulation study. Overall, these results demonstrate that the PAL learning network is acceptably reliable and, together with reaction times, may be more suitable to detect drug-induced effects than accuracy measures.

It is possible that reliability estimates may differ depending on which contrast is assessed; for example contrasts assessing the encoding and retrieval networks (including all learning phases) compared to the control phase may have given more robust results due to the possible subtlety of the learning effect. However, for the purpose of this chapter, whereby learning related effects were the focus, the contrast chosen was deemed the most appropriate.

7.4.3   The Effects of Ketamine on the PAL Task Network

Unlike previous studies that have investigated the effect of ketamine on the brain response to encoding and retrieval, cognitive processes essential to associative memory, this chapter found no significant effects of the drug when compared to post-infusion conditions alone (G. D. Honey, Honey, O'Loughlin, et al., 2005; Northoff et al., 2005). This may be due to the fact that the analysis performed in this chapter attempted to isolate more subtle effects of ketamine in terms of what occurs to encoding and retrieval processes as learning proceeds, compared to what occurs when learning is not required. It is possible that if one were to investigate the influence of ketamine on encoding and retrieval irrespective of learning an effect would be found, however this would not provide any insight into how NMDAR antagonism affects associative learning per se and whether fMRI is sensitive to an effect.

Other additional reasons could also be responsible for a lack of effect; (1) the dose of ketamine used was too low to produce a detectable effect, although even the administration of higher doses in monkeys did not disrupt their ability to learn during a visuo-spatial PAL task (Taffe, Davis, Gutierrez, & Gold, 2002); (2) the PAL task itself is

not ideally suited to detect a potential effect and (3) NMDAR antagonism occurred over too short a time-frame for learning to be affected; for example, only chronic administration may induce learning impairments (Freeman et al., 2009). With respect to the PAL task, and considering that the volunteers included in this thesis were highly performing healthy individuals, the level of difficulty may have been too low, rendering the task-related brain response resistant to low dose ketamine. Thus, in order to detect any subtle effects of the drug it may have been beneficial to vary the levels of difficulty as has been done in previous studies (de Rover et al., 2011) but also retain the learning component of the task.

In summary, in the context of post-infusion conditions alone, low-level NMDAR antagonism in healthy individuals does not appear to significantly modulate the neural correlates of associative learning. Nonetheless, when pre-infusion placebo conditions are taken into account a pattern emerges whereby ketamine appears to attenuate learning-related increases in activation. Furthermore, the interaction between the ketamine and lamotrigine treatment arms described below also lends support to the notion that the glutamatergic system is somehow involved in visual associative processes.

7.4.4   Reversal of the Ketamine-induced Brain Response

Contrary to previous studies (Bubenikova-Valesova et al., 2008; Celikyurt et al., 2011; Didriksen et al., 2007; Ishiyama et al., 2007; McLean et al., 2010) and the proposed 5HT2A mechanism of action of risperidone, no reversal of the effects of ketamine in terms of performance or brain response was found. This may be due to the subtle effects of ketamine in the context of the PAL task, but may also suggest acute antagonism at the dopamine D2 and serotonin 5HT2A receptors does not impact associative learning (in contrast to the apparent effects of such antagonism in a WM context; see Chapter 6) as risperidone alone also has no detectable effects.

In contrast, lamotrigine appears to have a much stronger effect within the context of the PAL task with modulation of the effects of ketamine evident in the DLPFC, VLPFC, and inferior temporal lobe. On placebo, the pattern of response in these regions during encoding is characterised by a task-related increase in activity between the initial presentation of the stimuli and the last presentation, when stimulus-location associations have been acquired. In contrast, lamotrigine disrupts this pattern, with activity decreasing rather than increasing with learning phase. Furthermore, in the inferior temporal lobe the

brain response is sensitive to individual differences in performance, suggesting that changes in this region are not just due to "passive exposure to the task" ((Messinger, Squire, Zola, & Albright, 2001), p.12243). This then suggests that lamotrigine induces an alteration in coupling between this region and the task learning effects, as the performance-related brain response is opposite to what occurs during placebo. The task related decrease in activity across learning phase also occurs during ketamine administration; however when ketamine is combined with lamotrigine the pattern of activation normalises and resembles that which occurs under placebo.

However, it is difficult to ascertain whether the ketamine effect is a true drug-induced effect as no significant interactions were found between the placebo and ketamine treatment arms, indicating that what occurs in the ketamine arm does not differ significantly from what occurs over time (pre- versus post-infusion) in placebo conditions. In addition, it is also impossible to test whether the effect of lamotrigine differs from what occurs over time in placebo conditions as a placebo (pre-infusion)-lamotrigine (post-infusion) treatment arm was not included in the study design. On the other hand, the interaction between ketamine and lamotrigine treatment arms is only apparent in (1) certain regions considered to be essential for visual associational memory and learning (Browning, Easton, Buckley, & Gaffan, 2005; Eacott & Gaffan, 1992; Messinger et al., 2001; Ranganath et al., 2004; Takeda, Naya, Fujimichi, Takeuchi, & Miyashita, 2005) and (2) during encoding, a process more reliant on NMDARs in comparison to retrieval (Barker & Warburton, 2008). The specificity and cognitive relevance of this modulatory effect suggests that it is a result worthy of further investigation.

7.4.5 The Mechanism Underlying the Effects of Lamotrigine and Ketamine: A Role for the Glutamatergic System in Associative Learning?

As described in the introduction to this chapter, lamotrigine is considered to exert its effects through inhibition of sodium channels, decreasing neuronal activity and subsequent glutamate release (Kida et al., 2001). In animals, this inhibitory action has been hypothesised to underlie a decrease in BOLD signal seen in the somatosensory cortex following forepaw stimulation (Kida et al., 2001). Lamotrigine-induced modulation of BOLD signal has also been found in the prefrontal and temporal cortices in patients suffering from bipolar disorder and in healthy volunteers (Jogia, Haldane, Cobb, Kumari, & Frangou, 2008; X. Li et al., 2004). In addition, cerebral glucose metabolism in the

inferior temporal lobe has been found to be reduced by lamotrigine in individuals with epilepsy (Joo, Tae, & Hong, 2006). Thus, in this study, lamotrigine could be inhibiting the recruitment of certain task-related regions due to the lack of cortical excitation, glutamate release and subsequent NMDAR stimulation in such areas; neurobiological processes that are essential for the integrity of associational memory and learning (Fanselow & Poulos, 2005; G. Riedel et al., 2003).

With regards to ketamine, it appears to have a similar effect to lamotrigine in terms of brain response in the DLPFC, VLPFC and temporal lobe. However, this effect could be achieved via a different mechanism of action, i.e. direct antagonism of the NMDAR receptor, which as previous studies have shown, causes disruption to associational processes (Harborne et al., 1996). Why, if both ketamine and lamotrigine alone have detrimental effects on normal task response does the response normalise when these compounds are combined? It is perhaps possible that the inhibitory effects of lamotrigine on glutamate release and the stimulation of this release by ketamine, an effect previously found in cortical areas (Adams & Moghaddam, 1998; Moghaddam et al., 1997), may counteract each other and maintain normal responding.

Finally, why does this apparent glutamatergic disruption occur only during encoding conditions? As mentioned in the introduction to this chapter, NMDAR hypofunction may only be relevant to the acquisition of associations rather than their retrieval (Barker & Warburton, 2008; Bethus et al., 2010; Day et al., 2003), thus it is not entirely surprising that compounds which could effectively reduce glutamatergic transmission at this receptor have a more pronounced effect during encoding. This finding is also in agreement with previous studies that demonstrate that the effect of ketamine is more evident during encoding compared to retrieval (Hetem et al., 2000; G. D. Honey, Honey, Sharar, et al., 2005; C. J. Morgan, Mofeez, et al., 2004b; Oye et al., 1992; Rowland, Astur, et al., 2005). Nonetheless, it is important to note that no interactions between ketamine and lamotrigine treatment arms were found within the anterior hippocampus, an area of the brain supposedly recruited during successful encoding (Chua et al., 2007; Prince et al., 2005).

The findings summarised in this chapter do suggest that the glutamatergic system is relevant for associative learning in humans; however more in depth analysis is needed to

clarify how it is relevant and whether low dose ketamine is the ideal candidate to characterise its involvement.

7.4.6 Glutamatergic Effects on Visual Associational Memory and Learning Hubs: the Dorsolateral Prefrontal Cortex and Inferior Temporal Lobe

Although the exact molecular mechanisms of ketamine and lamotrigine cannot be elucidated within the context of an fMRI study, it is apparent that regions such as the DLPFC and ITL are particularly vulnerable to glutamatergic dysfunction perhaps due to the role that they play in visual associational processes. As Chapter 6 has described, the DLPFC is required for the manipulation and monitoring of information being maintained in memory (Owen et al., 2005), and specifically in "maintaining relations amongst objects" ((Ranganath & D'Esposito, 2001), p.177; (Ranganath, Minzenberg, & Ragland, 2008)). Furthermore, it exerts top-down modulation of the ITL in order to retrieve information from visual associative memory (Tomita, Ohbayashi, Nakahara, Hasegawa, & Miyashita, 1999). On the other hand, neurons within the ITL have not only been shown to exhibit delay-related activity, which is hypothesised to be required to link stimuli and establish associations (Yakovlev, Fusi, Berman, & Zohary, 1998), but also to respond in a similar fashion to stimuli that belong to the same pair, with this effect becoming more pronounced as learning evolves (Messinger et al., 2001). Communication between these two areas is also essential to visual associational memory, with animal lesion studies demonstrating severe impairments in associative learning if the prefrontal-inferior temporal connections are removed (Eacott & Gaffan, 1992; Gutnikov, Ma, & Gaffan, 1997).

This functional connection is highlighted by the fact that simultaneous activation of NMDARs within the prefrontal cortex and temporal cortex is essential for the long-term encoding of visuo-spatial associations (Barker & Warburton, 2008). It may thus be more relevant, in the context of associative learning, to identify whether ketamine affects the functional connectivity of these areas, rather than testing only isolated amplitude changes in BOLD signal. This analysis may also be suited to making parallels between the effects of ketamine and deficits in associative learning found in patients suffering from schizophrenia, characterised by a disruption in fronto-temporal connectivity specifically during encoding (Banyai et al., 2011; E. Murphy et al., 2008).

7.4.7   Caveats & Limitations

As with the N-Back task described in Chapter 6, the PAL task requires the use of various cognitive processes, including WM. It is thus difficult for example to attribute the recruitment of the DLPFC, a region with an established role in WM, to specific associative learning processes. Furthermore, as mentioned above, the subtlety of the ketamine-induced effects may be due to the PAL task being too easy for healthy volunteers or not providing sufficient learning phases to robustly ascertain the effects of acute NMDAR antagonism on associational processes across time. It is also possible, that the subtlety of effects may be due to the low doses of ketamine administered. Although the choice of dose was principled to ensure minimal side-effects, it may be important to conduct a study with a higher dose in order to confirm the effect of ketamine on associative learning processes.

7.4.8   Conclusions

This is the first study to investigate the effects of ketamine on an fMRI PAL task in healthy volunteers and the modulation of its effects by lamotrigine and risperidone. This chapter demonstrates that although acute administration of low-dose ketamine does not appear to have pronounced effects on paired associative learning performance and brain response, the interaction between between ketamine and lamotrigine in areas important for visual associational memory and learning, such as the DLPFC and ITL, does suggest that the glutamatergic system contributes to such processes in healthy human volunteers. Further analysis is needed to either confirm or refute these findings, for example with the use of functional connectivity techniques.

Chapter 8 Ketamine-induced Modulation of Functional Connectivity in the Context of Associative Learning

8.1 INTRODUCTION

Chapter 7 highlights the idea that communication between the PFC and ITL is essential for visual associational learning and memory processes and hints at an involvement of the glutamatergic system in this dynamic. However, the results with ketamine alone appear subtle, perhaps due to the nature of the image analysis technique used, which focuses on changes *within* localised areas rather than the functional connectivity *between* such areas. The idea of regional inter-communication may be more pertinent with regards to investigating the effects of ketamine, due to the established role of NMDARs in synaptic plasticity, neuronal communication and ensuing molecular learning and memory processes (Rebola et al., 2010; G. Riedel et al., 2003). Thus, in order to establish whether ketamine is a useful probe of the involvement of the glutamatergic system in associational processes it is perhaps better to turn to a technique that allows one to test "how brain regions interact in a task-dependent manner" ((McLaren, Ries, Xu, & Johnson, 2012), p.1277).

Psychophysiological interaction (PPI) analysis is such a method (K. J. Friston et al., 1997). This technique has the ability to detect brains regions whose coupling (or connection) with a 'seed' region of interest is modulated by the psychological context (O'Reilly, Woolrich, Behrens, Smith, & Johansen-Berg, 2012). For example, in the framework of the PAL task, a PPI analysis could identify areas whose coupling with a seed region changes depending on the encoding learning phase. It is important to remember however that a strengthening of a connection between two areas does not inform us on the question of causality, nor does it exclude the idea that the connection may be mediated by another brain area (O'Reilly et al., 2012).

In light of the fact that the PFC and the ITL appear to interact during associative learning (Eacott & Gaffan, 1992; Gutnikov et al., 1997; Tomita et al., 1999), and the role of both the DLPFC and ITL in promoting the encoding of associations (Blumenfeld, Parks, Yonelinas, & Ranganath, 2011; Murray & Ranganath, 2007; Yakovlev et al., 1998), the ITL region was chosen as the seed region in a PPI analysis of the PAL task and the

DLPFC as the area whose coupling with the ITL would be modulated by learning phase during placebo conditions. The administration of ketamine would be predicted to disrupt any learning-related changes in coupling between these two areas due to the importance of NMDARs for the encoding of visuo-spatial associations (Barker & Warburton, 2008). These hypotheses were tested only within the encoding phase of the PAL task following the theoretical reasoning outlined in the introduction to Chapter 7 and the subsequent drug-induced effects evident primarily during this phase.

8.2   METHODS

8.2.1   PPI Analysis

In order to investigate the effects of ketamine on learning-related changes in coupling during encoding, the PPI analysis was performed on the P_Saline and P_Ketamine data from the modulation study. The methodology was based on that described in the SPM8 manual (http://www.fil.ion.ucl.ac.uk/spm/doc/manual.pdf) and included 15 participants (robust data was absent within the ITL in one participant using the encode-learn contrast (Encode 3>Encode1)).

The right ITL (rITL) was defined as the seed ROI. A group-level peak coordinate [36, -2, -36] within this region was defined with a one sample t-test including the encode-learn contrasts from the P_Saline and P_Ketamine conditions. Timeseries, representing the 'physiological' component of PPI analysis (see Figure 8-1(a)), were then extracted from 6mm spheres around the rITL peaks in each individual subject (peaks no more than 10mm away from the group-level peak).
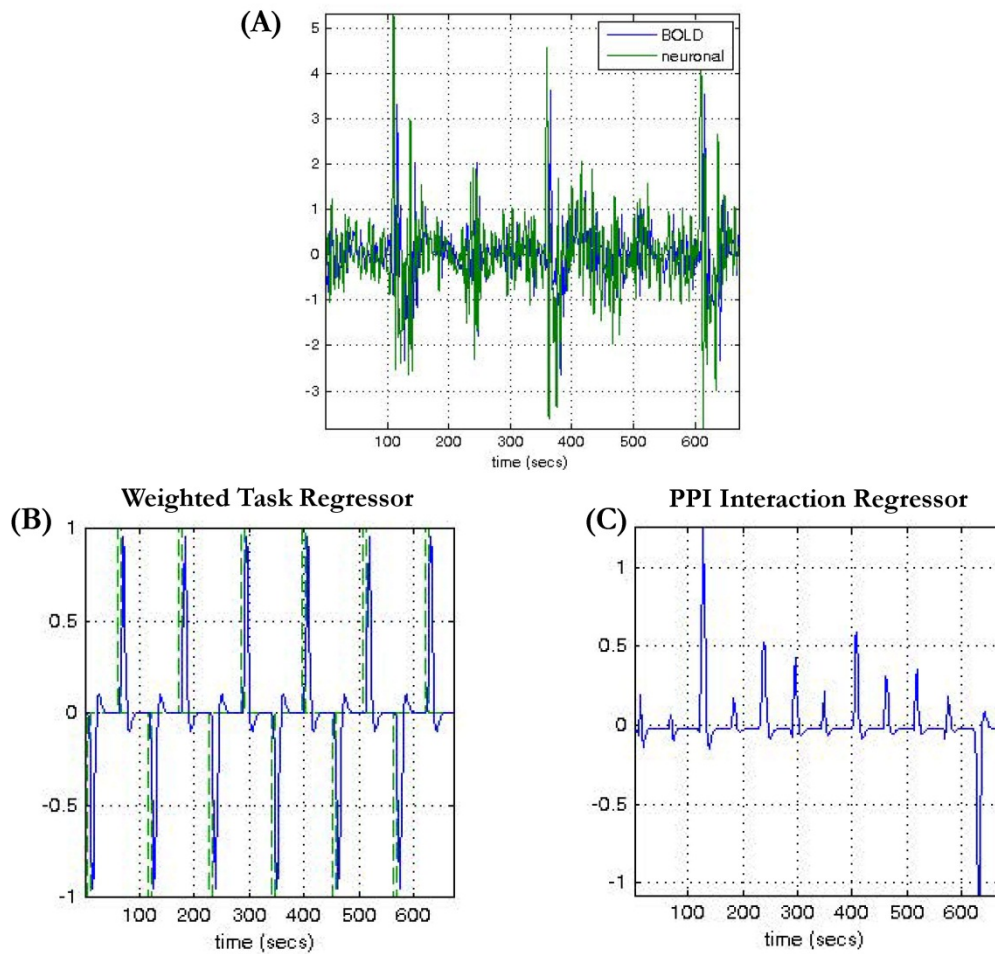
Figure 8-1 Creation of the PPI variable of interest for one participant

Following this, a deconvolution step was employed to produce a time-course of predicted neuronal activity which underlies the observed BOLD timeseries of the seed region. An interaction (PPI) variable was then created by multiplying the 'psychological' variable (representing the contrast between two task conditions i.e. the 'encode-learn' contrast; see Figure 8-1 (b)) and the predicted neuronal timeseries. The seed timeseries is deconvolved from the HRF as an interaction between physiological and psychological factors is assumed to occur at the level of neuronal activity rather than during subsequent haemodynamic processes (Gitelman, Penny, Ashburner, & Friston, 2003). The PPI variable thus represents the predicted neuronal response of a region whose coupling with the seed ROI is contextually modulated (i.e. modulated by encoding phase or learning; see Figure 8-1(c)).
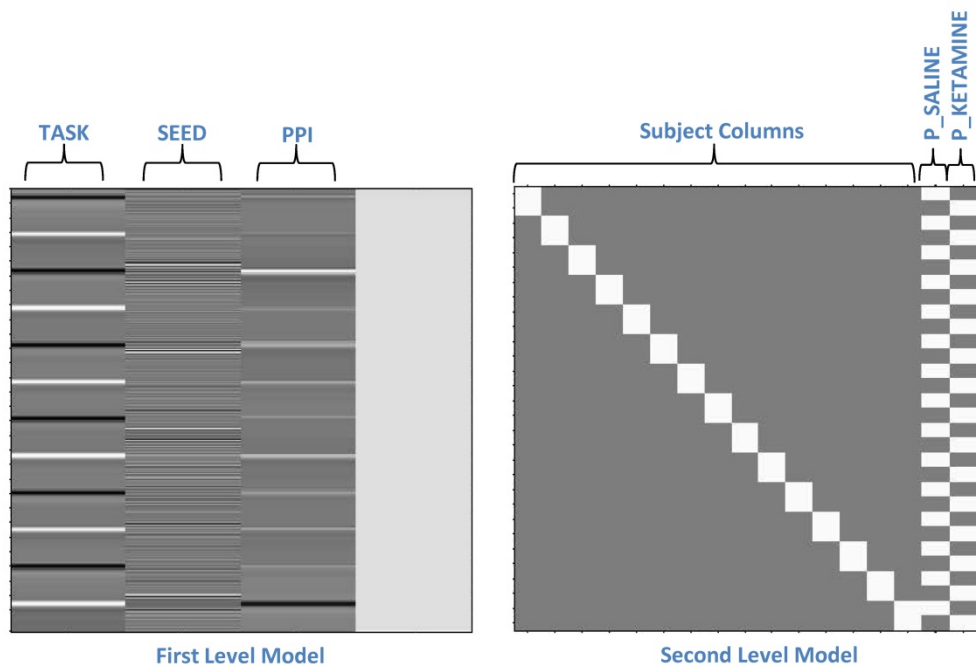
Figure 8-2 First and second level design matrices for the PPI analysis

The PPI variable is then reconvolved with the HRF (as is done in standard fMRI analysis, see Chapter 3) and entered into a first level model (see Figure 8-2). Regressors of no interest, consisting of the psychological and physiological variables used to create the PPI variable, are also entered into the model in order to ensure that "the PPI will only detect functional connectivity effects over and above the main effect of task […] and physiological correlation" ((O'Reilly et al., 2012), p. 606). First level models were constructed for all subjects in the P_Saline and P_Ketamine conditions. The individual PPI variable T-contrasts were then taken through to two one-sample t-tests to test for changes in coupling in placebo and ketamine conditions separately. These tests will show which brain areas demonstrate either learning-related positive coupling (correlation coefficient is more positive during encode3 than encode1) or negative coupling (the correlation coefficient is more negative during encode3 than encode1) with the ITL depending on what contrast is applied (see Figure 8-3 (a,b)). Contrasts were also entered into a second level paired t-test in order to test if any ketamine-induced changes in learning-related coupling during encoding were significant when compared to placebo (Figure 8-3 (c,d)).

ONE SAMPLE T TESTS
Saline or Ketamine

PAIRED SAMPLE T TEST
Saline vs Ketamine
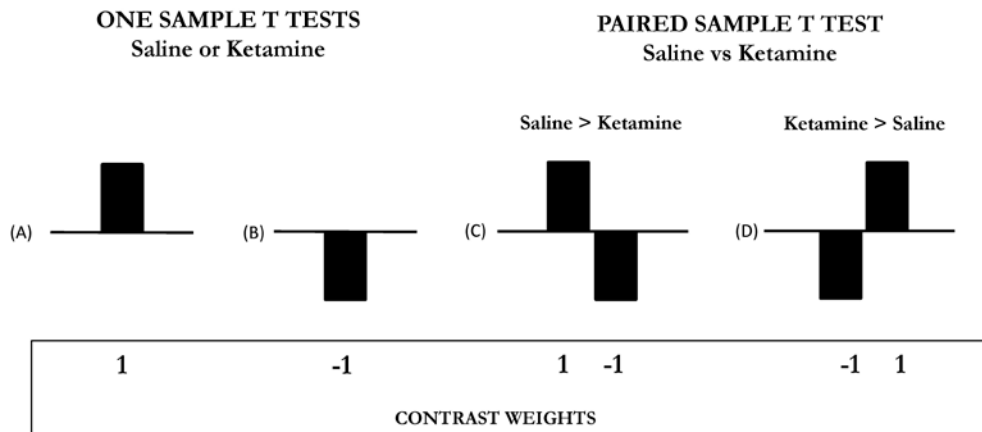
Figure 8-3 Contrasts for the one-sample and paired t-tests used for the PPI

analysis

A contrast (a) for either the P_Saline and P_Ketamine conditions that will display brain areas demonstrating learning-related positive coupling with the ITL; (b) for either the P_Saline and P_Ketamine conditions that will display brain areas that demonstrate learning-related negative coupling with the ITL; (c) that will display brain areas where the learning-related change in coupling (PPI) is more positive on placebo that on ketamine and (d) that will display brain areas where the change in coupling is more positive on ketamine than on placebo.

Any effects within the DLPFC were tested with small volume correction (FWE cluster-level threshold of $p<0.05$), within two pre-defined coordinate-based ROIs (right: [50,20,34]; left [-26,44,40]) (see Chapter 6 for details). Whole-brain changes in connectivity were also examined using an uncorrected threshold of $p<0.001$, family-wise corrected for multiple comparisons at $p<0.05$ on the basis of cluster extent.

8.3   RESULTS

Within the DLPFC ROIs or at a whole-brain level, no significant learning-related changes in coupling with the rITL were found to occur under placebo or ketamine conditions when considered separately. In contrast, the learning-related change in coupling under ketamine conditions was found to be significantly different from that seen with placebo within the right DLPFC (FWE corrected for small volumes at $p<0.05$) and the left parietal cortex (PC) (specifically the postcentral gyrus (BA3)) at a whole-brain cluster corrected level (see Figure 8-4)). More specifically, the learning-related change in coupling

between the ITL and DLPFC/PC is more positive under ketamine than placebo conditions. No areas demonstrated a significant effect in the opposite contrast where differences in coupling were more positive on placebo compared to ketamine.
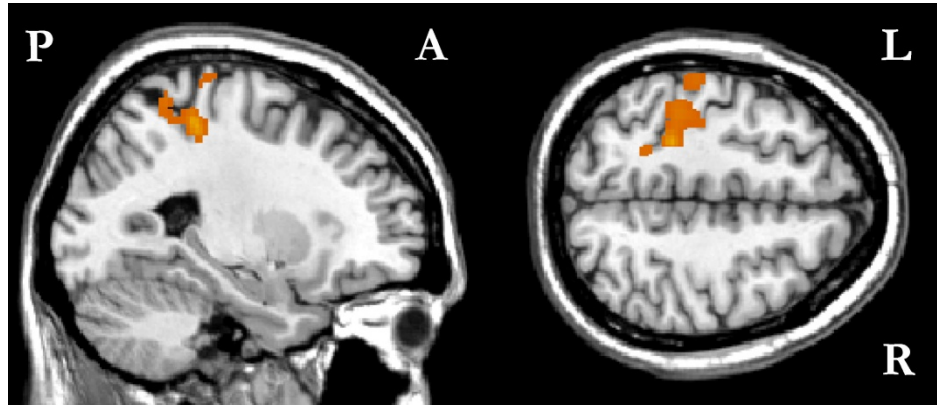
Figure 8-4 Ketamine-induced changes in functional connectivity

Ketamine-induced changes in functional connectivity of the left PC with the ITL which are significantly different to what occurs during placebo conditions. Display centred on MNI coordinates [-26, -36, 52] at an uncorrected threshold of 0.001, FWE corrected on the basis of cluster extent at p<0.05.

Although second level contrasts will inform us on whether there is a change in the nature of the coupling between the two drug treatments, it is difficult just with the associated beta parameter estimates to interpret the exact directionality of the effect. Thus, in order to interpret the ketamine-induced changes in coupling (when compared to placebo), timeseries were extracted for the right DLPFC and left PC, corresponding to the BOLD activity during encode 1 and encode 3 respectively. These were plotted against the timeseries of the ITL in order to establish how the correlation in activity between different regions changes depending on encoding phase and drug treatment (see Figure 8-5).
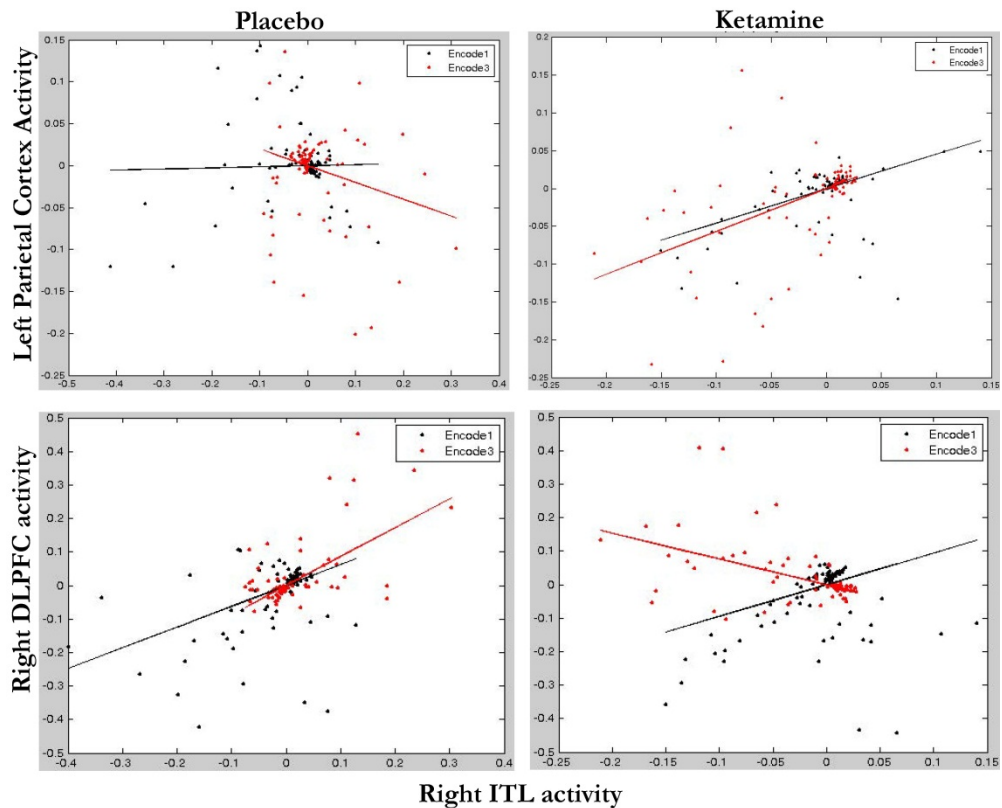
Figure 8-5 Neural activity correlations between the right ITL and right DLPFC and left PC

Correlations during each encoding phase of interest in both placebo and ketamine conditions averaged across participants. The best fit line for Encode 1 = black; the best fit line for Encode 3 = red (results in the least amount of difference between the observed data points and the line; Field (2009)).

As Figure 8-5 shows, during placebo conditions, activity in the right ITL and left PC appear uncorrelated during encode 1, and negatively correlated during encode 3. In contrast, the ITL and left PC are positively correlated during encode 3 under ketamine conditions. In the right DLPFC, ketamine also alters the correlation during encode 3, as the ITL and DLPFC are positively correlated during placebo conditions but negatively correlated post-ketamine infusion. Thus although the coupling between regions was not found to differ significantly between encoding phases when placebo and ketamine conditions were investigated separately, ketamine does significantly alter the pattern of coupling seen in both parietal and dorsolateral prefrontal cortices when compared to placebo.

8.4 DISCUSSION

8.4.1 Summary of Results

Contrary to the hypotheses outlined in the introduction to this chapter, the DLPFC did not demonstrate significant learning-related changes in coupling with the ITL in placebo or ketamine conditions when assessed separately. Nonetheless, as expected, ketamine did significantly alter the pattern of learning-related coupling evident in placebo conditions not only within the right DLPFC but also within the left PC.

8.4.2 Role of the Dorsolateral and Parietal Cortices in Associative Learning

Within the right DLPFC regional activity appears positively correlated with encoding-related ITL activity during early and late phases of learning, although the correlation appears stronger in the latter phase under placebo. Due to the interaction of these two regions during associative learning (Eacott & Gaffan, 1992; Gutnikov et al., 1997; Tomita et al., 1999) it would be unsurprising that under placebo conditions their functional connectivity increases with learning, demonstrating a "task-specific increase in the exchange of information" ((O'Reilly et al., 2012), p.604). In contrast, ketamine appears to disrupt this pattern of communication, with DLPFC and ITL activity being negatively correlated in the late phase of learning (i.e. as ITL activity increases, DLPFC activity decreases). Although this does not necessarily suggest that a decrease in information transfer occurs, it does suggest that the normal learning-related changes in coupling between these two areas are disrupted. The effect of ketamine on DLPFC connectivity could have consequences for consolidation of items within associative memory (Barker & Warburton, 2008; Crespo-Garcia, Cantero, Pomyalov, Boccaletti, & Atienza, 2010) but also for the top-down modulation of inferior temporal areas, essential for subsequent successful retrieval of associative memories (Tomita et al., 1999).

On the other hand, the left parietal cortex demontrates the opposite to what occurs in the DLPFC; under placebo conditions the PC and ITL are negatively correlated during the late learning phase whereas under ketamine conditions these regions are positively correlated. This again suggests that ketamine disrupts learning-related changes in coupling between brain areas. The involvement of parietal cortex connectivity in the context of visuo-spatial associative learning is appropriate considering its role in successful encoding

of associations in both animals and humans (Achim & Lepage, 2005; Fitzgerald, Freedman, & Assad, 2011; Robinson & Bucci, 2012; Sommer, Rose, Weiller, & Buchel, 2005). Anatomically, receiving input from multiple structures, it is also ideally placed to integrate information from different sensorimotor modalities and is aptly named as an 'association cortex' (Berryhill, Drowos, & Olson, 2009; Robinson & Bucci, 2012). However, it is important to note that it is the parietal cortex in the opposite hemisphere to the ITL seed that demonstrates this ketamine-induced modulation of coupling, thus the anatomical basis of this result is unclear.

Nonetheless, both electroencephalography (EEG) and fMRI studies highlight the importance of temporo-parietal connectivity in associative learning. For example, Buchel and colleagues (1999) demonstrate that learning of object-location associations occurs in parallel to increased effective (causal) connectivity between the ITL and PC. In a study by Crespo-Garcia and colleagues (2010), EEG theta oscillations between the PC and the parahippocampal gyrus and between the DLPFC and parahippocampal gyrus, were shown to become more synchronised during associative encoding, interpreted as an index of functional coordination between these regions. In addition, the DLPFC and PC were also found to be coupled suggesting that these regions, together with the temporal lobe, belong to a neural network essential to encoding visuo-spatial associations (Crespo-Garcia et al., 2010; Dudkin, Chueva, & Makarov, 2004; P. Fletcher et al., 1999; Mangels, Manzi, & Summerfield, 2010). Ketamine-induced disruption to parietal connectivity could potentially influence its role in spatial processing as part of the dorsal 'object-location' stream (Dudkin et al., 2004; Sommer et al., 2005) or its putative role in allocating attentional and organisational resources essential for encoding (Sommer et al., 2005; Uncapher & Wagner, 2009; Wendelken, Bunge, & Carter, 2008).

8.4.3   Mechanism of Disruption: The Effect of Ketamine on Synaptic Plasticity

Ketamine has previously been shown to disrupt functional coupling between different brain regions. Resting state changes in thalamocortical connectivity have been found in both animals and humans (Dawson et al., 2011; S. P. Kim, Hwang, Kang, Kim, & Choi, 2012), although only with the use of near-anaesthetic levels of ketamine. Ketamine-induced changes in connectivity have also been found in the context of pain and

depression, measured with resting state fMRI and magnetoencephalography respectively (Niesters et al., 2012; Salvadore et al., 2010). In this chapter, ketamine-induced changes in functional connectivity, assessed with BOLD fMRI, were found in the context of an associative learning task.

Through what mechanism does ketamine disrupt brain connectivity? As described in the introduction, NMDARs are intimately involved in the molecular mechanisms of synaptic plasticity, a process deemed to underlie patterns of connectivity between brain regions (K. Friston, 2003a). This is supported by the fact that, in this chapter, ketamine disrupts learning-related changes in coupling specifically by altering the relationship between brain regions in the later phases of learning – a period in which synaptic plasticity may be more likely to occur. Thus, NMDAR antagonism can result not only in changes to short-term synaptic plasticity but also to the functional integration of brain areas (Stephan, Baldeweg, & Friston, 2006). In fact, Schmidt and colleagues (2012) demonstrate, using a functional connectivity approach - dynamic causal modelling (DCM), that ketamine-induced disruption of an electrophysiological signal of learning (specifically prediction error) referred to as mismatch negativity, could be attributed to NMDAR-mediated impairments in short-term plasticity of long-range glutamatergic connections with accompanying changes in frontotemporal connectivity and cognitive function. It is interesting to note that NMDAR-mediated disruption to synaptic plasticity has also been hypothesised to underlie the functional disconnection syndrome evident in schizophrenia (K. Friston, 2003a; Stephan et al., 2006; Stephan, Friston, & Frith, 2009). Furthermore, specific disruption of functional connections between dorsolateral, inferior temporal and parietal regions has been found in schizophrenic patients in the context of associative learning (Banyai et al., 2011; E. Murphy et al., 2008).

8.4.4   Caveats & Limitations

Although the PPI seed-based analysis performed in this chapter demonstrates that ketamine affects fronto-temporal and parieto-temporal connectivity in the context of associative learning, it may be informative to supplement this analysis by the use of a technique that tests the *causal* structure of the network of implicated regions as a whole, such as DCM (Banyai et al., 2011; Schmidt et al., 2012). This would enable one to assess "whether activity in a given neuronal population [for example the DLPFC] modulates the

coupling between other populations [for example between the ITL and PC], in a task-specific fashion" ((Marreiros, Kiebel, & Friston, 2010), p.9568). This could bring the analysis of the effects of ketamine on associative learning one step further.

8.4.5 Conclusions

The findings of this chapter support the notion that acute administration of low-dose ketamine acts via NMDARs to disrupt synaptic plasticity and consequently, efficient communication between brain areas. Furthermore, in comparison to Chapter 7, whereby ketamine only appears to subtly affect the brain response underlying associative learning and memory, this chapter demonstrates, by using an analysis technique which is perhaps more suited to the potential effects of NMDAR antagonists and the organisation of the brain itself, that ketamine may indeed be an appropriate probe of the glutamatergic system and enlightening with regards to its role in associative learning.

Chapter 9 General Discussion

9.1   Overview

The aim of this thesis was to assess whether the brain response to ketamine could be informative with regards to the glutamatergic system and its role in human cognition. To assess this question this thesis has combined the administration of low-dose ketamine administration with imaging techniques such as ASL and fMRI in both resting state and cognitive task conditions. Based on the literature presented in Chapter 1, ketamine was hypothesised to induce increases in BOLD signal and CBF during resting state and disrupt working memory and associative learning brain networks. These ketamine-induced changes were also expected to be attenuated by both lamotrigine and risperidone. More specific hypotheses were outlined in the individual chapters (see Table 9-1). Together with the discussions to each individual chapter, the following discussion addresses whether these hypotheses have been satisfied and highlights the overarching issues brought to light by this thesis. Potential limitations are also described and are followed by a discussion on the future directions this work could take.

Table 9-1 Summary of Main Findings

Main Aims	Main Hypotheses	Main Findings
Chapter 4 Test-retest reliability of the BOLD phMRI response to ketamine and its modulation by lamotrigine and risperidone	Widespread ketamine-induced increases in BOLD response. Attenuation of this effect by risperidone and lamotrigine in the anterior, posterior and subgenual cingulate, thalamus and hippocampus.	Robust and reliable effects of ketamine. Attenuation of this response by lamotrigine found in all ROIs except the subgenual cingulate. Attenuation in all ROIs was found with risperidone.
Chapter 5 Test-retest reliability of the ASL phMRI response to ketamine and its modulation by lamotrigine and risperidone	Ketamine-induced increases in CBF in frontal, cingulate and thalamic areas. Attenuation of this effect in the anterior/posterior cingulate and thalamus by risperidone and lamotrigine.	Reliable effects of ketamine although the CBF response was not as robust as the BOLD. No modulatory effects of lamotrigine or risperidone were found.
Chapter 6 The effects of ketamine on the N-Back task and modulation by lamotrigine and risperidone	Ketamine-induced (1) increases in activation across all WM loads or (2) alterations in the WM BOLD load-response profile. Modulation of this response by risperidone and lamotrigine.	Ketamine-induced altered load-response profile in the dorsolateral prefrontal cortex. Risperidone-mediated effects were found in the hippocampus and posterior cingulate. No modulatory effects of lamotrigine were found.
Chapter 7 The effects of ketamine on the PAL task and modulation by lamotrigine and risperidone	Ketamine-induced disruption of learning-related changes in BOLD response in both encoding and retrieval. Modulation of this response by lamotrigine and risperidone.	No ketamine-induced changes in learning-related BOLD response were found. Lamotrigine-mediated learning effects were found in dorsolateral and ventrolateral prefrontal cortices and the inferior temporal lobe only during encoding.
Chapter 8 The effects of ketamine on frontotemporal connectivity in the context of associative learning	Ketamine-induced disruption of learning-related changes in frontotemporal coupling.	Ketamine-induced alterations in the pattern of learning-related coupling between the inferior temporal lobe and dorsolateral prefrontal and parietal cortices.

9.2 The Brain Response to Ketamine: A Platform for Testing Modulatory Compounds

A high degree of interest continues in the study of the central effects of ketamine due to its potential relevance to schizophrenia, antidepressant efficacy and anaelgesia (R. M. Berman et al., 2000; Prommer, 2012; W. J. Riedel, 2007). Thus, one of the main aims of this thesis was to examine whether the brain response to acute ketamine provides a valid pharmacological tool to investigate glutamatergic dysfunction with the added sensitivity to detect the modulatory effects of compounds known to indirectly regulate this neurotransmitter system. Chapters' 4 and 5 address this question with the use of resting-state BOLD and perfusion scans. The ketamine-induced increases in both BOLD response and CBF described in this thesis were found to be consistent with animal and

human literature (see Chapter 1, Section 1.3.2) and thus also with the hypotheses outlined in Chapters' 1, 4 and 5. However, the results suggest that, in the context of the initial response to low-dose ketamine, the BOLD response represents the more suitable technique for future repeated-measures interventional studies as the perfusion response to ketamine was not found to be sensitive to modulation. Nonetheless, ketamine-induced effects were found to be reliable with the use of both methods; an important finding with respect to the value of fMRI in general (McGonigle, 2012) and drug development research (Barch & Mathalon, 2011). It must be stressed however that the reliability measurements are only applicable to the sample or regions of interest tested, with perhaps a different outcome in a patient population (Barch & Mathalon, 2011).

There is a need in ketamine research to develop a more standardised methodology, particularly due to the differences in ketamine dose or infusion procedures previously used (see Chapter 1; Table 1-1 and 1-2). In this thesis, the reliability of the ketamine BOLD response was assessed with the use of (1) a dose which limits the psychotomimetic effects seen, (2) an infusion protocol demonstrated to be suited for low-dose infusions (Absalom et al., 2007), and (3) an analysis methodology which takes advantage of the temporal resolution within fMRI data. This represents progress towards a procedure which can be used not only across subjects but also across different sites of data acquisition, with the aim to develop a suitable marker which demonstrates mechanistic engagement of novel compounds that normalise aberrant glutamatergic activity in the brain.

9.3 The Brain Response to Ketamine and the Role of the Glutamatergic System in Cognition

Whilst many past studies have focused on the psychotomimetic effects of ketamine, Chapters' 6,7 and 8 of this thesis look at the neural effects of this drug in two cognitive tasks based on WM and associative learning. Furthermore, the effects of risperidone and lamotrigine on any ketamine-induced effects were also assessed. This was done in order to address the second aim of this thesis which was to further our knowledge on the role of the glutamatergic system in aspects of human cognition also known to be severely affected in schizophrenia (Barch & Ceaser, 2012). At first glance, and perhaps in contrast to the relevant hypotheses (see Table 9-1), the profile of ketamine-induced brain changes

in both the N-Back and the PAL tasks appears subtle suggesting that low-level NMDAR hypofunction, as assessed by fMRI, does not impact greatly on learning and memory processes in healthy humans. Nonetheless, when examined in more detail the results provide some interesting findings. For example, it appears that risperidone and lamotrigine have differential effects depending on the cognitive task at hand, with risperidone demonstrating a more prominent role in WM and lamotrigine in associative learning. Thus, contrary to what was hypothesised risperidone and lamotrigine do not attenuate the brain response to ketamine in a generalised manner, at least in the context of the cognitive tasks.

The dominance of each compound is also evident when their interactions with ketamine are assessed. Due to their dissimilar pharmacological mechanisms of action, one serotonergic/dopaminergic and the other glutamatergic, this illustrates the ability of cognitive tasks to dissociate the various neurochemical systems underlying such higher-order cognitive functions. More specifically, it appears that it is the dopaminergic effects of ketamine that are more crucial for WM whereas its hyperglutamatergic effects are more critical to associative learning and memory (Lindefors et al., 1997; Moghaddam et al., 1997; C. J. Morgan & Curran, 2006; Verma & Moghaddam, 1996). Nonetheless, it will be important to confirm these speculations with the use of more pharmacologically selective compounds. For example, due to the importance of NMDAR – dopamine D1 receptor interactions in the prefrontal cortex during WM (Arnsten, 2011; Frankle, Lerma, & Laruelle, 2003; Williams & Castner, 2006), the use of a dopamine D1 agonist or antagonist may help to elucidate the contribution of ketamine's dopaminergic effects to it effects in WM. Dopamine D1 agonists have been found to be beneficial in ketamine-induced WM deficits in animals (Roberts, Seymour, et al., 2010), however a selective D1 agonist has yet to be approved for human use. A proof of concept investigation could utilize the dopamine precursor, levodopa, which will impact both dopamine D1 and dopamine D2 receptors. However, given the higher concentration of dopamine D1 receptors in the frontal cortex compared to D2 receptors this could prove a promising strategy (Castner & Williams, 2007).

Another important point which one can glean from the results is that the choice of analysis technique is critical with regards to the decision on whether or not ketamine is an adequate probe of the glutamatergic system in cognition. More specifically, contrary to the

hypotheses stated in Chapter 7, ketamine has no robust effects during associative learning when one views brain regions as functionally segregated (Chapter 7), whereas effects were found on learning-related changes in functional connectivity between dorsolateral prefrontal/parietal cortices and the inferior temporal lobe (Chapter 8). This suggests that a technique that focuses on the functional coupling between areas may be more suitable to investigate the short-term (contextual) plasticity effects of ketamine. Finally, the response of the dorsolateral prefrontal cortex appears to be affected by ketamine in both cognitive tasks (although this effect is detected with the use of different analysis methodologies; Chapter 6 and 8), suggesting that this region may be of central importance to (1) understanding the effects of the drug and (2) for future studies investigating cognitive processes that are targeted by NMDAR hypofunction.

This latter finding may be important when considering that cognitive functions such as WM and associative learning and memory may share cognitive processes "generic to different mnemonic demands" ((Marklund & Nyberg, 2007), p.307). Thus the finding that ketamine targets the DLPFC in two different cognitive tasks suggests that it may be these 'subprocesses' that are affected by NMDAR hypofunction. Fractionating cognitive processes can be instructive (G. Honey, 2009), but it may sometimes be just as revealing to consider their commonalities.

In fact, WM and associative memory encoding and retrieval tasks have previously been found to be mediated by similar neural substrates including the DLPFC, VLPFC and the cingulate gyrus (Marklund & Nyberg, 2007; Owen, 2000). Task networks defined in chapters' 6 and 7 demonstrate these similarities although chapter 7 focuses primarily on learning-related changes in encoding and retrieval. Due to these findings, Marklund and Nyberg (2007) posit that the DLPFC is engaged in executive control processes including representation, storage and monitoring/manipulation of contextual information which is subsequently compared to what is held in long-term memory in order to guide behaviour. In fact, DLPFC activity has been found to be critical for the formation of relationships between items held in WM (Murray & Ranganath, 2007) and for the subsequent long-term memory of such inter-item associations (Blumenfeld & Ranganath, 2006). Together with the results from this thesis, these findings demonstrate that (1) the DLPFC-mediated cognitive processes critical to WM, are also essential to strengthen associative memory and (2) that such processes may be vulnerable to NMDAR hypofunction. Nevertheless,

although basic cognitive processes may well underlie both WM and associative memory function, one must not underestimate the importance of the psychological probe used in determining what specific processes are available for selective modulation by the drug administered.

A criticism highlighted in Chapter 1 (Section 1.3.4.1) and reiterated in the cognition-centred chapters is that the tasks used require multiple cognitive processes, which makes it more difficult to determine the effects of ketamine on specific subprocesses (see sections 6.4.7. and 7.4.7. for a more detailed discussion of this issue). Furthermore, the effects of ketamine described in this thesis appear to be slightly at odds with the ketamine-cognition literature, where increases in activation are most commonly seen (Chapter 1, section 1.3.4.2). This may be due to the dose of ketamine administered or the infusion protocol used, although previous studies have demonstrated ketamine-induced neural effects at plasma levels as low as 50ng/ml (G. D. Honey, Honey, O'Loughlin, et al., 2005). Nevertheless, the cognitive tasks chosen were able to robustly activate the functional networks thought to underlie WM and associative learning and memory functions. In addition, the analyses conducted were able to reveal the more subtle neural effects of ketamine.

In terms of performance, no strong ketamine-induced deficits were found in either the N-Back or the PAL task as hypothesised (see Chapter 1, 6 and 7). This can also be viewed as an advantage as drug-induced changes in brain activity in the absence of performance deficits suggests that the brain changes are indeed due to a disruption of underlying neural mechanisms rather than merely the correlates of poor performance ((R. A. Honey et al., 2004); see Chapter 1, Section 1.3.4.2). Furthermore, the BOLD response may be more sensitive to changes in effort or cognitive strategy compared to performance measures (G. Honey & Bullmore, 2004; Wise & Tracey, 2006). Overall, the choice of cognitive task is supported by the fact that they adequately elicited (1) activation of brain areas affected in schizophrenia and hyperglutamatergic states and (2) NMDAR-dependent learning and memory mechanisms. Furthermore, although the effects of ketamine are subtle they do support the notion that NMDAR function plays a role in both WM and associative learning in healthy humans, and that this can be detected with fMRI.

9.4   Wider Implications for Psychiatric Disorders

As stated in the introduction to this thesis the cognitive deficits seen in schizophrenia have increasingly become the focus of treatment research, due to their persistence over time, presence in first-degree relatives, and predictive value in terms of prognosis (Nuechterlein, Green, & Kern, 2009). In recent years, glutamatergic dysfunction has been implicated in such symptoms, with novel treatment strategies targeting various aspects of this system (Moghaddam & Javitt, 2012). The effects of ketamine and research on the contribution of NMDAR function to cognition may thus impact upon the utility of such glutamatergic treatments.

Overall, the findings from this thesis support the view that the effects of ketamine administration may well be informative with regards to the involvement of glutamatergic dysfunction in schizophrenia. For example, consistent with hypotheses, Chapter 4 demonstrates that the BOLD resting state response to ketamine is attenuated by risperidone, an atypical antipsychotic that has been shown to improve certain cognitive deficits in those suffering from schizophrenia (H. Y. Meltzer & McGurk, 1999). This suggests that 5HT2A antagonism and subsequent inhibition of glutamate release may play an important role in risperidone's mechanism of action in relation to the glutamatergic component of schizophrenia. Chapter 6 demonstrates that the effect of ketamine on the WM 'load-activation curve' within the DLPFC reflects a similar pattern of response seen in this region in schizophrenic patients (Callicott et al., 2003). Finally, Chapter 8 demonstrates a ketamine-induced change in learning-related coupling between inferior temporal and dorsolateral prefrontal and parietal cortices. Disruption of connectivity such as this has been found in schizophrenic patients in the context of WM (J. J. Kim et al., 2003; A. Meyer-Lindenberg et al., 2001; A. S. Meyer-Lindenberg et al., 2005), associative learning (Banyai et al., 2011; E. Murphy et al., 2008) and resting state (Zhou et al., 2007). It is also important to note that the findings summarised above implicate the DLPFC as one of the focal points of ketamine-induced disruption, a region which is also considered fundamental to the symptoms of schizophrenia (K. F. Berman, 2002; Bunney & Bunney, 2000). Overall, although the effects of ketamine are subtle in terms of the cognitive tasks, the pattern of effects is consistent with those seen in the schizophrenia literature. Future work however, will need to establish how this hypothesised glutamatergic dysfunction can be selectively targeted in patients.

The value of ketamine as a probe of the glutamate system is also relevant to other psychiatric disorders such as major depression and bipolar disorder whose neurobiological basis is also considered to involve glutamate (C. Zarate, Jr. et al., 2010). Acute administration of ketamine has been found to result in rapid antidepressant activity which has been shown to last from hours to several weeks in treatment-resistant patients (Cryan & O'Leary, 2010). This effect has also been found to be linked to anterior cingulate activity (C. Zarate, Jr. et al., 2010). A potential mechanism of this antidepressant effect involves ketamine-induced activation of the NMDAR-AMPAR mediated intracellular rapamycin (mTor) pathway which has been shown to result in synaptogenesis and increased serotonergic transmission in the prefrontal cortex (N. Li et al., 2010). This downstream process is not inconsistent with that of disinhibition of glutamate release, the mechanism hypothesised to underlie the ketamine-induced increases in BOLD response seen in Chapters' 4 and 5 (N. Li et al., 2010). As described in Chapter 4, risperidone but not lamotrigine attenuates the ketamine-induced BOLD response in the subgenual cingulate, suggesting that this response is due to a serotonergic mechanism, validating the results found in animals and supporting the mechanism of action put forward by Li and colleagues (2010).

However, due to the physical, perceptual and cognitive side effects of ketamine, it may not be applicable for long-term clinical use (Krystal, 2007). It is thus of paramount importance to determine whether compounds that are NMDAR-subunit selective, have similar antidepressant efficacy in the absence of side effects (Krystal, 2007). The resting state phMRI paradigm described in Chapter 4 may be of use in testing these novel compounds; the BOLD response to ketamine could represent an antidepressant 'brain signature' which the new drugs will have to demonstrate in order to be considered to have potential therapeutic value (Paulus & Stein, 2007). The differential response of individuals suffering from depression to this paradigm may also be of use in the classification of responders and non-responders to treatment, or different types of depressive disorder (Paulus & Stein, 2007). Finally, the effects of ketamine in the cognitive tasks may also be of relevance as dysfunctional glutamate transmission has also been found in the DLPFC of patients suffering from depression (Merkl et al., 2011).

9.5 Limitations and Future Work

9.5.1 Power Analysis

No formal power calculations were conducted for the studies presented in this thesis. This is due to the fact that standard statistical computations of power of detection are problematic for neuroimaging studies because the statistical inference is made from the computation of thousands of voxels in each subject. Although more specific guidelines have recently been introduced for the calculation of fMRI power analyses (Mumford, 2012), which have in fact been implemented in De Simoni et al., (2013), these were not available at the time of study design. Furthermore, guidelines regarding power calculations specifically for fMRI reliability studies still do not exist.

Nonetheless, preliminary investigations (Brammer et al., 2004) appear to indicate that BOLD related signal change as small as 1% at the individual level, are reliably detectable with groups of 10−12 individuals. The sample size of both the reliability and modulation study was based on this preliminary analysis and sample sizes used in previous studies. Caceres and colleagues (2009) have also shown that reliable activations can be detected with 8 volunteers.

The modulation study was also informed by previous pharmacological neuroimaging studies. In these studies, participant populations of 12−24 subjects have demonstrated statistically significant, drug induced differences in task-related and task-unrelated activity within groups, in cross-over designs. Of particular relevance to this study is Deakin et al. (2008) who used both ketamine and lamotrigine, two of the drugs used in this thesis. Similar brain imaging outcome measures were statistically significant within two experiments of 12 and 19 participants. Therefore, in order to allow for adequate statistical power and attrition due to potential drop outs a sample of 20 healthy volunteers was recruited in order to obtain complete data sets on 16 participants.

Nevertheless, it is possible that the lack of modulatory effects seen, for example, in the perfusion data or the lack of dose effect in the resting-state BOLD data, is due to inadequate sample sizes. Future studies should ensure that formal power calculations are carried out prior to data collection.

9.5.2   Saline Control

As a methodological standard, it is customary to include a placebo condition in psychopharmacology studies. As such, the modulation study was placebo-controlled. However a placebo visit was not included in the reliability study. This lack of placebo-control may thus lead to difficulties in ascertaining if the brain response seen is due to ketamine itself or extraneous variables, such as scanner drift (although this is partially resolved by the presence of a linear drift regressor within the first level model), movement or surprise at the infusion and physiological effects associated with a liquid solution entering the brain. In the future, if feasible, it may be beneficial to begin the resting-state phMRI scan when participants are already being infused with saline. This may engender a smoother transition between no-drug and drug states, removing some of the effects due to the confounding variables.

In addition, in the reliability study, both experimenters and participants were aware that they were receiving a ketamine infusion on both occasions, thus they were not blinded to the drug being administered. Although the participants were informed that the response to ketamine was variable between individuals, knowledge of ketamine's effects may have led to an altered response, due to certain expectations about the experience.

Nevertheless, the brain response to ketamine was validated through the modulation study. In these analyses, the ketamine brain response was contrasted with the saline brain response, demonstrating a result highly consistent with that from the reliability study. This suggests that the brain response seen is more likely to be due to the effects of ketamine itself compared to any confounding influences.

9.5.3   Psychometric Validity

The lack of significant effects on the subjective ratings in the modulation study may reflect a loss of psychometric validity, as only individual questions from the full questionnaires (PSI, CADSS and VAS) used in the reliability study were taken through to assess the acute effects of ketamine. The sensitivity and reliability of these specific questions were not formally compared with that of the questionnaires used or against other questionnaires assessing psychotomimetic symptoms, thus affecting their construct validity.

Nevertheless, this brief questionnaire was constructed mainly for practical purposes, as full questionnaire administration inside the scanner can be problematic and time consuming. Furthermore, the specific questions were chosen in a systematic way (i.e. based on their effect sizes within the reliability study), to ensure rapid assessment of the most common ketamine-induced subjective feelings. In addition, the full questionnaires were administered during the modulation study (data not reported) although these were administered at time-points which were somewhat removed from the initial ketamine bolus (see Chapter 3, Figure 3.3). Thus, future studies should strive to administer the full questionnaires at more appropriate time-points to increase the sensitivity of these measures to the effects of ketamine and any modulatory effects of the pre-treatment drugs.

9.5.4 Confirming Mechanism

As touched upon in Chapters' 2 and 4, due to the indirect nature of the BOLD signal, an important consideration in pharmacological imaging is the question of whether drug-induced changes in BOLD response are a true indicator of neuronal activity rather than non-specific changes in factors contributing to neurovascular coupling or direct effects of the drug on the vasculature (Stein, 2001; Wise & Tracey, 2006). The global effects of ketamine on the resting BOLD response described in Chapter 4, suggest it is a priority to control for such non-specific effects of the drug. Furthermore, the BOLD response was not found to be reliably correlated with subjective ratings, a result that would have been encouraging in terms of ascertaining whether the effects of ketamine are truly neuronal in origin (Schweinhardt et al., 2006). However, as previously mentioned, reasonable confidence can be had in stating that the effects of ketamine are neuronal as the effects of ketamine are consistent with the pattern of ketamine-induced changes in metabolism in animals and humans (Breier et al., 1997; G. E. Duncan, Moy, et al., 1998; Littlewood, Jones, et al., 2006; Vollenweider, Leenders, Scharfetter, et al., 1997) and low-dose ketamine has been shown not to induce decoupling between metabolism and blood flow (Langsjo et al., 2004). Furthermore, the results from Chapters' 6 and 7 demonstrate that ketamine does not cause global changes in the context of cognitive tasks, suggesting that it is unlikely that its effects are merely vascular (Wise & Tracey, 2006).

Nonetheless, several reasons suggest that more analysis and research is essential to ascertain that the effects of ketamine in the resting BOLD phMRI response are truly due to NMDAR blockade and subsequent increased glutamate release; (1) the resting state and cognitive task data were not acquired under identical conditions, i.e. the initial effects of ketamine could be due to different factors than during the maintenance steady-state period, (2) although the perfusion data is encouraging as it demonstrates that the effects of ketamine on CBF are not global, it does not unequivocally demonstrate the effects of ketamine are neuronal as the increases in CBF seen could also be due to non-specific factors, and (3) the resting state BOLD response to ketamine is highly robust compared to the more subtle effects seen in the cognitive tasks. Thus to investigate the mechanism of ketamine further, physiological parameters including respiration, blood pressure and heart rate which could affect BOLD measures, could be incorporated into the BOLD data analysis as nuisance variables as these were acquired during the ketamine infusion period. Incidentally, the heart rate and respiration parameters have in fact been incorporated into the resting phMRI analysis, with no change to the overall results (data analysed as part of a Master thesis project; (Felixson, 2012)). Another avenue would be to analyse the breath-hold fMRI paradigm also acquired within the context of the studies described in this thesis. This task requires the participant to alternate breathing normally with periods of holding their breath. During these periods, blood levels of carbon dioxide (CO_2) increase, causing vasodilation and increases in CBF (Magon et al., 2009; K. Murphy, Harris, & Wise, 2011). Drugs may affect this vascular reactivity by influencing the "vessel's ability to respond to a vasodilatory stimulus such as CO_2" ((K. Murphy et al., 2011), p.369) or by changing the respiration rate of the volunteer. By using the breath-hold paradigm one can thus assess if ketamine has an effect on vascular reactivity; if so, the supposed neuronal effects of the drug could be seen under a different light. Finally a potential method to confirm mechanistic engagement by ketamine is the use of simultaneous fMRI-EEG acquisition, which could demonstrate whether ketamine-induced changes in elecrophysiological activity occur in conjunction with changes in BOLD signal (Wise & Tracey, 2006).

An important finding of this thesis was that the resting-state BOLD response to ketamine was found to be attenuated by both risperidone and lamotrigine, compounds thought to act directly or indirectly on the glutamatergic system. This was achieved with the use of a

ROI-based univariate analysis approach (see Chapter 3 and 4). Although extremely informative this analysis could be supplemented with the use of multivariate techniques which, by being receptive to spatial patterns within the data, may be more sensitive to modulatory effects when the pharmacological interventions elicit correlated and distributed effects across brain regions.The outcome of this analysis would reduce effects across brain regions to a single outcome measure based on the whole-brain pattern and could be used to classify the data. A framework to benchmark the degree of attenuation of ketamine-induced BOLD signal changes was developed in collaboration with the image analysis research group within King's College London and was able to discriminate with high probability the saline and ketamine conditions (Doyle, De Simoni, Brammer, Schwarz, & Mehta, 2011). Furthermore, both lamotrigine and risperidone pre-treatment resulted in phMRI responses to ketamine that were more difficult to separate from the saline condition than ketamine alone. This method is particularly useful for pharmacological modulation applications as it enables one to place an individual phMRI response on a continuum to gauge the extent of the modulation relative to a control condition (Doyle et al., 2012).

An additional concern of the ROI-driven approach applied in this thesis is that of multiple comparisons. As described in Chapter 3, multiple comparisons correction was not applied across ROIs in order to follow a pragmatic approach that would enable the characterisation of the brain response to ketamine whilst avoiding false negative results. Nonetheless, one must acknowledge that this strategy may result in a proportion of the findings being due to chance. To ensure only statistically robust results are focussed upon, future manuscripts based on the work in this thesis will highlight the corrected results.

9.5.5   Linking Resting-State and Cognition

As mentioned above, a goal of this thesis was to validate the use of the ketamine BOLD phMRI response as a platform against which novel drugs could be assessed, for use in disorders with underlying glutamatergic dysfunction. Therefore, it would also be beneficial to know whether the brain areas elicited by the initial ketamine bolus are those altered in a sustained manner, in the context of a cognitive task. This would reinforce the value of the ketamine phMRI response as a drug assay as, if a novel drug were to modulate the effects of ketamine in these particular brain areas, it could have predictive value in terms of potential cognitive-enhancing effects of such a compound. Thus, potential further

analyses leading to cross-talk between the resting state and cognitive data acquired for this thesis, could include assessing the extent to which a particular brain region responding to the early effects of ketamine is correlated with its response during the cognitive task. This would suggest that individual differences in how one responds to ketamine in resting conditions may impact upon ensuing cognitive function.

9.5.6 Individual Differences

A related question is whether the neural effects of ketamine can predict subsequent 'clinical' changes, an important issue if the drug effects are to be relevant to existing psychiatric disorders. Although the dose of ketamine used in this thesis was chosen specifically in order to minimise any psychotomimetic side-effects, a potential limitation of this study design is the fact that both subjective effects and cognitive performance deficits are weak and show little between-subject variability. As a result, this reduces the ability of the brain changes to predict what occurs in terms of subjective experience. Research performed by Garry Honey and Phil Corlett (Corlett et al., 2006; G. D. Honey et al., 2008) has focused on this issue by linking cognition, brain activity and subjective response. For example, they showed that prefrontal recruitment during a WM task in subjects who had received a placebo, is predictive of subsequent ketamine-induced negative symptoms (G. D. Honey et al., 2008). Although this is informative it would be of interest to determine whether the ketamine (rather than placebo) response to a WM task was also predictive of or related to such symptoms. This would perhaps more effectively address the question of whether cognitive function and subjective response share a common underlying neurobiological mechanism. As described in Honey et al. (2008) this could be achieved by assessing the response to a cognitive task in healthy volunteers under low-dose ketamine and the clinical response when the dose has been increased to a level which has previously been shown to elicit psychotomimetic symptoms. Furthermore this design could be extended to include those suffering from depression, as it would also be important to know whether, within this cohort, frontocortical responses on a cognitive task were to predict an improvement in negative symptoms, and consequently used as a marker of antidepressant efficacy.

Another approach to investigate the impact of individual differences in the response to ketamine would be turn to the domain of pharmacogenetics (G. Honey & Bullmore,

2004). A successful fMRI pharmacogenetic study was conducted by Mattay and colleagues (2003) who demonstrated that an individual's brain response to amphetamine in the context of a WM task, was dependent on which polymorphism of the COMT gene they possessed, a gene encoding an enzyme used for the metabolism of dopamine (G. Honey & Bullmore, 2004). More pertinently, a study using transcranial magnetic stimulation, demonstrated that the degree of cortical excitability and plasticity in healthy human volunteers was influenced by the specific genetic polymorphism of the NMDAR NR2B subunit gene GRIN2B present in the individual (Mori et al., 2011). However this study lacks information on how these molecular changes impacted upon cognitive function. Thus, in the context of ketamine research and the role of NMDARs in cognitive function, one could determine whether polymorphisms evident in the NR2B gene affect the brain response to ketamine during a WM or learning task. The NR2B subunit is primarily expressed in the forebrain (Hedegaard, Hansen, Andersen, Brauner-Osborne, & Traynelis, 2012) and polymorphisms within its corresponding gene have been found to be associated with schizophrenia (D. Li & He, 2007). Furthermore, the NR2B subunits are considered to be important in defining the kinetic and functional properties of the NMDAR and "the rate-limiting factor in controlling NMDAR-mediated synaptic plasticity and memory formation" ((F. Li & Tsien, 2009), p.302; (Papouin et al., 2012)). This suggests that investigating the NR2B-gene may be ideal, with regards to the role of the NMDAR in learning and memory processes and its relevance for individually-tailored treatment (i.e. predict who would respond positively and who would respond detrimentally to ketamine administration in terms of cognition).

9.5.7 Acute, Multiple Dose and Chronic Ketamine Administration

As this thesis demonstrates, single dose studies can be enlightening; however it will be important to replicate its findings with the use of higher doses of ketamine. Conducting a dose-response study with ketamine may elucidate which effects of the drug are dose-dependent and which are not, as well as the nature of the dose dependence. Furthermore, the formation of a dose-response curve would enable one to determine at which stage NMDAR antagonism becomes detrimental to performance and associated neurocognitive circuits and at which stage in the process psychotomimetic symptoms appear. By assessing how the glutamatergic system responds to a range of ketamine doses, one may be more able to determine a 'critical' stage in NMDAR hypofunction.

An additional strategy that can be used to determine the effects of NMDAR hypofunction in cognition is to investigate chronic ketamine use. Long-term effects of NMDAR blockade may be more clinically relevant due to the fact that, for example, most schizophrenic patients requiring treatment will have suffered from the disorder for many years. In fact, chronic ketamine use and its effects have been compared to those seen with the acute administration of ketamine and have been suggested to better represent the cognitive deficits seen in schizophrenia (Jentsch & Roth, 1999). However, this advantage may instead depend on which cognitive function one is considering; with acute administration more effective at mimicking the deficit in WM whereas chronic treatment mimics that of episodic memory (C. J. Morgan & Curran, 2006).

For ethical reasons chronic, repeated ketamine administration would not be possible in healthy volunteers, however due to the increase in recreational use of the drug, a population of ketamine users is available for comparison (C. J. Morgan, Muetzelfeldt, et al., 2009). Although many behavioural studies have been conducted involving chronic ketamine users and how their cognitive performance is affected (Freeman et al., 2009; C. J. Morgan, Huddy, Lipton, Curran, & Joyce, 2009; C. J. Morgan, Muetzelfeldt, et al., 2009; C. J. Morgan, Muetzelfeldt, & Curran, 2010; C. J. Morgan, Rees, & Curran, 2008; C. J. Morgan, Rossell, et al., 2006), none as yet have used fMRI in this context. The comparison of the resting- or cognitive-state BOLD response to ketamine in chronic ketamine users set against that of healthy volunteers may elucidate which neural systems are the most vulnerable to NMDAR hypofunction, perhaps narrowing down the search to a more selective set of targets. Further studies could also investigate whether the chronic users who demonstrate a significant brain response to ketamine belong to the cohort of individuals who exhibit the strongest cognitive or psychotic effects or go on to develop a schizotypal disorder, although this development appears relatively rare (C. J. Morgan, Monaghan, et al., 2004). This could perhaps contribute to research regarding factors that predispose one towards or are protective against the effects of NMDAR hypofunction. Although very informative this research can be problematic as the population of chronic ketamine users will most often have a history of additional drug use and the purity of the ketamine taken will not have been tested (J. Krystal et al., 1999).

9.6 Final Conclusions

Glutamate is the main excitatory neurotransmitter in the brain, thus it is of paramount importance to further our understanding of the role it plays in human behaviour and cognition. Pharmacological research in healthy volunteers is essential to this endeavour as enhanced understanding of 'normal' glutamatergic function is necessary as a basis for improved treatment for those suffering from imbalances within this system. However, from a more basic neurobiological perspective, one must emphasise that "no single neurotransmitter is an island" ((Jenkins, 2012), p.1078), thus research with acute ketamine must continue to assess the effects of compounds which influence systems that interact with glutamate. Overall, the findings of this thesis contribute to research into the impact of the glutamatergic system on human cognition and endorse the use of ketamine as a model of glutamatergic dysfunction relevant to psychiatric disorders.

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Appendix A Subjective Ratings

Example scales included in the Visual Analogue Scales questionnaire:

Alert	▬▬▬▬▬▬▬▬▬▬▬▬▬▬▬	**Drowsy**
Calm	▬▬▬▬▬▬▬▬▬▬▬▬▬▬▬	**Excited**

Example questions included in the Psychotomimetic States Inventory questionnaire:

1. You enjoy mixing with people	3 2 1 0
2. You hesitate even when you know what you are going to say	0 1 2 3

Example questions included in the Clinician Administered Dissociative States Scale questionnaire:

1. At this moment in time: Do things seem to be moving in slow motion?

not at all	slightly	moderately	considerably	extremely
0	**1**	**2**	**3**	**4**

2. At this moment in time: Do things seem unreal to you as if you are in a dream?

not at all	slightly	moderately	considerably	extremely
0	**1**	**2**	**3**	**4**

The following figure (A-1) shows the effect sizes for the ketamine response for individual questions on the subjective rating scales. For convenience the 2 largest effect sizes are detailed for each session/questionnaire.
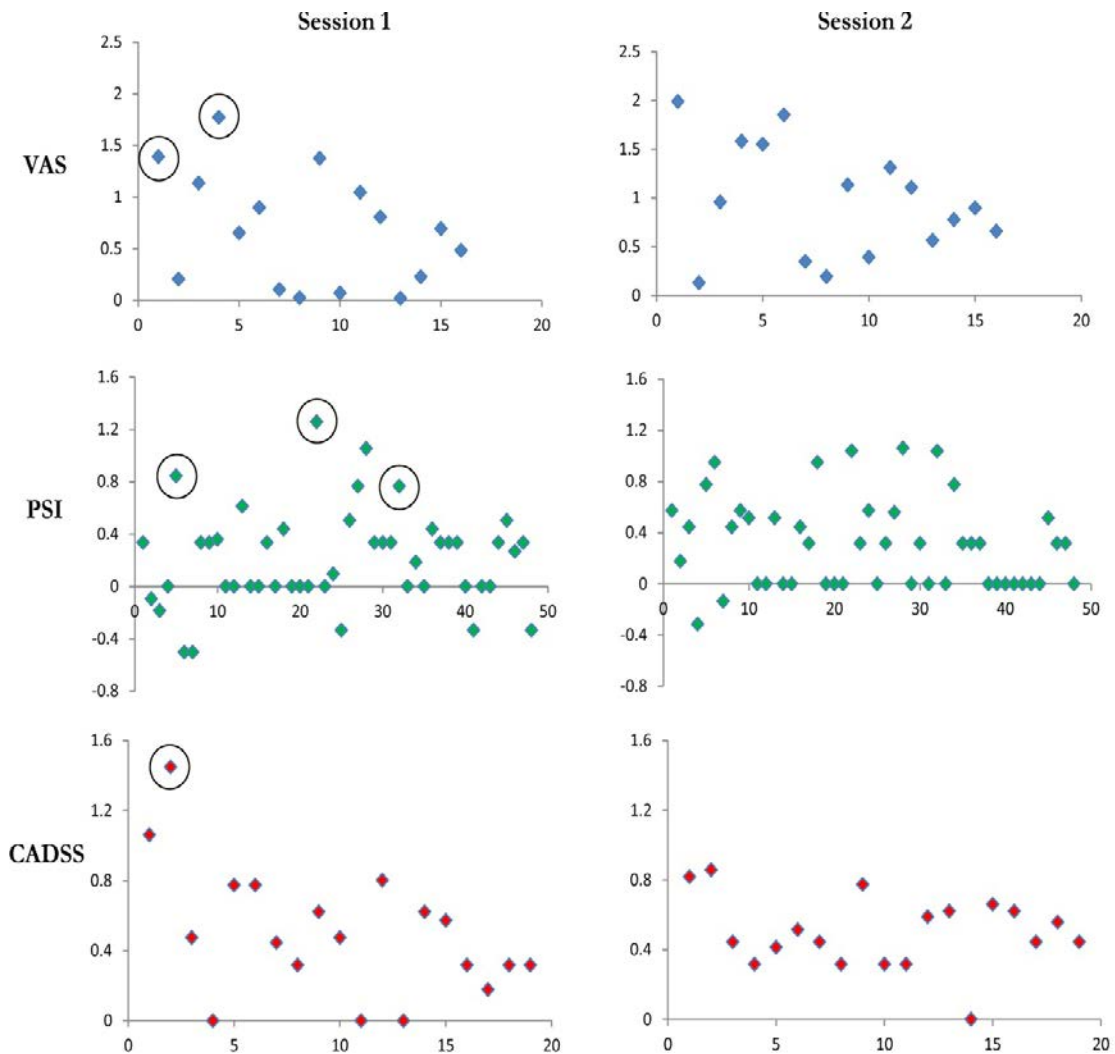
Figure A-1: Session 1 and 2 Cohen's d effect size for Visual Analogue Scales (VAS) 1 – 16 (top row), Psychotomimetic States Inventory (1-48) (middle row) and Clinician Administered Dissociative States Scale (1-19) (bottom row). Effect sizes circled in black indicate the questions chosen for the mini questionnaire.

Visual Analogue Scales (VAS) Points 1 – 16 on the x-axis of figure B-1 (top row) represent the 16 dimensions on the visual analogue scales. The two largest effects sizes are for alert-drowsy (question 1) and muzzy clear-headed (question 4). For session 2, the largest effect sizes were for alert-drowsy (question 1) and lethargic – energetic (question

6). Question 4 (muzzy – clear-headed) gave the next highest effect size and thus when sessions 1 and 2 were considered together questions 1 and 4 were identified for inclusion in the mini-questionnaire for the modulation study.

Psychotomimetic States Inventory (PSI) Points 1 – 48 on the x-axis of figure A-1 (middle row) represent the 48 statements in the PSI. The two largest effects sizes are for 'Your experience of time is unnaturally fast or slow' (question 22) and 'Your hearing has become very sensitive' (question 27). For session 2 the largest effect sizes were for 'Your experience of time is unnaturally fast or slow' (question 22), 'You find it difficult to think clearly' (question 28), and 'You feel as though your head, limbs or body have somehow changed' (question 32).

For the modulation study, questions 22 and 32 were chosen as these statements demonstrated one of the highest effect sizes in both reliability study sessions. Question 28 was not included due to its similarity to the questions provided by the visual analogue scales. Question 5 was thus included instead due to its high effect size in both session A and B.

Clinician Administered Dissociative States Scale (CADSS) Points 1 – 19 on the x-axis of figure A-1 represent the 19 statements in the CADSS. The two largest effects sizes are for 'Do things seem to be moving in slow motion?' (question 1) and 'Do things seem unreal to you as if you are in a dream?' (question 2). The same questions have the highest effect sizes in Session 2. Only question 2 was included for the questionnaire in the modulation study due to the similarity of question 1 to one of the chosen PSI questions.

Appendix B CASL ROI Analysis

Table B-1 Pre-Infusion - Effect of Treatment within the Left Anterior Cingulate

Pairwise Comparisons

Measure: MEASURE_1

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	-1.938	17.988	1.000	-56.555	52.680
	3	22.563	14.022	.771	-20.011	65.136
	4	32.875*	10.763	.048	.196	65.554
2	1	1.938	17.988	1.000	-52.680	56.555
	3	24.500	10.903	.241	-8.605	57.605
	4	34.813	16.724	.330	-15.966	85.591
3	1	-22.563	14.022	.771	-65.136	20.011
	2	-24.500	10.903	.241	-57.605	8.605
	4	10.313	13.023	1.000	-29.229	49.854
4	1	-32.875*	10.763	.048	-65.554	-.196
	2	-34.813	16.724	.330	-85.591	15.966
	3	-10.313	13.023	1.000	-49.854	29.229

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Table B-2 Pre-Infusion ANOVA - Effect of Treatment within the Right medial PFC

Pairwise Comparisons

Measure: MEASURE_1

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
					Lower Bound	Upper Bound
1	2	-4.438	21.520	1.000	-69.779	60.904
	3	34.375	20.437	.680	-27.678	96.428
	4	42.000	14.630	.070	-2.420	86.420
2	1	4.438	21.520	1.000	-60.904	69.779
	3	38.813	12.956	.054	-.525	78.150
	4	46.438	18.113	.130	-8.558	101.433
3	1	-34.375	20.437	.680	-96.428	27.678
	2	-38.813	12.956	.054	-78.150	.525
	4	7.625	15.189	1.000	-38.494	53.744
4	1	-42.000	14.630	.070	-86.420	2.420
	2	-46.438	18.113	.130	-101.433	8.558
	3	-7.625	15.189	1.000	-53.744	38.494

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Table B-3 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x4 repeated measures ANOVA

	Perfusion			
ROIs	**Full Model 2x4 ANOVA**			
	Main Effect of Time		**Time x Treatment Interaction**	
	F[1,15]	**P**	**F[3,45]**	**P**
rACC	26.534	P<0.01	0.761	P=0.522
lACC	23.443	P<0.01	1.919	P=0.140
rSubG	54.764	P<0.01	2.038	P=0.122
lSubG	28.201	P<0.01	1.485	P=0.232
RmPFC	26.479	P<0.01	0.885	P=0.456
LmPFC	35.262	P<0.01	0.971	P=0.415

Appendix C N-Back ROI Analysis

Table C-1 Pre-Infusion ANOVA: Left Caudate Treatment x Load Interaction

Pairwise Comparisons

Measure: MEASURE_1

Load	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference [b] Lower Bound	Upper Bound
1	1	2	-.261	.096	.094	-.552	.030
		3	-.349*	.097	.016	-.643	-.055
		4	-.268	.097	.085	-.561	.025
	2	1	.261	.096	.094	-.030	.552
		3	-.087	.075	1.000	-.316	.142
		4	-.007	.082	1.000	-.257	.243
	3	1	.349*	.097	.016	.055	.643
		2	.087	.075	1.000	-.142	.316
		4	.081	.100	1.000	-.222	.383
	4	1	.268	.097	.085	-.025	.561
		2	.007	.082	1.000	-.243	.257
		3	-.081	.100	1.000	-.383	.222
2	1	2	.041	.106	1.000	-.282	.364
		3	.013	.110	1.000	-.321	.348
		4	.048	.092	1.000	-.230	.327
	2	1	-.041	.106	1.000	-.364	.282
		3	-.028	.083	1.000	-.280	.224
		4	.007	.108	1.000	-.321	.335
	3	1	-.013	.110	1.000	-.348	.321
		2	.028	.083	1.000	-.224	.280
		4	.035	.098	1.000	-.262	.332
	4	1	-.048	.092	1.000	-.327	.230
		2	-.007	.108	1.000	-.335	.321
		3	-.035	.098	1.000	-.332	.262
3	1	2	-.206*	.063	.031	-.398	-.015
		3	.020	.093	1.000	-.263	.302
		4	-.044	.097	1.000	-.339	.250
	2	1	.206*	.063	.031	.015	.398
		3	.226	.091	.152	-.050	.502
		4	.162	.081	.381	-.084	.407
	3	1	-.020	.093	1.000	-.302	.263
		2	-.226	.091	.152	-.502	.050
		4	-.064	.120	1.000	-.427	.299
	4	1	.044	.097	1.000	-.250	.339
		2	-.162	.081	.381	-.407	.084
		3	.064	.120	1.000	-.299	.427

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

*The green circle highlights the variability between placebo conditions at 3-back. Treatment 1 = Placebo_S;Treatment 2 = Placebo_K;Treatment 3 = Risperidone_K;Treatment 4 = Lamotrigine_K

Table C-2 Pre-Infusion ANOVA: Right DLPFC Treatment x Load Interaction

Pairwise Comparisons

Measure: MEASURE_1

Load	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.ᵃ	95% Confidence Interval for Difference ᵃ	
						Lower Bound	Upper Bound
1	1	2	-.353	.201	.600	-.964	.258
		3	-.590	.262	.241	-1.386	.207
		4	-.435	.267	.738	-1.245	.374
	2	1	.353	.201	.600	-.258	.964
		3	-.237	.190	1.000	-.814	.340
		4	-.083	.203	1.000	-.700	.535
	3	1	.590	.262	.241	-.207	1.386
		2	.237	.190	1.000	-.340	.814
		4	.154	.189	1.000	-.419	.728
	4	1	.435	.267	.738	-.374	1.245
		2	.083	.203	1.000	-.535	.700
		3	-.154	.189	1.000	-.728	.419
2	1	2	.410	.207	.400	-.220	1.039
		3	.252	.231	1.000	-.448	.952
		4	.359	.240	.940	-.372	1.089
	2	1	-.410	.207	.400	-1.039	.220
		3	-.157	.186	1.000	-.721	.406
		4	-.051	.280	1.000	-.902	.800
	3	1	-.252	.231	1.000	-.952	.448
		2	.157	.186	1.000	-.406	.721
		4	.106	.296	1.000	-.792	1.005
	4	1	-.359	.240	.940	-1.089	.372
		2	.051	.280	1.000	-.800	.902
		3	-.106	.296	1.000	-1.005	.792
3	1	2	.201	.220	1.000	-.467	.868
		3	.243	.162	.923	-.248	.735
		4	-.137	.239	1.000	-.861	.587
	2	1	-.201	.220	1.000	-.868	.467
		3	.042	.237	1.000	-.678	.763
		4	-.338	.253	1.000	-1.106	.431
	3	1	-.243	.162	.923	-.735	.248
		2	-.042	.237	1.000	-.763	.678
		4	-.380	.297	1.000	-1.283	.523
	4	1	.137	.239	1.000	-.587	.861
		2	.338	.253	1.000	-.431	1.106
		3	.380	.297	1.000	-.523	1.283

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Table C-3 Pre-Infusion ANOVA: Right Thalamus Treatment x Load Interaction

Pairwise Comparisons

Measure: MEASURE_1

Load	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b] Lower Bound	Upper Bound
1	1	2	-.536	.200	.103	-1.143	.072
		3	-.761*	.219	.020	-1.425	-.097
		4	-.564	.203	.084	-1.181	.052
	2	1	.536	.200	.103	-.072	1.143
		3	-.225	.144	.826	-.662	.211
		4	-.029	.179	1.000	-.572	.515
	3	1	.761*	.219	.020	.097	1.425
		2	.225	.144	.826	-.211	.662
		4	.197	.129	.881	-.194	.587
	4	1	.564	.203	.084	-.052	1.181
		2	.029	.179	1.000	-.515	.572
		3	-.197	.129	.881	-.587	.194
2	1	2	.208	.161	1.000	-.279	.695
		3	.120	.172	1.000	-.402	.642
		4	.149	.198	1.000	-.452	.750
	2	1	-.208	.161	1.000	-.695	.279
		3	-.088	.168	1.000	-.597	.421
		4	-.059	.195	1.000	-.650	.532
	3	1	-.120	.172	1.000	-.642	.402
		2	.088	.168	1.000	-.421	.597
		4	.029	.197	1.000	-.571	.628
	4	1	-.149	.198	1.000	-.750	.452
		2	.059	.195	1.000	-.532	.650
		3	-.029	.197	1.000	-.628	.571
3	1	2	-.198	.147	1.000	-.645	.249
		3	-.062	.215	1.000	-.716	.592
		4	-.166	.178	1.000	-.707	.376
	2	1	.198	.147	1.000	-.249	.645
		3	.136	.252	1.000	-.629	.901
		4	.032	.166	1.000	-.471	.536
	3	1	.062	.215	1.000	-.592	.716
		2	-.136	.252	1.000	-.901	.629
		4	-.104	.161	1.000	-.594	.386
	4	1	.166	.178	1.000	-.376	.707
		2	-.032	.166	1.000	-.536	.471
		3	.104	.161	1.000	-.386	.594

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Table C-4 Post-Infusion ANOVA: Right DLPFC Treatment x Load Interaction

Pairwise Comparisons

Measure: MEASURE_1

Load	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a] Lower Bound	95% Confidence Interval for Difference[a] Upper Bound
1	1	2	-.435	.222	.413	-1.110	.239
		3	-.340	.181	.481	-.890	.210
		4	-.375	.236	.794	-1.090	.340
	2	1	.435	.222	.413	-.239	1.110
		3	.095	.189	1.000	-.479	.670
		4	.060	.241	1.000	-.672	.793
	3	1	.340	.181	.481	-.210	.890
		2	-.095	.189	1.000	-.670	.479
		4	-.035	.188	1.000	-.606	.536
	4	1	.375	.236	.794	-.340	1.090
		2	-.060	.241	1.000	-.793	.672
		3	.035	.188	1.000	-.536	.606
2	1	2	-.006	.233	1.000	-.713	.702
		3	-.008	.209	1.000	-.643	.627
		4	-.061	.246	1.000	-.808	.686
	2	1	.006	.233	1.000	-.702	.713
		3	-.002	.157	1.000	-.480	.476
		4	-.056	.149	1.000	-.508	.397
	3	1	.008	.209	1.000	-.627	.643
		2	.002	.157	1.000	-.476	.480
		4	-.053	.154	1.000	-.520	.414
	4	1	.061	.246	1.000	-.686	.808
		2	.056	.149	1.000	-.397	.508
		3	.053	.154	1.000	-.414	.520
3	1	2	.299	.217	1.000	-.361	.959
		3	.258	.152	.657	-.202	.718
		4	-.205	.239	1.000	-.930	.520
	2	1	-.299	.217	1.000	-.959	.361
		3	-.041	.182	1.000	-.593	.512
		4	-.504	.288	.605	-1.378	.371
	3	1	-.258	.152	.657	-.718	.202
		2	.041	.182	1.000	-.512	.593
		4	-.463	.222	.327	-1.137	.211
	4	1	.205	.239	1.000	-.520	.930
		2	.504	.288	.605	-.371	1.378
		3	.463	.222	.327	-.211	1.137

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

*Treatment 1 = P_Saline/Treatment 2 = P_Ketamine/Treatment 3 = R_Ketamine/Treatment 4 = L_Ketamine

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Table C-5 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x4x3 repeated measures ANOVA

ROIs	2x4x3 ANOVA (time)					
	Main Effect of Time		Time x Treatment Interaction		Time x Treatment x Load Interaction	
	F[1,15]	P	F[3,45]	P	F[6,90]	P
rDLPFC	3.704	p=0.073	0.25	p=0.861	0.754	p=0.608
lDLPFC	0.644	p=0.435	0.483	p=0.696	1.371	p=0.235
rCING	1.098	p=0.311	0.924	p=0.437	1.015	p=0.421
lCING	1.645	p=0.219	0.987	p=0.407	1.562	p=0.167
rCAU	10.777	p<0.01	1.233	p=0.309	0.951	p=0.463
lCAU	17.962	p<0.01	0.398	p=0.755	2.344	p<0.05
rTHAL	6.836	p<0.05	1.742	p=0.172	2.569	p<0.05
lTHAL	6.339	p<0.05	1.163	p=0.334	0.582	p=0.744
rHIPP	6.492	p<0.05	1.455	p=0.240	1.761	p=0.116
lHIPP	1.021	p=0.328	1.382	p=0.260	1.491	p=0.190

*Treatment 1 = Placebo Arm /Treatment 2 = Ketamine Arm/ Treatment 3 = Risperidone Arm/ Treatment 4 = Lamotrigine Arm

Table C-6 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x2x3 repeated measures ANOVA including the placebo and ketamine treatment arms – Left Cingulate Time x Treatment x Load Interaction

Pairwise Comparisons

Measure: MEASURE_1

Time	Load	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference [a]	
							Lower Bound	Upper Bound
1	1	1	2	-.154	.154	.334	-.482	.175
		2	1	.154	.154	.334	-.175	.482
	2	1	2	.333	.193	.104	-.077	.744
		2	1	-.333	.193	.104	-.744	.077
	3	1	2	.045	.130	.735	-.232	.322
		2	1	-.045	.130	.735	-.322	.232
2	1	1	2	.170	.149	.272	-.148	.488
		2	1	-.170	.149	.272	-.488	.148
	2	1	2	-.059	.102	.569	-.276	.158
		2	1	.059	.102	.569	-.158	.276
	3	1	2	.252	.121	.055	-.006	.509
		2	1	-.252	.121	.055	-.509	.006

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

*Treatment 1 = Placebo Arm /Treatment 2 = Ketamine Arm

Table C-7 and C-8 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x2x3 repeated measures ANOVA including the placebo and ketamine treatment arms – Right Thalamus Time x Treatment x Load Interaction

Pairwise Comparisons

Measure: MEASURE_1

Time	Load	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.^{b}	95% Confidence Interval for Difference^b	
							Lower Bound	Upper Bound
1	1	1	2	-.536*	.200	.017	-.962	-.109
		2	1	.536*	.200	.017	.109	.962
	2	1	2	.208	.161	.214	-.134	.550
		2	1	-.208	.161	.214	-.550	.134
	3	1	2	-.198	.147	.199	-.512	.116
		2	1	.198	.147	.199	-.116	.512
2	1	1	2	.049	.174	.784	-.322	.420
		2	1	-.049	.174	.784	-.420	.322
	2	1	2	.114	.187	.552	-.285	.514
		2	1	-.114	.187	.552	-.514	.285
	3	1	2	.116	.169	.502	-.244	.477
		2	1	-.116	.169	.502	-.477	.244

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Pairwise Comparisons

Measure: MEASURE_1

Treatment	Load	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.^{b}	95% Confidence Interval for Difference^b	
							Lower Bound	Upper Bound
1	1	1	2	-.468*	.135	.004	-.756	-.179
		2	1	.468*	.135	.004	.179	.756
	2	1	2	.255	.191	.202	-.152	.661
		2	1	-.255	.191	.202	-.661	.152
	3	1	2	.033	.187	.861	-.366	.433
		2	1	-.033	.187	.861	-.433	.366
2	1	1	2	.117	.130	.381	-.159	.393
		2	1	-.117	.130	.381	-.393	.159
	2	1	2	.161	.157	.322	-.174	.496
		2	1	-.161	.157	.322	-.496	.174
	3	1	2	.348	.247	.180	-.180	.875
		2	1	-.348	.247	.180	-.875	.180

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Table C-9 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x2x3 repeated measures ANOVA including the ketamine and risperidone treatment arms – Right Hippocampus Time x Treatment Interaction

Pairwise Comparisons

Measure: MEASURE_1

Treatment	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	1	2	.207*	.082	.024	.032	.383
	2	1	-.207*	.082	.024	-.383	-.032
2	1	2	.046	.064	.482	-.090	.182
	2	1	-.046	.064	.482	-.182	.090

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

*Treatment 1 = Ketamine Arm/ Treatment 2 = Risperidone Arm

Appendix D PAL ROI Analysis

Table D-1 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x4 repeated measures ANOVA including all treatment arms during encoding

	ENCODING			
ROIs	**Full Model 2x4 ANOVA**			
	Main Effect of Time		**Time x Treatment Interaction**	
	F[1,15]	**P**	**F[3,45]**	**P**
rDLPFC	0.478	P=0.500	2.090	P=0.115
lDLPFC	0.895	P=0.359	2.445	P=0.076
rVLPFC	0.282	P=0.603	1.768	P=0.167
lVLPFC	1.228	P=0.285	1.139	P=0.344
rPCC	1.330	P=0.267	2.044	P=0.121
lPCC	1.045	P=0.323	1.063	P=0.374
rPHIPP	0.540	P=0.474	0.738	P=0.535
lPHIPP	0.298	P=0.593	2.514	P=0.070
aRHIPP	2.941	P=0.107	1.006	P=0.399
aLHIPP	0.131	P=0.722	0.138	P=0.937

Table D-2 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x4 repeated measures ANOVA including the ketamine and lamotrigine treatment arms during encoding – Right DLPFC Time x Treatment Interaction

Pairwise Comparisons

Measure: MEASURE_1

Treatment	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
1	1	2	.301	.260	.265	-.253	.856
	2	1	-.301	.260	.265	-.856	.253
2	1	2	-.380	.256	.158	-.925	.165
	2	1	.380	.256	.158	-.165	.925

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Table D-3 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x4 repeated measures ANOVA including the ketamine and lamotrigine treatment arms during encoding – Left DLPFC Time x Treatment Interaction

Pairwise Comparisons

Measure: MEASURE_1

Treatment	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
						Lower Bound	Upper Bound
1	1	2	.609*	.240	.023	.098	1.120
	2	1	-.609*	.240	.023	-1.120	-.098
2	1	2	-.365	.214	.108	-.821	.090
	2	1	.365	.214	.108	-.090	.821

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Table D-4 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x4 repeated measures ANOVA including the ketamine and lamotrigine treatment arms during encoding – Right VLPFC Time x Treatment Interaction

Pairwise Comparisons

Measure: MEASURE_1

Treatment	(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[a]	95% Confidence Interval for Difference[a]	
						Lower Bound	Upper Bound
1	1	2	.149	.239	.543	-.361	.659
	2	1	-.149	.239	.543	-.659	.361
2	1	2	-.391	.208	.079	-.834	.052
	2	1	.391	.208	.079	-.052	.834

Based on estimated marginal means

a. Adjustment for multiple comparisons: Bonferroni.

Table D-5 The Influence of Pre-Infusion Conditions on Post-Infusion Modulation Effects: a 2x4 repeated measures ANOVA including all treatment arms during retrieval

	RETRIEVAL			
ROIs	Full Model 2x4 ANOVA			
	Main Effect of Time		Time x Treatment Interaction	
	F[1,15]	P	F[3,45]	P
rDLPFC	0.08	P=0.782	0.694	P=0.561
lDLPFC	0.844	P=0.373	0.598	P=0.620
rVLPFC	0.031	P=0.862	0.348	P=0.791
lVLPFC	0.280	P=0.604	0.334	P=0.801
rPCC	0.134	P=0.720	1.364	P=0.266
lPCC	0.002	P=0.961	0.284	P=0.837
rPHIPP	0.693	P=0.418	0.311	P=0.817
lPHIPP	0.424	P=0.525	1.520	P=0.222
aRHIPP	0.092	P=0.765	1.556	P=0.213
aLHIPP	0.890	P=0.360	1.844	P=0.146

APPENDIX E INFORMATION SHEETS AND ETHICS APPROVAL

I. **Information Sheet (Reliability Study)**

INFORMATION SHEET FOR HEALTHY VOLUNTEERS

STUDY TITLE

Pharmacological validation of an NMDA model of brain dysfunction

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A study conducted by the Centre for Neuroimaging Sciences at the Institute of Psychiatry and sponsored by Eli Lilly.

Principal Contact for general queries or in the event of adverse effects: **Dr Mitul Mehta,** Centre for Neuroimaging Sciences, Institute of Psychiatry, London SE5 8AF, Telephone: 020 3228 3053/3058

We would like to invite you to take part in a research study at the Centre for Neuroimaging Sciences. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. The information is separated into two parts. Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you do decide to take part, you will be given a copy of this document to take home with you. If you would like independent information on taking part in drug studies or to look at some questions to ask we can provide you with a leaflet or you can look at the following websites:

www.ccra.org.uk/CCRA/ParticipatingInTrials.htm, www.ctu.mrc.ac.uk/TakePart.asp

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PART I

Purpose of the research

Brain imaging is currently used for a number of reasons including understanding where in the brain medicines have their effects. The purpose of this study is to establish and confirm a brain imaging

methodology to assess novel drugs for treatment in psychiatric disorders such as schizophrenia. In order to achieve this we will use the prescription anaesthetic drug ketamine to activate particular regions of your brain. This is an established model for looking at features that are common in psychiatric disorders like schizophrenia. We plan to assess the sensitivity of different brain imaging techniques to ketamine. We are doing this study to help us determine how useful these brain imaging methods may be for assessing the effects of existing and future medicines on brain function in patients. Such methods could then be used in the development of new medicines and may help us understand how they might benefit patients with various psychiatric disorders.

Do I have to take part?

Participation in the study is voluntary and it is up to you to decide whether or not to take part. We will describe the study to you, and go through this information sheet, which you can take away with you. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason.

What is involved?

The study will involve three separate visits to the Centre for Neuroimaging Sciences. The first will last about 2-3 hours and the other two will last about 4 hours each. Each visit will be separated by at least one week. Your involvement in this study will take between 4 and 8 weeks (depending on your availability and the sessions available at the research centre). The overall study will be on-going for approximately 18 months. We aim to have 10 volunteers complete the study.

Screening Visit

The first visit is a screening visit. The study will be explained to you and you will be asked to sign the consent form. A copy of this form will be given to you to keep. The screening visit will include answering questions about your health, a physical examination, checking your blood pressure and pulse rate, taking a recording of your heart called an ECG (electrocardiogram) and measuring your weight. We will also need to take samples of your blood and urine which will be tested in the laboratory. The urine sample will be used to check for any drugs of abuse and we will also test your breath for alcohol. These tests are important to confirm that your body will handle the study medication we give you in a normal manner. We request that you take part in these screening procedures so we can gain an understanding of your state of health and to ensure your safety during the scanning phase of the study. The samples we collect from you will only be used for the purposes described and will not be stored.

On the screening visit we will also request that you take part in a structured clinical interview which will include questions about your mental health, and complete some subjective questionnaires about your mood. This is so that we can ensure that all volunteers are suitable for the study. In the study we will be using a magnetic field to help generate pictures of your brain. For that reason you must not have a scan if you have received metal-associated injuries to your eyes, had metallic objects (including clips)

inserted into your body during an operation, or if you have received a gun shot injury or have a heart pace maker. The study physician will go through a list of possible risks with you at screening as will the person conducting the scan before you go into the scanner. If you take part in the study you will be asked to complete some tasks on the computer. During screening there will be a practice session of these tasks in which you will receive instructions on what you will be asked to do while you are in the scanner. You will be asked to look at a computer screen and move a joystick in response to certain images. You will also be given the opportunity to lie in a mock-up scanner at the Centre for Neuroimaging Sciences before lying in the real scanner, to help you become accustomed to the scanning environment. These screening assessments together with specific questions we will ask, will confirm your eligibility for participation in the study.

Study days

If we are satisfied with the screening procedures and you are eligible to participate in the study we will invite you to take part in two subsequent scanning visits on two separate occasions at least 1 week apart. We will arrange these dates so that they are convenient for you. Each of these two study visits will include identical study assessments and procedures. On each visit we will first give you a brief interview to confirm that you are still suitable to take part and that you understand the study procedures, conduct a brief physical examination and test your urine for drugs of abuse and check the alcohol levels in your breath. If these are all normal we will continue with the visit. On each occasion we will give you one medicine, ketamine, delivered directly into the blood by a programmable intravenous infusion pump (see below for more information about the possible side effects of this medication). Ketamine is a short-acting anaesthetic drug, which works by decreasing the effects of glutamate, a substance that exists in the brain. In order to be able to connect the infusion pump we will insert a small plastic tube into a vein in your arm (a cannula). This may cause slight discomfort at the insertion site and occasionally bruising. All volunteers will receive the ketamine infusion. Both you and the investigators will know what you have taken on each day. We will plan the infusion to give steady drug levels in your blood of a maximum of 75ng/ml. This is much lower (less than 10%) than doses used to induce anaesthesia with this drug. Scanning will take place before and after you have been given the ketamine dose. During the study day you will also be asked to complete some brief questionnaires which assess your subjective state (mood, alertness, etc).

Each scanning session will consist of two parts, in each part you will undergo the same procedures and be asked to complete the same computer tasks. In the first part, which will last approximately one and a half hours, we will collect pictures (scans) of your head while you are resting and whilst you are performing some computer tasks involving memory and attention. These tasks will involve looking at letters or pictures and trying to remember them. You will not be given any ketamine during this first part. After performing the tasks there will be a short break during which the ketamine infusion pump

will be set up and attached to the cannula in your arm. The second part will then start. We will collect some more pictures of your head while you are resting, and this will not take longer than 30 minutes.

We use a very modern method of scanning known as Magnetic Resonance Imaging (MRI). This technique is commonly used to diagnose a number of diseases, but in this case it has been adapted to take images of which parts of your brain are active when you perform a task. When a part of your brain is more active, more blood flows to that region and this change is captured on the photos that we take. We will make a map of which parts of your brain has more blood flow than others.

In order for us to take pictures of your brain, you will have to lie as still as possible in the MRI scanner. The scanner consists of a powerful magnet, but you will not feel any force or special sensation inside a magnetic field because your body is insensitive to it. Because of the magnetic field, you must not have a scan if you have received metal injuries to your eyes, had metallic objects (including clips) inserted into your body during an operation, or if you have received a gun shot injury or have a heart pace maker. The radiographer will go through a list of possible risks with you before you go into the scanner, identifying with you any reasons why you should not be scanned. Please note that MRI scans do not involve any form of ionising radiation (X-rays), but the scanner itself can be quite claustrophobic; therefore please inform us if you have a fear of enclosed spaces.

All the time you are in the scanner there will be a microphone switched on so you can talk to us. We will talk to you regularly to explain what will happen next. Some people find the machine a little noisy, but the headphones we provide allow adequate noise protection for most people.

During each study visit we will take 2 blood samples which will be used to measure the levels of ketamine in your body. One of these samples will be collected while you are lying in the scanner. These samples will either be collected by simple needle into a vein or through the cannula in your arm (see above). The samples we collect from you will only be used for the purposes described and will not be stored. While you are in the scanner we will also monitor your heart rate and respiration rate. At intervals throughout the day we will also check your blood pressure and heart rate outside the scanner while you are lying down and again while you are standing up, and ask you to rate how you are feeling using some questionnaires. If you feel unwell for what ever reason during the course of the day you should let one of the study team know.

What will I have to do?

If you decide to take part in this research study we ask that you visit the Centre for Neuroimaging Sciences on 3 separate occasions. Before each visit we ask that you (1) do not drink alcohol, take products containing caffeine or engage in strenuous exercise (e.g. heavy lifting, aerobics) for 24 hours (2) eat nothing but a light breakfast (e.g. bowl of cereal, or two pieces of toast, nothing high in fat) between midnight and your arrival at the centre for neuroimaging sciences on the study days (not

screening) and (3) do not eat anything containing grapefruit from midnight the night before the study day (4) abstain from nicotine- or tobacco- containing products for at least 4 hours before arrival at the Centre. During the study day we will provide breakfast and lunch and ask you to abstain from nicotine-, tobacco- or caffeine- containing products until you are discharged home. After each study day we will ask you to avoid alcohol and driving or operating heavy machinery for at least 24 hours after receiving each dose because small levels of ketamine may still be present in your system.

If you fall ill or need to take any medication through the course of the study you should notify the researcher as soon as possible.

Importantly, if you have private health insurance you should contact the company to inform them that you are taking part in a research study to ensure it does not affect your cover.

Will I experience any side effects?

Clinical Procedures

Taking blood or siting the cannula are well-tolerated procedures, although you may experience some minor discomfort, minimal bleeding or bruising in your arm.

Ketamine is commonly used as an anaesthetic. You should be aware that it is sometimes used socially and can lead to dependence. Ketamine can affect your blood pressure so you will not be included in the study if you have a history of high blood pressure or other heart problems. When on the drug you may feel your heart beating faster. At high doses ketamine can also cause strange experiences (such as feeling disconnected from your body or other distortions of sense such as hallucinations). Importantly the doses to be used in this study have been used previously in healthy volunteers, are well-tolerated, and are much lower than the doses used in anaesthesia. The strange experiences typically occur at doses higher than those used in this study. If they should occur you may find them unpleasant and frightening. If this happens the infusion will be stopped immediately and any strange experiences should rapidly fade. You may also feel nauseous when we stop the infusion and slightly clumsy or drunk for about 1 hour. A qualified physician will always be present during the study periods, and will examine you before you leave for the day. In the unlikely event that you are unfit to leave the imaging centre you will be admitted to The Maudsley Hospital for overnight monitoring.

The drug information leaflet for ketamine has been included with this document. The document contains a more detailed list of potential side-effects which are more rare. If you do experience any side effects please contact Dr. Mitul Mehta on 0203 228 3084/3053 immediately.

Will I benefit from my participation?

We do not expect that you will draw any specific personal benefit apart from a payment of £250 to compensate for your time. If we decide that you are not suitable during the screening session we will pay you £50 for your time. Also, all your travel expenses will be reimbursed.

What do I do if I want to withdraw from the study?

From our previous experience in studies of this type we do not anticipate that you will have any problems but if you do, we want to assure you that you are free to withdraw from the study at any time you like. You will not be required to give us any reasons for withdrawal from the study but please inform us as soon as possible if you wish to do so.

Will my participation be kept confidential?

If you agree to take part in this study your General Practitioner (GP) will be contacted to inform them of your participation. You will not be identified in our computers by name but by a number, and all records obtained while your are in this study, including related health records will remain strictly confidential at all times . A copy of this 'Information Sheet' and of the signed 'Consent Form' will be given to you to keep. However, a copy of your consent will be made available to others working on the study at the Institute of Psychiatry and Eli Lilly, the Independent Ethics Committee members and Medicines Regulatory Authorities. More information on confidentiality is given in Part II of this information sheet.

In this trial, personal and in particular medical data will be collected, stored and analysed. The sponsor will use this study data for research purposes to support the scientific objectives of the study described in the consent document, to assess the safety of any drug or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies. This information will be handled in a strictly confidential manner by your doctor in charge and staff. The uses and disclosure of your personal health information follows legal requirements and needs your permission to this Data Privacy Statement (authorisation), i.e. without your permission you are not allowed to take part in this study.

Information from this study will be reported to the Sponsor and to official drug regulatory authorities, and governmental agencies in other countries, where the study drug may be considered for marketing approval.

Under the data protection laws Eli Lilly and Company is the controller of your personal data. Your personal data (including sensitive personal data) may need to be transferred within Eli Lilly and Company, to others working on their behalf and to the medicines regulatory authorities inside and outside the European Union (EU), including the USA, where data protection laws may not be as stringent as in the EU. Eli Lilly and Company will take steps to ensure your personal data is protected and by agreeing to take part in this study you give your permission for these transfers.

You may withdraw your permission at any time by providing written notice to the study doctor. The study doctor and staff would then no longer use or share your personal health information in connection with this study, unless it is essential to ensure that the study is scientifically reliable. However, the sponsor would still use study data that was collected before you withdrew your permission. In addition, you would no longer be able to participate in the study.

If you agree to participate in the research study, your personal health information will be used and shared in the following ways:

1. The study doctor and staff will send your study-related health information ("study data") to the sponsor of the study, its associated companies and its representatives ("the sponsor"). Because the sponsor conducts business related to clinical research in many countries around the world, this may involve sending your study data outside of the UK and Europe. The sponsor has joined the United States Safe Harbour program. Participation in the Safe Harbour program amounts to a commitment by the sponsor to the protection of personal information in accordance with the data protection principles set down by UK and European data privacy law.

2. The sponsor will use the study data for research purposes to support the scientific objectives of the study described in the consent document, to assess the safety or efficacy of any drug or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies.

3. Your study data, either alone or combined with data from other studies, may be shared with regulatory authorities in this country and other countries including the United States, and the Ethics Committee that reviewed this study.

4. Study data that does not identify you may be published in medical journals or shared with others as part of scientific discussions.

5. Your original medical records, which may contain information that directly identifies you, may be reviewed by the sponsor, the Ethics Committee that reviewed this study, and regulatory authorities in this country and/or other countries including the United States.

The sponsor works with business partners in drug development. The sponsor may share your study data with these business partners, but only if the business partner signs a contract that requires it to protect your study data in the same way as the sponsor.

You have the right to see and copy your personal health information related to the research study for as long as the study doctor or research institution holds this information. However, to ensure the scientific integrity of the study, you will not be able to review some of your personal health information related to the study, until after the study has been completed.

If you have any questions about matters related to the study please contact Dr. Mitul Mehta on 020 3228 3058/3053.

PART II

What if relevant new information becomes available?

Sometimes we get new information about medicines being used in research. This is unlikely for the drug to be used in this study because they have been in general use for many years. However, if this happens, a member of the research team will tell you and discuss whether you should continue in the study. If you decide to continue in the study he/she may ask you to sign an updated consent form. If the study is stopped for any other reason, we will tell you and explain the reasons why this has occurred.

Although unlikely, it is possible that whilst performing normal medical checks we may identify a significant abnormality that you didn't realise you had. If this occurs a study physician will discuss the finding with you and we will inform your GP. Eli Lilly and Company (the Sponsor), may stop the study or your participation in the study at any time, for any reason, without your consent. This may be due to an adverse reaction or other factors which relate to your safety or well-being. A full explanation will be given to you should this be necessary. MRI scans will be reviewed by a specialist and any significant abnormalities will be reported to the study investigators.

What will happen if I don't want to carry on with the study?

If you withdraw from the study we will retain and continue to use any data collected before such withdrawal of consent unless you request that you do not want us to use any data collected from you.

What if there is a problem?

While we do not expect you to suffer any health problems by taking part in this study, Eli Lilly, the study's sponsor may compensate anyone whose health suffers as a result of participation. You do not have to prove it was anyone's fault; if the health problem arose because of your participation in this study, you will be compensated.

We accept that this study is being conducted subject to the Association of the British Pharmaceutical Industry (ABPI) guidelines, "Clinical Trial Compensation Guidelines," relating to compensation for injury arising in the course of clinical trials, a copy of which is available on request.

If you have a concern about any aspect of this study, please ask to speak to any of the researchers. We will do our best to answer your questions. If you remain unhappy and wish to complain formally, you can write to the Dean of the Institute of Psychiatry. Please write to The Executive Assistant to the Dean, Institute of Psychiatry, Box P001, London SE5 8AF.

Will my taking part in this study be kept confidential?

With your consent your GP will be told that you have decided to take part in this study. All information obtained during the study, as well as related health records, will remain strictly confidential at all times. However, these may need to be made available to others working on the Institute of Psychiatry or Eli Lilly's behalf, the Ethics Committee members and medicines Regulatory Authorities.

By signing the consent form you agree to this access for the current study and any further research that may be done. However, the Institute of Psychiatry and Eli Lilly will take steps to protect your personal information and will not include your name on any sponsor forms, reports, publications, or in any future disclosures. If you withdraw from the study, we will no longer collect your personal information, but we may need to continue to use information already collected. The study information collected will be sent to other locations outside of the UK, but you will not be referred to by name or identified in any report or publication nor could the information be traced back to you. This will be for healthcare and/or medical research purposes only. Your data will only be shared with countries where data protection laws are comparable to those in the UK. However, the Institute of Psychiatry and Eli Lilly maintain high standards of confidentiality and protection. The Institute of Psychiatry (who will control the use of the data) will take steps to ensure your personal data is protected. These will include not sharing any information which could potentially lead to someone learning your identity. This information will be kept in accordance with the data protection act. Additional steps taken to ensure anonymity will include editing brain scanning data to ensure that no facial features could be reconstructed from the data.

YOUR RIGHTS UNDER ANY APPLICABLE DATA PROTECTION LAWS ARE NOT AFFECTED

What will happen to any samples I give?

We will ask you to provide a number of urine and blood samples for this study. During the screening visit we will take up to 20mL of blood from a vein in your arm and a urine sample. During subsequent visits we will take no more than 21mL of blood across all time points to measure the drug levels in your body. Overall we will not take more than 62mL of blood over the course of the study. Once the samples have been analysed, they will be destroyed.

What will happen to the results of the research study?

The results of this research will be published as scientific reports and maybe presented at meetings within the Institute of Psychiatry or Eli Lilly or at international scientific meetings. You will not be identified in any report or publication that results from this study.

Who is organising and funding the research?

The research is being organized as a collaborative study between the Institute of Psychiatry and the pharmaceutical company Eli Lilly, who is sponsoring the study. The researchers involved in conducting this study do not receive any financial incentives for including you in this study and do not benefit financially from this study.

Who has reviewed the study?

This research study has been looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Wandsworth Research Ethics Committee.

If you have any questions about matters related to the study please contact Dr. Mitul Mehta on 020 3228 3053/3058.

II. Information Sheet (Modulation Study)

INFORMATION SHEET FOR HEALTHY VOLUNTEERS

STUDY TITLE

Pharmacological validation of an NMDA model of brain dysfunction

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A study conducted by the Centre for Neuroimaging Sciences at the Institute of Psychiatry sponsored by Eli Lilly.

Principal Contact for general queries or in the event of adverse effects: **Dr Mitul Mehta,** Centre for Neuroimaging Sciences, Institute of Psychiatry, London SE5 8AF, Telephone: 020 7919 3053

We would like to invite you to take part in a research study at the Centre for Neuroimaging Sciences. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. The information is separated into two parts. Part 1 tells you the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. If you do decide to participate, you will be given a copy of this document to take home with you. If you would like independent information on taking part in drug studies or to look at some questions to ask we can provide you with a leaflet or you can look at the following websites:

www.ccra.org.uk/CCRA/ParticipatingInTrials.htm, www.ctu.mrc.ac.uk/TakePart.asp

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PART I

Purpose of the research

Brain imaging is currently used for a number of reasons including understanding where in the brain medicines have their effects. The purpose of this study is to establish and confirm a brain imaging methodology to assess novel drugs for treatment in psychiatric disorders such as schizophrenia. In order to achieve this we will use the prescription anaesthetic drug ketamine to activate particular regions of your brain. This is an established model for looking at features that are common in psychiatric disorders like schizophrenia. We plan to assess the sensitivity of different brain imaging techniques to ketamine and test the reversal of ketamine's effects with two marketed prescription drugs, lamotrigine used to treat fits (epilepsy) and risperidone used to treat psychiatric conditions. We are doing this study to help us determine how useful these brain imaging methods may be for assessing the effects of medicines on brain function in patients. Such methods could then be used in the development of new medicines and may help us understand how they might benefit patients with various psychiatric disorders.

Do I have to take part?

Participation in this study is voluntary and it is up to you to decide whether or not to take part. We will describe the study to you and go through this information sheet, which you can take away with you. We will then ask you to sign a consent form to show you have agreed to take part. You are free to withdraw at any time, without giving a reason.

What is involved?

The study will involve five separate visits to the Centre for Neuroimaging Sciences. The first will last about 3 hours and the other four will last about 7 hours each. Each visit will be separated by at least 10 days. Your involvement in this study will take between 5 and 15 weeks (depending on your availability and the sessions available at the research centre). The overall study will be on-going for approximately 18 months. We aim to have 16 volunteers complete the study.

Screening Visit

The first visit is a screening visit. The study will be explained to you and you will be asked to sign the consent form. A copy of this form will be given to you to keep. The screening visit will include answering questions about your health, having a physical examination, checking your blood pressure and pulse rate, taking a recording of your heart called an ECG (electrocardiogram) and measuring your weight. We will also need to take samples of your blood and urine which will be tested in the laboratory. The urine sample will be used to check for any drugs of abuse and we will also test your breath for alcohol. These tests are important to confirm that your body will handle the drugs we give you in a normal manner. We request that you take part in these screening procedures so we can gain an

understanding of your state of health and to ensure your safety during the scanning phase of the study. The samples we collect from you will only be used for the purposes described and will not be stored.

On the screening visit we will also request that you take part in a structured clinical interview which will include questions about your mental health, and complete some subjective questionnaires about your mood. This is so that we can ensure that all volunteers are suitable for the study. In the study we will be using a magnetic field to help generate pictures of your brain. For that reason you must not have a scan if you have received metal injuries to your eyes, had metallic objects (including clips) inserted into your body during an operation, or if you have received a gun shot injury or have a heart pace maker. The study physician will go through a list of possible risks with you at screening, as will the person doing the scan, before you go into the scanner. If you take part in the study you will be asked to complete some tasks on the computer. During screening there will be a practice session of these tasks in which you will receive instructions on what you will be asked to do while you are in the scanner. You will be asked to look at a computer screen and move a joystick in response to certain images. You will also be given the opportunity to lie in a mock-up scanner at the Centre for Neuroimaging Sciences before lying in the real scanner. This will help you become accustomed to the scanning environment. These screening assessments together with specific questions we will ask, will confirm that you are suitable to take part in the study.

If we are satisfied with the screening procedures and you are suitable to take part in the study we will invite you to take part in four subsequent scanning visits, on four separate occasions, at approximately 10–21 day intervals. We will arrange these dates so that they are convenient for you. Each of these four study visits will include identical study assessments and procedures, only the oral drugs that you will receive will differ. On each visit we will first give you a brief interview to confirm that you are still suitable to take part and that you understand the study procedures, conduct a brief physical examination and test your urine for drugs of abuse and check the alcohol levels in your breath. If these are all normal we will continue with the visit. On each scanning visit you will receive a single oral dose of one of the following treatments: placebo (2 visits) a dummy tablet with no active drug, lamotrigine (150mg or 300mg), or risperidone (1mg or 2mg). The treatment you receive at each visit will be decided by a random code (chance). In addition, you will be administered ketamine into a vein on three of the four scanning days, and a salt solution (saline) into a vein on one of the scanning days. The ketamine and saline will be delivered by a programmable intravenous infusion pump. In order to be able to connect the infusion pump we will insert a small plastic tube into a vein in your arm (a cannula). This may cause slight discomfort at the insertion site and occasionally bruising. We will plan the infusion to give steady drug levels of ketamine in your blood of a maximum of 75ng/ml. This is much lower (<10%) than doses used to induce anaesthesia with this drug.

The four drug combinations that all volunteers will receive will be:

1. Oral placebo and saline infusion,

2. Oral placebo and ketamine infusion,
3. Oral lamotrigine and ketamine infusion
4. Oral risperidone and ketamine infusion

You will not know which combination of treatments you receive at any visit. Neither you nor the investigators will know what you have taken on each day, although we can find out quickly if needed in the case of an emergency (See below for more information about the possible side effects of these medications).

Risperidone is a drug used to treat psychiatric conditions that reduces the effects of naturally occurring substances in your brain, dopamine and serotonin. A dose of 2mg is thought to be an effective dose for patients with schizophrenia with little potential for serious side effects like involuntary muscle contractions called dystonic reactions. It has been selected as the "high dose" that will be administered to the first 8 volunteers in this study. The second 8 volunteers will receive either a single oral dose of 2mg or a single oral dose of 1mg risperidone.

Lamotrigine is drug used to treat fits (epilepsy) and decreases the levels of a naturally occurring substance, glutamate, in the brain. The initial 8 volunteers of this study will receive a single oral dose of 300mg lamotrigine. The second 8 volunteers will receive either a single oral dose of 300mg or a lower single oral dose of 150mg. A dose of 300mg is typically used in patients and previous research studies have confirmed that this dose produces detectable changes in brain imaging scans.

If you are one of the eight subjects in the second group, the dose of lamotrigine or risperidone that you will receive will be decided after seeing how these drugs effect brain activity in the first 8 subjects. Which of the two groups of eights subjects that you will be in, will depend on when you join the study.

Each scanning session will consist of two parts; in each part you will undergo the same procedures and be asked to complete the same computerised tasks. The first part will take place approximately two hours after giving you an oral tablet of either risperidone, lamotrigine or placebo. Then over approximately one and a half hours, we will collect pictures (scans) of your head while you are resting and whilst you are performing some tasks involving memory and attention. These tasks will involve looking at letters or pictures and trying to remember them. After performing the tasks there will be a one hour break during which you will be given a light lunch and the infusion pump to give you ketamine or placebo will be set up and attached to the cannula in your arm. The second scanning session will then start. We will collect some more pictures of your head while you are resting and then while you are performing the same tasks as earlier in the day. This session will also take approximately one and a half hours. We use a very modern method of scanning known as Magnetic Resonance Imaging (MRI). This technique is commonly used to diagnose a number of diseases, but in this case it has been changed to take images of which parts of your brain are active when you perform a task. When a part of your brain is more active, more blood flows to that region and this change is captured on the photos that we take. We will make a map of which parts of your brain has more blood flow

than others. During the study day you will also be asked to complete some brief questionnaires which assess your subjective state (mood, alertness, etc).

In order for us to take pictures of your brain, you will have to lie as still as possible in the MRI scanner. The scanner consists of a powerful magnet, but you will not feel any force or special sensation inside a magnetic field because your body is insensitive to it. Because of the magnetic field, you must not have a scan if you have received metal injuries to your eyes, had metallic objects (including clips) inserted into your body during an operation, or if you have received a gun shot injury or have a heart pace maker. The person taking the scan will go through a list of possible risks with you before you go into the scanner, identifying with you any reasons why you should not be scanned. Please note that MRI scans do not involve any form of ionising radiation (X-rays), but the scanner itself can be quite claustrophobic; therefore please inform us if you have a fear of enclosed spaces.

All the time you are in the scanner there will be a microphone switched on so you can talk to us. We will talk to you regularly to explain what will happen next. Some people find the machine a little noisy, but the headphones we provide allow adequate noise protection for most people.

After the scanning session you will be asked to perform two more computer tasks. These will be learning tasks that involve comparing different pictures on a computer screen.

During each study visit we will take 9 blood samples which will be used to measure the levels of the study drugs in your body. One of these samples will be collected while you are lying in the scanner. These samples will either be collected by a simple needle into the vein or prior to the ketamine/placebo infusion through the cannula in your arm (see above). The samples we collect from you will only be used for the purposes described and will not be stored. While you are in the scanner we will also monitor your heart rate and respiration rate. At intervals throughout the day we will also check your blood pressure and heart rate while you are lying down and again while you are standing up, and ask you to rate how you are feeling using some questionnaires. . If you feel unwell for what ever reason during the course of the day you should let one of the study team know.

What will I have to do?

If you decide to take part in this research study we ask that you visit the Centre for Neuroimaging Sciences on 5 separate occasions. Before each visit we ask that you (1) do not drink alcohol, take products containing caffeine or engage in strenuous exercise (e.g. heavy lifting, aerobics) for 24 hours (2) eat nothing but a light breakfast (e.g. bowl of cereal, or a few pieces of toast, nothing high in fat) between midnight and your arrival at the centre for neuroimaging sciences on the study days (not screening) and (3) do not eat anything containing grapefruit from midnight the night before the study day (4) abstain from nicotine- or tobacco- containing products for at least 4 hours before arrival at the Centre. During the study day we will provide lunch and ask you to abstain from nicotine-, tobacco- or caffeine- containing products until you are discharged home. After each study day we will ask you to

avoid alcohol and driving or operating heavy machinery for at least 24 hours after receiving each dose because small levels of the medicine may still be present in your system.

If you fall ill or need to take any medication through the course of the study you should notify the researcher as soon as possible.

Importantly, if you have private health insurance you should contact the company to inform them that you are taking part in a clinical trial to ensure it does not affect your cover.

Will I experience any side effects?

Clinical Procedures

Taking blood or siting the cannula are well-tolerated procedures, although you may experience some minor discomfort, minimal bleeding or bruising in your arm.

Ketamine

Ketamine is commonly used as an anaesthetic. You should be aware that it is sometimes used socially and can lead to dependence. Ketamine can affect your blood pressure so you will not be included in the study if you have a history of high blood pressure or other heart problems. When on the drug you may feel your heart beating faster. At high doses ketamine can also cause strange experiences (such as feeling disconnected from your body or other distortions of sense). Importantly the doses to be used in this study have been used previously, are well-tolerated in healthy volunteers, and are well-below doses used in anaesthesia. The strange experiences typically occur at doses higher than those in this study. If they should occur you may find them unpleasant and frightening. If this happens the infusion will be stopped immediately and any strange experiences should rapidly fade. You may also feel nauseous when we stop the infusion and slightly clumsy or drunk for about 1 hour. Volunteers will all also be monitored using established and validated questionnaires and a qualified physician will always be present during the study periods.

Lamotrigine

Lamotrigine is a commonly used treatment for fits (epilepsy). There is a risk of a rash with this drug. The risk of rash is thought to increase with more rapid dose increases. In this study you will only receive a single dose. The risk is thought to be very low with single doses of the drug and research studies in healthy volunteers using 300mg of lamotrigine have not reported adverse events. In clinical practice this dose is commonly given although starting doses are much lower (25mg). Doses up to 300mg are considered safe for use in healthy volunteers. There is a very low risk of a hypersensitivity reaction. If this occurs it will be treated with antihistamines or subcutaneous adrenaline as clinically appropriate.

Risperidone

Risperidone is a commonly used treatment for psychiatric conditions. Doses of up to 4mg of risperidone have been administered to small numbers of healthy volunteers and have been well tolerated. The most common side effects for risperidone are mild and start during the course of daily treatment. In this study you will only receive a single dose. The possible side-effects include nausea, dizziness, low blood pressure, slowing of the heartbeat, tiredness, sleepiness, blurred vision and constipation.

The drug information leaflets for these drugs have been included with this document. These documents contain a more detailed list of potential side-effects which are more rare.

If you do experience any side effects please contact Dr. Mitul Mehta on 0203 228 3053 immediately.

Will I benefit from my participation?

We do not expect that you will draw any specific personal benefit apart from a payment of £710 to compensate for your time. If we decide that you are not suitable during the screening session we will pay you £50 for your time. Also, all your travel expenses will be reimbursed.

What do I do if I want to withdraw from the study?

From our previous experience in studies of this type we do not anticipate that you will have any problems but if you do, we want to assure you that you are free to withdraw from the study at any time you like. You will not be required to give us any reasons for withdrawal from the study but please inform us as soon as possible if you wish to do so.

Will my participation be kept confidential?

If you agree to take part in this study your General Practitioner (GP) will be contacted to inform them of your participation. You will not be identified in our computers by name but by a number, and all records obtained while your are in this study, including related health records will remain strictly confidential at all times . A copy of this 'Information Sheet' and of the signed 'Consent Form' will be given to you to keep. However, a copy of your consent will be made available to others working on the study at the Institute of Psychiatry or Eli Lilly, the Independent Ethics Committee members and Medicines Regulatory Authorities.

In this trial, personal and in particular medical data will be collected, stored and analysed. The sponsor will use this study data for research purposes to support the scientific objectives of the study described in the consent document, to assess the safety of any drug or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies. This information will be handled in a strictly confidential manner by your doctor in charge and staff. The uses and disclosure of your personal health information follows legal requirements and needs your permission to this Data Privacy Statement (authorisation), i.e. without your permission you are not allowed to take part in this study.

Information from this study will be reported to the Sponsor and to official drug regulatory authorities, and governmental agencies in other countries, where the study drug may be considered for marketing approval.

Under the data protection laws Eli Lilly and Company is the controller of your personal data. Your personal data (including sensitive personal data) may need to be transferred within Eli Lilly and Company, to others working on their behalf and to the medicines regulatory authorities inside and outside the European Union (EU), including the USA, where data protection laws may not be as stringent as in the EU. Eli Lilly and Company will take steps to ensure your personal data is protected and by agreeing to take part in this study you give your permission for these transfers.

You may withdraw your permission at any time by providing written notice to the study doctor. The study doctor and staff would then no longer use or share your personal health information in connection with this study, unless it is essential to ensure that the study is scientifically reliable. However, the sponsor would still use study data that was collected before you withdrew your permission. In addition, you would no longer be able to participate in the study.

If you agree to participate in the research study, your personal health information will be used and shared in the following ways:

6. The study doctor and staff will send your study-related health information ("study data") to the sponsor of the study, its associated companies and its representatives ("the sponsor"). Because the sponsor conducts business related to clinical research in many countries around the world, this may involve sending your study data outside of the UK and Europe. The sponsor has joined the United States Safe Harbour program. Participation in the Safe Harbour program amounts to a commitment by the sponsor to the protection of personal information in accordance with the data protection principles set down by UK and European data privacy law.

7. The sponsor will use the study data for research purposes to support the scientific objectives of the study described in the consent document, to assess the safety or efficacy of any drug or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies.

8. Your study data, either alone or combined with data from other studies, may be shared with regulatory authorities in this country and other countries including the United States, and the Ethics Committee that reviewed this study.

9. Study data that does not identify you may be published in medical journals or shared with others as part of scientific discussions.

10. Your original medical records, which may contain information that directly identifies you, may be reviewed by the sponsor, the Ethics Committee that reviewed this study, and regulatory authorities in this country and/or other countries including the United States.

The sponsor works with business partners in drug development. The sponsor may share your study data with these business partners, but only if the business partner signs a contract that requires it to protect your study data in the same way as the sponsor.

You have the right to see and copy your personal health information related to the research study for as long as the study doctor or research institution holds this information. However, to ensure the scientific integrity of the study, you will not be able to review some of your personal health information related to the study, until after the study has been completed.

If you have any questions about matters related to the study please contact Dr. Mitul Mehta on 0203-228-3058/3053.

PART II

What if relevant new information becomes available?

Sometimes we get new information about medicines being used in research. This is unlikely for the drug to be used in this study because they have been in general use for many years. However, if this happens, a member of the research team will tell you and discuss whether you should continue in the study. If you decide to continue in the study he/she may ask you to sign an updated consent form. If the study is stopped for any other reason, we will tell you and explain the reasons why this has occurred.

Although unlikely, it is possible that whilst performing normal medical checks we may identify a significant abnormality that you didn't realise you had. If this occurs a study physician will discuss the finding with you and we will inform your GP. Eli Lilly and Company (the Sponsor), may stop the study or your participation in the study at any time, for any reason, without your consent. This may be due to an adverse reaction or other factors which relate to your safety or well-being. A full explanation will be given to you should this be necessary.MRI scans will be reviewed by a specialist and any significant abnormalities will be reported to the study investigators.

What will happen if I don't want to carry on with the study?

If you withdraw from the study we will destroy all identifiable information about you. We will retain and continue to use any data collected before such withdrawal of consent unless you request that you do not want us to use any data collected from you.

What if there is a problem?

While we do not expect you to suffer any health problems by taking part in this study, King's College London, the study's sponsor may compensate anyone whose health suffers as a result of participation. You do not have to prove it was anyone's fault; if the health problem arose because of your participation in this study, you will be compensated.

We accept that this study is being conducted subject to the Association of the British Pharmaceutical Industry (ABPI) guidelines, "Clinical Trial Compensation Guidelines," relating to compensation for injury arising in the course of clinical trials, a copy of which is available on request.

If you have a concern about any aspect of this study, please ask to speak to any of the researchers. We will do our best to answer your questions. If you remain unhappy and wish to complain formally, you can write to the Dean of the Institute of Psychiatry. Please write to The Executive Assistant to the Dean, Institute of Psychiatry, Box P001, London SE5 8AF.

Will my taking part in this study be kept confidential?

With your consent your GP will be told that you have decided to take part in this study. All information obtained during the study, as well as related health records, will remain strictly confidential at all times. However, these may need to be made available to others working on the Institute of Psychiatry or Eli Lilly's behalf, the Ethics Committee members and Medicines Regulatory Authorities.

By signing the consent form you agree to this access for the current study and any further research that may be done. However, the Institute of Psychiatry and Eli Lilly will take steps to protect your personal information and will not include your name on any sponsor forms, reports, publications, or in any future disclosures. If you withdraw from the study, we will no longer collect your personal information, but we may need to continue to use information already collected. The study information collected will be sent to other locations outside of the UK, but you will not be referred to by name or identified in any report or publication nor could the information be traced back to you. This will be for healthcare and/or medical research purposes only. Your data will only be shared with countries where data protection laws are comparable to those in the UK. However, the Institute of Psychiatry and Eli Lilly maintain high standards of confidentiality and protection. The Institute of Psychiatry (who will control the use of the data) will take steps to ensure your personal data is protected. These will include not sharing any information which could potentially lead to someone learning your identity. This information will be kept in accordance with the data protection act. Additional steps taken to ensure anonymity will include editing brain scanning data to ensure that no facial features could be reconstructed from the data.

YOUR RIGHTS UNDER ANY APPLICABLE DATA PROTECTION LAWS ARE NOT AFFECTED

What will happen to any samples I give?

We will ask you to provide a number of urine and blood samples for this study. During the screening visit we will take about 20mL of blood from a vein in your arm and a small urine sample. During subsequent visits we take no more than 140 mL of blood across all timepoints to measure the drug levels in your body. Overall we will not take more than 580mL of blood over the course of the study,

which is slightly more than is taken at a single blood donation. Once the samples have been analysed, they will be destroyed.

What will happen to the results of the research study?

The results of this research will be published as scientific reports and maybe presented at meetings within the Institute of Psychiatry or Eli Lilly or at international scientific meetings. You will not be identified in any report or publication that results from this study.

Who is organising and funding the research?

The research is being organized as a collaborative study between the Institute of Psychiatry and Eli Lilly, who are sponsoring the study. The researchers involved in conducting this study do not receive any financial incentives for including you in this study and do not benefit financially from this study.

Who has reviewed the study?

This research has been looked at by an independent group of people, called a Research Ethics Committee to protect your safety, rights, wellbeing and dignity. This study has been reviewed and given favourable opinion by the Wandsworth Research Ethics Committee.

If you have any questions about matters related to the study please contact Dr. Mitul Mehta on 0203228 3058/3053.

III.   Ethics Approval Letter

NHS

National Research Ethics Service

Wandsworth Research Ethics Committee
South London REC Office (1)
Room 1.14, Jenner Wing
St Georges University of London
Cranmer Terrace
Tooting, London
SW17 0RE

Telephone: 0208 725 1558
Facsimile: 0208 725 1980

29 April 2009

Dr Mitul A Mehta
Senior Lecturer
Institute of Psychiatry
Centre for Neuroimaging Sciences (PO89)
DeCrespigny Park
London
SE5 8AF

Dear Dr Mehta

Full title of study:	EVALUATION OF THE SENSTITIVITY OF A KETAMINE-CHALLENGE PHARMACOLOGICAL FMRI ASSAY TO LAMOTRIGINE AND RISPERIDONE IN HEALTHY MALE VOLUNTEERS
REC reference number:	09/H0803/48

Thank you for your letter of 17 April 2009, responding to the Committee's request for further information on the above research and submitting revised documentation, subject to the conditions specified below.

The further information was considered in correspondence by a sub-committee of the REC. A list of the sub-committee members is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. *Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.*

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Shorter State Schizotypy		
Confidential ADDS		
Columbia Suicide Severity Rating Scale		
GP/ Consultant Information Sheet Part B	1	04 March 2009
Participant Consent Form: Part B	1	24 February 2009
Participant Consent Form: Part A	1	02 March 2009
GP/Consultant Information Sheets	Part A	02 March 2009
Advertisement		02 March 2009
Covering Letter		04 March 2009
Protocol	13	16 February 2009
Investigator CV		
Application	2.0	
Certificate of insurance	AIG	
Response to Request for Further Information		17 April 2009
Participant Information Sheet: Part B	2	14 March 2009
Participant Information Sheet: Part A	2	14 March 2009

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments

- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H0803/48	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Dr. Christine Heron
Chair

E-mail: Recwand@stgeorges.nhs.uk

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments
"After ethical review – guidance for researchers"

Copy to: Mr Matthew Dunn

Appendix F

Table F-1. Randomisation for the modulation study.

Order Number	Order of Treatment Arms	Participants
1	Lamotrigine, Ketamine, Placebo, Risperidone	1,6,11,14
2	Placebo, Lamotrigine, Risperidone, Ketamine	2,108,112,16
3	Ketamine, Risperidone, Lamotrigine, Placebo	3,107,10,15
4	Risperidone, Placebo, Ketamine, Lamotrigine	4,5,9,113

The randomization was fully balanced for order across 16 participants. Random number generator software generated the orders used and randomly distributed these across 16 participants (an equal number of participants got each order). The design used is not a full Latin Square as such a design is problematic, due to fixed sequence effects (e.g. placebo always follows ketamine).

In contrast, the design used controls for (1) order, similarly to the Latin Square design and (2) for sequence effects, by ensuring that every treatment is administered both before and after every other treatment equally often. Thus, although not all possible sequences are used, each group of participants receives a different sequence (e.g. placebo does not always follow ketamine).