Neural and cognitive mechanisms underpinning novel treatments for depression

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Declaration

I, Níall Lally, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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“I almost wish I hadn't gone down that rabbit-hole — and yet — and yet — it's rather curious, you know, this sort of life!” – Lewis Carroll

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Abstract

Depression is a prevalent and debilitating psychiatric condition. However, currently available pharmacological treatments are ineffective for almost a third of all depressed patients. Moreover, even when effective, standard treatments suffer from substantial therapeutic lag, often taking weeks-to-months to reach maximum efficacy. Thus, the need for faster acting treatments for depression is high. Evidence of novel glutamatergic pharmacological and brain stimulation antidepressant treatments has been reported. However, it is unknown how exactly these treatments, namely transcranial direct current stimulation and ketamine, work at either the biological or cognitive level. The aim of this thesis was to provide a cognitive and systems level biological explanation for the efficacy of these treatments on two important symptoms of depression, anhedonia and cognitive control. Following a general introductory chapter, the first experimental chapter explores the effect of tDCS on cognitive control in healthy volunteers. The second experimental chapter explores the reliability of 7 Tesla (T) proton magnetic resonance spectroscopy (^{1}H-MRS) as a technique to quantify glutamate and glutamine levels in the healthy human brain. The third experimental chapter explores the relationship between levels of glutamatergic metabolites, one purported mechanism to which ketamine and tDCS elicit their antidepressant response, and anhedonia in medication-free depressed patients and healthy individuals at baseline and following ketamine and placebo. The fifth and final experimental chapter explores whether ketamine alters the behaviour and neural activity underlying cognitive control in patients with depression. The results suggest that tDCS may induce improvements in cognition in healthy volunteers and that ketamine may improve levels of anhedonia and mood, but not cognition, in depressed patients. Surprisingly, a decrease in 7T ^{1}H-MRS measured glutamine, but not glutamate, levels was found post-ketamine; moreover, baseline levels of glutamine, but not glutamate, were associated with the antidepressant and anti-anhedonic response to ketamine. The final chapter discusses the experimental results in light of cognitive and neural mechanisms thought to underpin depression and its treatment.

List of abbreviations

^{1}H-MRS	Proton magnetic resonance spectroscopy
ACC	Anterior cingulate cortex
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
BD	Bipolar disorder
BDI	Beck depression inventory
BDNF	Brain-derived neurotrophic factor
BOLD	Blood-oxygen level dependent
CBT	Cognitive behavioural therapy
Cho	Choline
Cre	Creatine
CRLB	Cramér-Rao lower bounds
CV	Coefficient of variation
DLPFC	Dorsolateral prefrontal cortex
DSM-V	Diagnostic and Statistical Manual of Mental Disorders
ECT	Electroconvulsive therapy
EEfRT	Effort Expenditure for Rewards Task
EPI	Echo planar imaging
FDA	Food and drug administration
FID	Free induction decay
fMRI	functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
GLM	General linear model
Gln	Glutamine
Glu	Glutamate
GSH	Glutathione
HDRS	Hamilton depression rating scale
Hz	Hertz
ICC	Intraclass correlation coefficient
IQ	Intelligence quotient
MADRS	Montgomery-Åsberg Depression Rating Scale

MBSR	Mindfulness based stress reduction
MDD	Major depressive disorder
MRI	Magnetic resonance imaging
NAA	N-acteylaspartate
NAAG	N-acetylaspartylglutamate
NaSSA	Noradrenaline and specific serotonergic antidepressants
NBS	Non-invasive brain stimulation
NMDA	N-methyl-D-aspartate
NRI	Noradrenaline reuptake inhibitor
PET	Positron emission tomography
pgACC	Pregenual anterior cingulate cortex
PPM	Parts per million
PRESS	Pont resolved spectroscopy
ROI	Region of interest
RT	Reaction time
rTMS	Repetitive transcranial magnetic stimulation
SHAPS	Snaith-Hamilton Pleasure Scale
SNR	Signal-to-noise ratio
SNRI	Serotonin and noradrenaline reuptake inhibitors
SPM	Statistical Parametric Mapping
SSRI	Selective serotonin reuptake inhibitor
STEAM	Stimulated echo acquisition mode
T	Tesla
tDCS	transcranial direct current stimulation
TEPS	Temporal Experience of Pleasure Scale
TE	Echo time
TI	Inversion time
TMS	Transcranial magnetic stimulation
tNAA	Total n-acetylaspartate
TR	Repetition time
WASI	Wechsler Abbreviated Scale of Intelligence
WHO	World Health Organization

Note to examiners

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1 General Introduction

1.1 Clinical characteristics of major depressive disorder

Major depressive disorder (MDD; otherwise known as unipolar depression) is a complex psychiatric illness. To reach criteria for the diagnosis of MDD, an individual must have experienced a two-week episode comprising at least one of two cardinal symptoms, low mood or anhedonia, the loss of interest or pleasure, most of the day, nearly every day. Additionally, the person must experience at least four of the following seven symptoms nearly every day over the same two-week minimum period (only three are required if both cardinal symptoms are present): insomnia or hypersomnia, unintentional weight or appetite changes, fatigue or anergia, psychomotor agitation or retardation, feelings of worthlessness or excessive guilt, cognitive difficulties or indecisiveness and suicidal ideation or self-injurious behaviour. Collectively, the symptoms should also represent an impairment in functioning from previous non-depressed levels and must not be drug induced. Bipolar depression (BD; otherwise known as manic depression) comprises the same symptoms as MDD during the depressive phase but also requires at least a single one-week period of consistent mania (BD I) or four-days of hypomania (BD II). The sweeping flexibility of the MDD diagnosis has undoubtedly led to difficulties in successfully treating the disease, resulting in its high societal pervasiveness and deleterious impact (Hidaka, 2012, Kessler *et al.*, 2007).

MDD is a prevalent and debilitating mental illness; over 350 million people annually are affected worldwide (approximately 5% of the world's total population); it is the number one cause of global disability (World Health Organization, 2012). The impact of MDD on society is large; for example, MDD affects around 30 million European Union citizens, costing around €92 billion (Olesen *et al.*, 2012). The burden of lost years associated with depression is particularly apparent when statistics related to suicide are considered. Suicide remains the biggest killer of men under 50 years of age in the UK (Office for National Statistics, 2014) and depression is one of the strongest predictors of suicidal ideation (Nock *et al.*, 2010). Suicidal patients tend to be severely depressed (Birtchnell, 1970). Up to 6% of MDD patients

will commit suicide (Inskip *et al.*, 1998). Thus, the need for successful understanding and treatment of MDD is very high.

1.2 Common treatments for MDD and their efficacy

Presently, only two common treatment methods for MDD are widely available, psychological 'talking' and pharmacological therapy; they are equally effective (Cuijpers *et al.*, 2013, DeRubeis *et al.*, 2008) but have a purported additive effect when combined (Hollon *et al.*, 2014). A third treatment, electroconvulsive therapy (Group, 2003), is typically reserved for individuals who fail to respond to initial treatment with medication and psychotherapy. A fourth and final treatment option, ablative neurosurgery, typically a dorsal anterior cingulotomy, is used, albeit rarely, in very severe treatment-resistant depression, with some success (Shields *et al.*, 2008).

1.2.1 Psychotherapy

The term psychotherapy derives from the ancient Greek word "psyche" meaning "breath", "spirit" or "soul" and "therapeia" meaning "healing". Psychotherapy is the application of clinical methods and psychological principles for the purpose of assisting people to modify their behaviours, cognitions, emotions and characteristics (APA, 2012). In most instances, psychotherapy takes the form of verbal communication between a therapist and a patient or group of patients. A huge variety of different types of effective psychotherapies for MDD exist (see Hollon and Ponniah (2010) for an extensive review), including: behavioural activation therapy, cognitive behavioural therapy (CBT), interpersonal therapy, mindfulness based stress reduction (MBSR) and psychodynamic therapy, amongst others. To date, there is little evidence for one form of psychotherapy being more effective than another in treating MDD patients (Baardseth *et al.*, 2013, Leichsenring, 2001, Leichsenring *et al.*, 2015); head-to-head comparisons are rarely conducted. Typical remission rates (usually classified as a Hamilton depression rating scale score (HDRS; Hamilton (1960)) of seven or less) for MDD patients following a 16 week course of CBT, the most widely-used psychotherapy, are approximately 40% (DeRubeis *et al.*, 2005). There is also some evidence that CBT may provide some enduring long term benefit

in the prevention of recurrent depressive episodes (Hollon *et al.*, 2005) with lower relapses than other treatments (Steinert *et al.*, 2014).

However, the resource (e.g. financial and time) demands associated with psychotherapy have made it a much less prevalent and attractive form of treatment than medication (Marcus and Olfson, 2010). In addition to the demand on resources, the significant time required to reach maximal therapeutic efficacy (16-weeks) should be noted. Moreover, the fact that typically over 40% of depressed patients do not reach response (a 50% reduction in symptoms) criterion following CBT (DeRubeis *et al.*, 2008) makes psychotherapy a substantially less than perfect treatment option. Furthermore, concerns regarding the effectiveness of CBT for MDD in well controlled studies have also emerged, with reports of only a small effect size when the CBT group is compared to a placebo pill group (Lynch *et al.*, 2010), as opposed to, the more lax and typical, waiting list group comparison. Therefore, the need for more efficacious and alternative treatments to psychotherapy is greatly needed. As the focus of this thesis is on novel brain stimulation and pharmacological interventions for depression, we shall focus on these methodologies herein.

1.2.2 Pharmacotherapy

Currently available pharmacological therapies for MDD mostly target the monoaminergic neurotransmitter system, primarily augmenting extracellular serotonin, next noradrenaline, and finally, dopamine levels in the central nervous system (Delgado, 2000). The monoaminergic treatment of depression centres on the idea that the core symptoms of major depression, dysphoria and anhedonia, arise due to a neurochemical imbalance (Asberg *et al.*, 1976, Schildkraut, 1965). This crude neurobiological theory of major depression arose serendipitously when terminal patients with tuberculosis were given a novel experimental drug (iproniazid) to treat their tuberculosis symptoms; patients exhibited a remarked improvement in mood and vigour (Selikoff *et al.*, 1952). The new agent was found to exhibit its effects via inhibition of monoamine oxidase, an enzyme implicitly involved in the breakdown of monoamines; by reverse inference therefore, depression was thought to be caused by

low levels of monoamines. Pharmaceutical development for the treatment of depression since has mainly focussed on the creation of compounds that increase, directly or indirectly, specifically or non-specifically, levels of monoamines in the brain. Despite convincing evidence of the efficacy of such antidepressant medications in animal models, progress in the development of effective treatments for depression in humans has been extremely slow.

There are over 30 commonly prescribed medications for MDD, with six main classes (Kupfer *et al.*, 2012): selective serotonin reuptake inhibitors (SSRIs), serotonin and noradrenaline reuptake inhibitors (SNRIs), noradrenaline and specific serotoninergic antidepressants (NaSSAs), noradrenaline reuptake inhibitors (NRIs), tricyclics and monoamine oxidase inhibitors (although these are not as frequently prescribed now due to dangerous food and drug interaction potential). Medications typically prescribed for psychotic disorders (e.g. schizophrenia; both the typical and atypical antipsychotics), which mainly modulate the dopaminergic system, are also frequently administered as adjunctives (Spielmans *et al.*, 2013). One report profiled the effectiveness of 12 new-generation antidepressants against each other in a large multiple-treatment meta-analysis and found that mirtazapine (NaSSA), escitalopram (SSRI), venlafaxine (SNRI) and sertraline (SSRI) were significantly more efficacious than five of the other antidepressants, with sertraline and escitalopram evidencing the greatest patient acceptability (Cipriani *et al.*, 2009). Nevertheless, the effectiveness of standard antidepressants over placebo in relieving depressive symptomatology has come under intense scrutiny in recent years (Fountoulakis and Moller, 2011, Fournier *et al.*, 2010, Kirsch, 2008).

Increasingly, evidence suggests that the effect size of standard antidepressants is small, particularly for trials where an active placebo is used as a comparison (Moncrieff *et al.*, 2004). Researchers have also questioned the clinical significance of such small effects (Moncrieff and Kirsch, 2015). Interestingly, standard antidepressant medications appear to be most effective for individuals currently in a very severe depressive episode (Fournier *et al.*, 2010); although this may be due to a reduced placebo response. Indeed, the UK's National Institute for Health and Care Excellence (NICE, 2009) guidelines indicate that antidepressants be

a first line treatment for patients in a moderate or severe depressive episode or those in a persistent mild depressive episode. However, a meta-analysis found that the antidepressant benefits of fluoxetine and venlafaxine were not superior for patients more depressed at baseline (Gibbons *et al.*, 2012), suggesting that certain antidepressants may be more favourable for different depression severities or symptoms (Nutt, 2008). The combination of multiple antidepressant drugs appears to be the most effective approach for patients to reach remission from depression across a 6-week period (Blier *et al.*, 2010). Of the antipsychotics prescribed as adjunctives for patients with MDD, quetiapine and aripiprazole have the strongest antidepressant efficacy evidence base (Zhou *et al.*, 2015). In addition to their questionable efficacy over placebo conditions, a number of other critiques surrounding the effectiveness of antidepressants have been voiced.

Commonly prescribed antidepressants, e.g. SSRIs, can take from weeks to months before any substantial reduction in depressive symptoms occurs (Trivedi *et al.*, 2006), although significant signs of improvement can be seen as early as one week following administration in some cases (Smagula *et al.*, 2015, Taylor *et al.*, 2006). Additionally, their administration has been associated with increases in suicidal ideation and behaviour (Moller *et al.*, 2008) and increased chance of manic or hypomanic episode induction (Frye *et al.*, 2009). Most importantly, they are almost completely ineffective in more than one-third of MDD patients (Trivedi *et al.*, 2006). Specifically, a large open-label investigation, the STAR*D study, found that remission rates following 14-weeks of treatment with citalopram, a prominent SSRI, reach approximately 30 % (Trivedi *et al.*, 2006). Amongst the standard antidepressant medications, there is little evidence to delineate one from another for specific symptoms, despite their apparently different mechanisms of action. Typically, one of the main differentials amongst standard antidepressants is in the side effect and risk category. Of the patients who discontinue treatment with an SSRI, 15% do so because of side effects (Montgomery *et al.*, 1994). Notably, both SSRIs and SNRIs can induce significant sexual side effects in individuals, with SSRIs appearing to be the worst of these two typical classes (Gregorian *et al.*, 2002); bupropion, however, has a more favourable sexual side effect profile (Thase *et al.*, 2006). However, sexual dysfunction may be a side effect of all antidepressant

medication (Taylor *et al.*, 2006). In recent years, there has been a particular focus from the pharmaceutical industry on the reduction of side effects associated with earlier medications, particularly with the creation of the second (SSRIs) and third (non-SSRIs) generation antidepressants (Olver *et al.*, 2001). In general, their safety profile is good, with a very low chance of fatality (Hawton *et al.*, 2010).

In sum, standard antidepressants appear to have some efficacy in relieving depressive symptoms, particularly for those in a severe depressive episode and when administered in combination with other antidepressants medications or psychotherapy. However, a number of issues have been raised regarding both the time taken to induce meaningful treatment impact and also the side effects associated with their administration. Most importantly however, standard antidepressants provide no meaningful improvement for a substantial proportion of MDD patients.

1.2.3 Electroconvulsive therapy

ECT, formerly known as electroshock therapy, is the most effective antidepressant treatment available (Fava, 2003, Pagnin *et al.*, 2004); it has long been considered the gold standard technique for MDD patients who are treatment refractory (Fink, 1990). Indeed, meta-analyses have revealed that remission rates following ECT are 51-90% for treatment resistant MDD patients (Dierckx *et al.*, 2012, Group, 2003). ECT trumps other treatments on at least two levels. First, evidence suggests the probability of responding to ECT is substantially higher than antidepressants. Second, over 50% of patients treated with ECT show improvements within one week (Husain *et al.*, 2004), with 66% reaching response and 53% remission criteria following 6 sessions (Khalid *et al.*, 2008). ECT, as the name suggests, involves the use of electricity, which is applied to the brain, to induce a seizure in anesthetized patients; patients are also administered muscle relaxants. Electrodes can be placed either unilaterally or bilaterally. Patients are typically stimulated two-to-three times per week until symptoms remit, with a two-to-four week treatment schedule frequently used. The risk profile of ECT has however caused concern and it remains a controversial treatment with severe restrictions in some European countries.

As ECT is typically administered under a general anaesthetic, treatment with this technique is unsurprisingly associated with some confusion and memory loss. However, for most patients ECT results in significant temporary cognitive impairment, which includes both retrograde (the most persistent effect) and anterograde amnesia (Lisanby *et al.*, 2000). For some patients, the effects of ECT may induce permanent memory loss, which may extend to months and years of retrograde amnesia from the time of treatment. Bilateral stimulation has been shown to induce significantly more memory impairment that unilateral but is more effective as a treatment (Lisanby *et al.*, 2000). Despite these findings, ECT has not been found to induce structural brain damage in MDD patients (Devanand *et al.*, 1994) and remains an important tool in the treatment of MDD.

1.2.4 Summary of standard treatments and their efficacy for MDD

Psychotherapy and antidepressant medications are effective methodologies for the treatment of MDD, with consistent, albeit small, effects for both treatments found in randomised controlled trials. However, both treatments are associated with significant lag in their time to reach maximal efficacy. Additionally, while psychotherapy is costly, antidepressants are associated with significant side effects for some patients. Moreover, both treatments are ineffective for a substantial portion of MDD patients. Only a third of patients administered an antidepressant medication (citalopram) will reach remission status after 14-weeks of treatment (Trivedi *et al.*, 2006). Furthermore, despite their apparent efficacy, patients administered either of these treatments will typically relapse within 12 months, with lower relapse rates for cognitive therapy (31%) than antidepressant medication (76%; Hollon *et al.* (2005)), suggesting an enduring effect of psychotherapy over medication. The combination of both psychotherapy and antidepressant medication appears to be the most effective typical approach in treating MDD (Hollon *et al.*, 2014, Schramm *et al.*, 2007). While ECT is the most effective treatment for MDD in general, with particular efficacy in treatment resistant individuals, and works quicker than either of the other two standard treatments, there are substantial side effects that warrant its use only in severe cases of MDD. Without additional active treatment however, virtually all remitted patients will relapse within six months following ECT (Sackeim *et al.*,

2001). In sum, all of the aforementioned treatments have advantages and disadvantages. There is still an unmet need for a safe, quick-acting treatment that is effective in the majority of MDD patients and is not associated with substantial long-term side effects. A better understanding of current treatments and their biological bases is needed to drive forward the generation of novel, alternative and more effective treatments for MDD.

1.3 Models of MDD and its treatment

From the clinical evidence for the aforementioned MDD treatments, a number of models of MDD and its typical treatment have been proposed. Here we will discuss a few particularly prominent theories at the neurotransmitter, cellular and psychological levels of treatment action.

1.3.1 Monoaminergic model

Stemming from the fact that effective standard antidepressant medications mainly modulate the serotonin, noradrenaline and dopamine neurotransmitter systems, it has been posited, via *ex juvantibus* reasoning, that abnormalities in either the levels or functionality of the monoamine systems may underlie MDD (Coppen, 1967, Schildkraut *et al.*, 1965). However, and despite almost 50 years of research, there is little consistent evidence to date that irregularities in these neurotransmitter systems are apparent in MDD (Lacasse and Leo, 2005). A handful of positron emission tomography (PET) studies examining serotonin receptor and transporter binding potentials and densities in MDD patients have found some evidence for alterations (Cannon *et al.*, 2007, Kaufman *et al.*, 2015, Murrough *et al.*, 2011); however, these studies are potentially confounded, similarly to the earlier studies examining plasma levels of serotonin, by the patient's previous medication usage (Karege *et al.*, 1994). Interestingly, Parsey and colleagues (2006) found that antidepressant naïve patients had the lowest serotonin transporter binding potential; this finding remains to be replicated. Nevertheless, the strongest evidence for the monoamine model of MDD comes from dietary depletion studies, where central nervous system levels of either serotonin or the catecholamines (noradrenaline and dopamine) are temporarily lowered (Cowen and Browning, 2015).

Recovered MDD patients show a tendency towards a transient relapse following depletion of tryptophan (Ruhe *et al.*, 2007), the precursor to serotonin, and α–methyl-paratyrosine (Bremner *et al.*, 2003, Homan *et al.*, 2015), a rate limiting inhibitor of the catecholamines, but not tyrosine (McTavish *et al.*, 2005, Roiser *et al.*, 2005), the precursor to dopamine. One possible explanation for this effect however is that patients administered monoamine modulating medications may have a direct or indirect iatrogenic disposition to these depletion studies; performing monoamine depletion experiments on antidepressant medication naive patients following recovery from psychotherapy only would be an interesting avenue to examine this hypothesis. Nevertheless, depleting levels of tryptophan or tyrosine does not reliably induce depressive symptomatology in healthy volunteers (Cowen and Browning, 2015). Additionally, giving extremely large doses of tryptophan does not lead to an antidepressant response in MDD patients (Mendels *et al.*, 1975). Finally, one of the primary critiques of the monoamine model of MDD is that although antidepressant medications alter levels of neurotransmitters within hours, meaningful clinical effects in humans typically take weeks-to-months to take place; they occur more rapidly in rodents. Taken together, these findings suggest a potentially important, but insufficient, role for monoamines in the neurobiology of MDD (Cowen, 2008, Massart *et al.*, 2012); very little work to date has examined the effects of either psychotherapy or ECT on brain monoamines. Interestingly, preclinical and clinical work examining cellular and systems level neurobiological effects of administration of standard antidepressant medications has found evidence for plasticity enhancing properties of these drugs (Boldrini *et al.*, 2013, Dranovsky and Hen, 2006, Duman *et al.*, 1999, Maya Vetencourt *et al.*, 2008, Normann *et al.*, 2007, Sheline *et al.*, 2003), which may explain the treatment lag and provide key insights into the aetiology of MDD.

1.3.2 Cellular plasticity model

The time from increased levels of monoamines to clinical effects suggests that slow adaptive changes in downstream neurobiological signalling and plasticity may underlie the mechanism of successful antidepressant treatment. Evidence for a cellular plasticity hypothesis of MDD and its treatment comes from many sources.

First, one of the most robust findings in depression research is that of neuronal atrophy, specifically, reduced hippocampal volume in MDD patients in comparison to healthy volunteers (Schmaal *et al.*, 2015); with this effect being driven by the number of recurrent major depressive episodes. There is a consistent body of evidence suggesting that depression is associated with gross reductions in grey matter volume (Grieve *et al.*, 2013). This evidence is also borne out in post-mortem studies where specific reductions in glial cell, namely astrocytes, density and neuronal size have been found recurrently in the prefrontal cortex and hippocampi of depressed patients (Drevets *et al.*, 2008, Miguel-Hidalgo and Rajkowska, 2002, Rajkowska, 2000).

Second, depression is considered a stress-related illness and animal models of depression, which often use stress as a manipulation, reliably induce specific behaviours, which are argued to reflect some aspects of the human condition. Interestingly, depressed rodents also exhibit atrophy and loss of neurons and glia (Duman and Li, 2012), providing cross species validation. Further, brain-derived neurotrophic factor (BDNF), a plasticity protein involved in neuronal support and growth, has been implicated in both stress and depression, with lower serum levels found in MDD patients (Sen et al., 2008). Persistent stress induced BDNF decreases eventually lead to hippocampal atrophy in rodents, suggesting a mechanistic link between stress and depression. Interestingly, BDNF has been found to be important for memory (Bekinschtein *et al.*, 2007), a process commonly impaired in MDD patients (Zakzanis *et al.*, 1998) and known to strongly depend on the hippocampus (Squire, 1992). BDNF is also involved in synaptogenesis, dendritogenesis, and neurogenesis, the creation of new synapses, dendrites, and adult neurons, respectively (Leal *et al.*, 2014, Lu *et al.*, 2013). Neurogenesis in the human brain occurs in at least two locations in adults, the dentate gyrus of the hippocampus and the subventricular zone of the lateral ventricles (Ming and Song, 2011). Stress has been shown to inhibit adult neurogenesis in the hippocampus (Anacker, 2014). Snyder and colleagues (2011) provided the first direct evidence for the role of adult neurogenesis in buffering the impact of stress and preventing depressive behaviour in rodents. Thus, it appears that plasticity is a critical step in the resilience to, and potentially also the treatment of, depression.

There is increasing evidence that effective antidepressant medications may increase neuroplasticity (Duman *et al.*, 1999, Pittenger and Duman, 2008). For example, chronic treatment with fluoxetine, an SSRI, enhanced BDNF mediated synaptic plasticity in the amygdala, a key brain region implicated in depression, which permitted, when combined with extinction training, fear erasure in rodents (Karpova *et al.*, 2011). Administration of SSRIs and SNRIs has been shown to increase neurotrophic factors (e.g. BDNF and vascular endothelial growth factor) expression (Banasr *et al.*, 2011). Furthermore, there is evidence that SSRIs increase neurogenesis in the adult rat (Malberg *et al.*, 2000), non-human primate (Perera *et al.*, 2007, Perera *et al.*, 2011) and human (Anacker *et al.*, 2011) hippocampus. The time required from initial hippocampal neurogenesis to maturation and integration is slow (8-weeks) and mirrors the time taken for typical antidepressants to reach peak clinical efficacy, suggesting a link between these two processes (Schoenfeld and Cameron, 2015). Indeed, research has found that neurogenesis is in fact a vital mediating step in the antidepressant response to some medications in animal models (Santarelli *et al.*, 2003), and may also be important for the efficacy of other antidepressant treatments (Schoenfeld and Cameron, 2015).

There is evidence that ECT may also induce neurogenesis in rodents (Madsen *et al.*, 2000, Malberg *et al.*, 2000, Scott *et al.*, 2000) and non-human primates (Perera *et al.*, 2011). Intriguingly, a recent investigation found that ECT in MDD patients was associated with volumetric increases in the hippocampus and amygdala, with volume increases post-ECT positively correlating with clinical improvement (Joshi *et al.*, 2015). Interestingly, both ECT and SSRIs increase plasma levels of BDNF (Marano *et al.*, 2007) and pre-treatment serum levels of BDNF predict SSRI response (Wolkowitz *et al.*, 2011). Patients treated with antidepressant medications tend to have less pronounced in-vivo (Sheline *et al.*, 2003) and post-mortem (Boldrini *et al.*, 2013) hippocampal volumetric decreases. At least two studies have examined neuronal glucose metabolism changes, an indirect measure of plasticity, induced by psychotherapy in MDD patients using 18-fluorodeoxyglucose PET. Brody et al. (2001) found that interpersonal therapy increased right prefrontal cortex and left anterior cingulate and temporal lobe metabolism. Goldapple et al. (2004) found that response to CBT was associated with increases in hippocampal and dorsal

anterior cingulate metabolism and decreases in dorsal, ventral and medial prefrontal cortex. To date however, there is no published research examining changes in either brain volume or plasma levels of BDNF in MDD following psychotherapy, although one would expect robust and detectable neurobiological changes to underpin alterations in behaviour. One model, however, has sought to intersect aspects of both the monoamine and plasticity model to explain how antidepressants and potentially also psychotherapy, work at a more psychological level.

1.3.3 Cognitive neuropsychological model

The cognitive neuropsychological model of MDD and its treatment suggests that depression is associated with a deeply ingrained dysfunctional negative schema of both the world and the patient themselves, which are instantiated by negative affective processing biases (Harmer *et al.*, 2009a, Roiser *et al.*, 2012). This model stems from earlier cognitive models (Beck, 1967, 1976), which focussed on the importance of early negative life experiences in generating negative schemata, overgeneralization and arbitrary inference, which lead to biased information processing and ultimately, depression, and, drove the development of some psychotherapeutic approaches, such as CBT. However, the focus on early life experiences and high-level psychological constructs make objectively testing these earlier models difficult. In contrast, the cognitive neuropsychological model suggests that negative affective processing biases, which may be related to environmental or genetic factors, drive the onset of depression; importantly, this model can be tested through objective behavioural measures and/or neural responses.

The cognitive neuropsychological model suggests that standard therapies for depression exert their beneficial influence by attenuating (medications) or deconstructing (psychotherapy) negative biases allowing an eventual reconceptualization of the world from the patient's perspective (Harmer, 2008). In particular, the model purports that the monoaminergic system plays a key role in the maintenance of the aforementioned negative biases, but does not affect mood directly. Rather, the monoaminergic system is believed to underlie the emotional colour with which information is coded. For example, a depressed patient may

misinterpret a neutral facial expression as a negative stimulus due to aberrant levels of one or more of the monoamines. The effect of weakening or deconstructing negative biases is a gradual improvement in the patient's model, which may explain why both standard antidepressant medications and psychotherapy take several weeks to months to reach their peak clinical efficacy, despite medications increasing synaptic levels within hours (Roiser *et al.*, 2012). Evidence for this model comes from a number of sources.

First, there is now substantial evidence that MDD is associated with negative affective biases (Gotlib and Joormann, 2010, Harmer, 2008). For example, there has been a wealth of studies examining facial affect in patients with MDD. While findings are somewhat inconsistent, in general there is a reported bias (both reaction time (RT) and accuracy differences in comparison to healthy volunteers) in MDD patients away from positive (e.g. happy) facial stimuli and toward more negative (sad, angry) facial images (Roiser *et al.*, 2012). Second, the effects of antidepressant administration are visible implicitly using cognitive tasks and functional neuroimaging within hours, long before they reach observable changes on psychometric scales in MDD patients. For example, Harmer et al. (2009b) found that a single dose of reboxetine, an NRI, but not a placebo, was enough to ameliorate negative perceptual biases (both memory and RT) towards affective facial stimuli, despite no change in reported mood. Additionally, similar changes in biases were also apparent in healthy volunteers following acute administration of citalopram (Harmer *et al.*, 2003a) and reboxetine (Harmer *et al.*, 2003b, Harmer *et al.*, 2009b). Third and finally, the model predicts that depressed patients showing the greatest early changes in such implicit tasks will also evidence the best clinical improvement following administration of the medication. Indeed, Tranter et al. (2009) found that the patients who exhibited the greatest change in their affective biases within two-weeks of citalopram or reboxetine treatment displayed the greatest antidepressant response at six-weeks. This last finding has been reinforced by similar effects at the neural level; changes in brain activity of MDD patients at seven-days post-acute treatment with escitalopram predicted response at six-weeks (Warren *et al.*, 2015).

1.3.4 Summary and limitations of models of MDD and its treatment

In summary, evidence exists for at least three different accounts of how depression manifests and how standard treatments may work in treating this illness. While the overly simplistic monoamine hypothesis has now fallen out of favour, it is clear that monoamine-modulating medications provide some benefit for depressed patients. It may be that more complex theories such as the cognitive neuropsychological and neuroplasticity models are more applicable both to understanding the complexity of the illness and developing new treatments. While each of the models provide different accounts of how antidepressants work and what the underlying dysfunction in MDD is, they are far from mutually exclusive and can all, to some degree, be true. A parsimonious account of depression and its treatment may involve all three models. However, these models are not without their limitations. While they all provide explanations for how some depression treatments work, the plasticity model fails to account for the effects of psychotherapy and how this treatment may work at the neural level and the same can be argued for the cognitive neuropsychological model and ECT. Moreover, there are inconsistent and contradictory findings for each of the models (Cowen and Browning, 2015, Hanson *et al.*, 2011, Roiser *et al.*, 2012, Schoenfeld and Cameron, 2015, Warren *et al.*, 2015). For example, disruption of neurogenesis does not induce depressive symptoms or increase chronic stress sensitivity in rodents (Jayatissa *et al.*, 2009, Surget *et al.*, 2008). Furthermore, none of the models attempt to explain why some MDD patients do not respond to standard treatments and what the underlying biology or psychology may be in these individuals. Thus, the need for alternative and more comprehensive models of depression is high. Fortunately, evidence from several new potential treatments for depression provides some novel clues to the underlying mechanisms of MDD, and, may eventually themselves become established techniques for improving the lives of people suffering from this debilitating illness.

1.4 Novel plasticity enhancing treatments for MDD

1.4.1 Brain stimulation as a treatment for depression

One alternative approach to pharmaceutical and psychotherapeutic intervention for depression is the use of non-invasive brain stimulation (NBS) techniques; ECT is considered an invasive procedure due to the associated risks (Stevens *et al.*, 1996, Tharyan and Adams, 2005). Notably, both transcranial magnetic stimulation (TMS) and transcranial direct-current stimulation (tDCS) have received attention as potential cognitive and mood enhancers for patients with MDD. Anthony Barker invented TMS in 1985 (Barker *et al.*, 1985) as an attempt to create a brain stimulation methodology that could painlessly and non-invasively induce regionally specific action potentials. The technique uses a rapidly changing magnetic field to focally induce an eddy current in the brain. tDCS is a much older and simpler technique than TMS; despite having been used since the 18th century, tDCS is currently undergoing its second scientific renaissance since the turn of the millennium. tDCS works by creating an electrical current flowing circuit, whereby electrons flow from the excitatory anodal to the inhibitory cathodal electrode. Purportedly, the area of the brain under the anodal electrode increases in excitability and the area under the cathode is inhibited. Importantly, both NBS techniques (TMS and tDCS) are capable of producing plastic changes at both the neuronal and behavioural level that can last for hours after their discontinued administration (Fritsch *et al.*, 2010, Huang *et al.*, 2005).

Both forms of NBS have been used to modulate cognition and mood with varying degrees of research endeavour and success. Researchers have attempted to utilise repetitive TMS (rTMS), where stimulation pulses are applied repeatedly at a frequency of 1 hertz (Hz) or quicker, to treat depression for almost 20 years, with moderate success culminating in its Food and Drug Authority (FDA) approval to treat MDD in 2008. The evaluation of tDCS to treat depression has thus far yielded very variable results (Kalu *et al.*, 2012). However, the use of tDCS to modulate behavioural and neural performance has received a large amount of research attention, due to the low research costs and risks associated with the device. Both

NBS techniques are promising avenues of research to enhance the lives of patients suffering from depression.

1.4.1.1 Transcranial magnetic stimulation (TMS)

TMS was first used to treat depression in the mid-nineties; researchers found that rTMS, delivered at a high pulse delivery frequency (10-20Hz), thought to cause an increase in cortical excitability, applied to the left dorsolateral pre-frontal cortex (DLPFC) caused an improvement in mood in treatment resistant MDD patients (George *et al.*, 1995, Pascual-Leone *et al.*, 1996). Low frequency rTMS (1Hz), thought to cause a down regulation in regional activity, applied to the right DLPFC has also been successful in reducing dysphoria in depressed patients (Klein *et al.*, 1999). The rTMS frequency and laterality discrepancy between these findings has led some researchers to conclude that depression might be caused by hypoactivity in the left and hyperactivity in the right DLPFC (Brunoni *et al.*, 2013a, Hecht, 2010); however, such *ex juvantibus* logic mirrors that underpinning the monoamine hypothesis of depression and has not been substantiated through research (Speer *et al.*, 2014). A well designed randomised sham-controlled assessment of rTMS in a large sample of patients confirmed the therapeutic efficacy of excitatory left DLPFC rTMS as a treatment for MDD; George et al. (2010) found that approximately 14% of patients achieved remission status following active stimulation versus 5% for sham, a highly comparable remission differential statistic to placebo controlled evaluations of SSRIs (Thase *et al.*, 2001). Highly similar remission rates were also found in an earlier industry sponsored double-blind study exploring the antidepressant effects of six-weeks of left DLPFC rTMS for MDD (O'Reardon *et al.*, 2007).

1.4.1.2 Transcranial direct current stimulation (tDCS)

Excitatory tDCS has been successfully used as a method to improve performance in a variety of cognitive domains in healthy volunteers, including: numerical competence (Cohen Kadosh *et al.*, 2010), decision-making (Hecht *et al.*, 2010), motor learning (Reis *et al.*, 2009) planning (Dockery *et al.*, 2009), target detection (Clark *et al.*, 2012) and working memory (Zaehle *et al.*, 2011). However, questions

remain regarding the optimal stimulation parameter settings for cognitive enhancement; the parameter space includes the following variables: current intensity and blinding (O'Connell *et al.*, 2012), stimulation frequency (Horvath *et al.*, 2015), stimulation duration (Nitsche *et al.*, 2008), and electrode positions (Miranda *et al.*, 2006); these parameters can all strongly influence the interpretation of alterations in performance. However, despite the apparent efficacy of tDCS for cognitive improvement, recent reviews and meta-analyses have cast doubt on some of the earlier enhancement findings (Horvath *et al.*, 2015). There is a possibility that tDCS may be particularly suited to remediating cognitive deficits in clinical populations and there is now a burgeoning field of research examining the effects of tDCS for depression more generally.

The use of tDCS in the treatment of depression has only been evaluated in appropriately designed randomized sham controlled clinical trials in the past six-years, with few well-designed studies published thus far. While some studies have yielded very large effect sizes (Boggio *et al.*, 2009, Brunoni *et al.*, 2013a) others have found little to no difference in remission rates between sham and active stimulation (Kalu *et al.*, 2012, Loo *et al.*, 2012, Palm *et al.*, 2012). However, a recent factorial between-subjects investigation compared the effects of active DLPFC tDCS in conjunction with an SSRI versus tDCS with placebo, versus sham tDCS with drug and finally against sham tDCS and placebo. The results demonstrated that tDCS had antidepressant efficacy versus both sham stimulation and placebo, however, the antidepressant effect of tDCS when co-administered with sertraline (SSRI) was significantly greater than either one alone (Brunoni *et al.*, 2013a), possibly indicating a concomitant increase in plasticity from the combined treatments and corroborating previous work indicating a role for serotonin and enhanced tDCS efficacy (Nitsche *et al.*, 2009). Nevertheless, the most recent reviews of the antidepressant effects of tDCS do not support its use in treatment resistant depression (Meron *et al.*, 2015) and question its use as a direct stand-alone mood-enhancing treatment for MDD patients (Berlim *et al.*, 2013). As the use of tDCS to significantly improve mood in patients with MDD has proved very variable, a more tractable approach may involve the use of tDCS as a cognitive enhancer, which has arguably been a more successful endeavour thus far in healthy volunteers, to boost the cognitive deficits seen in MDD

patients. Interestingly, in the cognitive neuropsychological model, cognitive impairment is proposed to play a causal role in the development of depressive symptoms and their treatment, as it potentially makes schemata harder to break down and psychotherapy more difficult to engage with (Roiser *et al.*, 2012). Thus, if tDCS can treat cognitive impairment in MDD patients, other treatments may subsequently be more effective.

The possibility of using tDCS as a cognitive enhancer to improve cognitive difficulties in MDD patients has received some attention thus far (Tortella *et al.*, 2014). A number of studies have demonstrated an increase in cognitive task performance in MDD patients following tDCS (Oliveira *et al.*, 2013, Wolkenstein and Plewnia, 2013). For example, Loo et al. (2012) found that patients showed improvements in both attention and working memory following a single session of tDCS. Moreover, Wolkenstein and Plenia (2013) found that excitatory DLPFC tDCS improved both cognitive control, a central factor implicated in depression, and working memory in MDD patients. However, whether these behavioural enhancements are directly related to improvements in mood or are independent is unclear (Tortella *et al.*, 2014). Furthermore, much like studies using tDCS to induce cognitive enhancement in healthy volunteers, the parameter space for optimality in MDD patients has not been systematically assessed; for example, all cognitive enhancement studies conducted in MDD patients have used the left DLPFC as the region of excitatory anodal stimulation, with inconsistent placement of the reference electrode. Nonetheless, questions remain about the use of tDCS as a cognitive enhancer in general; very few double-blind experiments have been conducted. Whether tDCS could be used effectively as a cognitive enhancer for MDD patients, which could thereafter enhance mood or facilitate other treatments, such as antidepressant medication (Brunoni *et al.*, 2013a) or psychotherapy, remains inconclusive.

1.4.2 Ketamine as a rapid acting antidepressant

Research surrounding the potential involvement of glutamate, the predominant and excitatory mammalian neurotransmitter, in depression has soared in the past 15 years

thanks, in part, to an influential first report demonstrating that ketamine, a non-competitive ionotropic *N*-methyl-D-aspartate (NMDA) receptor antagonist and anaesthetic, rapidly alleviated depressive symptomatology in a double-blind, placebo controlled investigation (Berman *et al.*, 2000). Berman and colleagues (2000) found that levels of depression in their small sample of patients with MDD (N = 7) were significantly reduced within 72-hours following a single sub-anaesthetic intravenous infusion of ketamine. Subsequent investigations of ketamine using larger samples and more rigorous investigations in both treatment resistant patients with MDD (Ibrahim *et al.*, 2012, Zarate *et al.*, 2006) and BD (Diazgranados *et al.*, 2010a, Zarate *et al.*, 2012), including active control drugs (e.g. midazolam, Murrough *et al.* (2013a)), and, across multiple sites in MDD patients (Mathew *et al.*, 2010, Valentine *et al.*, 2011), have yielded consistent findings of ketamine's antidepressant effect (Dutta *et al.*, 2015, Naughton *et al.*, 2014). Furthermore, ketamine appears to have some efficacy in alleviating depression in MDD patients who fail to respond to ECT (Ibrahim *et al.*, 2011), the most effective standard treatment for treatment-resistant depression.

These studies not only confirmed the antidepressant efficacy of ketamine but found that the improvement in depressive symptoms was statistically significant within two-hours of a single intravenous infusion even in treatment refractory patients (Ibrahim *et al.*, 2012, Zarate *et al.*, 2006). However, although the antidepressant effect of a single infusion of ketamine is rapid, it is also transient, lasting on average one week before symptoms return to baseline levels. Nevertheless, the antidepressant effect of ketamine has heralded a new conceptualization and benchmark for psychiatry, the rapid acting medicine, and launched an increase in research centred on glutamatergic modulating pharmaceuticals for depression (Duman and Aghajanian, 2012). Due to its potential for abuse and harm (Liao *et al.*, 2011, Liao *et al.*, 2010, Morgan *et al.*, 2014), ketamine is not considered by some to be a viable standard treatment for the majority of MDD patients (Sanacora and Schatzberg, 2015, Schatzberg, 2014). However, research has sought to utilise it as a model to further understand the biological underpinnings of depression and its rapid treatment. It is hoped that with further understanding of the clinical and biological mechanisms with which ketamine exerts its rapid-acting antidepressant effect, better

treatments can be created and, furthermore, that specific treatments for symptoms and subtypes be delineated.

1.4.2.1 Clinical symptoms associated with successful treatment with ketamine

The rapid-acting nature of ketamine is a huge benefit over standard treatments for MDD. In particular however, it is the symptoms that ketamine has been shown to quickly improve that have caused such great enthusiasm for the drug. Suicide and suicidal ideation are a huge burden associated with MDD; due to the nature of these behaviours, rapid resolution of these symptoms is of the utmost importance. However, there is currently no approved treatment specifically designed to rapidly decrease suicidal ideation in MDD, although clozapine is recommended for suicidal patients with schizophrenia. It has now been demonstrated several times that ketamine can cause a rapid reduction in suicidal ideation (Ballard *et al.*, 2014, DiazGranados *et al.*, 2010b, Price *et al.*, 2009b); at least one study has examined the efficacy of ketamine in treating suicidal depressed patients in a naturalistic emergency department setting, finding similarly consistent anti-suicidal effects (Larkin and Beautrais, 2011).

In addition to causing a significant reduction in suicidal ideation, ketamine may be particularly beneficial for MDD patients who have anhedonia. There is mounting evidence that standard medication treatments for MDD patients are ineffective at relieving anhedonia (Boyer *et al.*, 2000) and may even induce anhedonic symptoms (Hindmarch, 1998, Price *et al.*, 2009a). Nierenberg and colleagues (1999) found that anhedonia was one of the most prevalent residual symptoms in MDD patients who responded to fluoxetine; importantly, residual symptoms are thought to precipitate relapse (Paykel, 2008). Moreover, the presence of anhedonia is associated with poorer treatment response to both pharmacological therapy (Uher *et al.*, 2012) and novel therapeutics such as rTMS (Downar *et al.*, 2014). Recent evidence suggests that ketamine may be effective at improving levels of anhedonia in both MDD (DeWilde *et al.*, 2015, Lally *et al.*, 2015b) and BD (Lally *et al.*, 2014b) patients. In particular, Lally and colleagues (2014b) found that ketamine rapidly improved levels of anhedonia in treatment refractory BD patients, with the anti-anhedonic effect still present even when the other depressive symptoms

were controlled for, suggesting that ketamine may specifically improve anhedonia in depressed patients.

1.4.3 Purported antidepressant mechanisms of action of tDCS and ketamine

How do tDCS and ketamine exert their behavioural and neural changes? Intriguingly, recent research has posited a prominent role for glutamatergic based BDNF-dependent plasticity in the treatment of depression (Duman and Aghajanian, 2012, Nosyreva *et al.*, 2013), through which, both of these agents may work. Fritsch et al. (2010) found that tDCS plasticity was dependent upon BDNF and also increased levels of BDNF in rodents. Haile et al. (2014) found that plasma levels of BDNF were highly correlated with improvements in depression score following ketamine, but not post-midazolam. Moreover, Lepack et al. (2015) found that BDNF release was a required component of the antidepressant actions of ketamine in rodents. Autry and colleagues (2011) further examined the neural mechanisms behind ketamine's response in a series of elegant experiments in mice, demonstrating that ketamine's antidepressant effects, as well as another NMDAR antagonist (MK-801) that elicits a rapid-acting antidepressant response in rodents, are dependent upon the rapid translation, but not transcription, of BDNF. Furthermore, healthy volunteers and MDD patient carriers of the *met* allele of the BDNF single nucleotide polymorphism rs6265 exhibit attenuated responses to tDCS (Fritsch *et al.*, 2010) and ketamine (Laje *et al.*, 2012). Importantly, tDCS is also NMDA receptor dependent (Fritsch *et al.*, 2010), further linking its mechanisms of action with the glutamatergic system and plasticity.

Preclinical evidence suggests that the neural effect of ketamine is mediated by increases in glutamatergic activity via disinhibition of inhibitory interneurons in the prefrontal cortex (Homayoun and Moghaddam, 2007). Administration of both excitatory tDCS and sub-anaesthetic doses of ketamine have been shown to cause acute alterations in Glx (a glutamatergic composite marker of glutamate and glutamine) and gamma-amino butyric acid (GABA)-ergic levels, as measured by proton magnetic resonance spectroscopy (^{1}H-MRS) in healthy volunteers (Clark *et al.*, 2011, Stagg *et al.*, 2009, Stone *et al.*, 2012). Importantly, intravenous infusion of

a sub-anaesthetic dose of ketamine has very recently been shown to acutely increase, by nearly 40%, levels of both GABA and Glx in the medial prefrontal cortex of MDD patients (Milak *et al.*, 2015), providing further evidence for a glutamatergic and plasticity-related theory of depression and its treatment; however, similar studies in healthy humans (Rowland *et al.*, 2005, Stone *et al.*, 2012, Taylor *et al.*, 2012) and non-acutely in depressed patients (Valentine *et al.*, 2011) reported mixed findings and smaller effects. Interestingly, Li et al. (2010) demonstrated that ketamine caused an increase in both protein signalling and the number and function of spine synapses in the prefrontal cortex of rats and this increase in plasticity was dependent upon the glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. Thus, it appears that NBS techniques and NMDA receptor antagonism may share a common neural mechanism in relieving depressive symptoms; depression appears to be improved by techniques that enhance neuroplasticity via glutamatergic and BDNF dependent mechanisms.

1.4.4 Cognitive symptoms improved by ketamine

Cognitive processing post-ketamine administration has been studied far less than following standard antidepressant treatment in patients with MDD. In an active comparator infusion study comparing sub-anaesthetic doses of ketamine and midazolam in treatment refractory MDD patients, Murrough et al. (2015) found improvements in neurocognitive performance from baseline at seven days post-infusion for both drugs, but no performance differences between ketamine and midazolam at seven days post-infusion. Furthermore, there was no association between the magnitude of the antidepressant response to ketamine and the improvements in cognition. The lack of an association between treatment response and behaviour change might suggest that ketamine may not have specific cognitive enhancing capabilities and that performance improvements may be mediated by indirect effects on other symptoms, for example anhedonia or mood; the results may also be partially explained by practice effects or the comparable cognitive enhancing capabilities of midazolam. Interestingly, the slowest performing patients improved the most following ketamine, replicating an earlier finding (Murrough *et al.*, 2013b), which may suggest that ketamine causes the greatest improvements in the most

impaired or depressed patients, much like other antidepressant and psychotherapeutic interventions. One study (Price *et al.*, 2009b) examined whether open-label ketamine treatment might, in addition to improving scale scores related to suicidal ideation, improve levels of both explicit and implicit suicidality, as measured by a cognitive task; a death related implicit association test was administered both pre- and post-infusion to a small sample (N = 10) of MDD patients. Price et al. (2009b) found that implicit suicidality was reduced following intravenous ketamine and that the magnitude of this reduction was correlated with the anti-suicidal impact of the sub-anaesthetic infusion of ketamine. Further research on the cognitive benefits of treatment with ketamine is needed to understand who might benefit most from this treatment. In particular, it will be important to understand what specific components of complex symptoms, such as anhedonia, are improved by treatment with ketamine.

1.4.5 Can current models of antidepressant treatment explain the effects of tDCS and ketamine?

The antidepressant effects of both tDCS and ketamine can be explained in terms of changes in neuroplasticity, a system that appears to be dysfunctional in MDD. Can the monoamine and cognitive neuropsychological models also explain their antidepressant or cognitive enhancing capabilities? While there is very little evidence for monoaminergic effects of tDCS there is an increasing amount of studies examining such systems in relation to ketamine. A PET study in healthy rhesus macaques revealed that administration of a sub-anaesthetic dose of ketamine was associated with increased serotonin receptor (5-HT1B specifically) and decreased serotonin transporter binding in the nucleus accumbens and ventral pallidum (Yamanaka *et al.*, 2014); the effect of ketamine on serotonin receptor binding, but not transporter, was blocked by administration of an AMPA receptor antagonist, which blocks the antidepressant effect of ketamine in rodents, implicating an interaction between the glutamatergic and serotonergic systems in ketamine's mechanism of action. A combined PET and microdialysis study in healthy macaques revealed that administration of a sub-anaesthetic dose of ketamine was associated with increased serotonergic transmission in the prefrontal cortex via inhibition of serotonin transporter activity (Yamamoto *et al.*, 2013). Interestingly, the

antidepressant effect of ketamine, as assessed by immobility time in the forced swim test, was blocked in rats that underwent serotonin depletion (Gigliucci *et al.*, 2013), suggesting the monoamine system may be critical for ketamine to exert an antidepressant effect in rodents. It has been shown that ketamine exhibits behaviour suggestive of a partial agonist of the dopaminergic D2 receptor (Kapur and Seeman, 2002) and causes increases in levels of dopamine in the striatum (Vollenweider *et al.*, 2000). Interestingly, a recent report provided the first evidence that the antidepressant effect of ketamine is partially dependent on the dopaminergic system in rodents (Belujon and Grace, 2014); additional evidence suggests that the D2/D3 receptor, but not D1, may be particularly important for the antidepressant effect of ketamine in rodents (Li *et al.*, 2015b).

Interestingly, the administration of ketamine to healthy volunteers is a model of schizophrenia (Corlett et al., 2007), a disorder treated primarily by dopamine modulating medication. While the recent preclinical evidence purports that dopamine is important for the antidepressant effects of ketamine, clinical evidence in depressed patients suggests that the psychosis-like effects of ketamine may not be clinically relevant to its antidepressant response. Luckenbaugh and colleagues (2014) found that the dissociative, but not the psychotomimetic, side effects during a sub-anaesthetic intravenous ketamine infusion positively correlated with the antidepressant effects at both 230 minutes and seven days post-infusion in a mixed sample (N = 108) of depressed patients (MDD and BD); however, a study with a smaller sample (N = 27) found a relationship between the psychotomimetic, but not the dissociative, side effects and the antidepressant response to ketamine (Sos *et al.*, 2013). Preclinical evidence suggests that levels of presynaptic glutamate release or cycling to glutamine may mediate the dissociative side effects of ketamine (Anand *et al.*, 2000), a hypothesis that could be partially testable via ^{1}H-MRS investigations. Nevertheless, the dissociative side effects are one the strongest predictors of an antidepressant response to ketamine in treatment-refractory patients, (Luckenbaugh *et al.*, 2014), suggesting an important psychological component to the antidepressant efficacy of ketamine. A thought-provoking report by Dakwar et al. (2014a) found that sub-anaesthetic intravenous infusions of ketamine in cocaine dependent individuals caused an increase in mystical thinking, which was found to mediate the

motivation to quit cocaine 24-hours post-infusion (Dakwar *et al.*, 2014b). Thus, a strong psychological experience, such as an increase in mystical thinking driven by dissociative effects, may be important for the rapid-acting antidepressant efficacy of ketamine.

Whether tDCS is an effective antidepressant by itself remains to be seen; hypothetically, it may exert its effects by increasing DLPFC functionality, allowing for greater executive function or cognitive control and thus might work in a similar manner to CBT.

1.5 Thesis aims, hypotheses and predictions

The overall aim of this thesis was to investigate the neural and cognitive underpinnings of two novel antidepressant treatments, tDCS and ketamine. Both treatments are thought operate in part by increasing glutamatergic transmission and, via somewhat similar cellular and molecular mechanisms of action, by enhancing plasticity. However, it is unknown if either technique improves cognitive impairments found in depression or how these induced improvements may translate at the neural level. These questions will be addressed through four experimental chapters.

1.5.1 Chapter 2

In Chapter 2 we assessed whether excitatory fronto-extraencephalic DLPFC tDCS could improve performance in healthy volunteers on a working memory task on which MDD patients show reliable deficits, the n-back, in a double-blind, multi-stimulation, sham-controlled investigation. The aim was to probe the efficacy of tDCS to function as a cognitive enhancer first in healthy volunteers, with the hope that a successful outcome could then be applied to MDD patients. The rationale for focusing on DLPFC stemmed from its suggested involvement in affective disorders (Fales *et al.*, 2009, Rajkowska *et al.*, 1999), and clear evidence for deficits in MDD patients on cognitive tasks tapping its function (Snyder, 2013). Research has identified DLPFC as a node that is hyperactive in MDD patients while performing the n-back, despite comparable behavioural performance to control subjects (see

Wang *et al.* (2015) for a meta-analysis), suggesting that MDD patients may need to exert increased cognitive resources to maintain similar working memory performance. We hypothesized that excitatory anodal tDCS, applied in conjunction with a cognitive task, would up-regulate (and possibly increase the efficiency of) DLPFC function, which is known to underlie working memory performance; DLPFC activity scales linearly with cognitive load during the n-back in healthy volunteers (Yun *et al.*, 2010). It is thought that tDCS increases neuronal excitability by pumping in positive ions beneath the anodal stimulated area (Nitsche and Paulus, 2000), thus DLPFC in theory should participate at a higher level during active stimulation. tDCS may also increase plasticity in the region via increased levels of glutamate transmission (Clark *et al.*, 2011) and BDNF secretion (Fritsch *et al.*, 2010). Thus, tDCS stimulated DLPFC neurons may contribute more to the network of brain regions implicated in the task and permit greater performance enhancements for those receiving active, than sham, stimulation. Therefore, we predicted that excitatory tDCS would cause an enhancement in working memory performance over sham stimulation in healthy volunteers. Additionally, we predicted that there would be a linear improvement of the enhancement effect of active stimulation over sham with repeated tDCS sessions.

1.5.2 Chapter 3

In Chapter 3 we evaluated the reliability of an adapted ^{1}H-MRS pulse sequence (An *et al.*, 2015) to quantify levels of neural glutamate and glutamine at 7 Tesla (T). Specifically, we explored within- and between-session variability of glutamate and glutamine levels in healthy volunteer brains. This assessment allowed us to test the consistency of our pulse sequence in measuring neural glutamate and glutamine levels before using this methodology in a subsequent experiment (Chapter 4). The aim was to evaluate the sequence for within- and between-session reliability to permit an accurate estimation of the measurement noise prior to using the pulse sequence in clinical and pharmacology studies. We expected that the novel ^{1}H-MRS sequence would allow excellent detection of both glutamate and glutamine due to their resolved nature with this sequence and the high field strength used here; these

factors should also facilitate reliable repeated measurement of these metabolites within- and between-scanning sessions.

1.5.3 Chapter 4

In Chapter 4 we assessed whether there were baseline differences in anhedonia-related psychometric scales, reward tasks and, using the evaluated ^{1}H-MRS sequence from Chapter 3, medial prefrontal cortex glutamate and glutamine levels, between medication free patients currently in a major depressive episode and healthy volunteers. We then examined these metrics again in the same patients following intravenous infusion of a sub-anaesthetic dose of ketamine and placebo (exactly two-weeks between infusions) and explored whether changes in depressive symptoms following ketamine were related to behavioural and glutamatergic neurotransmitter changes. We hypothesized that ketamine would cause an acute increase in prefrontal cortex glutamate levels via disinhibition of GABA interneurons and that this elevation would normalize these measures to healthy volunteer levels in successfully treated patients leading to an antidepressant response. The upshot to an increase in glutamate levels would be a cascade of events leading to protein synthesis and neural plasticity, namely an up-regulation of AMPA receptor activation, which would cause downstream increases in expression of plasticity dependent proteins, such as BDNF (Autry *et al.*, 2011). The result of an activation of BDNF may be an activation of mammalian target of rapamycin, which could lead to the reported increases in synaptic signalling proteins and new spine synapses (Li *et al.*, 2010). In particular, glutamatergic normalization in the medial prefrontal cortex is thought to underlie changes in levels of anhedonia following treatment with ketamine (Lally *et al.*, 2014b, Lally *et al.*, 2015b). By improving levels of anhedonia, potentially via a glutamatergic boost, we predicted that depressed patients would then respond more similarly to healthy volunteers on the anhedonia questionnaires and reward tasks.

We predicted that there would be baseline differences between patients and controls on anhedonia psychometric scales, reward tasks and medial prefrontal cortex ^{1}H-MRS measured glutamate levels. We aimed to replicate and extend a previous finding of a relationship between pre-treatment levels of a surrogate marker

of glutamine and response to ketamine in MDD patients (Salvadore *et al.*, 2012); the improved resolution permitted by the combination of our novel sequence and higher MRI field should permit a more accurate assessment of the relationship between glutamatergic metabolites at baseline and response to ketamine. We predicted that ketamine would also serve as a cognitive and motivational enhancer and improve performance on our reward tasks, increase ^{1}H-MRS measured glutamate levels and improve levels of anticipatory anhedonia. Finally, we predicted that the improvements in psychometric scales, reward tasks and medial prefrontal cortex ^{1}H-MRS measured glutamate or glutamine levels following ketamine would be interrelated, with relative increases in medial prefrontal cortex glutamate or glutamine positively relating to the magnitude of improvements on tasks and scales.

1.5.4 Chapter 5

In Chapter 5 we assessed whether there were baseline differences in blood-oxygen-level dependent (BOLD) contrast imaging between the same medication free depressed patients and healthy volunteers as Chapter 4, while both groups performed the aforementioned (Chapter 2) n-back task. There is strong evidence to suggest that working memory deficits may play a prominent role in depression (Christopher and MacDonald, 2005, Joormann *et al.*, 2011, Pelosi *et al.*, 2000, Rose and Ebmeier, 2006). Similarly to Chapter 4, we again examined cognition and neural activity (using BOLD contrast imaging) changes following intravenous ketamine and placebo (again, two-weeks apart) and related these to changes in depressive symptoms. The goal was to assess whether ketamine causes an improvement in working memory performance in patients and what are the mediating structures underlying response to the rapid acting antidepressant. We hypothesized that ketamine would improve cognitive control associated neural network activity via the aforementioned enhancement in plasticity in depressed patients who respond to the treatment. Specifically, we hypothesized that DLPFC would be more efficient two days post-ketamine leading to a corresponding reduction in the required levels of activity required to complete the task. We predicted that depressed patients would be worse than healthy volunteers at performing the n-back task at baseline and that their underlying BOLD activity associated with this task, particularly in the DLPFC

region, would be greater (Wang *et al.*, 2015). We also predicted that ketamine would normalize DLPFC BOLD activity and task performance in depressed patients who respond to the treatment. Finally, we predicted that response to ketamine would be related to neural activity, particularly in the DLPFC region, elicited during the baseline performance of the n-back.

2 Does excitatory fronto-extracerebral tDCS lead to improved working memory performance in healthy volunteers?

2.1 Abstract

Evidence suggests that excitatory tDCS may improve cognitive performance in both healthy volunteers and depressed patients. Recent reports also suggest that tDCS may possess general antidepressant potential. However, questions remain regarding the exact efficacious stimulation parameters and the precise nature of the mood and cognitive enhancements. Here, using a double-blind between-subjects design, we explored whether 1 mA excitatory (anodal) left DLPFC stimulation with a contralateral extracerebral reference electrode, leads to enhanced working memory performance across two days, relative to sham stimulation, in healthy volunteers (N = 21). Participants performed the 3-back, a test of working memory, at baseline, and during and immediately following stimulation on two days, separated by 24-to-48-hours. Active stimulation did not significantly enhance performance versus sham over the course of the entire experiment. However, exploratory comparisons revealed a significant effect (which survived correction for multiple comparisons) of stimulation group on performance during the first stimulation phase only, with active stimulation recipients performing better than sham. While these results do not fully support the hypothesis that excitatory DLPFC tDCS boosts working memory, they raise the possibility that its effects may be greatest during early learning stages, which could have implications for the treatment of depression.

2.2 Introduction

tDCS has been utilised as a non-invasive brain stimulation methodology to improve performance on a variety of cognitive tasks in healthy volunteers, including decision-making (Hecht *et al.*, 2010), planning (Dockery *et al.*, 2009) and working memory (Andrews *et al.*, 2011, Ohn *et al.*, 2008). Due to the minimal risk profile, arising as function of the very low current delivered to the scalp, and the relatively inexpensive nature of the device, it has high potential as a tool for cognitive enhancement in clinical populations. Indeed, a growing body of evidence suggests that tDCS may be effective in specifically enhancing cognition in patients with depression (Bueno *et al.*, 2011, Oliveira *et al.*, 2013, Wolkenstein and Plewnia, 2013). As cognitive impairment is a prevalent and frequently difficult to treat symptom in depression (Austin *et al.*, 2001), the apparent cognitive enhancements induced by tDCS may underpin the reported, yet tentative, antidepressant effects of the stimulation (Brunoni *et al.*, 2014, Brunoni *et al.*, 2013b, Meron *et al.*, 2015). However, the optimal stimulation parameters are presently unknown. Questions remain over stimulation condition blinding (O'Connell *et al.*, 2012), stimulation frequency (Hoy *et al.*, 2013) and appropriate electrode placement (Miranda *et al.*, 2006) as these parameters can strongly influence the efficacy of the stimulation device, the induced neuronal activity and moreover, the interpretation of stimulation effects on cognitive performance. Questions have also been raised about appropriate behavioural and stimulation controls (Walsh, 2013).

tDCS kits comprise two polarised stimulation electrodes, both of which are connected to a battery-powered device that delivers constant current. The application of the excitatory tDCS electrode to the scalp is thought to cause an increase in neuronal excitability in the stimulated area by altering the resting potential (Nitsche and Paulus, 2000). To complete the electrical circuit, the reference or inhibitory cathodal electrode must be placed somewhere on the head or body being stimulated. The majority of studies exploring cognitive enhancement using tDCS have targeted DLPFC as their region of excitation while the inhibitory electrode has typically been placed on the contralateral supra-orbit (or DLPFC). For example, in a single blind investigation using this electrode montage, Ohn et al. (2008) found that 30 minutes

of 1 mA tDCS while participants performed the n-back (Kirchner, 1958), a cognitive task commonly used to assess aspects of executive function and thought to engage working memory in particular, led to significant improvements in task performance over sham during stimulation only. However, placing the reference electrode on the scalp introduces a potential confound in the interpretation of any resulting behavioural effects: these could arise as a result of excitation, inhibition, or a combination of the two electrodes. The location of the reference electrode, whether extra or intra -cephalic, has been show to play a prominent role in the efficacy of the excitatory electrode (Moliadze *et al.*, 2010).

A single-blind within-subjects investigation by Zaehle et al. (2011) demonstrated that 15 minutes of 1 mA excitatory tDCS applied to left DLPFC during rest, with the inhibitory electrode placed ipsilaterally at the mastoid, resulted in enhanced post-stimulation performance on the 2-back task in comparison with cathodal, but not sham, stimulation. Importantly, Zaehle et al. (2011) utilized a fronto-extracephalic montage, which attenuates interpretational difficulties as the reference electrode and its position can affect the efficacy of tDCS and the underlying neuronal activity (Moliadze *et al.*, 2010). Furthermore, it remains to be determined whether stimulation during a task or while at rest is more beneficial. Andrews et al. (2011) found tentative evidence to suggest that DLPFC tDCS applied concurrently with a cognitive task may provide more robust effects on subsequent working memory performance that stimulation during rest.

Working memory dysfunction, and executive function deficits more broadly, have been found in depression (Snyder, 2013). Indeed, executive function performance has been identified as a tractable endophenotype to explore in depression (Hasler *et al.*, 2004). Thus, there is significant potential for non-invasive brain stimulation techniques such as tDCS to be applied clinically to ameliorate cognitive dysfunction. The n-back task has frequently been used in the context of functional neuroimaging experiments in both healthy volunteers (Owen *et al.*, 2005) and patients with depression (Snyder, 2013). The results of these studies consistently implicate a network of brain regions including parietal cortex and DLPFC, which are engaged with increasing cognitive load during n-back performance (Owen *et al.*,

2005). Importantly, altered DLPFC function is associated with depression (Pizzagalli, 2011). For example, a recent meta-analysis of working memory fMRI research in MDD, 60% of which used the n-back task, identified the DLPFC as a region of consistent hyperactivity in MDD patients, despite no between group behavioural differences to healthy volunteers (Wang *et al.*, 2015), suggesting that MDD patients may need to use greater neural resources to achieve the same level of performance. Enhancing DLPFC efficiency in depressed patients is one potential avenue towards cognitive enhancement. However, in order to establish whether tDCS has the potential to improve clinical conditions through modulatory effects on executive function, it is important to first establish the effects of specific stimulation parameters in healthy volunteers.

Here, we sought to build on prior research (Andrews *et al.*, 2011, Ohn *et al.*, 2008, Zaehle *et al.*, 2011) by conducting a double-blind between-subjects experiment to examine whether excitatory DLPFC tDCS applied across two days would lead to enhancement of n-back performance during and post-stimulation. Specifically, we assessed whether excitatory fronto-extracerebral DLPFC tDCS, with the reference on the contralateral cheek, could improve performance on the 3-back in healthy volunteers across two stimulation days. We predicted that those receiving active stimulation would have greater task performance improvement, relative to baseline, in comparison with sham stimulation recipients.

We hypothesized that anodal (excitatory) tDCS applied to the left DLPFC during the n-back would up-regulate neuronal functionality in this region. In healthy volunteers, DLPFC activity scales linearly with cognitive load during the n-back (Yun *et al.*, 2010). tDCS is thought to increase neuronal excitability beneath the stimulated area of the anodal electrode by lowering the action potential threshold (Nitsche and Paulus, 2000), thus DLPFC neurons should in theory be more activated during anodal stimulation. Consequently, tDCS stimulated DLPFC neurons may participate more in the neuronal network elicited by the n-back and permit greater performance enhancements for those receiving active, than sham, stimulation. Therefore, we predicted that excitatory tDCS would elicit a working memory performance enhancement, over sham stimulation, in healthy volunteers. Moreover,

we predicted that a linear improvement of the performance enhancement effect of active stimulation, over sham, would occur with repeated tDCS sessions.

2.3 Methods

2.3.1 Participants

Twenty-one (14 females, M = 23.09 years, SD = 3.95) right-handed participants were recruited from the Psychology subject pool at University College London, UK. Participants self-reported no history of mental or neurological illness, current psychiatric medication use, no prior or current participation in another brain stimulation experiment within the previous 24-hours and had normal or corrected to normal vision. There were no significant age ($t_{(19)} = 0.211$, $P = 0.835$) or gender ($X^2_{(1)} = 1.527$, $P = 0.361$) differences between the two stimulation groups (active and sham). All participants provided written informed consent and were compensated for their time. The study was approved by the UCL ethics committee.

2.3.2 Task

The n-back (**Figure 2.1**) consisted of a continuous sequence of 300 (150 for baseline) centrally presented consonants (500 ms) interleaved with fixation crosses (1500 ms). Participants were instructed to respond to every appearance of a letter (button pressing did not affect stimulus timing), pressing the 'H' key only when the letter onscreen matched the letter 3-back, and pressing the 'F' key for all other instances. It is thought that this version of the n-back may afford increased sensitivity to working memory performance than versions that focus solely on hits (Haatveit *et al.*, 2010, Zaehle *et al.*, 2011). Matches (one-fifth of all stimuli) and non-matches to 3-back stimuli were randomized in order but their ratio was fixed throughout the experiment. The task was coded in MATLAB (release 2008b for Windows; Mathworks, Natick, MA, USA) using the Cogent Toolbox (http://www.vislab.ucl.ac.uk/cogent_2000.php) and is available for free online (https://sites.google.com/site/nialllally/home/code/. The code is also permanently available at 10.5281/zenodo.7148.

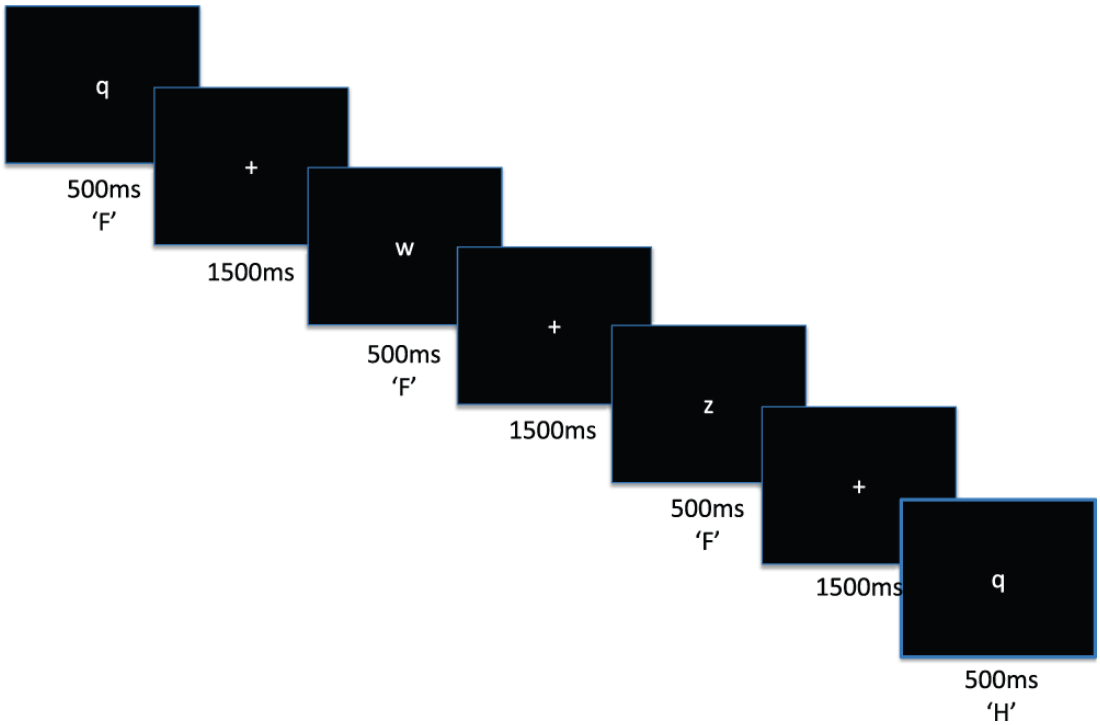

Figure 2.1. Schema of 3-back task. Stimuli (consonants) were presented centrally for 500ms and followed by a fixation cross for 1500 ms. Participants were instructed to respond to every stimulus, indicating whether the stimulus matched the letter 3-back ('H') or not ('F').

2.3.3 tDCS

tDCS was administered continuously at 1 mA using the Neuroconn DC-Stimulator (Neuroconn, Germany) via a pair of rubber electrodes (7 cm × 5 cm) housed in small synthetic sponges dampened with salt water to increase conductivity. The excitatory (anodal) electrode was placed over F3 (**Figure 2.2A**), corresponding to the left DLPFC, while the reference (cathodal) electrode was placed on the contralateral cheek (Berryhill and Jones, 2012, Tseng *et al.*, 2012). F3 was located using a 10-20 electroencephalography cap and demarcated using a removable marker. Left DLPFC was chosen as the anodal electrode position as this region has been consistently implicated in working memory paradigms (Owen *et al.*, 2005). Additionally as the task involved processing static letters, the left side of the brain was considered most appropriate (Mull and Seyal, 2001). Once the area was located, the electrodes were fastened in position using two headbands (a polyester hairband across the forehead and a rubber band beneath the jaw and around the circumference of the head; **Figure 2.2B**).

Before arrival, participants were randomized to one of two brain stimulation conditions using MATLAB, active (N = 10) or sham (N = 11); participants remained in their stimulation group (active or sham) throughout the experiment i.e. active tDCS recipients on Day 1 received active tDCS on day 2. Specific codes were selected from the tDCS device manual by an independent researcher not involved with the study and were assigned to each condition and randomized to each participant. Importantly, utilizing the 'study mode' of the device allowed the stimulation-administering researchers to remain blinded to the condition participants were in as the readout on the stimulation apparatus was identical for both active and sham stimulation. However, the integrity of the blinding was not assessed. The administered current was applied for 10 minutes with an additional 15-second fade-in and fade-out ramping period to minimize discomfort and facilitate participant blinding. Sham stimulation was limited to small pulses of 100–200 µA every 400–550 ms between a 15-second fade-in and fade-out voltage ramp (Palm *et al.*, 2013).

2.3.4 Study design

During the baseline session on day 1 (D1), participants were first trained on the n-back with a brief exposure to 1, 2 and finally 3-back. Thereafter, participants completed a 5-minute version of the 3-back, which served as a baseline pre-stimulation measure of performance. Immediately after, tDCS was administered for 10 minutes while participants performed the 3-back task (D1 tDCS; **Figure 2C**). Following this, participants completed a further 10-minute session of the 3-back (D1 post-tDCS) without stimulation. Participants were instructed that continuation to day 2 was dependent on D1 post-tDCS task performance but were not given feedback until the end of day 1. Continuation to day 2 was dependent on above chance performance on the D1 post-tDCS assessment only, which was any positive d' value:

$$d' = Z(\text{hit rate}) - Z(\text{false alarm rate}) \quad (1)$$

where hit and false alarm rate are the number of correct or incorrect 'H' responses, respectively, divided by the total number of opportunities (1/5 or 4/5 of total stimulus letters) and Z is the inverse of the cumulative Gaussian distribution. Participants received £10 for their participation on day 1 irrespective of task performance. Day 2

(24-to-48-hours later) consisted of one 10-minute task run with stimulation (D2 tDCS) and one post-stimulation (D2 post-tDCS). Participants were told that performing better than the test phase of day 1 would result in a bonus of £10 on top of the £5 basic payment on day 2. Thus, participants had a performance incentive for the post-tDCS assessment only on each day.

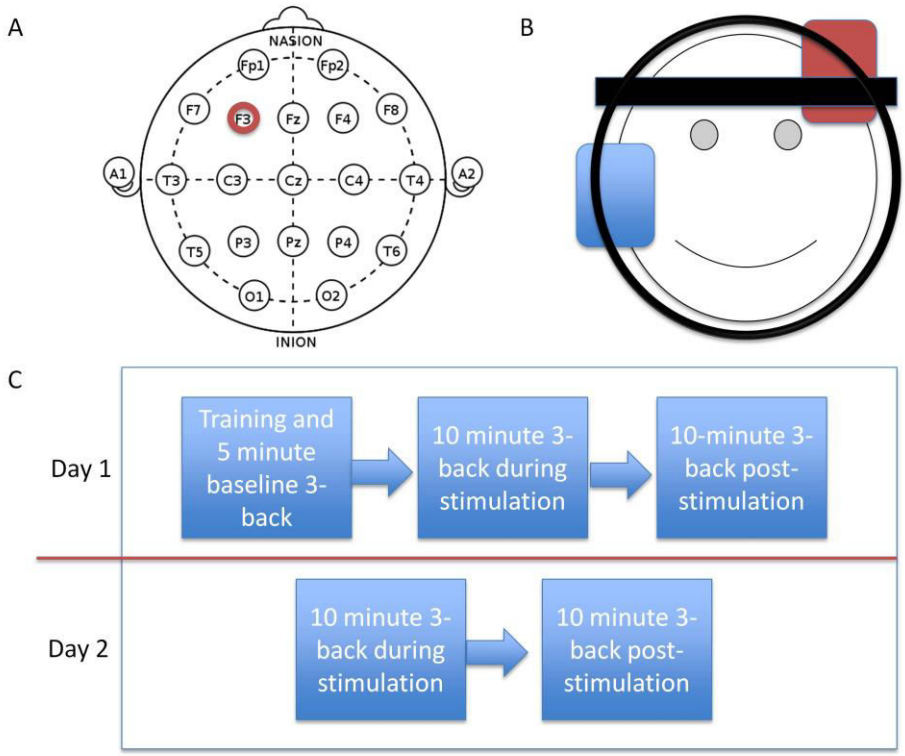

Figure 2.2. tDCS electrode montage and study design. A, B) The excitatory anodal electrode (red) was positioned using a 10-20 standard electroencephalography electrode cap under F3, which corresponds to dorsolateral prefrontal cortex. The inhibitory cathodal electrode (blue) was positioned on the contralateral cheek. C) Timeline of events in the study. Participants performed the 3-back on five separate occasions, once at baseline on day 1, twice during and twice following stimulation on both days.

2.3.5 Statistical analyses

To assess the effect of active stimulation versus sham over time, we conducted a linear mixed model in SPSS, version 21 (IBM Corp New York 2012). The dependent variable was d'. Follow up models also were conducted using hit rate, correct rejection rate (1 - false alarm rate) and reaction time for both of these variables. The four testing sessions after baseline (D1 tDCS, D1 post-tDCS, D2 tDCS, D2 post-

tDCS) were entered as a fixed effect of time; tDCS and sham stimulation were entered as a fixed effect of group; and their interaction (time-by-group) was also entered as a fixed effect. Participant number was entered as a random effect and baseline performance was entered as a covariate. A heterogeneous first order autoregressive covariance structure was employed. Bonferroni corrected tests between the groups at each time point were conducted using linear contrasts to assess between-group differences. Follow up assessments of significant points were assessed using a general linear model with baseline performance entered as a covariate. Performance differences at baseline were assessed using an independent samples *t*-test. Based on our sample size we had 80% power to detect a large effect size (d = 1.3) at $P = 0.05$ (two-tailed) between the stimulation groups.

2.4 Results

One participant (sham group) scored a negative d' value for day 1 and did not participate in session 2, but their data were included in the linear mixed model. Additionally, the testing computer malfunctioned during the day 1 post-tDCS assessment for 1 participant (active group), approximately 40% through the task; these data were included in the model and the participant completed a further post-tDCS test, which was used only to determine progress to day 2. There was no significant difference in d' performance between the groups at baseline ($t_{(19)} = 1.044$, $P = 0.309$; **Figure 2.3**). As expected, there was a significant main effect of time ($F_{(3,36)} = 7.669$, $P < 0.001$) on d' performance, reflecting improvement across both groups with increasing exposure to the task. However, contrary to our hypothesis, no main effect of stimulation group was identified ($F_{(1,16)} = 2.228$, $P = 0.155$) and there was no group × time interaction ($F_{(3,36)} = 1.339$, $P = 0.277$).

Exploratory Bonferroni corrected pairwise comparisons were carried out to assess group performance differences at the four post-baseline time points. A significant difference between active sham stimulation was identified at the day 1 tDCS time point ($F_{(1,13)} = 10.747$, $P = 0.006$; controlling for baseline performance and Bonferroni corrected for multiple comparisons), indicating a large effect size (Cohen's $d = 1.427$, $r^2 = 0.337$). No other stimulation group differences in d' were found at other time points (all $F < 1.2$, $P > 0.3$; see **Table 2.1** for group performance across sessions and task components). Further analyses of performance during stimulation on day 1 (including baseline as a covariate) revealed that both the hit rate ($F_{(1,18)} = 4.454$, $P = 0.049$, $\eta p^2 = 0.198$) and correct rejection rate ($F_{(1,18)} = 3.680$, $P = 0.071$, $\eta p^2 = .170$) of active stimulation recipients were significantly, or at trend level, better than sham. However, no significant reaction time differences were found for this time point for either hits ($F_{(1,18)} = 0.010$, $P = 0.923$, $\eta p^2 = 0.001$) or correct rejections ($F_{(1,18)} = 0.202$, $P = 0.659$, $\eta p^2 = 0.011$).

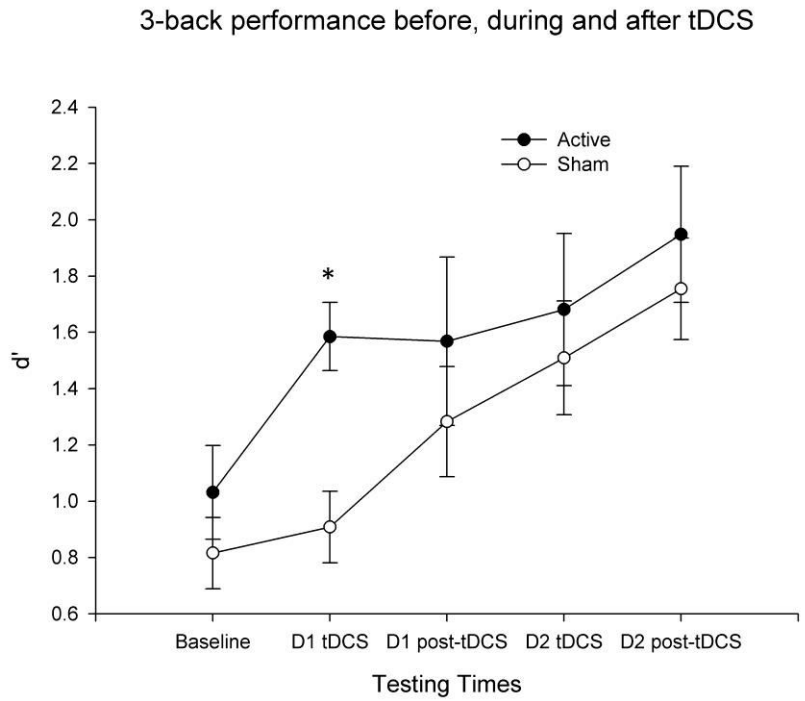

Figure 2.3. 3-back d' performance (mean values) across testing times and days. The active stimulation group always performed better than the sham group but only statistically significantly so during stimulation on day 1 (D1 tDCS), denoted by an asterisk (*). Baseline performance did not differ between the groups but was included in the model as a covariate. Error bars represent ±1 standard error of the mean.

Table 2.1. Means and standard deviations of each 3-back session per group.

		Baseline	D1 tDCS	D1 post-tDCS	D2 tDCS	D2 post-tDCS
Anodal	HR	0.4167	0.5250	0.5333	0.5533	0.6067
		(0.1694)	(0.1336)	(0.2278)	(0.2131)	(0.1994)
	CRR	0.8933	0.9296	0.9317	0.9283	0.9425
		(0.0355)	(0.0385)	(0.0486)	(0.0500)	(0.0389)
	Hit RT	0.6848	0.6651	0.6300	0.6052	0.6118
		(0.2587)	(0.2358)	(0.2467)	(0.2279)	(0.2609)
	CR RT	0.6702	0.6566	0.6180	0.5948	0.5939
		(0.2422)	(0.2159)	(0.2214)	(0.2229)	(0.2237)
Sham	HR	0.3909	0.4030	0.4879	0.5030	0.5333
		(0.1106)	(0.1197)	(0.1959)	(0.2048)	(0.2196)
	CRR	0.8614	0.8720	0.9049	0.8186	0.8390
		(0.0429)	(0.0577)	(0.0383)	(0.2757)	(0.2821)
	Hit RT	0.7026	0.6816	0.6345	0.5546	0.5714
		(0.2062)	(0.1763)	(0.1770)	(0.2339)	(0.2734)
	CR RT	0.7159	0.6780	0.6365	0.5727	0.5592
		(0.2017)	(0.1715)	(0.1630)	(0.2448)	(0.2685)

D1 = day 1, HR = hit rate, CRR = correct rejection rate; RT, reaction time, CR = correct rejection.

2.5 Discussion

Contrary to our hypothesis, no main effect of tDCS on task performance was identified in this study. However, exploratory tests suggested that active stimulation was associated with enhanced performance relative to sham stimulation during the first stimulation period on day 1 only. Our results may therefore indicate that the performance enhancement effects of excitatory tDCS may be limited to earlier stages of learning (Meinzer *et al.*, 2013). They also suggest that reports of improvements after one session of tDCS – the most common report in enhancement studies – may not translate to continual improvement with additional stimulation. Indeed, Martin *et* al. (2013) using an extracephalic electrode montage with the reference placed on the deltoid muscle, found in a relatively large university sample that, in comparison to sham stimulation, 10 sessions of DLPFC tDCS did not lead to a significant improvement in task performance on a variant of the n-back task when controlling for baseline performance. However, uncorrected contrasts, without controlling for baseline, revealed a benefit of active tDCS, in comparison to sham, at the first and eighth tDCS sessions only; a result largely consistent with our findings here of attenuated effects of tDCS on task performance with repeated sessions.

Our results do not support the hypothesis that excitatory tDCS applied to DLPFC results in post-stimulation improvement on the n-back task across multiple days. This result is consistent with some previous research; in comparison with sham stimulation, neither Zaehle et al. (2011) nor Ohn et al. (2008) demonstrated significant performance enhancements on the n-back task immediately following excitatory DLPFC tDCS. Nevertheless, we found evidence for a specific improvement in performance during stimulation on day 1 only, an outcome consistent with results from Ohn et al. (2008) and others (Fregni *et al.*, 2005). Andrews et al. (2011) found that DLPFC excitatory tDCS applied during a working memory task (n-back) led to significant improvements in post-stimulation performance in comparison with baseline on an alternative working memory task (digit span forward but not backward). The improvements found (Andrews *et al.*, 2011) were not present for either sham stimulation in conjunction with task performance or stimulation without task performance. Behavioural data were not reported for the task during stimulation and an intracerebral reference electrode was

used, limiting direct comparison with the present study. Furthermore, Hoy et al. (2013) found that 1 mA excitatory tDCS applied to DLPFC at rest resulted in an enhancement in 2-back RTs 40 minutes post-stimulation, but found no improvement in accuracy. However, other reports have found evidence for more enduring cognitive enhancement following tDCS (Cohen Kadosh *et al.*, 2010, Dockery *et al.*, 2009) (but see also Walsh (2013)).

This discrepancy between results may reflect the different tasks, stimulation parameters, sample sizes and study designs used. For example, it is possible that the payment schemes that served as a performance motivator here limited the potential to observe performance enhancing effects of tDCS. As there was no monetary motivation during the stimulation phase on day 1, participants may not have exerted themselves fully and thus the effects of stimulation may have had greater impact; while on day 2, participants in both groups may have reached a level whereby any potential for further enhancement of performance through tDCS was limited. Whilst the sample size used here is low for a between-subjects study, few tDCS studies have thus far been conducted using large sample sizes, and future studies should address the issue of stimulation parameter optimization using large sample sizes. Nevertheless, our results suggest that tDCS may be particularly sensitive to earlier stages of learning (Meinzer *et al.*, 2013).

In theory, the beneficial effects of tDCS may be most pronounced in poorer performers. Indeed, there is some evidence that tDCS may be particularly useful as a cognitive enhancer with lower performing individuals (Tseng *et al.*, 2012). As the population utilised here primarily comprised students from University College London, between-group differences arising as a function of tDCS may have been attenuated due to high initial baseline ability. As depression is associated with substantial deficits in executive function task performance (Snyder, 2013), which is akin to a lower baseline performance rate in comparison to healthy volunteers, depressed patients in particular may benefit from the tDCS cognitive enhancement shown here. Recent research has indeed shown that excitatory DLPFC tDCS can enhance cognitive control, a component of executive function, in major depressive disorder (Wolkenstein and Plewnia, 2013); however, long-lasting cognitive

ameliorative effects of stimulation in depression have yet to be demonstrated. If our finding that tDCS advances early learning holds true, tDCS could in theory be used to advance patients cognitive state to facilitate other therapeutic interventions, such as CBT.

The electrode montage used here (fronto-extracerebral) may have also played a significant part in the efficacy of the stimulation. While DLPFC is one of the most frequent site selections for anodal tDCS, placing the reference (cathodal) electrode on the contralateral cheek is a relatively novel occurrence (Berryhill and Jones, 2012, Tseng *et al.*, 2012). Current modelling, a technique to determine the amount of electrical current cerebrally induced as a function of the electrode positioning and other parameters, has been performed for various anodal DLPFC montages, with the reference electrode placed on the contralateral supraorbit, DLPFC and deltoid. The DLPFC and contralateral cheek montage however has yet to be modelled, thus it is unknown if it is more or less efficacious than other electrode configurations. Nevertheless, the distance between the anodal and reference electrode is negatively correlated with the tDCS induced after-effect magnitude and duration in healthy volunteers (Moliadze *et al.*, 2010); in theory, the cheek may be preferential to the deltoid and trapezius muscles, however this remains to be assessed. Furthermore, the majority of early investigations of tDCS for task performance enhancement used an electrode montage with both electrodes positioned proximal to the cerebrum (e.g. DLPFC and the contralateral supraorbit), which limits the interpretation as to which brain region was primarily modulated as both electrodes could contribute, and may in fact do so in opposing ways, to alterations in task performance. Nevertheless, widespread changes in neuronal activity outside of the sites of stimulation have been consistently identified (Nord, 2013, Stagg *et al.*, 2013), making the issue of electrode placement complex. In sum, electrode positioning is a major potential confound when comparing tDCS effects on both brain activity and behaviour across studies, further research addressing the biological and behavioural consequences of differing electrode placement is needed.

The results of this experiment require replication and extension to validate the potential role for tDCS in executive function enhancement. In particular, the

evaluation of result specificity represents a prominent hole in the current literature. Few studies thus far have contrasted active stimulation results in comparison with control tasks and active stimulation of control site locations on the scalp (Walsh, 2013); such measures would be beneficial in assessing the findings here and across the field. Additionally, it could be fruitful to replicate this experiment without the monetary incentive. Testing a larger and more representative sample including non-university students would also be informative. Furthermore, while performance improvements under stimulation are important, the clinical utilization of tDCS may necessitate long lasting effects once stimulation has ceased. Finally, evidence suggests that individual differences in genotype may play a large part in susceptibility to the plasticity enhancing capabilities of tDCS. Fritsch et al. (2010) found that tDCS was more efficacious in both mice and humans possessing the homozygous Val/Val genotype of the BDNF polymorphism (rs6265), than Met carriers, though we did not have a sufficiently large sample to explore such moderators in the current study.

In conclusion, our results do not support the hypothesis that excitatory tDCS applied to the left DLPFC using a contralateral fronto-extracerebral electrode reference produces consistent improvements in executive function beyond the period of stimulation. Nonetheless, we found a beneficial effect of tDCS during task performance only when the task was relatively novel, which could be interpreted as indicating that this particular electrode montage, stimulation voltage and study design may be best suited to early stages of learning.

3 Reliability of 7T ^{1}H-MRS Measured Human Prefrontal Cortex Glutamate, Glutamine, and Glutathione Signals Using an Adapted Echo Time Optimised PRESS Sequence: A Between- and Within-Sessions Investigation

3.1 Abstract

Evidence suggests that aberrant brain glutamatergic signalling plays a role in depression. ^{1}H-MRS can non-invasively measure brain glutamatergic metabolite levels. Until recently, overlapping glutamatergic signals (glutamate, glutamine, and glutathione) could not easily be separated. However, the advent of novel pulse sequences and higher field magnetic resonance imaging (MRI) allows more precise resolution of glutamatergic signals. To ascertain the underlying mechanisms of depression and its treatment using ^{1}H-MRS, sequence-specific within- and between-session estimates of reliability are first required. At 7T, we acquired ^{1}H-MRS data from the medial pregenual anterior cingulate cortex of healthy volunteers (N = 26) twice on two separate days. An adapted echo time optimised point resolved spectroscopy sequence, modified with the addition of a J-suppression pulse to attenuate N-acetyl-aspartate multiplet signals at 2.49 parts per million, was used to excite and acquire the spectra. In house software was used to model glutamate, glutamine, and glutathione, amongst other metabolites, referenced to creatine. Intraclass correlation coefficients (ICCs) were computed for within- and between-session measurements. Within-session measurements of glutamate, glutamine, and glutathione were generally reliable (ICCs $\geq$ 0.7). As anticipated, ICCs for between-session values of glutamate, glutamine, and glutathione were slightly lower but nevertheless acceptable (ICC > 0.62). A negative correlation was observed between glutathione concentration and age ($r_{(24)}$ = -0.37; P < 0.05), and a gender effect was noted on glutamine and glutathione. The adapted sequence provides good reliability to measure glutamate, glutamine and glutathione signals at 7T and thus supports its use in the investigation of the underlying biology of depression and its treatment.

3.2 Introduction

Preclinical and clinical evidence suggests abnormal brain glutamatergic transmission may contribute to the pathophysiology of depression (Niciu *et al.*, 2014a, Paul and Skolnick, 2003, Sanacora *et al.*, 2012). ^{1}H-MRS provides a non-invasive estimate of at least three glutamatergic system components, glutamate, glutamine and glutathione. Glutamate is the most abundant amino acid and principal excitatory neurotransmitter in the human brain (Maddock and Buonocore, 2012). Glutamine, amongst other functions, is a precursor and principal metabolite of glutamate and is involved in the astrocytic cyclical regulation of glutamate (Maddock and Buonocore, 2012). Glutathione, the most abundant brain anti-oxidant, provides a reservoir of neuronal glutamate (Koga *et al.*, 2011) and serves important immunological roles. Studies using ^{1}H-MRS have linked abnormalities in the regulation of the glutamatergic system to depression (Hasler *et al.*, 2007, Lapidus *et al.*, 2014a, Luykx *et al.*, 2012, Walter *et al.*, 2009, Yuksel and Ongur, 2010), however others have found no differences (Abdallah *et al.*, 2014a, Godlewska *et al.*, 2015). Consistent with evidence of glutamatergic dysfunction in depression, drugs that modify this system, e.g. ketamine (Stone *et al.*, 2012), have been reported to rapidly improve depressive symptoms (Dutta *et al.*, 2015).

The potential for ^{1}H-MRS to help determine the mechanisms underpinning depression and its treatment remains both tantalising and tangible. However, to evaluate potential treatments using ^{1}H-MRS, its accuracy and reliability—both within- and between-sessions—must be determined (e.g. Cai *et al.* (2012)). Scanner field strength is particularly important for accurately measuring glutamatergic signals using ^{1}H-MRS. A recently reported ^{1}H-MRS sequence, evaluated at 7T (An *et al.*, 2015), affords enhanced detection of glutamate, glutamine, and glutathione. This sequence applies an extra excitation pulse to an echo time (TE) optimised point resolved spectroscopy (PRESS) sequence to weaken the contribution of the N-acetyl-aspartate (NAA) multiplet signal at 2.49 parts per million (ppm) via suppression of J-coupled NAA signals at 4.38 ppm; however, the reliability of this specific sequence (An *et al.*, 2015) has yet to be quantified. Hence, the purpose of the present study was to assess the within- and between-session reliability of this specific sequence (other sequences have been evaluated at 7T; (Cai *et al.*, 2012, Stephenson *et al.*,

2011, Wijtenburg *et al.*, 2013)) to detect neural glutamate, glutamine, and glutathione from a single medial prefrontal cortex voxel in healthy volunteers. Due to their resolved nature with this sequence and the high field strength (7T) used here, we expected that the adapted ^{1}H-MRS PRESS sequence would allow excellent detection of both glutamate and glutamine. The increased spectral resolution should also facilitate reliable within- and between-scanning sessions repeated measurement of glutamate and glutamine.

3.3 Methods

3.3.1 Participants

Twenty-six healthy volunteers (10 females and 16 males; mean age of all participants = 31.58 years, SD = 9.32, range = 20-54) were recruited to participate. Participants self-reported no history of head trauma, substance abuse or dependence, psychiatric or neurological illnesses. They were medically healthy as determined by physical and neurological examination and blood and urine laboratory tests. Psychiatric evaluation was performed by an experienced clinician using the structured clinical interview for DSM-IV (First, 2002), and confirmed by an unstructured interview with a board certified, practicing psychiatrist. The Combined Neuroscience Institutional Review Board at the NIH approved the study, and all participants provided written informed consent.

3.3.2 Design

Participants underwent MRI scanning once on two separate days (one session per day, each comprising two ^{1}H-MRS scans). The average time between each scanning session was 8.08 days (SD = 5.50, range = 2-21). Where possible, participants were scanned on the same day of the week and at the same time of day on the subsequent week or two-weeks later.

3.3.3 MRI

All MRIs were acquired using a Siemens 7T scanner. Images were collected using a 32-channel head coil. Standard 1 mm^3 isotropic resolution magnetization-prepared rapid gradient echo sequence images (repetition time (TR) = 3 s, TE = 3.9 ms, matrix = 256 × 256 × 256, inversion time (TI) = 1500 ms) were acquired on each of the two scanning days and used to create an anatomical brain image; this image was used to plan the location of the MRS voxel.

3.3.4 ^{1}H-MRS

Using the anatomical image, a 2 × 2 × 2 cm^3 voxel was placed in the medial pregenual anterior cingulate cortex (pgACC; **Figure 3.1A**). This region of the brain has been implicated in numerous neuropsychiatric disorders, particularly affective conditions such as major depressive disorder (MDD) (Hasler *et al.*, 2007, Walter *et al.*, 2009) and bipolar disorder (Ellison-Wright and Bullmore, 2010, Lally *et al.*, 2014b). The region was localized by placing the midpoint of the voxel at the midline between the two hemispheres in the axial view and the edge of the voxel adjacent to the genu of the corpus callosum in the sagittal view, permitting maximal grey, and minimal white, matter concentration. To allow for consistency in voxel positioning between scanning sessions, a screenshot of the voxel placement on the first scanning day, comprising sagittal, axial and coronal voxel viewpoints (**Figure 3.1A**), was created, and this was used to guide the second scanning session, which occurred on a different day.

Voxel-specific first and second order B_0 shim coefficients were adjusted using a fast, automatic shimming technique by mapping along projections (FASTMAP; (Gruetter, 1993)) sequence. Next, a water FID (free induction decay) was acquired to calculate and correct for frequency drift, an important determinant of spectral quality (Near *et al.*, 2014). The B_1 field was optimized using a stimulated echo sequence consisting of α^o - 90° - 90° - acquisition. The α^o pulse has no localization gradient and the two 90° pulses and acquisitions are localized. Hence, a one-dimensional bar going through the centre of the voxel was acquired and parameters were adjusted so that a null signal was found at the voxel centre. After these calibrations, water-suppressed MRS data were acquired using a TE-optimized PRESS pulse sequence modified by inserting a J-suppression pulse (An *et al.*, 2015). Water suppression was accomplished using eight RF pulses of ~350 Hz bandwidth. The residual water signal amplitude was smaller than the NAA peak amplitude in most cases. Only one data set was excluded due to failed water suppression, a hardware issue, where the residual water signal amplitude was more than 10 times larger than the NAA peak amplitude.

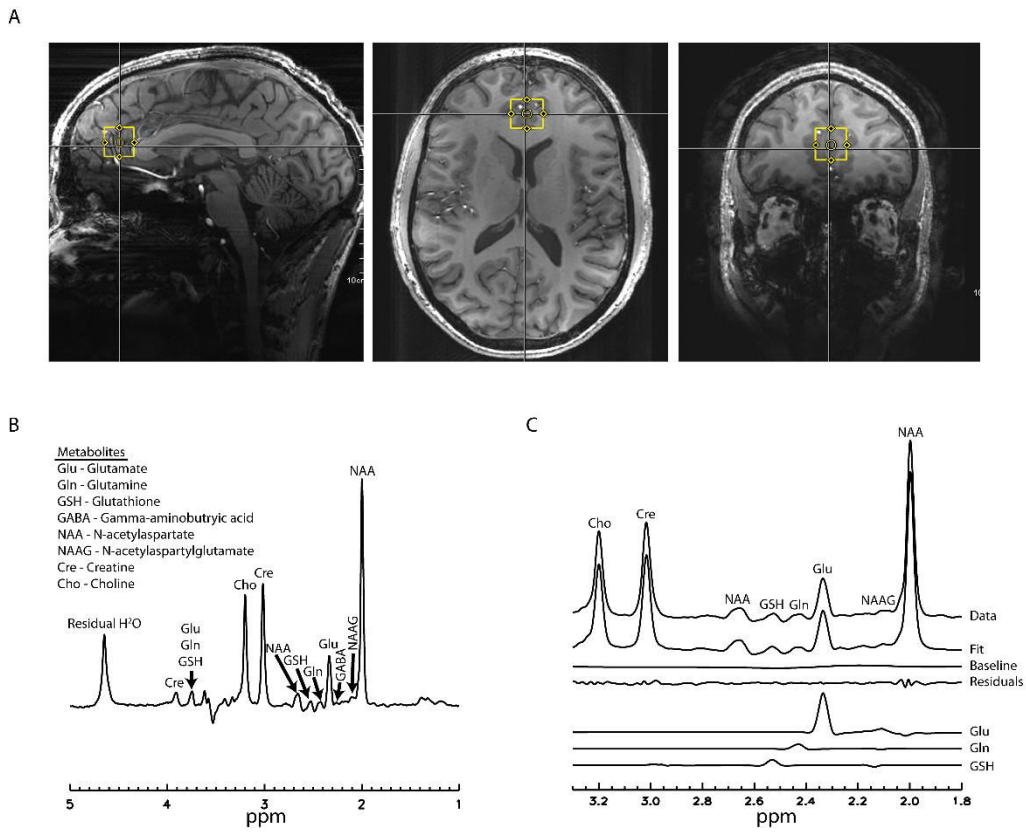

Figure 3.1. MRS voxel location, spectrum example showing metabolites and the corresponding model fit for our metabolites of interest. A) Sagittal, axial and coronal viewpoints showing the medial prefrontal cortex voxel positioning in yellow. B) A typical spectrum acquired from a study participant using our sequence. The x-axis is measured in ppm. C) The same data as (B), showing the corresponding model fit, baseline, and residual from our in-house software analysis.

The J-suppression pulse is a frequency-selective radiofrequency pulse placed at the resonance frequency of the aspartyl CH proton of N-acetyl-aspartate (NAA) at 4.38 ppm, thereby altering the J-evolution of the NAA aspartyl CH_2 multiplet at 2.49 ppm. The parameters TE_1 = 69 ms, TE_2 = 37 ms, and J-suppression pulse flip angle = 90° resulted in minimal NAA multiplet signals at 2.49 ppm while retaining near-maximum peak amplitudes for the C4 proton resonances of glutamate and glutamine using this sequence (An *et al.*, 2015), and were thus used here. The J-suppression pulse combined with optimized TE values minimized the interference of the NAA multiplet signals at 2.49 ppm to the detection of the glutamine signals at 2.45 ppm and glutathione signals at 2.54 ppm, thus enhancing our ability to resolve these peaks. Other parameters for the modified PRESS sequence were: TR = 2.5 s, spectral width = 4000 Hz, number of data points = 2048 and number of transients = 128.

For each scanning session (two ^{1}H-MRS scans per session, one session per day), participants were inside the MRI scanner for approximately 100 minutes. ^{1}H-MRS data were acquired once following the acquisition of the anatomical image at the beginning and once again at the end of the session (note, there was only one scanning session on each day) following echo-planar imaging (EPI) functional MRI (to be reported elsewhere). Once the calibrations were complete, the total time for each ^{1}H-MRS scan was approximately six minutes. Due to the long gap between the first and second MRS scan (approximately one-hour), when necessary, re-shimming was undertaken for the second scan. Precisely the same procedure was repeated on the second scanning day.

3.3.5 ^{1}H-MRS Modelling

The time-averaged 32-channel FID signals were merged into a combined single-channel metabolite FID using a generalized least squares method (An *et al.*, 2013). The combined FID was Fourier transformed into the frequency domain to generate the spectrum, which was thereafter processed using a custom written linear combination fitting program to estimate metabolite levels. Basis sets included glutamate, glutamine, glutathione, γ-aminobutryic acid (GABA), NAA, N-acetylaspartylglutamate (NAAG), choline, and creatine. A Levenberg-Marquardt least square minimization algorithm was used in spectral fitting. The basis functions of the metabolites were scaled, apodized using a Voigt lineshape, frequency shifted, zero-order phase corrected, Fourier transformed to the frequency domain, and added with a spline baseline with eight control points to fit the spectral data between 1.8 and 3.3 ppm. Each metabolite could have different linewidth but was constrained to have the same Lorentzian/Gaussian ratio. The frequency of each metabolite was constrained to vary within +- six Hz from its theoretical value. The zero-order phase of the spectral data was allowed to vary without any constraint. Metabolites were referenced to levels of creatine and are hereafter referred to by their metabolite names. Referencing to creatine reduces the need for tissue content correction because, similar to glutamatergic signals, creatine is only detected from the tissue and experiences the same BOLD effects as other metabolites (Lally *et al.*, 2014a). NAA was summed with NAAG, henceforth known as total NAA (tNAA), for

statistical analyses as the sequence used here is not optimal for reliable detection of NAAG (for an enhanced sequence to detect NAAG see (An *et al.*, 2014)). The TE values used by this sequence suppressed GABA signals, and quantification of GABA was thus compromised. Cramér-Rao lower bounds (CRLB) expressions of parameter variance were computed (Cavassila *et al.*, 2000). We computed the reduced chi-squared statistic for each spectrum to examine the fit of our model to the data; an arbitrary threshold of 12 or less for this statistic was set as an inclusion criterion for statistical analyses. Additionally, a water proton peak-line broadening criterion of 16 Hz was selected and only spectra with less than this value were included in the analyses.

3.3.6 Statistical Analyses

To rule out any systematic changes in our metabolites of interest (glutamate, glutamine, glutathione) we first assessed whether there was a main effect of scanning day or scan number, or an interaction between these two factors. We conducted a linear mixed model with compound symmetry selected as the covariance matrix and three fixed effects: scanning session (Day 1 or 2), scan number (1 or 2), and the interaction between these variables.

To determine the within- and between-session metabolite measurement reliability, we computed intraclass correlation coefficients (ICCs) in SPSS 21 (IBM SPSS, 2010, Chicago, IL, USA) with two-way random effects selected. Because we identified no effect for scan or day number on our metabolites of interest, we selected the more stringent absolute agreement (as opposed to consistency) option. We calculated reliability by comparing metabolites from the first and second scan (Scan 1 vs. Scan 2) within each day as well as the between-session reliability for each scan number (Day 1/Scan 1 vs. Day 2/Scan 1 and Day 1/Scan 2 vs. Day 2/Scan 2). The average (as opposed to single) measures ICC was selected from the SPSS output due to the averaging of both the FIDs and head coil channels conducted in the pre-processing stages. For consistency with previous neuroimaging studies (Nugent *et al.*, 2013), we used an ICC of 0.7 as our acceptable level. Specifically, poor, fair, good, and excellent reliability were arbitrarily defined as an average ICC value of < 0.6, 0.6 - 0.7, 0.7 - 0.8, and ≥ 0.8, respectively (Brandt *et al.*, 2013). Additionally, we

computed the coefficient of variation (CV; calculated as the standard deviation of the mean difference between two data points, divided by the mean of the two data points) to make our assessment more comparable to other 7T reliability studies. For completeness, we also determined the ICCs, CVs and systematic variation for other metabolites quantifiable using our pulse sequence, namely, tNAA and choline. We also examined GABA to demonstrate that this sequence is not suitable for reliably detecting GABA. Because we used creatine as the reference for all metabolites, it was not possible to examine its reliability. The reliability quantification and assessment of systematic change for tNAA, choline, and GABA was the same as for the analysis of glutamate, glutamine and glutathione.

We additionally assessed whether the number of days between each scanning day affected absolute change in any of our glutamatergic metabolites using Spearman's rho correlations due to the non-normal distribution. Finally, we also performed secondary exploratory analyses to examine previous reports of associations between our metabolites of interest and both gender and age. Higher glutamate levels (from a dorsolateral prefrontal cortex voxel) have been reported in men (O'Gorman *et al.*, 2011), and a negative correlation between age and glutamate (hippocampal and anterior cingulate cortex; ACC) has also been found (Schubert *et al.*, 2004). We expected our enhanced sequence to provide excellent spectral resolution to examine the relative quantity of glutamate and glutamine and to confirm previously reported associations. Although no association between ^{1}H-MRS-measured glutathione and age has been reported, we predicted that such a relationship would exist because intracellular responses to redox intermediaries are known to be less efficacious with age (Erden-Inal *et al.*, 2002). We also assessed possible gender effects related to ^{1}H-MRS-measured glutathione. These associations were assessed with linear mixed models with either gender or age entered as a fixed main effect and the dependent variable entered as glutamate, glutamine, or glutathione, averaged across scan number and day. Pearson product-moment correlations were used to assess the strength and direction of significant correlations. All statistical tests were two-tailed, with a significance threshold of $P < 0.05$.

3.4 Results

Some participants were missing at least one ^{1}H-MRS spectrum for the following reasons: withdrawal from the study before the second scanning day (n = 6 spectra); poor data quality (unsuppressed water linewidth > 16 Hz; n = 14 spectra); inability to acquire spectra due to scanner hardware, software or participant problems (n = 4 spectra); or our software's inability to accurately fit the data ($\chi^2 > 12$; n = 2 spectra). Thirteen participants had sufficient data quality for all four measurements. In total, there were 22 spectra for Day 1/Scan 1, 19 for Day 1/Scan 2, 20 for Day 2/Scan 1 and 17 for Day 2/Scan 2. The average water linewidth for all included spectra was 12.14 Hz (SD = 1.48), indicating high spectral resolution; a typical spectrum from one subject (**Figure 3.1B**) and the corresponding model fit (**Figure 3.1C**) is shown. Mean metabolite values (ratio to creatine) are presented in **Table 3.1**, and CRLB values are presented in **Table 3.2**.

Table 3.1. Average raw metabolite means (standard deviations), relative to creatine, from each day and scan number.

Metabolite	Day 1/Scan 1	Day 1/Scan 2	Day 2/Scan 1	Day 2/Scan 2
Glu/Cre	1.37 (0.13)	1.33 (0.13)	1.38 (0.13)	1.38 (0.14)
Gln/Cre	0.30 (0.06)	0.29 (0.05)	0.30 (0.06)	0.29 (0.05)
GSH/Cre	0.25 (0.03)	0.24 (0.03)	0.25 (0.03)	0.25 (0.03)
GABA/Cre	0.20 (0.06)	0.19 (0.05)	0.20 (0.05)	0.18 (0.03)
tNAA/Cre	1.62 (0.12)	1.62 (0.11)	1.61 (0.14)	1.61 (0.14)
Cho/Cre	0.30 (0.04)	0.30 (0.04)	0.30 (0.03)	0.29 (0.03)

Abbreviations: Glu, glutamate; Gln, glutamine; GSH, glutathione; GABA, γ-aminobutyric acid; tNAA, total N-acetyl-aspartate; Cho, choline Cre, creatine.

Table 3.2. Average metabolite percentage CRLB (standard deviations) for each metabolite.

Metabolite	Day 1/Scan 1	Day 1/Scan 2	Day 2/Scan 1	Day 2/Scan 2
Glu	1.58 (0.40)	1.54 (0.27)	1.47 (0.38)	1.35 (0.20)
Gln	6.76 (2.20)	6.86 (1.78)	6.16 (1.74)	5.90 (0.96)
GSH	6.14 (1.20)	6.13 (1.38)	5.83 (2.25)	5.35 (0.82)
GABA	6.69 (2.93)	6.39 (1.57)	5.99 (1.95)	5.89 (1.10)
NAA	0.76 (0.32)	0.69 (0.18)	0.71 (0.27)	0.64 (0.18)
NAAG	6.03 (3.42)	5.73 (3.93)	6.07 (4.54)	4.33 (1.24)
Cre	0.76 (0.23)	0.76 (0.18)	0.72 (0.23)	0.66 (0.18)
Cho	0.92 (0.29)	0.91 (0.22)	0.85 (0.30)	0.79 (0.19)

Abbreviations: CRLB, Cramér-Rao Lower Bound; Glu, glutamate; Gln, glutamine; GSH, glutathione; GABA, γ-aminobutyric acid; NAA, *N*-acetyl-aspartate; NAAG, *N*-acetylaspartylglutamate; Cre, Creatine; Cho, choline.

No main effects were observed for Day, Scan, or their interaction on levels of glutamate ($F_{(1,52)} = 0.29$, $P = 0.59$; $F_{(1,51)} = 1.06$, $P = 0.31$; $F_{(1,50)} = 0.11$, $P = 0.74$), glutamine ($F_{(1,56)} = 0.36$, $P = 0.55$; $F_{(1,53)} = 1.26$, $P = 0.28$; $F_{(1,52)} = 0.03$, $P = 0.86$), glutathione ($F_{(1,56)} = 0.08$, $P = 0.78$; $F_{(1,53)} = 0.07$, $P = 0.79$; $F_{(1,51)} = 0.15$, $P = 0.70$), GABA ($F_{(1,61)} = 0.55$, $P = 0.46$; $F_{(1,56)} = 1.68$, $P = 0.20$; $F_{(1,54)} = 0.10$, $P = 0.75$), or tNAA ($F_{(1,53)} = 0.61$, $P = 0.44$; $F_{(1,52)} = 0.02$, $P = 0.97$; $F_{(1,52)} = 0.44$, $P = 0.51$). Scan number significantly affected choline levels ($F_{(1,51)} = 5.09$, $P = 0.03$), but scanning day did not, nor was there any effect from their interaction ($F_{(1,51)} = 0.74$, $P = 0.39$; $F_{(1,51)} = 2.95$, $P = 0.09$, respectively). The significant main effect of Scan number found for choline reflected lower levels for Scan 2 than Scan 1.

Time between the two scanning days did not significantly affect metabolite levels for glutamate ($r_{s(19)} = 0.26$, $P = 0.28$), glutamine ($r_{s\,(19)} = 0.28$, $P = 0.25$), or glutathione ($r_{s\,(19)} = 0.31$, $P = 0.20$).

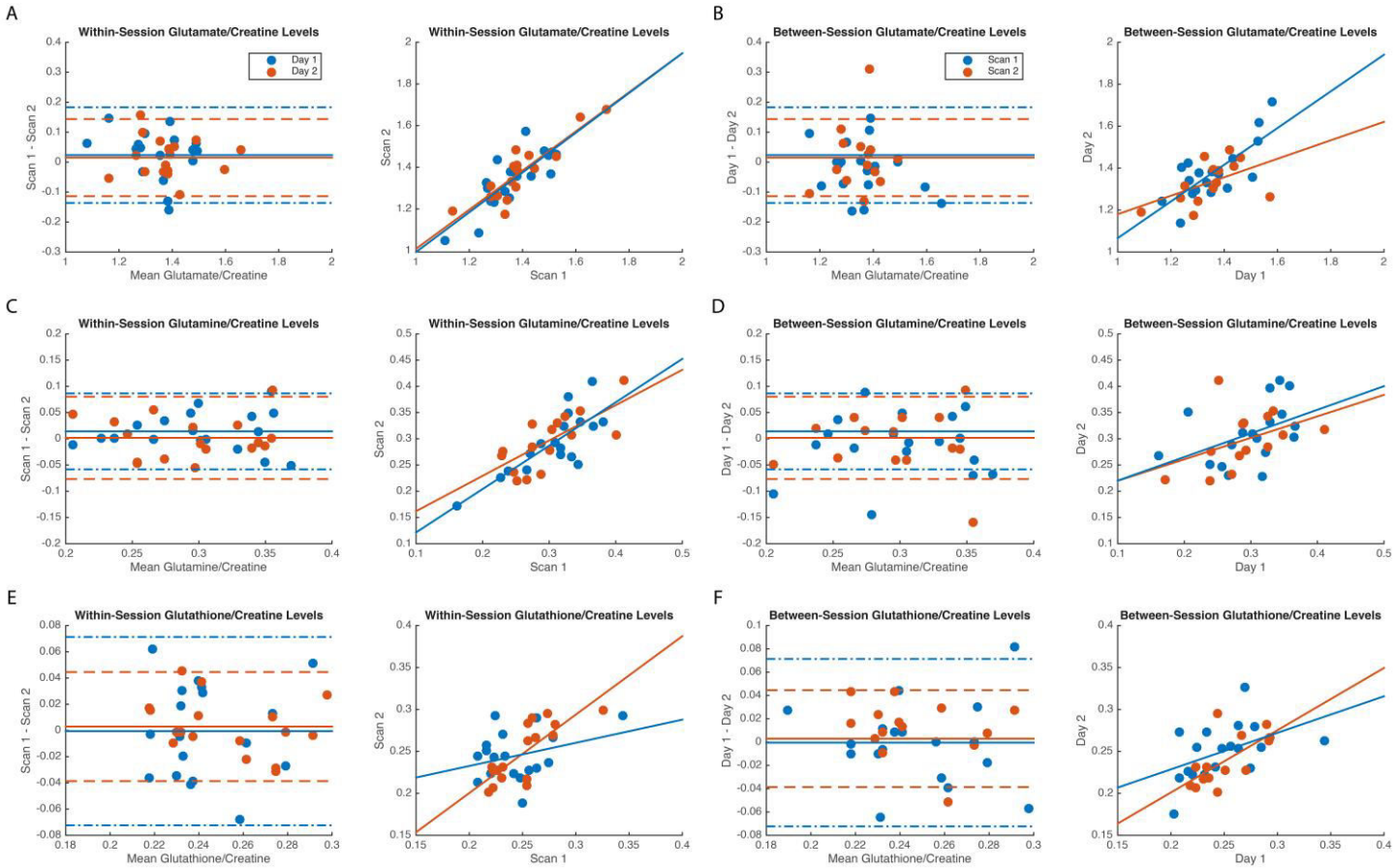

Figure 3.2. Bland–Altman and scatter plots depicting the within- and between-sessions reliability for glutamate (A, B), glutamine (C, D), and glutathione (E, F). Dashed lines indicate the agreement intervals, or 1.96 standard deviations greater than and less than the correspondingly coloured mean. Means are depicted by non-dashed lines for each respective condition in the Bland–Altman plots (left; Day or Scan). Coloured lines in the scatter plots indicate the lines of best fit for each respective day or scan number. All metabolites are referenced to creatine.

As anticipated, ICC values for the within-session measures were higher than for the between-session measures for all metabolites (except glutathione, which was on average comparable both between- and within-sessions; **Table 3.3**). Bland-Altman and scatter plots depicting the four relationships (within scanning days 1 and 2 and between scan numbers 1 and 2) for each of our metabolites of interest are presented in **Figure 3.2A-F**; Bland-Altman plots (Bland and Altman, 1986) may more easily depict systematic biases than scatter plots via the deviation from zero of the Y-axis mean difference lines (solid coloured lines). The corresponding CVs are also presented in **Table 3.3**. The reliability results obtained with our modified PRESS sequence may be summarized as follows. 1) On average, there was excellent within- (**Figure 3.2A**) and between-session (**Figure 3.2B**) reliability for glutamate. 2) Within-session reliability for glutamine was excellent (**Figure 3.2C**), but between-session reliability was only fair (**Figure 3.2D**). 3) Reliability for glutathione was on average fair within- and good between-sessions (**Figure 3.2E-F**). 4) As expected, the reliability of GABA was poor (**Table 3.3**). 5) Reliability for tNAA and choline were excellent both within- and between-sessions (**Figure 3.3A-D**). As choline was not a metabolite of interest per se, the more stringent absolute agreement ICC option was selected here despite the significant effect of scan number. CVs values were similar to ICCs with excellent reliability demonstrated using this statistic for glutamate, NAA and choline and fair-to-good reliability for glutamine and glutathione, both within- and between-session measurements (**Table 3.3**).

Table 3.3. Intraclass correlation coefficient and coefficient of variation (CV, %) values between- and within-scanning sessions.

Metabolites	**Within Day 1 (Scan 1 vs. Scan 2; n = 18)**		**Within Day 2 (Scan 1 vs. Scan 2; n = 17)**		**Between-Session (Scan 1; n = 18)**		**Between-Session (Scan 2; n = 14)**	
	ICC	CV	ICC	CV	ICC	CV	ICC	CV
Glu/Cre	0.88	6.00	0.94	4.77	0.86	6.48	0.68	7.95
Gln/Cre	0.87	12.44	0.84	13.40	0.63	15.53	0.62	9.38
GSH/Cre	0.49	14.95	0.88	8.54	0.65	11.45	0.76	8.25
GABA/Cre	-0.17	36.89	0.37	29.19	-0.26	30.92	0.21	15.43
tNAA/Cre	0.94	3.38	0.93	4.79	0.88	4.60	0.90	2.09
Cho/Cre	0.94	5.77	0.97	2.82	0.93	4.75	0.91	3.39

Abbreviations: ICC, intraclass correlation coefficient; CV, coefficient of variation; Glu, glutamate; Cre, creatine; Gln, glutamine; GSH, glutathione; GABA, γ-aminobutyric acid; tNAA, total *N*-acetyl-aspartate; Cho, choline.

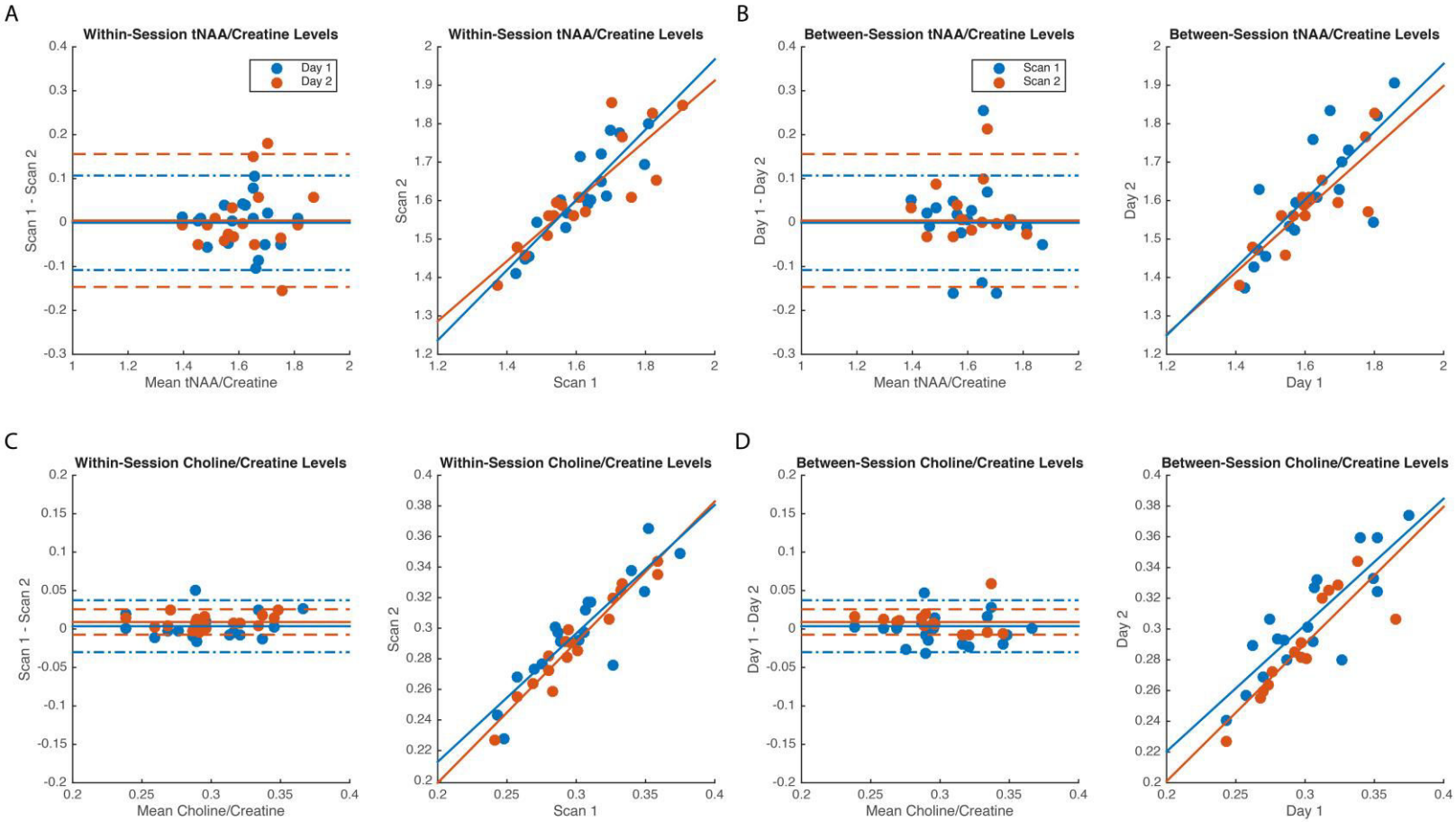

Figure 3.3. Bland–Altman and scatter plots depicting the within- and between-sessions reliability for total tNAA (A, B), and choline (C, D). Dashed lines indicate the agreement intervals, or 1.96 standard deviations greater than and less than the correspondingly coloured mean. Means are depicted by non-dashed lines for each respective condition (left; Day or Scan). All metabolites are referenced to creatine.

Finally, in our age- and gender-related analyses, we found a significant main effect of age on glutathione ($F_{(15,\ 7)}$ = 3.47, P = 0.049), reflecting a negative correlation using the average across all scans ($r_{(24)}$ = -0.37), but no relationship between age and glutamate ($F_{(15,\ 8)}$ = 1.58, P = 0.27) or glutamine levels ($F_{(15,\ 8)}$ = 0.62, P = 0.80). In contrast to previous reports (O'Gorman *et al.*, 2011), there was no main effect of gender on glutamate ($F_{(1,22)}$ = 0.29, P = 0.59). However, we found a significant effect of gender on glutamine ($F_{(1,23)}$ = 8.55, P = 0.008) and glutathione ($F_{(1,21)}$ = 4.32, P = 0.050); females had higher levels of glutathione, but lower levels of glutamine, than males.

3.5 Discussion

The present study used an adapted ^{1}H-MRS PRESS sequence (An *et al.*, 2015) in a group of healthy volunteers and found that measures of medial pgACC glutamate, glutamine, and glutathione showed on average good-to-excellent within-session reliability and fair-to-excellent between-session reliability. The precise measurement of neurochemistry is vital to improving our understanding of the pathophysiology and treatment of depression. Given that ^{1}H-MRS is the only non-invasive technique capable of measuring glutamatergic metabolites, it is important that its reproducibility be characterised.

Glutamate in particular, on average, showed excellent within- and between-session reproducibility, with high stability as evidenced by the low CRLB values. Corroborating the original report of this sequence (An *et al.*, 2015), the high signal-to-noise ratio (SNR) for glutamate detection evident in our data likely arose from both the excellent separation afforded by the high-field MRI and the TE-optimized pulse sequence. The high reliability of glutamate measurements in healthy volunteers is promising with regard to the methodological approach presented here, and particularly important given the recent surge in interest in developing and evaluating glutamate-modulating pharmacological compounds for psychiatric and neurological conditions (Kalia *et al.*, 2008, Zarate *et al.*, 2010).

For glutamine, within-session reproducibility was excellent, but between-session reproducibility was only fair. Glutamine concentrations in the brain are much lower than those of glutamate (~40%, (Govindaraju *et al.*, 2000)), thus the SNR is poorer and the metabolite harder to resolve accurately; these factors make quantification errors more likely than with glutamate. Nevertheless, our methodological approach may still be robust enough to permit pharmacological investigation of glutamine-modulating agents; measurement of ^{1}H-MRS glutamine provides an important and distinct biological signal to glutamate. In a cyclical fashion, glutamate is enzymatically converted into glutamine by glutamine synthetase in astrocytes, where it is then transported back for subsequent reconversion to glutamate and packed into synaptic vesicles. Thus, ^{1}H-MRS measurement of glutamine may provide an index of astrocytic functioning relating to this particular cycle; the ratio of glutamine-to-glutamate may be particularly salient.

Notably, alterations in glutamine (Maddock and Buonocore, 2012) and astrocyte function (Niciu *et al.*, 2014b) have been associated with a number of psychiatric conditions.

On average, pgACC glutathione levels showed fair reliability for within- and good for between-session measurements. Few studies have quantified neural glutathione with ^{1}H-MRS, as it is typically not resolvable at MRI scanner field strengths of 3T or lower; however it is readily detectable at 7T using sequences such as ours. Moreover, it remains an intriguing metabolite to explore clinically due to its functionality. Glutathione is the brain's primary antioxidant and is involved in inflammatory responses (Shungu *et al.*, 2012); indeed, aberrant ^{1}H-MRS glutathione concentrations have been found in several clinical conditions. For instance, Shungu and colleagues (2012) found that depressed patients had significantly lower levels of occipital glutathione than healthy volunteers. Reductions in glutathione have also been observed in amyotrophic lateral sclerosis (Weiduschat *et al.*, 2014) and chronic fatigue syndrome (Shungu *et al.*, 2012), suggesting that ^{1}H-MRS glutathione levels may be an index of oxidative stress, which commonly occurs in psychiatric and neurological conditions. We also noted a decrease in glutathione with age, which is particularly interesting because ageing is associated with impaired immunity. Moreover, given the association between the glutathione redox system and age (Erden-Inal *et al.*, 2002), it appears that ^{1}H-MRS-measured glutathione could be a sensitive marker of both cerebral ageing and immune function.

Our analyses suggest that tNAA and choline level measurements were also highly reliable, both within and between-sessions, using the sequence evaluated here. Given that these peaks are prominent within the standard ^{1}H-MRS spectrum, and are easily resolved at lower MRI field strengths, their high reliability here is reassuring and suggests that the reproducibility metrics for glutamate, glutamine and glutathione are appropriate. In contrast, the main effect of scan number on choline levels was surprising; lower levels were observed for the second ^{1}H-MRS acquisition on each day. Because choline is involved in cellular membrane turnover (Maddock and Buonocore, 2012), this decrease may reflect decreased functional activity in this region.

At least three studies have examined within- or between-session reliability of ^{1}H-MRS at 7T in healthy volunteers. Wijtenburg and colleagues (2013) explored the

between-session reliability of anterior cingulate cortex and DLPFC metabolites in a small sample (N = 4) using two distinct pulse sequences: STEAM and MEGA-PRESS-IVS. Both sequences demonstrated good measurement reliability, particularly for GABA. Stephenson and colleagues (2011) assessed the between-session reproducibility of ACC metabolites and within- and between-session reliability of insula metabolites using STEAM, in 12 healthy males, and reported good reliability for both regions. Finally, Cai and colleagues (2012) calculated the between-session (both same day and two-weeks apart) reproducibility of their sequence in male volunteers at 7T as an adjunct to a pharmacological investigation. They found that mean levels of occipital metabolites, collected separately using MEGA-PRESS and PRESS sequences, were similar (i.e. not significantly different) between scanning sessions, while drug administration significantly increased GABA levels.

The CV values found here using our sequence are comparable, for both the between and within session measurements, to these first two investigations (Stephenson *et al.*, 2011, Wijtenburg *et al.*, 2013), with much less than 10% variation for glutamate, NAA and choline and on average, less than 15% for glutamine; Cai and colleagues do not report any reliability statistics. However, none of these three aforementioned reliability studies assessed the reproducibility of their metabolite quantification using typical reliability statistics, such as ICC, making precise comparisons between studies somewhat difficult. Moreover, none of the aforementioned studies reported metabolite values for glutathione, suggesting that the sequence used here is specifically able to estimate this important signal. Furthermore, the CRLBs observed here in our measurements and analyses are, on average, lower for all metabolites than for those reported using STEAM (Wijtenburg *et al.*, 2013), suggesting that our sequence may offer enhanced sensitivity to detect most metabolites at 7T.

Although the data presented here suggest that our methodological approach provides fair-to-excellent reliability for detecting glutamatergic metabolites and excellent spectral resolution in general, several limitations and asides should be acknowledged. First, some loss of data quality may have occurred due to subject movement, partially arising from the long scan duration. Indeed, poor data (linewidth > 16 Hz) for a number of excluded spectra were likely due to subject movement; no

methods exist to measure intra- and inter-^{1}H-MRS scan movement. However, our long scan time also reflects the realistic length of time required for intra-scanner drug administration studies. Second, the results presented here are specific to the modified pulse sequence and scanner strength used; it remains unknown whether different field strengths using our sequence would yield similar results. It is also unknown if another scanner of the same strength and brand using the same sequence would yield highly comparable results, as significant variation likely exists across MRI scanners and the precise hardware factors that influence ^{1}H-MRS measurement remain undetermined. Third, although 26 individuals participated in the study, our final dataset included many participants missing at least one data point. Future studies would benefit from a larger sample size and more complete scan set.

Several other factors should also be mentioned. First, our metabolites were referenced to creatine, which should fully afford tissue relative concentrations without the need for tissue correction; however, this remains to be tested empirically and appropriate concentration referencing remains a controversial topic. Indeed, Wijtenburg and colleagues (2013) found a 12% variation of creatine between two scanning sessions, suggesting that creatine, or at least the measurement of this metabolite, may not be an appropriately reliable reference. Furthermore, referencing to creatine may not be applicable in comparisons between healthy volunteer and patient groups where creatine is potentially altered, for example in tumours; however, Maddock and Buonocore (2012) note that consistent evidence for abnormal creatine levels in major psychiatric illnesses such as MDD or schizophrenia has not been found. Second, the gender effects noted for glutamine and glutathione are hard to explain. Some authors have suggested that gender differences in glutamatergic metabolites may result from differing neuroactive steroids (e.g. oestrogen, progesterone, and testosterone (O'Gorman *et al.*, 2011)). If these gender effects are replicated in a larger sample, then studies should incorporate strict between-group gender matching when appropriate. Third, several of our findings may actually be type 1 (false positive) errors, including the significant effect of scan timing on choline, the trend for glutamate, the effects of age and gender on glutathione, and the effects of gender on glutamine. Specifically, because these were exploratory analyses and numerous control tests were also completed, Bonferroni correction for multiple comparisons was not performed. Additionally, due to the low number of subjects and

the lack of wide and systematic variation in age, these results remain tentative and require careful independent replication.

In conclusion, we used an adapted echo time optimised PRESS pulse sequence at 7T to measure glutamate, glutamine and glutathione signals in the healthy human brain, and found that these measurements were on average reliable both within- and between-sessions. Taken together, our results suggest that this novel 7T ^{1}H-MRS sequence is a useful and reliable tool for measuring brain glutamate, glutamine and glutathione signals. In sum, this novel 7T ^{1}H-MRS sequence is well placed to assess both glutamatergic differences between depressed patients and controls and antidepressant treatment effects.

4 Anhedonia, Reward Processing and Medial Prefrontal Glutamate in Major Depression: A 7T ^{1}H-MRS and NMDA Receptor Antagonist Treatment Investigation in Medication Free, Treatment-Resistant, Depressed Patients

4.1 Abstract

Anhedonia, the loss of pleasure or interest in enjoyable activities, is a prevalent and debilitating symptom in depression. Yet, little is known about the underlying neurobiology of anhedonia in depressed patients and there are no approved pharmacological treatments specifically for this symptom. Recent evidence suggests that ketamine, an *N*-methyl-D-Aspartate receptor antagonist, may have some efficacy in improving anhedonic symptomatology in patients diagnosed with major depressive or bipolar disorders. However, the precise clinical improvement in anhedonia caused by ketamine and its neuronal mechanisms of action in alleviating this symptom remain undetermined. To better understand the specific components of anhedonia improved by ketamine and the corresponding underlying neurobiological mechanisms, we administered anhedonia scales, reward-processing tasks and 7T ^{1}H-MRS scans, to healthy volunteers and medication-free patients currently in a major depressive episode. Scales, tasks and scans were all acquired at baseline and 24-hours after intravenous infusions of a sub-anaesthetic dose of ketamine and placebo, with two interim weeks, in a randomized, placebo-controlled, crossover study. We replicate previous findings of a ketamine-induced improvement in anhedonia levels. However, no changes in reward task performance or glutamate levels were found post-ketamine (N = 15 depressed patients) and changes in psychometric scales were not related to changes in behaviour or glutamate levels. Nonetheless, there was an association between baseline glutamine levels and the antidepressant and anti-anhedonic effects of ketamine. Moreover, a trend towards a reduction in glutamine levels post-ketamine was found (N = 12 depressed patients); however, post-infusion glutamine level alterations were not related to anhedonia changes. Further research exploring the mechanisms by which ketamine improves anhedonia is required.

4.2 Introduction

Anhedonia, the loss of interest or enjoyment in pleasurable activities, is one of two cardinal symptoms needed to diagnose a major depressive episode. The presence of anhedonia in depressed patients predicts both suicidal ideation (Winer *et al.*, 2014) and proximal suicide completion within one year (Fawcett *et al.*, 1990). Despite the apparent importance of anhedonia, there are currently no approved pharmacological interventions for this specific symptom. Highlighting the need for an anhedonia specific treatment, anhedonic depressed patients are less likely to respond to standard medications (Uher *et al.*, 2012) and some antidepressants may even cause symptoms of anhedonia, such as emotional blunting (Opbroek *et al.*, 2002, Price *et al.*, 2009a). Recent evidence tentatively suggests that intravenous sub-anaesthetic ketamine may, in addition to rapidly improving depressive symptoms generally (Berman *et al.*, 2000, Diazgranados *et al.*, 2010a, Dutta *et al.*, 2015, Zarate *et al.*, 2006), be effective at quickly alleviating anhedonia in treatment refractory patients diagnosed with major depressive or bipolar disorders (DeWilde *et al.*, 2015, Lally *et al.*, 2014b, Lally *et al.*, 2015b). However, precisely how ketamine improves levels of anhedonia and what specific components of the symptom are remedied, remains unknown.

At the clinical level, anhedonia is a complex multifaceted symptom comprising many factors (Treadway and Zald, 2011). For example, the diagnostic and statistical manual (DSM-V) specifies that there must be either diminished pleasure (i.e. consumption) or interest (i.e. anticipation) in enjoyable activities to meet criterion for this symptom (American Psychiatric and American Psychiatric Association, 2013). Importantly, these two psychological components, interest and pleasure, have distinct neurobiological bases (Der-Avakian and Markou, 2012) suggesting that conflating them for diagnostic purposes may hinder the identification of the neural mechanisms driving this symptom, as well as efforts to identify effective behavioural and pharmacological treatments (Treadway and Zald, 2011). Moreover, even the attenuated interest component of the clinical definition could reflect numerous cognitive processes, such as amotivation, impaired learning or valuation, pessimistic expectation, or an inability to fully anticipate a rewarding stimulus, which may also have distinct neural correlates. Compounding the issue of a

lack of clinical specificity, researchers have proposed a "decisional anhedonia" in depression, in which a deficit in value-based choice drives anhedonia (Treadway and Zald, 2011); this decisional anhedonia may be driven by broad deficiencies in reward processing, e.g. aberrant reward and punishment valuation systems, in depressed patients (Eshel and Roiser, 2010, Robinson *et al.*, 2012). However, the precise underlying deficits in reward processing associated with depression, and anhedonia in particular, are unclear as the majority of previous studies investigating these processes are confounded by patient medication status (Eshel and Roiser, 2010). The co-administration of anhedonia focussed psychometric scales and reward processing cognitive tasks in medication free patients before and after treatments could permit the exploration of what specific anhedonic processes are suitable targets for improvement in depressed patients and what treatments better what symptoms.

Early evidence suggests that a single infusion of sub-anaesthetic ketamine may improve levels of anticipatory anhedonia, as measured by the Snaith Hamilton Pleasure Scale (SHAPS; Snaith *et al.* (1995)), in depressed patients (Lally *et al.*, 2014b, Lally *et al.*, 2015b). Lally and colleagues (2014b) found that intravenous sub-anaesthetic ketamine improved levels of anticipatory anhedonia even after controlling for the overall improvement in general depressive symptoms in BD patients, suggesting specific anti-anhedonic properties for this treatment. Impressively, the specific anti-anhedonic effect that was still apparent two-weeks following the infusion. Non-anticipatory anhedonic components have yet to be examined in depressed patients following ketamine; however, preclinical evidence in rodents suggests other anhedonia components may be implicated. Autry and colleagues (2011) found that ketamine improved chronic stress-induced decreases in sucrose intake (a rodent index of consummatory anhedonia) in mice; this finding has been replicated in rats (Garcia *et al.*, 2009, Li *et al.*, 2011, Wang *et al.*, 2011) and mice (Ma *et al.*, 2013, Walker *et al.*, 2013) and using different doses of ketamine and routes of administration. However, Donahue et al. (2014) found that mice that were exposed to chronic social defeat stress showed attenuated social avoidance but no decrease in heightened lateral hypothalamic intracranial self-stimulation threshold (another rodent index of consummatory anhedonia) following ketamine. Taken together, these results suggest that ketamine may be effective for anticipatory, social

and some types of consummatory anhedonia. The question of how exactly ketamine may exert these anti-anhedonic changes and whether other components than anticipatory anhedonia are improved in depressed patients still remains unanswered.

Ketamine is classified as a non-competitive NMDA receptor antagonist, implicating the glutamatergic system in its effects. Administration of a sub-anaesthetic dose of ketamine is associated with acute increases in glutamate levels in the prefrontal cortex of rodents (Moghaddam *et al.*, 1997) and in the medial anterior cingulate cortex of both healthy volunteers (Stone *et al.*, 2012) and patients with depression (Milak *et al.*, 2015), assessed using ^{1}H-MRS acquired at 3T; however, see Taylor *et al.* (2012) for a contradictory ^{1}H-MRS finding at 3T of no acute effect of ketamine on glutamate levels in healthy volunteers. It should be noted that ^{1}H-MRS investigations at field strengths lower than 4T suffer from an inability to accurately quantify levels of both of glutamate and glutamine (Ramadan *et al.*, 2013). To date, Rowland and colleagues (2005) have conducted the only human ^{1}H-MRS investigation of the effects of ketamine at field strength > 3T. At 4T MRI, they found that acute administration of sub-anaesthetic ketamine caused increases in glutamine in the anterior cingulate cortex of healthy volunteers. Nevertheless, using ^{1}H-MRS at 3T, Salvadore *et al.* (2012) found that pre-treatment levels of a surrogate marker of glutamine (glutamate + glutamine (Glx) / glutamate) in the medial prefrontal cortex predicted the magnitude of the antidepressant response to ketamine in depressed patients. Glutamine may be a particularly important marker of astrocytic glutamate cycling as glutamate is processed, converted to glutamine and released by astrocytes (Ramadan *et al.*, 2013). Interestingly, astrocytic blockade of glutamate uptake in the prefrontal cortex induced an anhedonia-like phenotype in rats (Bechtholt-Gompf *et al.*, 2010, John *et al.*, 2012). Finally, using ^{1}H-MRS at 3T, Walter and colleagues (2009) found significantly lower levels of pregenual glutamine levels in depressed patients with anhedonia, but no statistical difference between those without, in comparison to healthy controls. Taken together, the evidence discussed above suggests that medial prefrontal cortex glutamate, and potentially glutamine, may contribute to depression and anhedonia and their treatment with ketamine.

To assess the neural basis of the anti-anhedonic effect of ketamine, we administered anhedonia-specific psychometric scales, reward-processing tasks and high field (7T) ^{1}H-MRS scans to healthy volunteers and depressed patients currently in a major depressive episode. The majority of the patients were medication free and treatment refractory. Reward tasks were administered to probe distinct components of reward processing, including reward motivation and learning. ^{1}H-MRS scans were conducted using a novel pulse sequence (See Chapter 3) shown to reliably distinguish and quantify levels of both glutamate and glutamine (An *et al.*, 2015, Lally *et al.*, 2015a). The scales, tasks and scans were acquired at baseline and 24-hours following sub-anaesthetic intravenous infusions of ketamine and placebo in a double-blind, randomized, placebo-controlled, crossover, treatment investigation.

We hypothesized that ketamine would cause a disinhibition of GABA interneurons and lead to an acute increase in prefrontal cortex glutamate levels; we believed that successful antidepressant treatment with ketamine would lead to an increase to healthy volunteer levels of glutamate. In particular, medial prefrontal cortex glutamatergic increases are thought to underlie post-ketamine improvements in anhedonia levels in depressed patients (Lally *et al.*, 2014b, Lally *et al.*, 2015b). We predicted pre-treatment differences between depressed patients and controls on anhedonia psychometric scales, reward tasks and medial prefrontal cortex ^{1}H-MRS measured glutamatergic metabolite levels. We aimed to replicate and extend an association between baseline levels of a surrogate marker of glutamine, acquired at 3T, and the antidepressant response to ketamine in MDD patients (Salvadore *et al.*, 2012); the improved resolution permitted by 7T MRI and our adapted pulse sequence should permit a more accurate assessment of the relationship between baseline glutamatergic metabolite levels and the antidepressant response to ketamine. We predicted that ketamine would serve as a motivational and cognitive enhancer and improve performance on our reward tasks and levels of anticipatory anhedonia. We predicted that these hedonic improvements would co-occur with increases in glutamate levels post-ketamine. Finally, we predicted that the alterations in our dependent variables (psychometric scales, reward tasks and medial prefrontal cortex ^{1}H-MRS measured glutamate or glutamine levels) post-ketamine would be interrelated, with relative increases in medial prefrontal cortex glutamate or

glutamine positively relating to the magnitude of improvements on tasks and scales. By improving anhedonia levels, potentially via a glutamatergic metabolite boost, we predicted that depressed patients would then respond more similarly to healthy volunteers on the anhedonia questionnaires and reward tasks.

4.3 Methods

4.3.1 Participants

Thirty-seven healthy participants (16 women) and 32 currently depressed patients (16 women) diagnosed with either major depressive disorder (MDD; N = 26, 12 women) without psychotic features or BD (N = 6, two men) were enrolled in this study, which was conducted at the National Institutes of Health (NIH), Bethesda, Maryland, USA. BD patients were diagnosed with BD I (N = 1 woman) or BD II (N = 5, two men). Healthy participants and patients were evaluated and diagnosed, respectively, via the structured clinical interview for DSM-IV Axis I disorders and an unstructured interview with a board certified psychiatrist. All patients were currently in a major depressive episode lasting at least four-weeks. At the time of testing, all participants were medication-free for at least 10 days with the exception of three of the BD patients (91%). Consistent with our previous protocols (Diazgranados *et al.*, 2010a, Zarate *et al.*, 2012), these three BD patients were administered a mood stabilizer (two patients received lithium and one valproate, only) at treatment levels (serum lithium, 0.6-1.2 mEq^{-1}; or valproic acid, 50-125 $\mu g\ ml^{-1}$), which was ineffective at alleviating the depressive episode. All participants were physically healthy and were free of any serious medical conditions and comorbid substance abuse (within the preceding three months) and lifetime dependence (excepting caffeine and nicotine); the latter two were lifetime exclusionary criteria for healthy volunteers. Pregnancy and nursing were not permitted and female subjects in the ketamine study (see below) were required to use approved methods of birth control. Comorbid axis I anxiety disorders were permitted for patients if they were not the primary diagnosis within the preceding 12 months. Healthy volunteers were excluded if any first-degree relatives had received a diagnosis of a major psychiatric disorder (N = 1 man). Participants were initially enrolled under a screening protocol with a view to undertaking further research at NIH. However, the primary protocol concerned in this instance evaluated the effect of intravenous anaesthetic ketamine as a treatment for depression, to which a subgroup of the total study patients and healthy participants enrolled. Studies were

approved by the combined neuroscience institutional review board of NIH and all subjects provided written informed consent before study entry.

4.3.2 Study Design

Medication free participants not enrolled or planning to enrol in ketamine studies were assessed at baseline only during a post-screening assessment. This assessment included the administration of psychometric scales, computerised reward tasks and, where possible, 7T MRI scans to acquire a ^{1}H-MRS spectrum; this non-ketamine dataset was used for the case-control comparison analyses (see below). Patients and healthy volunteers typically then proceeded to other non-ketamine studies.

4.3.2.1 Ketamine Study Design

Following the initial screening, a subgroup of depressed patients (N = 23, 5 BD) and healthy participants (N = 11) were admitted to an inpatient psychiatric unit at the NIH to participate in a ketamine mechanism of antidepressant action investigation. Details of participant disposition across measures are presented in **Table 4.1**. The study utilised a randomized, double-blind, placebo-controlled, crossover design study (**Figure 4.1**). Following a medication taper and a two week drug-free period (5-weeks for those taking fluoxetine) for patients (other than the three BD patient exceptions noted above who were maintained on a mood stabilizer), participants were administered one intravenous infusion of a sub-anaesthetic dose of ketamine hydrochloride (0.5 mg/kg) and one infusion of placebo (0.9% saline solution), with two interim weeks between infusions, which were counterbalanced across participants. Infusions were administered by an advanced cardiac life support licensed practitioner over 40 minutes using a Baxter infusion pump; identical injections were used to ensure blinding. A Montgomery-Åsberg Depression Rating Scale (MADRS; (Montgomery and Åsberg, 1979) score ≥ 20 at the time of screening and also prior to the first infusion was an inclusion criteria for depressed patients. The majority (22/23) of patients enrolled in the ketamine study were refractory to pharmacological treatment; treatment resistance was defined as failure of two or more adequate antidepressant trials, as assessed by the Antidepressant Treatment History Form (Sackeim, 2001). Aside from monotherapy with a mood stabilizer for

three of the BD patients, no psychotherapy or other treatment was permitted during the entire trial period. A number of measures (7T MRI scans, computerized decision-making tasks, and psychometric scales) were acquired repeatedly throughout this month-long period (**Figure 4.1**).

Table 4.1. Disposition of participants (N) across measures.

	Healthy Volunteers	**Patients**	
Baseline		MDD	BD
Psychometrics	37	26	6
EEfRT	34	21	5
Scene Choose	35	19	5
^{1}H-MRS	27	15	2
Ketamine			
Psychometrics	NA	18	4
EEfRT	NA	10	5
Scene Choose	NA	10	5
^{1}H-MRS	NA	10	2

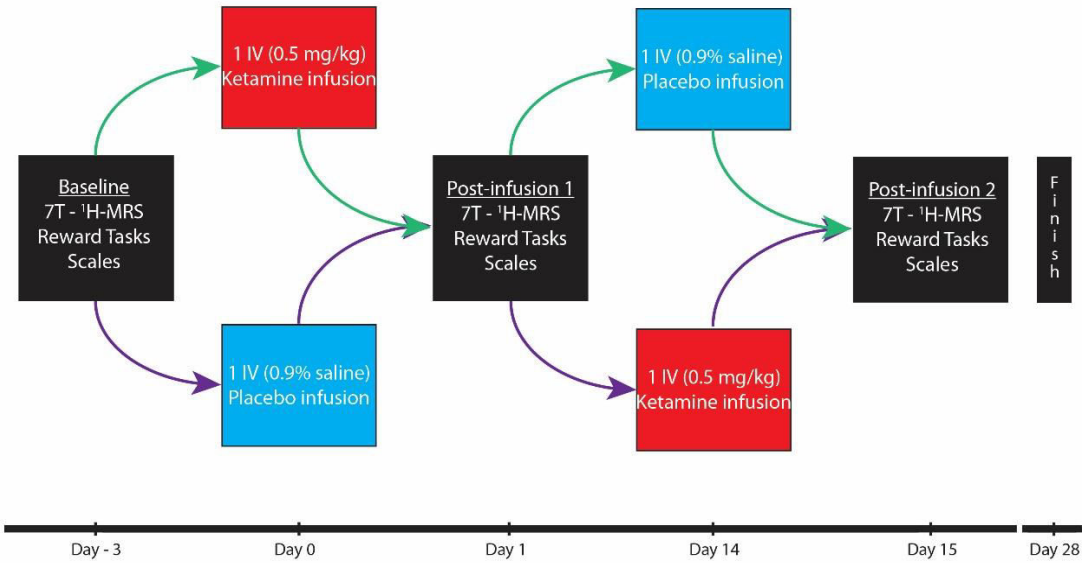

Figure 4.1. Ketamine Study Design. Approximately 3 days prior to the first infusion, all participants in the ketamine trial were scanned in a 7T MRI scanner where a ^{1}H-MRS spectrum was collected using a sequence optimized to detect glutamate and glutamine (An *et al.*, 2015). Additionally, reward processing tasks that assessed, amongst other variables, motivation and reward learning, were administered along with anhedonia specific psychometric scales on the same day. At day 0, participants received their first infusion. Participants received one ketamine and one placebo infusion in a randomized, counterbalanced fashion (days 0 and 14) and were scanned and tested on the same reward tasks as baseline the subsequent day (days 1 and 15, respectively).

4.3.3 Psychometric Scales

A number of psychometric scales (see Diazgranados *et al.* (2010a) for a list of other psychometric outcome measures) were administered at baseline, one-hour before the study began, and then again at 40, 80, 120 and 230 minutes and 1, 2, 3 7, 10 and 14 days follow the infusion commencement; at the time of writing, data from the scales of interest were only available up to day 3 post-infusion, however. Our primary scales of interest for this study were the MADRS, the Snaith-Hamilton Pleasure Scale (SHAPS; Snaith *et al.* (1995)) and Temporal Experience of Pleasure Scale (TEPS; Gard *et al.* (2006)). While the MADRS assesses general depressive symptomatology, both the SHAPS and TEPS focus specifically on anhedonia, the primary topic of interest in this chapter. The SHAPS measures anticipatory anhedonia via 14 hypothetical questions centred on socializing, hobbies and sensory stimuli (e.g. "during the past 24-hours, I would have been able to enjoy my favourite meal"). The scoring for the SHAPS ranges from 14-56 with higher scores indicating greater levels of anhedonia. The TEPS attempts to distinguish between two of the facets of anhedonia, namely anticipatory and consummatory processes. Scoring on the TEPS ranges from 1-6 with lower scores indicating less enjoyment (i.e. more anhedonia). Importantly, the scales were administered on the same day (except the MADRS, which was administered on day 0, not day -3) as the other dependent variables (MRI scans and reward tasks). A 50% reduction in MADRS scoring was defined as response criterion.

4.3.4 Reward Tasks

Participants were administered a battery of up to five computerized cognitive tasks (4 reward processing and one simple object recognition) in a randomized order. Participants who completed the infusion portion of the study completed the tasks again 24-hours after each infusion and in the same order as baseline. Details and data for two of the reward processing tasks only are presented below (data from the other tasks will be presented elsewhere).

Participants were administered the effort expenditure for rewards task EEfRT (Treadway *et al.*, 2009): the EEfRT is designed to assess levels of reward motivation

and probe aspects of decisional anhedonia. In brief, following a fixation cross (**Figure 4.2A**), participants were presented with two options, an easy or hard task (**Figure 4.2B**). The easy option involved making 21 repeated button ('L' for right and 'S' for left handers) taps with the index finger of the dominant hand within 7 seconds. The hard task entailed making 100 button taps with the pinky of the non-dominant hand within 21 seconds (**Figure 4.2D**). The easy task was always worth $1 but the hard task varied from trial to trial in value between $1.21 and $4.21. Additionally, the probability of receiving a reward was randomly varied amongst three independent probability categories: low (12%), even (50%), and high (88%). The number of button taps was reflected by an onscreen white vertical bar, which filled with red from bottom to top with each correct button tap (**Figure 4.2E-F**). Following each trial completion, participants were informed of their success or failure and the monetary outcome gleaned (**Figure 4.2G-H**). Participants initially completed 4 trials as training where one easy and one hard trial were encouraged to be selected and then played the task for 20 minutes, completing as many trials as possible. The sequence of reward probabilities and hard task value was random during each administration. The hard task took twice as long as the easy task; participants were informed of this before training. Subjects were paid $10 for total task completion and could win a further $20 (six trials were randomly selected and summed and the monetary outcome from these trials was added to the $10 basic payment).

Participants also completed an adapted reinforcement-learning task (Gold et al., 2012), named here as Scene Choose. The goal of this task was to explore participants' ability to learn the relationship between stimuli and feedback. Briefly, participants were presented with four pairs of scenes (randomized to either mountains, beaches or forests, only), one pair at a time. Two pairs were associated with potential gain (**Figure 4.2I-J**) and two with loss (**Figure 4.2K-L**). The correct response was reinforced 90% of the time in one pair and 80% of the time in the other for both gain and loss avoidance stimuli. From the 8 stimuli it was possible to construct four categories: frequent winners (FW), frequent losers (FL), frequent loss avoiders (FLA) and infrequent winners (IW). Each of the four pairs was shown 40 times during this training phase; the presentation of the stimuli was divided equally such that each pair was presented 10 times per quarter block. After training, a 64 trial

transfer test phase was presented where 24 novel pairings were shown twice alongside the original four pairings (each presented four times); no feedback was provided during the transfer phase. Stimuli were unique to each participant's session, such that if a subject was presented with beach scenes at baseline, session 2 comprised either mountains or forests with the final session stimuli coming from the remaining stimulus set. Subjects were again paid $10 for task completion and could win a further $20 via transfer phase performance; participants were paid a dollar for every optimal decision made above the chance criterion.

Both tasks were programmed in Cogent 2000 (www.vislab.ucl.ac.uk/Cogent), and/or the Psychophysics Toolbox (Brainard, 1997), stimulus presentation toolboxes for MATLAB (version 7.1).

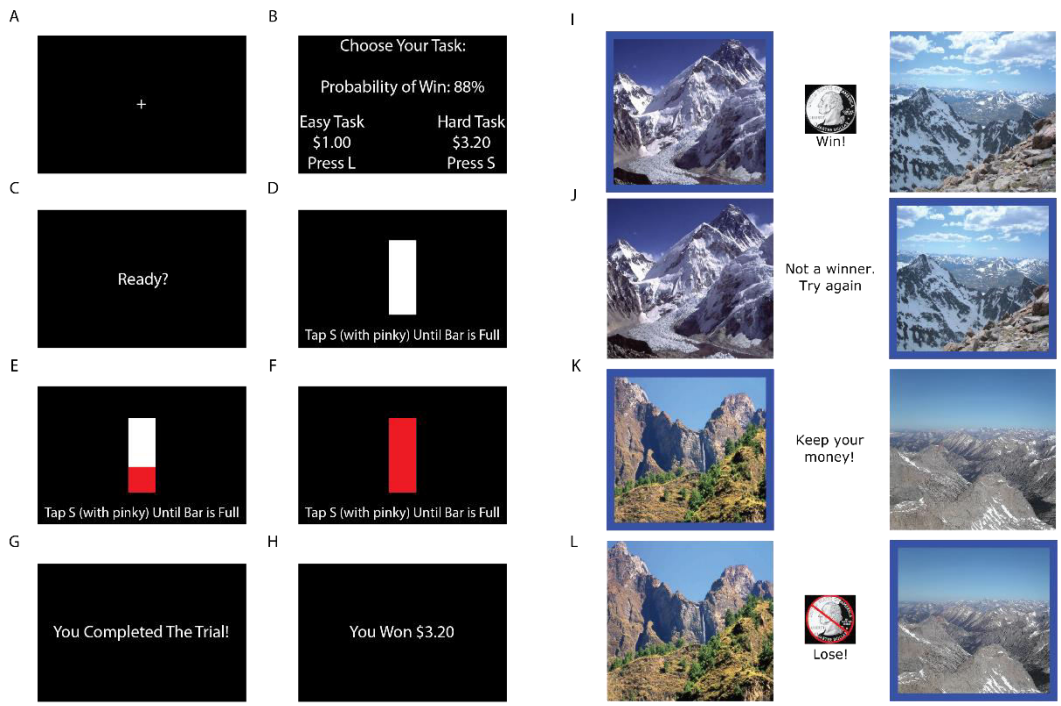

Figure 4.2. Reward Processing Tasks. Beginning with a fixation cross (A), the experimental effort for reward task (EEfRT) required participants to choose between an easy and a hard task (B). The easy task was always worth $1 while the hard task varied randomly between $1.21 - $4.21. The probability of winning money on a given trials also varied independently between low (12%), medium (50%) and high (88%) and was shown on screen. For right hand dominant individuals, the hard task (D) required participants to tap the S key 100 times with the pinky of their non-dominant hand; the easy task required these participants to press the L key 21 times in 7 seconds with the index of their dominant hand. Keys were reversed for left-hand dominant participants. Irrespective of the task chosen, the goal was to fill up a white (D-F) bar, which increased with red after each correct button tap. Once the time elapsed or the bar was full, participants received feedback on their performance (G)

and whether they won money (H). The Scene Choose Task comprised the presentation of four pairs of scenes (either mountains, forests or beaches). The goal was to learn by trial and error which scenes were winners (I), not winners (J), neutral (K), and, losers (L). Two pairs were associated with winners and two with losses. For both win and lose associated stimuli, the probability of correct feedback was modulated such that one of the two pairs was 90% and the other 80%. Following a stimulus learning phase, participants were tested on novel combinations of stimuli to ascertain the extent of learning and valuation between stimulus categories. No feedback was provided during this test phase.

4.3.5 ^{1}H-MRS

Participants (N = 20 patients and 28 controls) were scanned at baseline, and, for those participating in the ketamine mechanism of action trial (N = 21, 7 HVs), again 24-hours post-ketamine and post-placebo infusions. ^{1}H-MRS scanning occurred in the same Siemens 7T MRI scanner and followed precisely the same hardware, software, acquisition, location and pre-processing procedures as described in chapter 3 (see methods in 3.3). However, only one spectrum was acquired per session in this experiment. In brief, the same novel adapted point resolved spectroscopy sequence (PRESS; (An *et al.*, 2015) was used to acquire a single spectrum from the medial pregenual anterior cingulate region, an area frequently found to have decreased glutamatergic metabolites in depressed (Horn *et al.*, 2010, Luykx *et al.*, 2012) and anhedonic (Walter *et al.*, 2009) patients. Again, 16 Hz was set as the maximum line width criterion for all spectra included in our analyses. Data from Chapter 3 (first available spectrum for each participant only), the reliability investigation of a novel ^{1}H-MRS sequence in healthy volunteers (Lally *et al.*, 2015a), were included here as part of our healthy control sample for case-control comparisons. Four healthy volunteers from the reliability study also subsequently participated in the ketamine mechanism of action study and their baseline metabolite values from the ketamine study only were included in the analyses here. Again, all metabolite values were referenced to creatine.

Due to the preclinical (Moghaddam *et al.*, 1997) and clinical evidence supporting a glutamatergic mechanism for ketamine (Milak *et al.*, 2015, Stone *et al.*, 2012), our primary metabolite of interest was glutamate. Moreover, our sequence showed evidence of good reliability to detect glutamate levels in this region in the repeatability investigation (see Chapter 3) (Lally *et al.*, 2015a). Due to the difficulty

in separating glutamate from glutamine at lower field strengths, we speculated that glutamine may also play a role in the effect of ketamine. While the between-session reliability of this metabolite was not as high as glutamate, we reasoned that an investigation of its role in the mechanisms of action of ketamine was warranted given its strong relationship with glutamate (Ramadan *et al.*, 2013) and the aforementioned evidence positing a role for this metabolite in anhedonic depression (Walter *et al.*, 2009) and mechanisms of action of ketamine in humans (Rowland *et al.*, 2005, Salvadore *et al.*, 2012).

4.3.6 Statistical Analyses

Due to the lack of healthy volunteers with post-infusion data (N = 5), these participants were not included in the analysis of the effects of drug or post-drug group comparisons.

At baseline, we assessed psychometric scale differences between the two groups using independent samples *t*-tests and the non-parametric equivalent (Mann-Whitney U test, denoted by a U) when assumptions were violated. Normality was assessed using the Shapiro-Wilk test. A single factor repeated-measures ANOVA was used to assess the between and within group differences in TEPS scale component (i.e. anticipatory vs. consummatory anhedonia) scores at baseline, with group entered as a between-subjects factor; follow up post-hoc tests were conducted to assess significant differences. To calculate the effects of drug (i.e ketamine vs. placebo) on general depression and anhedonia specific scale scores, linear mixed models, with compound symmetry covariance structure, were performed, with baseline score on the scale of interest entered as a covariate; scale scores acquired at 40, 80, 120, 230 minutes and 1, 2 and 3 days post-infusion were all included in the model. Our main time point of interest post-infusion, however, was day 1, which has been shown in previous studies to be the time of the maximal antidepressant and anti-anhedonic effect of ketamine (Lally *et al.*, 2014b, Zarate *et al.*, 2006). Day 1 is also the time of the administration of both the reward tasks and the 7T ^{1}H-MRS scans and therefore should be the time most sensitive to detecting related changes in biology and behavior. We performed uncorrected simple effects tests within the aforementioned linear mixed models, again including the baseline score as a

covariate, to examine the effect of drug at the 24-hour time point post-infusion on the MADRS, SHAPS and TEPS. In a supplementary analysis of the SHAPS, we attempted to replicate our previous finding whereby a main effect of drug was still present on the SHAPS when changes in MADRS score were entered as a covariate (Lally *et al.*, 2014b).

For the EEfRT case-control comparison, participants were excluded if they only made easy or only made hard task selections. We first conducted analyses to assess that variations in reward probability and magnitude elicited motivational effects on task performance. We performed a repeated-measures analysis of variance (ANOVA) with a three level factor, the percentage of hard task selection split by the reward probability (three levels: 12%, 50% and 88% probability) to test for an effect of reward probability on decisions. To compute the effect of hard task reward magnitude on the decision to choose the hard task (denoted as high cost/high reward; HC/HR), we ran a general linear model using a binary logistic regression in MATLAB using the glmfit command and extracted the beta values and then estimated if there was a significant difference from 0 across all participants using a one-sample *t*-test in SPSS. A value different from 0 indicates there was a relationship. To compute the effect of reward magnitude on hard task decision reaction time and the time to complete the hard task, we fit a general linear model regression between these measures across all eligible trials for each individual subject using the glmfit command in MATLAB and extracted a beta weight for each participant. Again, a one-sample *t*-test from 0 on all participants' beta weights was used to test whether there was an association between magnitude of the hard task reward and the RT and the time to complete the trial.

Next, we evaluated if the two groups (depressed patients and controls) were comparable in the amount of trials undertaken and completed using independent samples t- and Mann-Whitney U-tests, respectively. To test for the previously demonstrated group difference between depressed patients and healthy volunteers on the mean percentage hard trials selected (Treadway *et al.*, 2012), we conducted a univariate general linear model (GLM) with group entered as a between-subjects factor. To test the effect of probability of reward on decisions across groups, we conducted a repeated-measures ANOVA with a three level factor (low [12%], mid [50%], high [88%]), and group entered as a between-subjects factor. Univariate

GLMs were used to test for between-group differences in the effect of reward magnitude on hard task decisions, RT and the time to complete the hard task. For the post-infusion analyses, we conducted similar control analyses to the case-control comparison assessment to validate adequate post-infusion task performance. A paired samples t- and a Wilcoxon signed ranks test were used to assess if similar numbers of trials were undertaken and completed, respectively, during the post-infusion sessions. To test the effect of drug on decisions made, we performed a two by three repeated measures ANOVA, with drug (ketamine and placebo) and reward probability (12%, 50% and 88%) entered as factors. The effects of drug on the relationship between the reward magnitude and the decision, RT and time to complete the hard task were also assessed using a single two level factor (ketamine vs. placebo) repeated measures ANOVA.

For the Scene Choose task case-control comparison, we conducted a two (Probability, 90% and 80%) by two (Valence, gain and loss avoidance) by four (Block, 1-4) repeated-measures ANOVA with group entered as a between-subjects factor to analyze the stimulus learning phase. To assess the transfer test phase, performance on comparisons between FW (90% and 80% gain) and FL (90% and 80% loss), IW (10 and 20% gain), and FLA (90 and 80% loss avoidance) and FLA and IW were grouped, and a repeated-measures ANOVA (with 4 levels) and group as a between-subjects factor was performed (Gold et al., 2012). To test the effect of drug on the stimulus learning phase, we ran a two (Drug, post-placebo and post-ketamine) by two (Probability) by two (Valence) by four (Block) repeated-measures ANOVA. To assess the effect of drug on the transfer test, we ran a two (drug) by four-level (as above) repeated-measures ANOVA.

To assess baseline differences in levels of medial prefrontal glutamate and glutamine in depression, we performed a univariate general linear model (GLM) with metabolite as the dependent factor and group entered as a fixed factor. For completeness, we then performed univariate GLMs on all the other metabolites modeled in our spectrum (glutathione, GABA, tNAA, and choline; Lally *et al.* (2015a)). We additionally explored previously found associations between baseline glutamatergic variables and response to ketamine using spearman's rho correlations due to violations in normality. Specifically, we assessed whether baseline glutamine and glutamate levels were predictive of the antidepressant response to ketamine. To

assess the effect of drug on levels of glutamate and glutamine, we performed a single factor repeated-measures ANOVA with post-infusion metabolite values entered in a single factor (drug); baseline metabolite value entered as a covariate. Finally, we examined whether changes in psychometric scales were related to changes in significant glutamatergic metabolites using spearman's rho correlations due to the non-normality of the variables.

If significant post-infusion changes were found on any of the three measures, these were then explored in the context of changes in the other measures to further understand the mechanisms of action.

For all case-comparison analyses, significantly different between-group demographic variables (age and years of education) were mean corrected and entered as covariates. If the variables entered as covariates had a significant, or trended toward a main effect, it was retained in the model or otherwise dropped to allow for increased degrees of freedom; unless otherwise stated, results of covariates were not significant and dropped from the model to increase the available degrees of freedom. Order of drug administration was entered as a between-subjects factor for all post-infusion analyses. Where applicable, if there was a significant, or trend level, drug by order interaction, order was retained in the model, or, otherwise, dropped to increase the degrees of freedom.

All statistical analyses were two tailed, conducted in SPSS (Armonk, NY, USA; version 21) or MATLAB (MathWorks, Natick, MA, USA) and a $P < 0.05$ was considered statistically significant and $P < 0.1$ was considered a trend towards significance. Post-hoc corrections are uncorrected unless otherwise stated. Huynh-Feldt correction was applied if the assumption of sphericity was violated during repeated-measures ANOVA.

4.4 Results

4.4.1 Case-control comparison

Demographic details are presented in **Table 4.2**. Of note, there were significant differences in both age ($t_{(59)}$ = 4.09, P < 0.001) and number of years of education ($t_{(59)}$ = -2.29, P < 0.001) between our groups, with patients being on average 11 years older and having two years less education than healthy volunteers.

Table 4.2. Participant demographics and psychometric scale scores at baseline.

	Healthy Volunteers (N = 37)		**Patients (N = 30; 24 MDD, 6 BD)**		**Statistical Differences**	
	Mean	SD	Mean	SD	*T*	*P*
Age	28.46	6.82	40.18	11.81	4.09	< 0.001
Age of onset	NA	NA	17.53	7.29		
Baseline BDI	1.17	1.42	28.32	8.64	16.70	< 0.001
Baseline MADRS	1.48	2.34	31.46	3.90	35.31	< 0.001
Baseline SHAPS	18.38	3.97	36.74	5.30	15.60	< 0.001
Baseline TEPS-A	4.75	0.58	2.66	0.77	-12.22	< 0.001
Baseline TEPS-C	4.78	0.63	3.52	0.79	-6.93	< 0.001
IQ	118.11	10.14	113.07	10.31	-1.35	0.19
Length of current episode (months)	NA	NA	30.94	41.53		
Years of education	18.42	1.98	16.14	2.57	-2.29	0.03
	N	%	N	%	X^2	
Gender	16	42	16	50	0.68	0.47
TRD	NA	NA	24	75		

BDI: Beck Depression Inventory; MADRS: Montgomery-Åsberg Depression Rating Scale; SHAPS: Snaith-Hamilton Pleasure Scale; TEPS-A: Temporal Experience of Pleasure Scale-Anticipation; TEPS-C: Temporal Experience of Pleasure Scale Consummatory; TRD: Treatment Resistant Depression; IQ: Intelligence Quotient.

4.4.1.1 Psychometric Scales

Within the depressed sample, strong positive correlations were found between total MADRS (BDI, $r_{(30)}$ = 0.74; SHAPS, $r_{(30)}$ = 0.59), BDI (SHAPS, $r_{(30)}$ = 0.57) and SHAPS scores. However, only the SHAPS was significantly related to the TEPS

(TEPS-A, $r_{(28)}$ = -0.69; TEPS-C, $r_{(28)}$ = -0.64), suggesting the importance of this scale in measuring unique aspects of depression, and specifically anhedonia, in our patient population. There was a main effect of TEPS subscale ($F_{(1,54)}$ = 31.88, P < 0.001) and a group by TEPS subscale interaction ($F_{(1,54)}$ = 26.43, P < 0.001). Post-hoc tests revealed that that levels of anticipatory anhedonia were greater than consummatory anhedonia in the depressed sample ($t_{(27)}$ = -7.25, P < 0.001) but not in the healthy controls ($t_{(30)}$ = -0.37, P = 0.71), suggesting the anticipatory component may be more problematic than the consummatory for depressed patients (**Figure 4.3A**).

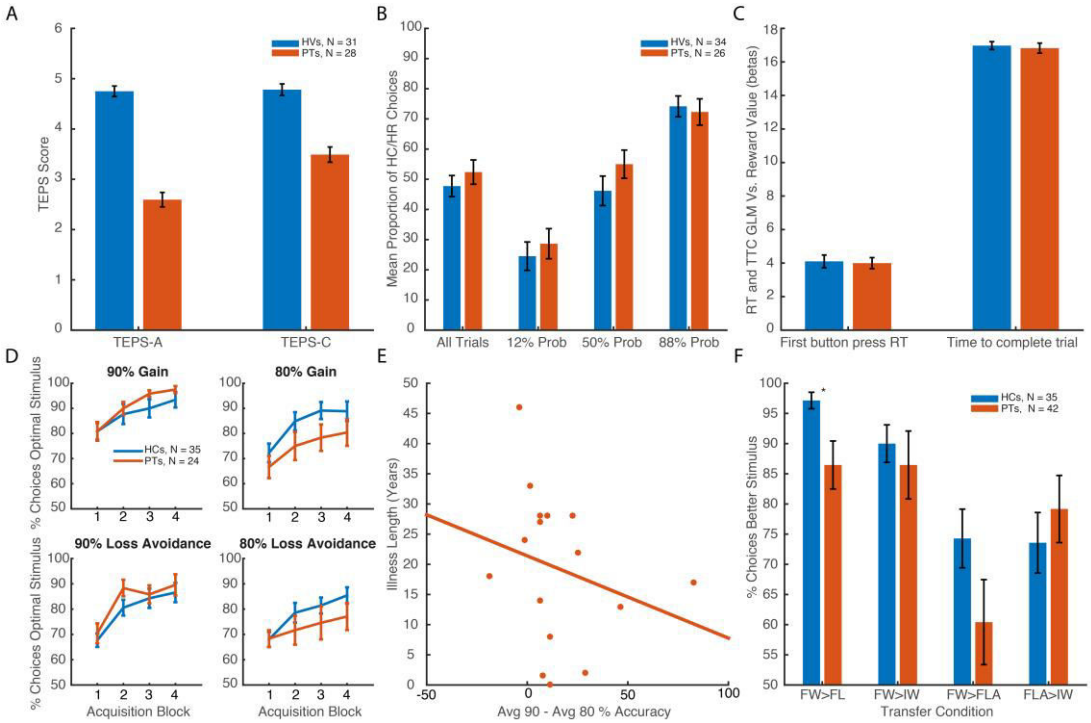

Figure 4.3. Psychometrics and reward tasks at baseline. (A) Levels of anhedonia were higher (a lower score indicates lower pleasure) in patients (red) than healthy controls (blue) on the temporal experience of pleasure scale (TEPS). TEPS anticipatory anhedonia was significantly greater than consummatory in patients. (B) There was no between group performance differences on the fraction of high cost/ high reward (HC/HR) decisions on average or across probabilities (prob) during the EEfRT (C) Reaction time (RT) or time to complete the hard trials to increasing rewards. There was an interaction between feedback probability and performance on the Scene Choose task (D), which was related to the number of years patients had been ill (E). The transfer phase revealed that patients showed impairment in the representation of either frequent winners (FW) or frequent losers (FL; F) in the non-feedback transfer test phase.

4.4.1.2 Reward Tasks

EEfRT: one participant was excluded from each group for only having selected hard trials, all other participants mixed their choices. There was a main effect of reward probability ($F_{(2,118)}$ = 105.43, P < 0.001) and reward magnitude ($t_{(58)}$ = 7.99, P < 0.001) on the decision to select the hard over the easy task, with higher probability of reward and greater hard task reward value eliciting more hard task choices; one participant was excluded from this analysis due to an inability of the model to fit the data. RT (the time taken to make the decision) to hard task choices ($t_{(59)}$ = 15.81, P < 0.001) and the time to complete all button presses during hard tasks ($t_{(59)}$ = 94.06, P < 0.001) also scaled with reward magnitude, suggesting that the task provided a good index of participant motivation.

Importantly, there was no group difference in the number of trials undertaken ($t_{(58)}$ = 0.22, P = 0.64; HVs M = 51.92, Depressed M = 50.85) or completed (U = 465, Z = -0.09, P = 0.93; HVs M = 99.6%, Depressed M = 99.6%). Nevertheless, contrary to previous reports (Treadway *et al.*, 2012), there were no group differences in the percentage of total hard trials selected ($F_{(1,58)}$ = 0.76, P = 0.39; **Figure 4.3B**), the association of reward magnitude on either hard task decision ($F_{(1,57)}$ = 0.42, P = 0.52), RT ($F_{(1,58)}$ = 0.04, P = 0.84; **Figure 4.3C**) or the time to complete the hard trials ($F_{(1,58)}$ = 0.16, P = 0.69; **Figure 4.3C**). There was no group by reward probability interaction on the percentage of hard trials selected ($F_{(2,108)}$ = 2.22, P = 0.11); there was a trend toward a main effect of years of education on the percentage of hard trials selected in this model ($F_{(1,54)}$ = 2.90, P = 0.094), but the main effect of group was not significant ($F_{(1,54)}$ = 0.06, P = 0.81). No other significant effects were found.

Scene Choose: there were main effects of probability ($F_{(1,57)}$ = 11.32, P = 0.001), valence ($F_{(1,57)}$ = 10.65, P = 0.002) and block number ($F_{(3,55)}$ = 26.72, P < 0.001) were found for performance during the stimulus training phase, indicating robust task performance across participants. Valence by block ($F_{(3,171)}$ = 0.35, P = 0.79), probability by block ($F_{(3,171)}$ = 0.74, P = 0.53) and probability by valence by block ($F_{(3,171)}$ = 1.42, P = 0.24) interactions were not significant. A significant interaction between probability and group ($F_{(1,57)}$ = 4.77, P = 0.03), but no others,

was found; the main effect of group was also not significant ($F_{(1,57)} = 0.42$, $P = 0.52$). Post-hoc analyses revealed a significant difference between probabilities (i.e. feedback sensitivity) for depressed patients ($F_{(1,57)} = 12.98$, $P = 0.001$), but not healthy volunteers ($F_{(1,57)} = 0.85$, $P = 0.36$), with patients performing worse on 80% than 90% stimuli. The sensitivity discrepancy in depressed patients showed a trend towards a relationship to illness length (the number of years since the first major depressive episode; $r_{s(16)} = -0.47$, $P = 0.07$), but not anhedonia levels ($r_{s(24)} = -0.10$, $P = 0.63$), as measured by the SHAPS.

There was a main effect of stimulus comparison performance during the transfer test phase ($F_{(3,171)} = 15.87$, $P < 0.001$) and a trend towards an interaction between group and performance ($F_{(3,174)} = 2.36$, $P = 0.098$). Note however, these data are not normally distributed and there is no non-parametric equivalent that permits a repeated-measures interaction effect (i.e. one cannot run a group by performance interaction). Nevertheless, follow up tests revealed that, in comparison to healthy controls, depressed patients did not select frequent winners over frequent losers as often (U = 302.5, $Z = 2.49$, $P = 0.012$). Again, however, patient performance on this measure did not relate to levels of anhedonia ($r_{s(24)} = -0.12$, $P = 0.59$).

4.4.1.3 ^{1}H-MRS

^{1}H-MRS scans were generally of high quality. Mean linewidth for all baseline scans was 12.26 Hz, with no additional baseline scans excluded due to poor quality, other than those omitted from the reliability analysis (Chapter 3). There was a trend toward significantly better (i.e. lower) linewidth in depressed patients (M = 11.76 Hz) than healthy controls at baseline (M = 12.57 Hz; $t_{(43)} = 1.85$, $P = 0.09$). Cramer Rao Lower Bounds were lower than 20% for every metabolite for all scans included. Baseline mean metabolite levels for patients and healthy volunteers are listed in **Table 4.2**. There was no significant main effect of group on baseline levels of medial pregenual cingulate glutamate levels ($F_{(1,43)} = 2.69$, $P = 0.11$). Our secondary analysis revealed no baseline group differences in levels of pregenual glutamine ($F_{(1,37)} = 0.08$, $P = 0.78$). For completeness, we also report the between group comparisons for the other metabolites modelled in our spectrum, glutathione, GABA,

tNAA and choline (**Table 4.3**); there was a significant effect of age on tNAA ($F_{(1,40)}$ = 4.14, P = 0.049) but both covariates were not significant for all other metabolite analyses.

Table 4.3. Baseline mean and standard deviations for metabolite levels, relative to creative, across the two groups.

	Healthy Volunteers (N = 27)		**Depressed Patients (N = 17, 2 BD)**		**Statistical Differences**	
Metabolite	Mean	SD	Mean	SD	$F_{(1,42)}$	P
Glu/Cre	1.37	0.12	1.29	0.17	2.67	0.11
Gln/Cre	0.30	0.05	0.28	0.08	0.64	0.43
GSH/Cre	0.25	0.03	0.23	0.04	1.63	0.21
GABA/Cre	0.20	0.05	0.19	0.06	0.10	0.75
tNAA/Cre	1.62	0.11	1.55	0.12	0.87	0.36
Cho/Cre	0.29	0.04	0.31	0.03	0.69	0.41

Glu, glutamate; Cre, Creatine; Gln, glutamine; GSH, glutathione, GABA, γ-aminobutyric acid; tNAA, total *N*-acetyl-aspartate; Cho, choline.

4.4.2 Post-Ketamine

Owing to the low numbers of healthy volunteers who were successfully tested at baseline and following both infusions (N = 5), these participants were not included in any post-infusion analyses.

4.4.2.1 Psychometric Scales

Relative to placebo, only three out of 17 (17%) patients reached response criterion at 24-hours post-ketamine, which is much lower than other reports (Milak *et al.*, 2015, Zarate *et al.*, 2012, Zarate *et al.*, 2006), with a mean improvement of 23% on the MADRS at this time point.

There was a significant interaction effect between drug, time and drug order ($F_{(6,183)}$ = 2.25, P = 0.040) on the MADRS and a drug by drug infusion order interaction for the SHAPS ($F_{(1,16)}$ = 5.34, P = 0.034), thus order of drug administration was retained in these models. A main effect of drug was found on MADRS ($F_{(1,186)}$ = 43.54, P < 0.001; **Figure 4.4A**) and SHAPS scores ($F_{(1,187)}$ = 23.97, P < 0.001; **Figure 4.4B**), with post-ketamine scores lower than post-placebo, replicating the previously found antidepressant and anti-anhedonic effect of

ketamine. However, the effect of drug on SHAPS scores was non-significant when controlling for total MADRS score ($F_{(1,188)}$ = 0.19, P = 0.66), contrasting with our previous finding (Lally *et al.*, 2014b).

There was a significant interaction between drug infusion order and drug on TEPS-A ($F_{(1,13)}$ = 11.68, P = 0.005) and TEPS-C ($F_{(1,15)}$ = 5.04, P = 0.039). However, there was no significant effects of drug on TEPS-A ($F_{(1,146)}$ = 2.47, P = 0.12; **Figure 4.4C**) or TEPS-C ($F_{(1,147)}$ = 0.54, P = 0.46; ; **Figure 4.4D**).

Post-hoc simple effects tests revealed that there was a significant difference between post-placebo and post-ketamine scores at our time point of interest, day 1, for the MADRS ($F_{(1,183)}$ = 10.34, P = 0.002) and the SHAPS ($F_{(1,183)}$ = 6.99, P = 0.009). However, there was no significant difference between post-placebo and post-ketamine scores at day 1 on TEPS-A ($F_{(1,142)}$ = 1.45, P = 0.23) or TEPS-C ($F_{(1,144)}$ = 0.06, P = 0.81).

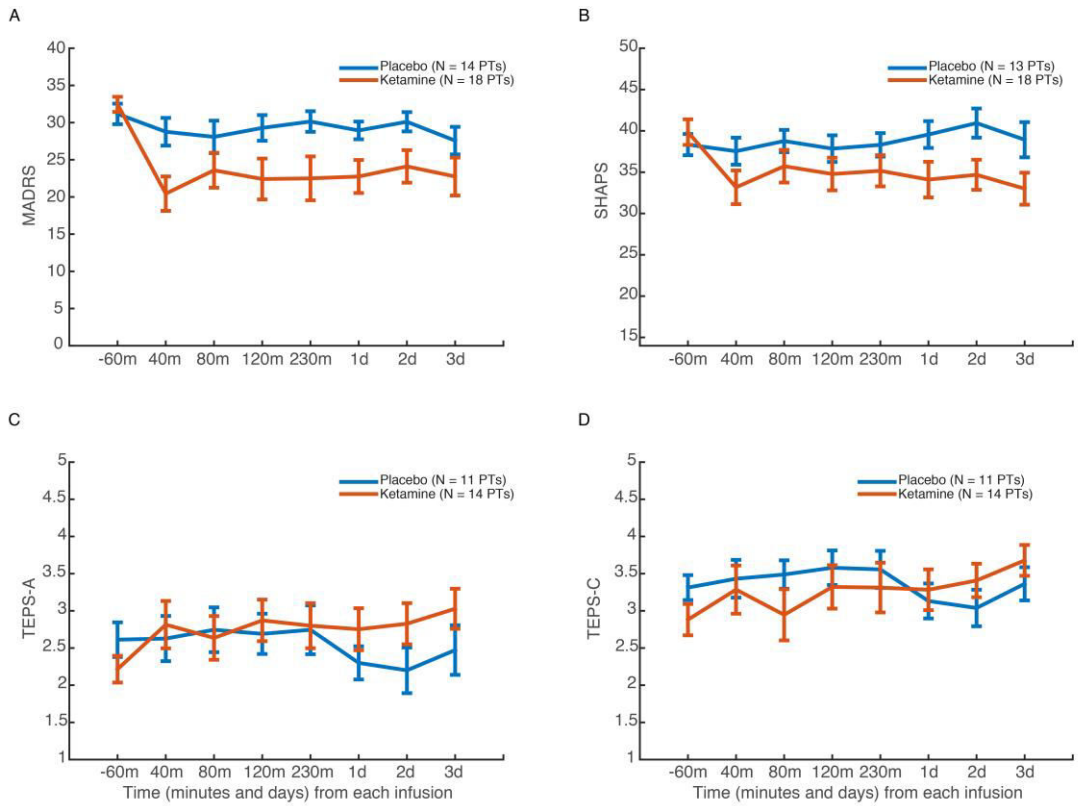

Figure 4.4. The effect of ketamine on depression and anhedonia psychometric scales. Relative to placebo, ketamine had a pronounced effect on (A) MADRS and (B) SHAPS scores, indicating general antidepressant and anti-anhedonic (anticipatory) effects. However, relative to placebo, there was no significant improvement in either anticipatory or consummatory anhedonia levels following ketamine, as measured by the TEPS-A (C) or TEPS-C (D).

Consistent with previous research (Salvadore *et al.*, 2012), there was a trend towards a significant relationship between baseline levels of glutamine ($r_{s(12)}$ = -0.52, P = 0.087; **Figure 4.5A**), but not glutamate ($r_{s(12)}$ = -0.02, P = 0.95), and the general antidepressant response to ketamine at day 1. This correlation reflected a greater antidepressant response to ketamine in depressed patients with greater levels of glutamine. There was also a similar trend towards a significant relationship between baseline levels of glutamine and change in SHAPS at 24-hours post-infusion ($r_{s(12)}$ = -0.57, P = 0.054), but not glutamate ($r_{s(12)}$ = -0.10, P = 0.75). Corroborating this evidence, baseline glutamine was a significant predictor of the general antidepressant response to ketamine at 48-hours post-infusion ($r_{s(12)}$ = -0.81, P = 0.002; **Figure 4.5B**).

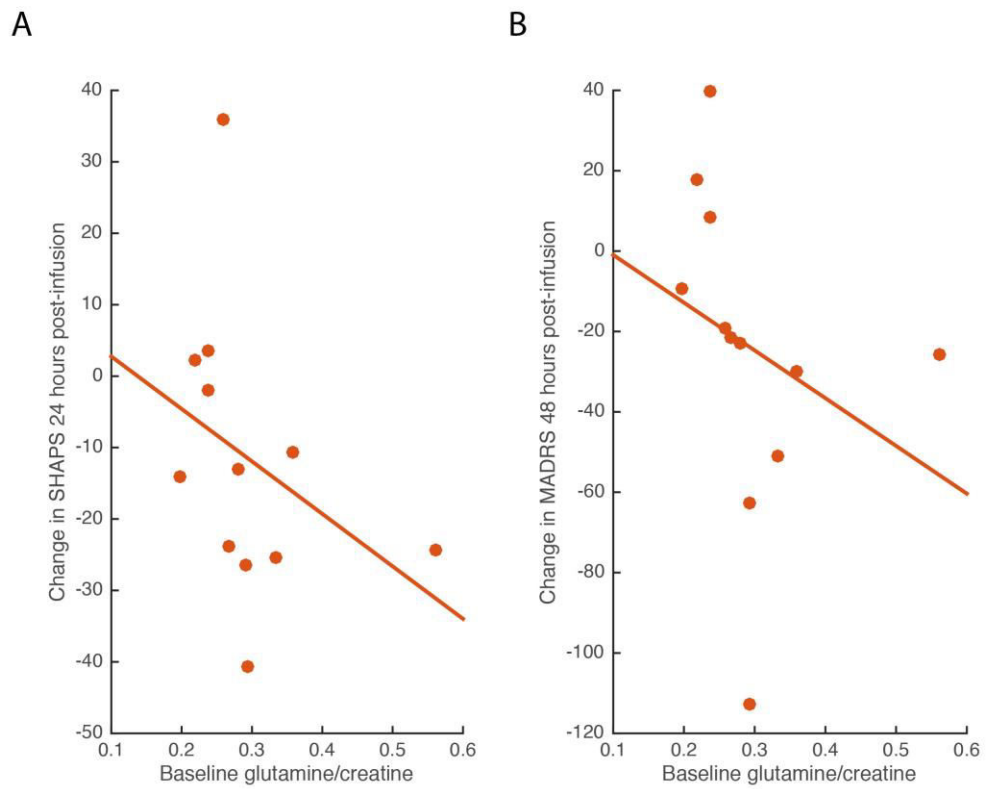

Figure 4.5. Relationship between baseline glutamine levels and the antidepressant response to ketamine. (A) There was a trend towards a significant relationship between baseline levels of glutamine and change in SHAPS post-ketamine. (B). There was a significant relationship between baseline

4.4.2.2 Reward Tasks

EEfRT: two participants were excluded from the post-infusion analyses, one for selecting all easy and the other for selecting all hard tasks for both post-infusion sessions, leaving a sample size of 15 depressed patients for the drug effect analysis. Importantly, there were no differences in the number of trials undertaken post-placebo (M = 48.40) or post-ketamine (M = 49.60; $t_{(14)}$ = -1.05, P = 0.31) or completed (Z = -0.68, P = 0.49; post-placebo M = 99.2%, post-ketamine M = 99.6%). Again, there was a main effect of reward probability on the decision to choose the hard task ($F_{(2,28)}$ = 33.52, P < 0.001). However, there was no main effect of drug ($F_{(1,14)}$ = 1.05, P = 0.32) or a drug by probability interaction ($F_{(2,28)}$ = 0.18, P = 0.84; **Figure 4.6A**) on the decision to choose the hard over the easy task. There was no effect of drug on the relationship between reward magnitude and hard task selection ($F_{(1,14)}$ = 0.04, P = 0.84; **Figure 4.6B**), RT ($F_{(1,14)}$ = 0.29, P = 0.59; **Figure**

4.6B), or the time to complete the hard task button pressing ($F_{(1,14)} = 0.36$, $P = 0.56$). No other significant effects were found.

Scene Choose: there was a significant main effect of block number ($F_{(3,42)} = 13.36$, $P < 0.001$) but the effects of probability ($F_{(1,14)} = 0.01$, $P = 0.92$), valence ($F_{(1,14)} = 0.11$, $P = 0.75$), and drug ($F_{(1,14)} = 0.16$, $P = 0.69$) were not significant. There was a trend toward a significant drug by block ($F_{(3,42)} = 2.54$, $P = 0.084$) and drug by block by valence ($F_{(3,42)} = 4.16$, $P = 0.011$) interaction. However, post-hoc tests on the interaction between drug and block interaction revealed no significant differences between placebo and ketamine for any of the four blocks (all $F_{(1,14)} < 3$ and $P > 0.10$). Follow up tests on the drug by block by valence interaction revealed a trend toward a valence by block interaction post-ketamine ($F_{(3,42)} = 2.55$, $P = 0.069$) but not post-placebo ($F_{(3,42)} = 2.11$, $P = 0.11$). Post-hoc tests, corrected for multiple comparisons, did not reveal any significant differences within the valence by block interaction post-ketamine ($F < 2.2$, $P > 0.16$). All other interactions for the stimulus learning phase were non-significant. There was a main effect of stimulus comparison type ($F_{(3,42)} = 3.95$, $P = 0.04$) during the transfer phase but no main effect of drug ($F_{(1,14)} = 0.66$, $P = 0.43$) or a drug by performance interaction on performance ($F_{(3,42)} = 0.01$, $P = 0.98$; **Figure 4.6D**). No other significant effects were found.

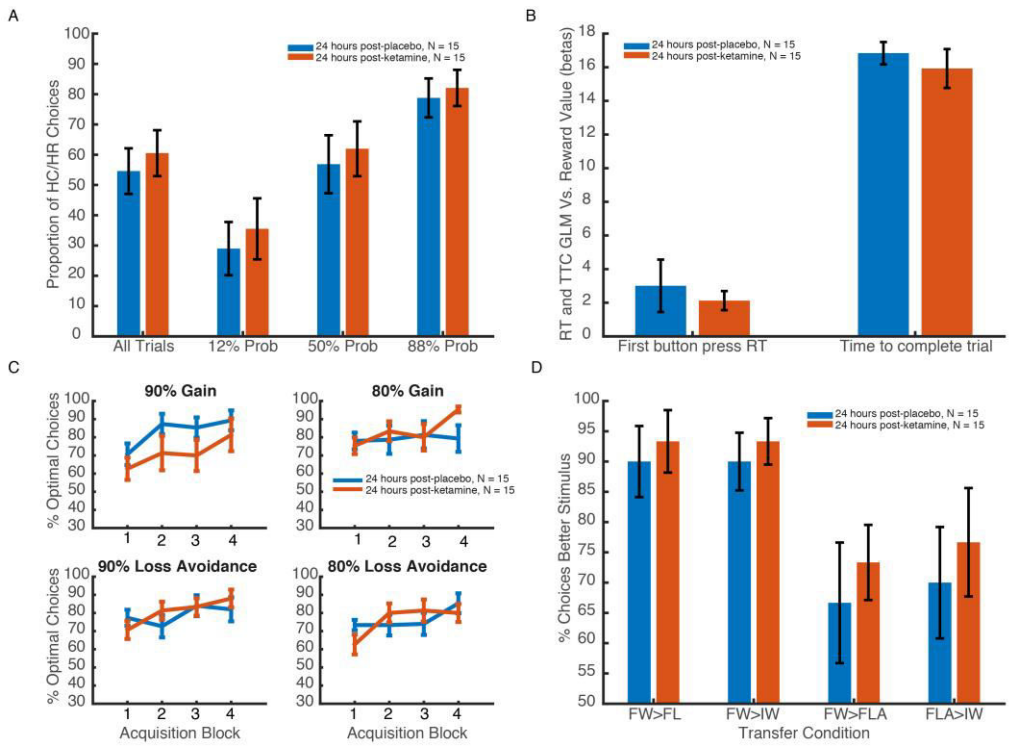

Figure 4.6. The effect of ketamine on reward processing tasks. There were no differences in EEfRT (high cost/high reward; HC/HR) behaviour 24-hours post-placebo and 24-hours post-ketamine for either decisions made (A) or the speed of decisions (RT) or the time to complete tasks (B). There was also no change on levels of reward learning as indicated by performance on the Scene Choose task. Participants performed similarly 24-hours post-placebo (blue) and 24-hours post-ketamine (red) on both the learning of stimulus outcomes during the acquisition stage where there was feedback (C) and the test phase where there was no feedback (D).

4.4.2.3 ^{1}H-MRS

Due to attrition (N = 1) and MRI scanner hardware (N = 2) and software problems (N = 2), data were successfully acquired for 12 patients only at baseline and both post-infusion infusion ^{1}H-MRS scans. Linewidth for the 12 subjects was however on average excellent for the two post-infusion scans (M = 11.62 Hz).

For our primary analysis, there was no significant interaction between glutamate levels and order of drug administration ($F_{(1,9)} = 0.06$, $P = 0.81$), thus this factor was removed from the final model. The effect of drug on levels of pregenual glutamate levels was not significant ($F_{(1,10)} = 1.08$, $P = 0.32$; **Figure 4.7A**). However, there was a trend towards an effect of drug ($F_{(1,9)} = 4.30$, $P = 0.068$) on levels of glutamine, reflecting, relative to placebo levels, a decrease in this metabolite following ketamine when baseline values were controlled for, as assessed by post-

hoc pairwise comparisons ($F_{(1,9)} = 5.11$, $P = 0.048$; **Figure 4.7B**). There was a trend towards a significant interaction between drug order and drug on glutamine levels ($F_{(1,9)} = 3.39$, $P = 0.099$), causing us to retain drug administration order as a factor in this final model. However, the relationships between relative variations ([ketamine-placebo]/baseline) in glutamine and changes in anhedonia ($r_{s(11)} = -0.17$, $P = 0.61$) or depression levels ($r_{s(11)} = -0.14$, $P = 0.69$) 24-hours following ketamine were not significant. No other significant effects were found. A summary of the main results from the chapter is presented in **Table 4.4**.

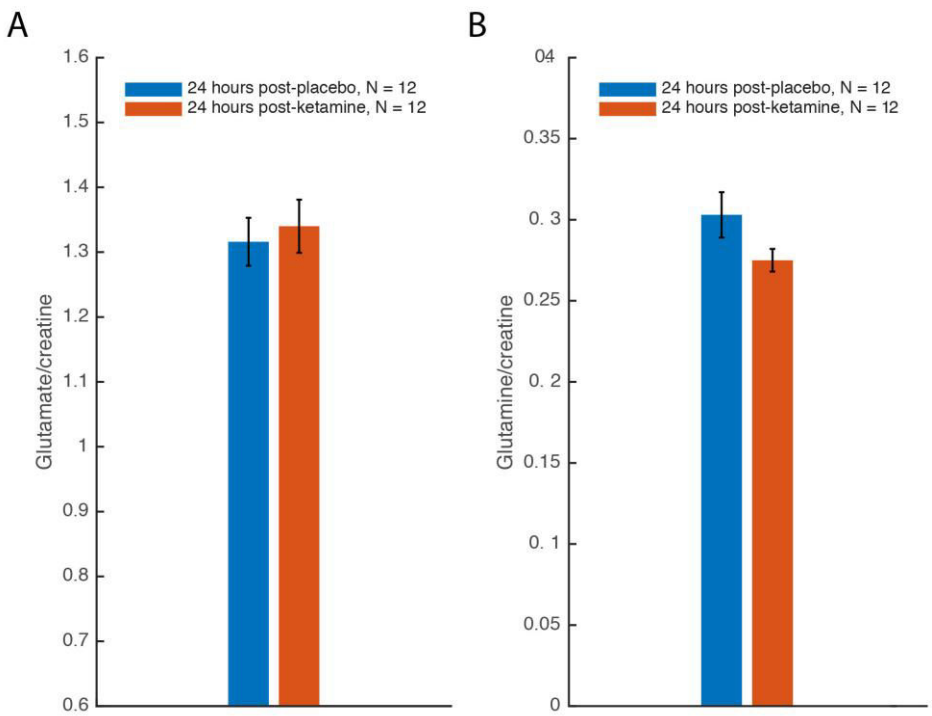

Figure 4.7. The effect of ketamine and placebo on levels of glutamate and glutamine. (A) In comparison to placebo and relative to baseline levels, there was no change in levels of glutamate 24-hours post-ketamine, but there was a significant decrease in glutamine levels at this time point (B).

Table 4.4. Summary of results from the case-control comparison between healthy volunteers and depressed patients and analyses comparing post-placebo and post-ketamine in depressed patients, only.

	Outcome
Baseline HVs vs. Patients	
Psychometrics	Sig (patients more anhedonic & depressed)
EEfRT	NS
Scene Choose	Sig (patients poorer learning variable stimuli)
^{1}H-MRS	NS
Ketamine vs. Placebo	
Psychometrics	Sig (improvement in depression post-ketamine)
EEfRT	NS
Scene Choose	NS
^{1}H-MRS	Sig (glutamine decreased post-ketamine)

HV, healthy volunteer; EEfRT, effort experimental for rewards task; ^{1}H-MRS, proton magnetic resonance spectroscopy; Sig, significant; NS, non-significant.

4.5 Discussion

The goal of this study was to replicate and extend recent reports that ketamine may exert anti-anhedonic effects in patients with depression (Lally *et al.*, 2014b, Lally *et al.*, 2015b) and to understand the mechanisms underlying these effects. In particular, we desired to examine in detail the precise clinical, cognitive and neural substrates of the improvements in anhedonia associated with treatment following ketamine in depressed patients. In addition to improving general depression scores, we found that ketamine improved levels of anhedonia on one particular scale, the SHAPS, which measures anticipatory anhedonia. However, no such effect was found for the TEPS, which measures both anticipatory and consummatory anhedonia separately. Furthermore, performance on our reward processing tasks, which probed levels of motivation and reward learning, showed no changes following treatment with ketamine. The antidepressant and anti-anhedonic response to ketamine was predicted by baseline levels of glutamine, replicating and extending an earlier finding. Additionally, in comparison to placebo and relative to baseline levels, ketamine decreased levels of glutamine post-infusion; however changes in glutamine did not relate significantly to the antidepressant or anti-anhedonic response to ketamine. No effect of drug was found on levels of glutamate 24-hours post-infusion, suggesting a more proximal examination of these metabolite effects may be required. Our results are suggestive of a particular role for ketamine in improving anticipatory anhedonia, but not reward motivation or learning, or consummatory processes, which may be potentially mediated by baseline levels of glutamine, implicating glutamatergic cycling and astrocytic function in the anti-anhedonic effect of ketamine.

The administration of ketamine was associated with an improvement in general depression and anticipatory anhedonia levels, as measured by the SHAPS, but not the TEPS and no improvement in consummatory anhedonia levels, again as measured by the TEPS. Our results contrast with evidence that treatment with ketamine in rodents improves stress induced deficits in consummatory behaviour (Walker *et al.*, 2013, Wang *et al.*, 2011). However, the majority of research examining the differing types of anhedonia in depressed patients finds a greater role for anticipatory rather than consummatory anhedonia (Treadway and Zald, 2011). For example, Sherdell et al. (2012) found that depressed patients rated cartoons

equally as funny as healthy volunteers but were less willing to exert effort to witness the stimuli. Similarly, Dichter and colleagues (2010) found that MDD patients rated sugar water as pleasant as healthy volunteers in a sweet taste test. Consistent with these reports, levels of anticipatory anhedonia at baseline were significantly greater here than consummatory levels in depressed patients, but the same effect was not apparent in healthy volunteers. Nonetheless, consummatory anhedonia levels were significantly higher in patients than in healthy volunteers here, suggesting that in treatment refractory depressed populations, consummatory processes may still be disrupted. However, it should be acknowledged that this is the first reported use of the TEPS in a treatment study so questions could also be raised of its validity for this form of investigation and whether it is sensitive to detect changes on this time scale.

We administered a reward decision and motivation task and a reward-learning task to further understand the potential anti-anhedonic effects of ketamine. To our surprise, the improvement in anhedonia levels, as measured by psychometric scales, was not mirrored by changes in reward task behaviour following ketamine. However, examination of the baseline effects revealed no significant differences in the EEfRT, contrasting a previous finding using this exact task (Treadway *et al.*, 2012). Several differences between our study and the original may explain why no baseline differences, and subsequent treatments effects, were found using this measure. First, our task was administered as part of a battery of tasks, which may have affected energy levels of both healthy participants and patients and thus confounded their decision behaviour and our results; it is not known how many tasks were administered during the original study. Secondly, the majority of patients in the original study (85%) were medicated with an SSRI (75%) or SNRI, whereas the majority of patients in this study were medication free. Evidence suggests that SSRIs are associated with emotional blunting of responses to stimuli in healthy volunteers (McCabe *et al.*, 2010) and patients (Opbroek *et al.*, 2002). It would interesting to perform a pharmacological manipulation to see if SSRI medication impacts performance of this task in healthy volunteers and patients. Finally, the differences in patient population exclusion criteria were substantial. The original study excluded patients who had any history of stimulant abuse or substance dependence and any past use of dopaminergic medications. As the population investigated here were mainly treatment refractory, these criteria would have precluded the majority of

patients' participation; thus, the patient sample differences may also explain why no effects were found here.

The second of our reward tasks, Scene Choose, did however reveal baseline between group performance differences. We found that patients performed comparably well to healthy volunteers on both loss avoidance and gain stimuli where the correct feedback was presented 90% of the time. However, performance on the stimuli, irrespective of valence, presented at a lower probability of correct feedback (80%) was worse than healthy volunteers. However, this sensitivity variable (90% - 80% performance) was related to the number of years since patient's first major depressive episode, but not levels of anhedonia, suggesting this difference was not related to state levels of subjective anticipatory anhedonia. Chronic depression is associated with hippocampal volume loss and the hippocampus is a key region responsible for memory. Thus, one potential explanation for this between group differences is that illness length leads depressed patients to become more sensitive to more variable environmental statistics. Interestingly, Browning et al. (2015) recently found that healthy individuals with high trait anxiety scores have difficulty learning the causal statistics of aversive environments (non-aversive stimuli were not tested) but did not show a deficit to false feedback probability, as shown here. In addition to a deficit in learning from noisier stimuli, depressed patients also displayed poor performance in differentiating frequent winners from frequent losers during the transfer phase where no feedback was provided. However, these results are likely explainable by poorer performance during the learning phase.

In an attempt to understand the neural basis of the anti-anhedonic effects of ketamine, we scanned patients at 7T using a ^{1}H-MRS sequence at baseline and 24-hours post-infusions, long after the psychotomimetic effects of ketamine had subsided. While the 24-hour post-infusion time point may have missed the direct mechanism of action of ketamine, the neural glutamatergic correlates associated with changes in anhedonia levels could have been visible and pointed in the direction of a potential mechanism of action. As ketamine primarily works on the glutamatergic system, and previous research has shown ketamine effects on this neurotransmitter at lower MRI field strength (Milak *et al.*, 2015, Stone *et al.*, 2012), our main metabolite of interest was glutamate. Our secondary analyses focussed on glutamine due to preclinical evidence positing a role for this metabolite in the mechanism of action of

ketamine and evidence suggesting its role in anhedonia in particular (Rowland *et al.*, 2005, Walter *et al.*, 2009). As this was the first study to examine neural metabolite levels in depression using ^{1}H-MRS at 7T, we first sought to confirm previous findings of a deficit in glutamate levels.

To our surprise, there were no significant differences in baseline glutamate or glutamine levels between depressed patients and healthy volunteers. Moreover, no significant changes in glutamate levels were found post-ketamine. Furthermore, glutamate levels were not predictive of the antidepressant or anti-anhedonic response to ketamine. However, our secondary analyses on glutamine found that ketamine caused a significant decrease in glutamine levels, but these decreases were not related to the improvement in anhedonia or depression. Additionally, we found that baseline levels of glutamine were predictive of the anti-anhedonic and antidepressant effects of ketamine. Taken together, our results suggest that glutamine, but not glutamate, is implicated in the treatment mechanism of depression with ketamine.

At first glance, the results of our ^{1}H-MRS investigation are surprising. Given that 7T permits excellent separation and the patients scanned were very depressed, medication refractory, and medication free, we expected that previous findings of a reduction in medial prefrontal cortex glutamate in depression would appear in this instance. Although non-significant, our results are consistent with previous findings and trends, in particular meta-analyses, of a pre-treatment reduction in glutamate levels in depressed patients in comparison to healthy volunteers (Hasler *et al.*, 2007, Luykx *et al.*, 2012, Yuksel and Ongur, 2010); however, a number of recent reports have found no evidence for decreased levels of baseline glutamate in depression in comparison to healthy volunteers (Abdallah *et al.*, 2014a, Godlewska *et al.*, 2015) suggesting the relationship may be variable. Our finding of a relationship between baseline glutamine and the anti-anhedonic effect of ketamine and a reduction in glutamine levels post-ketamine is interesting. At least one study has detected a large decrease in pregenual glutamine levels with successful treatment of depressed patients using lamotrigine (Frye *et al.*, 2007); however, this study was conducted at 1.5T and in BD patients so the ^{1}H-MRS changes are hard to interpret in the context of our results here. The glutamine findings suggest a role for astrocytes in the mechanisms of ketamine and treating depression. Accumulating evidence suggests that glial cells are dysfunctional in depression (Niciu *et al.*, 2014b) and their

influence can induce anhedonia (Bechtholt-Gompf *et al.*, 2010). One mechanism that could result in a decrease in levels of glutamine, but no change in glutamate, is an inhibition of extracellular glutamate release mediated through astrocytic signalling (Mitterauer, 2012). However, the role of astrocytes in the function of ketamine as a depression treatment remains to be tested. The antidepressant effects of sleep deprivation, the only other rapid acting antidepressant treatment to ketamine, are dependent on astrocyte mediated signalling (Hines *et al.*, 2013), suggesting a common pathway mechanism may be possible.

A number of limitations of this study merit comment. First, our patient sample was small and mixed, comprising both patients with MDD and BD. Future studies should both expand the sample and parse up the diagnoses as differences in reward processing and ^{1}H-MRS metabolite levels between depressive disorders may exist (Taylor, 2014). Second, three of our BD patients were medicated; future research would benefit by examining only medication free BD patients. Third, due to technical difficulties, only one ^{1}H-MRS voxel was acquired; the effects of ketamine on glutamate may be apparent in other brain regions not contained in our region of interest. Fourth, changes in glutamate following ketamine may occur acutely (i.e. during the infusion) and thus the timing of the ^{1}H-MRS scan here (24-hours post-infusion) may have been too late to detect changes in glutamate. Indeed, previous research has identified an acute change (within 40 minutes) in ^{1}H-MRS measured glutamate (Stone *et al.*, 2012) and glutamine (Rowland *et al.*, 2005) levels in healthy volunteers and patients following ketamine. However, ^{1}H-MRS investigations at 3T in healthy volunteers have also reported no acute effects of ketamine on glutamate or glutamine levels (Taylor *et al.*, 2012) and no effects in MDD patients three and 48-hours post-infusion (Valentine *et al.*, 2011). Fifth, in comparison to previous reports (Milak *et al.*, 2015, Zarate *et al.*, 2012, Zarate *et al.*, 2006), the antidepressant efficacy of ketamine was relatively limited in this sample, with only three out of 17 patients reaching response criterion. This lack of response may have negatively impacted our ability to detect differences in behaviour and brain activity post-infusion and to find relationships between measures. One possible reason for the lack of antidepressant efficacy of ketamine here is that the depressed patients recruited were particularly treatment refractory; additionally, media reports about ketamine may have raised patient expectation to unrealistic levels, which may have hindered

drug efficacy. Finally, due to the number of comparisons conducted here, our results require careful replication.

In summary, we found an effect of ketamine in improving levels of anhedonia, replicating previous findings. However, we found no baseline differences in reward processing that related to levels of anhedonia and no improvements in reward processing following ketamine. ^{1}H-MRS scans revealed that medial prefrontal cortex glutamine, but not glutamate, levels were predictive of the anti-anhedonic response to ketamine, replicating an earlier finding of predictability of baseline metabolites and the response to ketamine. While there was a significant decrease in glutamine levels post-ketamine, results were not associated with anhedonia or depression changes. Further research is required to better understand the cognitive and neural correlates of the anti-anhedonic effects of ketamine.

5 Working Memory in Major Depression: An fMRI and NMDA Receptor Antagonist Treatment Investigation in Medication-Free, Treatment-Resistant, Depressed Patients

5.1 Abstract

Working memory impairment is frequently reported in neuropsychological studies comparing depressed patients with healthy volunteers and neuroimaging studies often suggest load-related hyperactivity in DLPFC, an area involved in cognitive control, in MDD patients. Recent evidence suggests that ketamine, an NMDA receptor antagonist, has rapid-acting antidepressant properties and may have tentative cognitive enhancing capabilities for depressed patients. The NMDA receptor is strongly implicated in learning and memory but there is little work examining whether ketamine may improve cognitive impairments found in depression. Thus, we examined the effects of a single infusion of ketamine on working memory and its associated neural correlates in a randomised placebo-controlled trial. Treatment-resistant, medication-free depressed patients (N = 20) and healthy volunteers (N = 18) performed the n-back, a working memory task, during fMRI at baseline and 2 days following each infusion (N = 12 patients only; ketamine or saline). Our *a priori* region of interest was the left DLPFC. There was no difference in task performance at baseline. In contrast with previous research, depressed patients did not exhibit greater prefrontal baseline neural activity than healthy volunteers; however, healthy volunteers displayed a trend toward higher superior parietal lobule activity. Ketamine caused a robust antidepressant response, but this was not associated with changes in either task performance or BOLD activity. Our results do not support the hypothesis that treatment with ketamine is associated with an improvement in working memory performance as measured by the n-back or task-elicited changes in neural activity. Further research with a larger cohort is required to confirm these preliminary results.

5.2 Introduction

Although the true prevalence is unknown (Trivedi and Greer, 2014), cognitive dysfunction is a frequently reported, debilitating and hard to treat symptom of depression (Fava *et al.*, 2006). Evidence suggests that the presence of cognitive dysfunction is a mediator of functional disability in patients with MDD (Lam *et al.*, 2014). In particular, working memory, the ability to maintain and manipulate concurrent information online, is thought to be especially impaired in depression (Christopher and MacDonald, 2005, Joormann *et al.*, 2011, Pelosi *et al.*, 2000, Rose and Ebmeier, 2006). Standard antidepressants appear to provide limited benefit in alleviating cognitive dysfunction (Trivedi and Greer, 2014), and residual cognitive impairments remain one of the most common complaints (Fava *et al.*, 2006) and predict poorer treatment response (Dunkin *et al.*, 2000, Majer *et al.*, 2004). The recent evidence that ketamine, an NMDA receptor antagonist, may rapidly improve general depression levels in both unipolar and bipolar treatment-refractory patients (Diazgranados *et al.*, 2010a, Zarate *et al.*, 2006) raises the question of whether the mechanism by which this occurs may be through improvements in cognitive functioning; e.g. enhancements in cognition may lead to better daily functioning and/or decreases in anhedonia and thus potentially also improvements in mood.

To date, only three studies have investigated whether ketamine may improve cognitive functioning in depressed patients, none of which used objective measurements in blinded placebo-controlled designs. Murrough et al. (2015) administered a battery of cognitive tests to treatment-refractory unmedicated depressed patients at baseline and seven days post-infusion of both ketamine and midazolam (active control) in a randomized controlled trial. Although performance improved from baseline to seven days post-ketamine, a similar improvement was also seen seven days post-midazolam suggesting that the effect was non-specific and may reflect practice effects and/or symptomatic improvement. Nevertheless, Murrough et al. (2015) also found that slower baseline processing speed was predictive of the greatest antidepressant response to ketamine at 24-hours post-infusion, replicating an earlier finding (Murrough *et al.*, 2013b). In a retrospective analysis, DeWilde et al. (2015) found that treatment with ketamine was associated

with an average of a 58% improvement on the concentration item of the MADRS at 24-hours post-infusion; the authors did not account for change in general depression scores however. Notably, Shiroma et al. (2014) found that repeated open label infusions of ketamine were associated with improvements in working memory; however, these improvements were accounted for by changes in depressive symptoms, potentially suggesting a lack of a specific cognitive enhancement with ketamine. However, Shiroma et al. (2014) also found that lower attention scores, but not processing speed, at baseline predicted greater antidepressant response to ketamine. Taken together, these studies suggest that the cognitive enhancing properties of ketamine may be limited; nevertheless, poorer baseline pre-treatment cognitive levels may predict greater antidepressant response to ketamine.

At the neural level, Salvadore et al. (2010) found that working memory task-elicited (n-back) brain activity (the association between task performance and response was not assessed) in the pregenual anterior cingulate cortex strongly predicted the general antidepressant response to ketamine in an open label magnetoencepahalography (MEG) investigation. While the neural correlates of cognitive dysfunction in depression are not well understood, a consistent body of evidence suggests that hyperactivation of brain regions, particularly in the prefrontal cortex, may underpin the impaired cognitive task performance reliably found in depressed patients (Harvey *et al.*, 2005, Matsuo *et al.*, 2007). A recent functional magnetic resonance imaging (fMRI) meta-analysis (N = 10 studies, 6 of which used the n-back) by Wang et al. (2015) identified greater left DLPFC activity during working memory task performance in depressed subjects in comparison to healthy controls, irrespective of task performance, age, sex or medication status. Hyperactivity in depressed patients during comparable task performance to healthy volunteers has been suggested to reflect "neural inefficiency" in the depressed brain. DLPFC activity is associated with increasing cognitive demands, such as growing working memory load (Owen *et al.*, 2005). Interestingly, DLPFC is thought to play an important role in antidepressant treatment response (DeRubeis *et al.*, 2008). Notably, at least three studies (Brody *et al.*, 2001, Fales *et al.*, 2009, Kennedy *et al.*, 2001) have demonstrated a normalization of DLPFC activity in depressed patients following treatment, suggesting that this brain region may be sensitive to treatment

effects. Indeed, Ritchey et al. (2011) found that higher baseline DLPFC activation was strongly predictive of the improvement in symptoms following CBT.

The cognitive enhancing capabilities of ketamine remain unknown. Moreover, the precise systems-level biomarkers of the antidepressant response to ketamine and its cognitive mechanisms of action are yet to be determined. Tentative evidence suggests that pre-treatment working memory task-elicited brain activity may be predictive of the antidepressant response to ketamine (Salvadore *et al.*, 2010), potentially connecting this process to its mechanism of action. Therefore, we scanned healthy volunteers and depressed patients while performing the n-back, a task shown to robustly recruit prefrontal circuits implicated in treatment response, including DLPFC. Participants were scanned at baseline and 48-hours post-infusion with ketamine and placebo in a randomized, placebo-controlled investigation.

We hypothesized that improvements in depressive symptoms following ketamine would relate to alterations in neural networks recruited by working memory. Specifically, we hypothesized that DLPFC activity levels during task performance would be reduced 48-hours post-ketamine resulting in normalization to the levels observed with healthy volunteers. Given the treatment-refractory and medication-free nature of our patient sample here, we predicted that depressed patients would perform the n-back task worse than healthy volunteers at baseline and that the underlying BOLD activity associated with this task, particularly in the DLPFC region, would be greater. Finally, we predicted that the antidepressant response to ketamine would be related to pre-treatment and post-treatment n-back associated activation, particularly in the DLPFC.

5.3 Methods

5.3.1 Participants

Eighteen healthy participants (8 women) and 20 currently depressed patients (7 men) diagnosed with either major depressive disorder (MDD; N = 18, 6 men) without psychotic features or BD II (N = 2, one man) were enrolled in this study. The investigation was conducted at the National Institutes of Health (NIH), Bethesda, Maryland, USA. Healthy participants and patients were evaluated and diagnosed, respectively via the structured clinical interview for DSM-IV Axis I disorders and an unstructured interview with a board certified psychiatrist. All patients were currently in a major depressive episode lasting at least four-weeks. At the time of the initial testing, all participants, including the BD patients, had been medication free for at least 10 days. All participants were physically healthy and were free of any serious unstable medical conditions and comorbid substance abuse (three month duration) and dependence (excepting caffeine and nicotine); abuse and dependence were lifetime exclusion criteria for healthy volunteers. Pregnancy and nursing were not permitted and female subjects in the ketamine study (see below) were required to use approved methods of birth control. Comorbid axis I anxiety disorders were permitted for patients if they were not the primary diagnosis within the preceding 12 months. Healthy volunteers were excluded if any first-degree relatives had received a diagnosis of a major psychiatric disorder. Studies were approved by the combined neuroscience institutional review board of NIH and all subjects provided written informed consent before study entry.

5.3.2 Study Design

Following the initial screening, depressed patients and a subgroup of healthy participants (N = 12) were admitted to an inpatient psychiatric unit at the NIH to participate in a ketamine mechanism of antidepressant action investigation. The study had a randomized, double-blind, placebo-controlled, crossover design (**Figure 5.1**). Following a medication taper and a two-week drug-free period (5-weeks for those taking fluoxetine) for patients, participants were administered one intravenous infusion of a sub-anaesthetic dose of ketamine hydrochloride (0.5 mg/kg) and one

placebo (0.9% saline solution) infusion, with two-weeks between infusions (**Figure 5.1**). Infusions were administered by an advanced cardiac life support licensed practitioner over 40 minutes using a Baxter infusion pump; identical injections were used to ensure blinding. A Montgomery-Åsberg Depression Rating Scale (MADRS; (Montgomery and Åsberg, 1979) score ≥ 20 at the time of screening and also prior to the first infusion was an inclusion criteria for depressed patients. The majority (19/20) of patients enrolled in the ketamine study were refractory to pharmacological treatment; treatment resistance was defined as failure of two or more adequate antidepressant trials, as assessed by the Antidepressant Treatment History Form (Sackeim, 2001). No psychotherapy or other treatment was permitted during the entire trial period; BD patients were medication free throughout the study period. A number of measures (3T fMRI scans, and, psychometric scales) were acquired repeatedly throughout this month-long period (**Figure 5.1**).

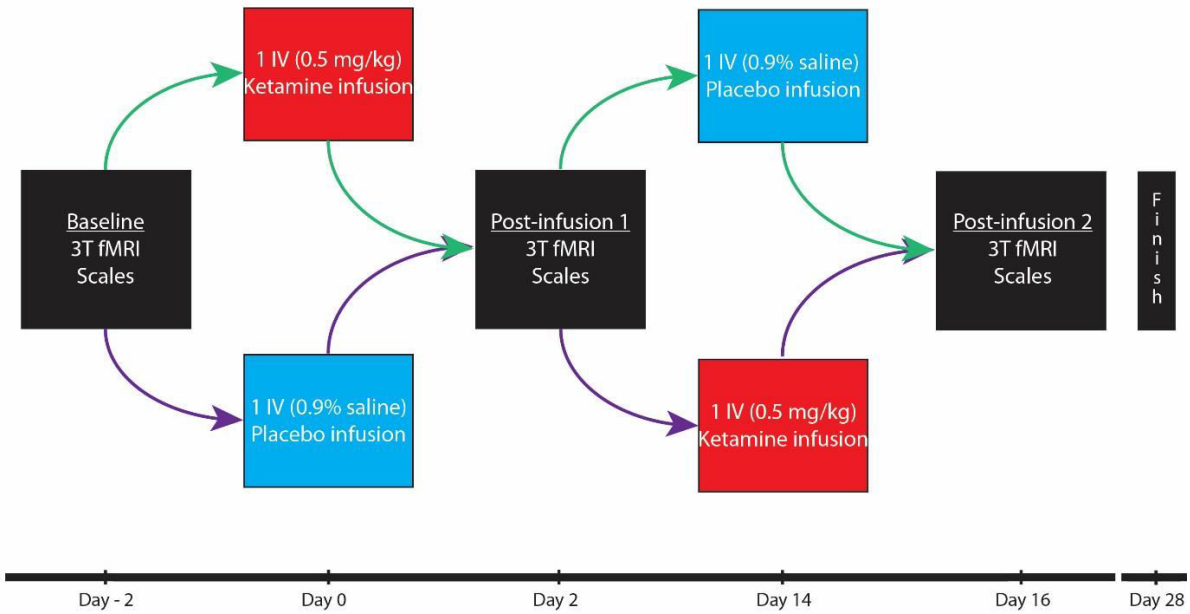

Figure 5.1. Study design. Approximately 2 days prior to the first infusion, all participants in the ketamine trial were scanned in a 3T MRI scanner where fMRI was collected during performance of the n-back task. At day 0, participants received their first infusion. Participants received one ketamine and one placebo infusion in a randomized, counterbalanced fashion (days 0 and 14) and were scanned using fMRI with the same n-back task as baseline 48-hours after each infusion (days 2 and 16, respectively).

5.3.3 N-back Task

The n-back task was adapted for fMRI from one described in detail previously in Chapter 2 (Lally *et al.*, 2013) and programmed in Cogent 2000 (www.vislab.ucl.ac.uk/Cogent), a stimulus presentation toolbox for MATLAB (MathWorks, Natick, MA, USA; version 7.1). Briefly, following an initial fixation cross (which lasted 0.9s in duration) and block condition instructions (1s; "1-back" or "3-back"; **Figure 5.2A;** these remained onscreen for the rest of the block), participants were presented with a randomized sequence of 12 lower case consonants (1s; "t", "f", "p", "v", "g" or "h"), between a blank screen (0.5s), save for the task instruction; alternatively, a fixation cross was presented continuously and participants were instructed to rest. The aim was for participants to make a button press when the stimulus on screen was the same as the stimulus n-back. Participants were only presented with 1- and 3-back active blocks and rest. Examples of 1-back and 3-back occurrences are shown (**Figure 5.2B**). Blocks, of which there were 18, were randomized in order, with six of each condition presented (rest, 1-back and 3-back). Each block lasted 19.1 seconds from the time of the instructions to the interim fixation cross, with the task lasting six minutes in total. Only one run was undertaken during each scanning session. Participants received extensive training on the n-back task, completing 0-, 1-, 2-, and, 3-back examples, prior to performing the task inside the MRI scanner.

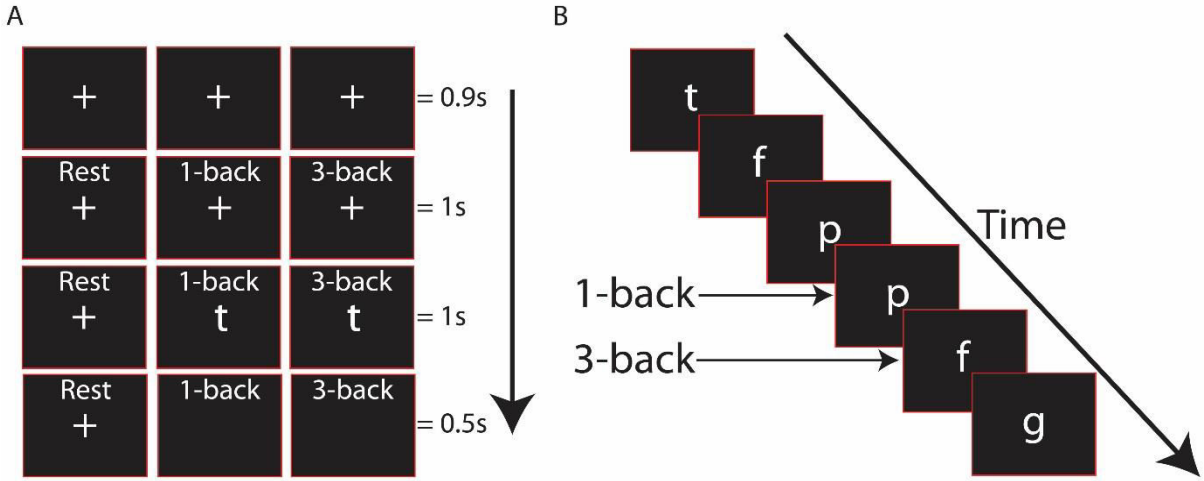

Figure 5.2. n-back task. (A) Outline of the three different task conditions; each column represents a condition (rest, 1-back or 3-back). Following an initial fixation cross, which lasted 0.9 seconds, participants were presented with either a rest or task block (either 1 or 3-back, only), which began with instructions that lasted 1 second and remained onscreen throughout the block. Twelve consonants were presented centrally (1s each) and were interspersed with null events, a blank screen aside from the task instructions, which lasted 0.5 seconds. (B) An example of a 1-back and 3-back stimulus. Here the letter 'p' is repeated directly once so represents an instance of 1-back. The letter 'f' reappears after 2 intervening stimuli, thus represents an instance of 3-back.

5.3.4 MRI scan acquisition

All MRIs were collected using a 3T General Electric scanner (GE Signa) and an 8-channel phased-array head coil. The task was presented via a head coil mirror and a front-of-bore projection system. One hundred and forty-eight T2* weighted echo-planar imaging (EPI) volumes (45 slices per volume, Slice thickness = 3.5 mm; gap between slices = 3.5 mm; slice repetition time (TR) = 56 ms, volume TR = 2.5 s; echo time (TE) = 23 ms, flip angle = 90°, field of view = 192 mm) were collected per session, totalling 6 minutes and 10 seconds in scan duration time. The first four volumes from the run were discarded to allow for T1 equilibrium effects, leaving 144 volumes per session. A 3D T1-weighted anatomical scan (FSPRG BRAVO; 176 slices; slice thickness = 1 mm; gap between slices = 1 mm; TR = 8.836 ms; TE = 3.50 ms; inversion time = 450; FA = 13; field of view = 256 x 256 mm^2; matrix size = 256 × 256; voxel size = 1×1×1 mm resolution) was acquired at the end of each scanning session.

5.3.5 fMRI preprocessing

EPIs were pre-processed prior to analysis using Statistical Parametric Mapping (SPM; Wellcome Trust Centre for Neuroimaging, London) 8 (release 4010; www.fil.ion.ucl.ac.uk/spm) in MATLAB. Images were spatially realigned (to the first available session volume) to correct for motion distortion. Volumes corrupted due to movement (0.03% of all volumes) were excluded and replaced by linear interpolation of the surrounding images. Images were then normalized to Montreal Neurological Institute (MNI) co-ordinate space and smoothed with a Gaussian kernel of 8 mm isotropic full-width at half-maximum (FWHM) Gaussian kernel.

5.3.6 Statistical analyses

For the case-control comparison analyses, unless otherwise specified, demographic variables were included in the statistical models as covariates if the variable was significantly different between groups. Three demographic variables, age, IQ and years of education were significantly different between the two groups (see below for details); however, due to missing IQ (N = 6) data, only age and years of education were included as covariates. If either age or years of education was found not to show a main effect, either significantly or at trend level, it was removed from the analyses to increase the available degrees of freedom.

Due to the low number of healthy volunteers with post-infusion data (N = 8), these participants were not included in analyses pertaining to the effects of drug. For all post-infusion analyses, order of drug administration was entered as a between-subjects factor and baseline performance or neural activity was entered as a covariate. Where applicable, if there was a significant drug by order interaction, order was retained in the model, or otherwise dropped to increase the degrees of freedom.

Where covariates were included in the model, they were first mean corrected. All statistical analyses were two-tailed, conducted in SPSS (Armonk, NY, USA; version 21) and a $P < 0.05$ was considered statistically significant and $P < 0.1$ was considered a trend towards significance. Huynh-Feldt correction was applied if the

assumption of sphericity was violated during repeated-measures ANOVA. Variables were assessed for normality using Shapiro-Wilk tests; where appropriate, non-parametric tests were used.

5.3.6.1 Psychometrics analyses

Our primary psychometric variable of interest for this chapter was the MADRS. As the effect of ketamine on the MADRS has already been explored in the majority of the sample of patients included here (Chapter 4), our aim here was to evaluate the antidepressant effect of ketamine at the time of the fMRI scan (48-hours post-infusion). We calculated the ketamine to placebo change in MADRS score at 48-hours relative to the respective ketamine and placebo pre-infusion baseline MADRS scores ([placebo baseline / (placebo baseline – 48-hours post-placebo)] - ([ketamine baseline / (ketamine baseline – 48-hours post-ketamine)]).

5.3.6.2 Behavioural analyses

Accuracy on the n-back was calculated as the percentage of correct 1-back and 3-back responses (hits) out of the total number of respective opportunities per condition, minus the percentage of commissions out of the total number of opportunities. Responses to the first stimulus on 1-back and the first three stimuli on the 3-back were not included in the commission opportunity statistic. For the case-control comparison, a single within-subjects factor repeated measures analysis of variance (ANOVA) was used to calculate the effect of difficulty (1- or 3-back) on accuracy and reaction time (RT) at the baseline time point, with group entered as a between subject's factor. In the case that no accurate response was made, these subjects were excluded from the RT analysis.

For the post-infusion analyses (in MDD patients only), repeated measures ANOVAs on accuracy and RT were examined with order of drug administration entered as a between-subjects factor and baseline performance (calculated as the difference between 1- and 3-back conditions) entered as a covariate. If the drug administration order by treatment interaction was non-significant, this factor was removed from the model. To limit the number of analyses calculated and the number

of baseline performance covariates included, the difference in accuracy and RT between the 1-back and 3-back conditions was used as our metric for these post-infusion analyses.

Based on previous findings (Murrough *et al.*, 2015, Murrough *et al.*, 2013b), we calculated whether baseline performance predicted change in depression levels, as measured by MADRS, score 24-hours post-infusion ([placebo baseline / (placebo baseline – 24-hours post-placebo)] - ([ketamine baseline / (ketamine baseline – 24-hours post-ketamine)]), a previously identified maximal time point of the antidepressant effect of ketamine (Zarate *et al.*, 2006). We also examined the relationship between change in behavioural performance and change in general depression using the MADRS at 48-hours post-ketamine. The time point of 48-hours was chosen, as this was when the fMRI task was performed. Pearson product moment correlation coefficients were used to examine these two relationships.

5.3.6.3 fMRI analyses

5.3.6.3.1 First-level analysis

A simple box car block design was fit at the first-level analysis stage. Regressors for 1- and 3-back conditions were constructed with rest blocks constituting an implicit baseline (all 19.1 seconds in duration). Our primary contrast of interest was 3-back > 1-back; contrasting these conditions allows the examination of neural mechanisms underpinning working memory as the 3-back requires greater working memory processes than the 1-back. Three other contrasts were conducted: n-back (1- + 3-back) > rest, 1-back > rest, and, 3-back > rest. The six realignment parameters were also included in the model. Estimation incorporated a high-pass filter at 1/128 Hz and serial correlations intrinsic to the fMRI time series were accounted for using an AR(1) model. Following estimation, subject-level contrast images entered into group-level one-sample *t*-tests.

5.3.6.3.2 Second-level analysis

Our group-level analyses comprised a region of interest and whole brain exploratory approaches. Second level models (see below) were constructed to explore basic task effects, group comparisons, effects of drug and the relationship between neural

activity and antidepressant response. Unless otherwise specified, we applied an initial threshold of $P = 0.005$ (uncorrected) and then family-wise error (whole brain; P_{WB}) correction for multiple comparisons at the cluster-level. Given our *a priori* hypotheses, we conducted a region of interest (ROI) analyses based on the left DLPFC coordinates identified by Wang et al. (2015) in their meta-analysis (MNI coordinates, [x = -46, y = 20, z = 31]). These coordinates were converted from Talairach to MNI coordinate space using the tal2mni algorithm (Brett *et al.*, 2002) and a 1cm sphere was created around this centre of mass and data were extracted from our second level model using the MarsBaR tool for SPM (http://marsbar.sourceforge.net/).

5.3.6.3.3 Models

First, we constructed two models to explore the effects of the n-back task generally, and, specifically, to examine the neural effects of increasing working memory demands of the n-back. We explored what brain regions were activated by the task by comparing task > rest blocks across all subjects. Next, the aim was to locate the neural regions that scaled with the increasing working-memory demands of our task, thus we compared 3-back > 1-back blocks across all participants. As anticipated, these analyses revealed very robust responses and, for these comparisons only, we set our threshold such that only voxels that reached whole-brain voxel-level family-wise error correction $P_{WB} < 0.05$ survived. For the 3-back > 1-back analysis only, we examined the appropriateness of our ROI selection by comparing extracted beta values (average across the ROI) from our two task conditions (1- and 3-back) across all subjects using a paired samples *t*-test. If the ROI is sensitive to task-related working memory demands, activity in this region should be higher during 3-back than 1-back blocks.

Second, we examined pre-treatment baseline group differences between depressed patients and healthy volunteers. We conducted an independent samples *t*-test in SPM with our primary contrast, 3-back > 1-back, as the dependent variable; significantly different group demographic variables (age and years of education) were included as covariates in the model. A univariate general linear model was used to explore the group differences in our left DLPFC ROI, with group entered as a

between-subjects effect and years of education and age entered as covariates. The same procedure was undertaken with the peak voxel from clusters identified as significantly different between groups as it is not straightforward to include interaction effects with covariates in SPM; however, the statistics for this analysis will be inflated due to the lack of correction for multiple comparisons. In the event of a significant baseline group difference in whole-brain corrected activity, we decided to use these regions as further ROIs in subsequent analyses exploring the effect of drug.

Third, we explored the effects of treatment with ketamine on brain activity during task performance in patients only. Difference images were calculated (post-ketamine – post-placebo) for our primary contrast of interest (3-back > 1-back) only. We extracted beta values from both our *a priori* and baseline difference identified ROIs. A single factor (drug: post-ketamine or post-placebo brain activity) repeated measures ANOVA with order entered as a between-subjects factor and baseline activity entered as a covariate was computed in SPSS for each post-infusion ROI. For exploratory whole-brain analyses, a one-sample *t*-test on the ketamine vs. placebo difference images was used to assess effects, with order entered as a covariate. Beta values were extracted from regions in which a significant effect of ketamine was identified and control analyses were computed to assess the interaction between order, baseline and post-treatment brain activity, as SPM cannot process a baseline image as a covariate. Note, for these methodological reasons the chances of a type II error are increased.

Follow-up analyses of regions identified using the case-control comparison model examined whether baseline activity levels in either our ROI or significant whole-brain cluster corrected regions (using the extracted peak voxel from significantly different clusters from the 3-back>1-back between groups contrast) were predictive of the antidepressant response to ketamine, in depressed patients only, at 24-hours post-infusion (a previously identified time of maximal antidepressant effect). We also explored in depressed patients only whether any brain region at baseline was correlated with the magnitude of the antidepressant response to ketamine at 24-hours post-infusion using an exploratory whole-brain approach.

Finally, we explored whether the post-infusion change in activity in our ROIs (using Pearson product moment correlation coefficient) or whole-brain difference image was related to the magnitude of antidepressant response to ketamine at 48-hours, the time of each post-infusion MRI scan.

5.4 Results

5.4.1 Case-control comparison

Participant demographic information is presented in **Table 5.1** Of note, significant differences in IQ ($t_{(29)}$ = -2.85, P = 0.008), age (U = 109.50, Z = 2.06, P = 0.04), and number of years of education (U = 102.50, Z = -2.39, P = 0.02) were found between patients and healthy volunteers, with patients less educated, displaying a lower IQ and older.

Table 5.1. Participant demographic information and psychometric scale scores at baseline.

	Healthy Volunteers (N = 18)		**Patients (N = 20; 18 MDD, 2 BD)**		**Statistical Differences**	
	Mean	SD	Mean	SD	t/Z	*P*
Age	32.22	9.90	38.45	11.01	2.06	0.04
Age of onset	NA	NA	16.44	8.43		
Baseline BDI	0.67	0.98	30.47	8.63	4.65	< 0.001
Baseline MADRS	0.31	0.48	31.67	4.00	4.75	< 0.001
IQ	121.5	7.29	111.89	10.86	-2.85	0.008
Current episode length (months)	NA	NA	29.64	42.32		
Years of education	18.71	1.69	16.90	2.61	-2.39	0.02
	N	%	N	%	X^2	
Female	8	44	13	65	1.62	0.33
TRD	NA	NA	19	95		

BDI: Beck Depression Inventory; MADRS: Montgomery-Åsberg Depression Rating Scale; TRD: Treatment Resistant Depression; IQ: Intelligence Quotient.

5.4.1.1 Behavioural data

There were no significant main effects of years of education or age on baseline task accuracy (hits minus commissions) so these variables were dropped from the model. There was a main effect of difficulty ($F_{(1,36)}$ = 172.97, P < 0.001), but no main effect of group ($F_{(1,36)}$ = 2.62, P = 0.11), or an interaction between difficulty and group ($F_{(1,36)}$ = 2.06, P = 0.16) on baseline task accuracy (**Figure 5.3A**). The main effect of difficulty reflected lower accuracy on the 3-back (M = 39%) in comparison to the 1-

back (M = 96%). Again, there were no significant main effects of age or education years on baseline hit RT and again these covariates were dropped from the model. Three patients had no correct 3-back responses and thus no RT and therefore were not included in this analysis. There was a main effect of difficulty on correct RTs ($F_{(1,33)} = 29.99$, $P < 0.001$) but no main effect of group ($F_{(1,33)} = 0.01$, $P = 0.92$) or a difficulty by group interaction ($F_{(1,33)} = 0.04$, $P = 0.85$; **Figure 5.3B**). The main effect of difficulty on RTs reflected faster correct responses to the 1-back (591 ms) than the 3-back (778 ms) task condition.

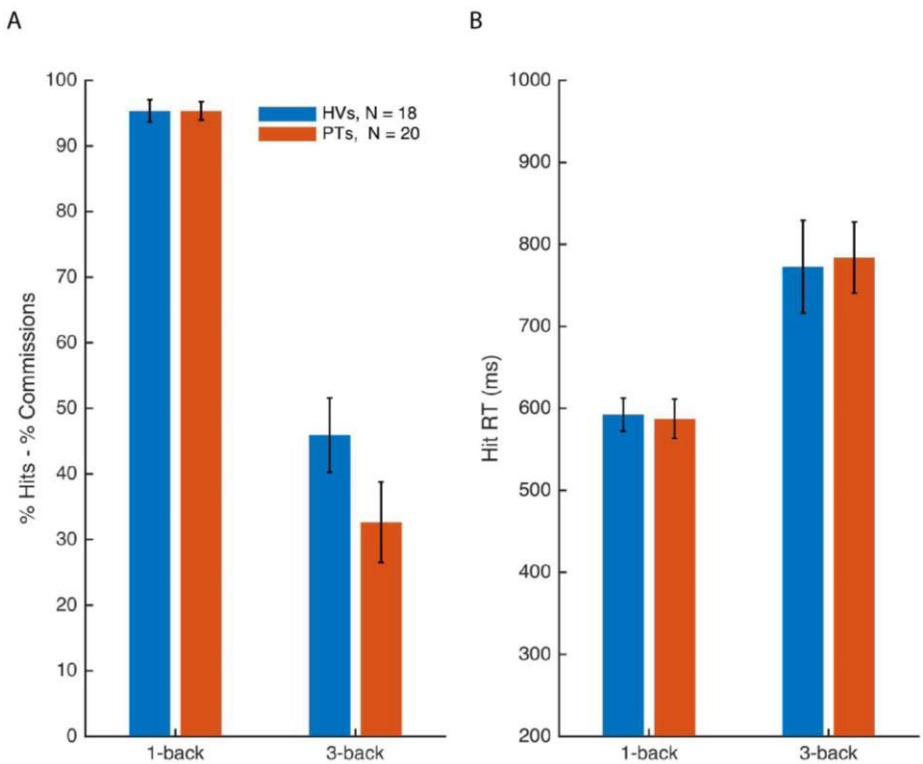

Figure 5.3. Case control comparison of n-back performance. Task accuracy (A) and reaction time (B) were comparable across group (healthy volunteers and depressed patients) for both the 1- and 3-back. Error bar indicate one standard error of the mean.

5.4.1.2 fMRI data

The n-back task activated a network of regions reliably found in previous studies using this task (Owen et al., 2005), including, motor, striatal, insular, parietal and dorsolateral prefrontal cortices (all $P_{WB} < 0.05$ at the voxel-level; **Figure 5.4A**; **Table 5.2**). Our main contrast of interest, 3-back > 1-back, also activated a number

of regions thought to be involved in increasing cognitive load using this task (Owen et al., 2005), including the cerebellum, parietal lobule and DLPFC (all $P_{WB} < 0.05$ at the voxel-level; **Table 5.2**). Supporting our ROI selection, across both groups combined, 3-back performance elicited significantly greater activation in the left DLPFC ROI than 1-back performance ($t_{(37)} = -7.27$, $P < 0.001$; **Figure 5.4B**).

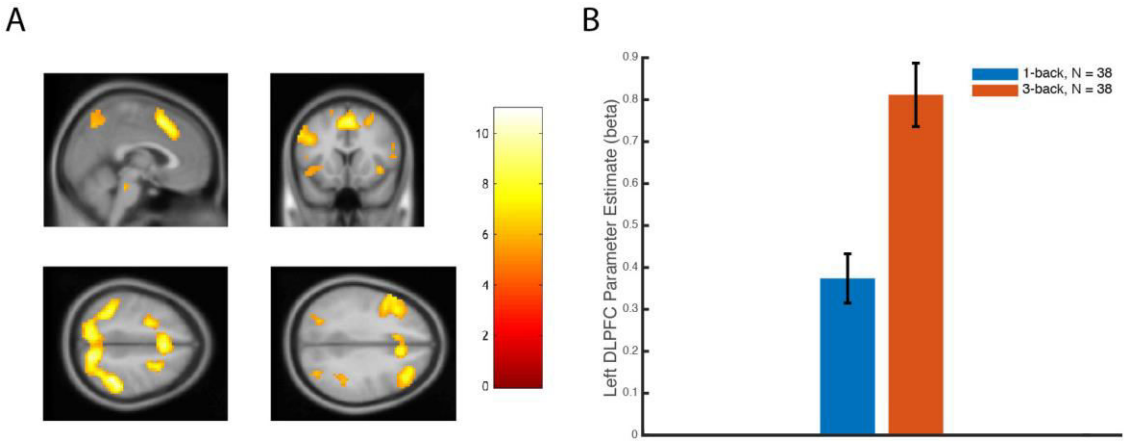

Figure 5.4. Neural responses to the n-back task. A) In comparison to rest, the n-back task reliably activated a network of task-related regions across all participants, including the motor, (top left) insular (top right), parietal (bottom left) and dorsolateral prefrontal (bottom right) cortices; all $P_{WB} < 0.05$ at the voxel-level. B) Beta values averaged across our region of interest, the left DLPFC, revealed that activity during the 3-back was greater than activity during the 1-back across the two groups combined. Error bar indicate one standard error of the mean. Colour bar indicates t-value.

Table 5.2. Regions activated during n-back task performance and during our primary contrast of interest (3-back > 1-back), both across all subjects (healthy volunteers and patients) and threshold $P_{WB} < 0.05$ voxel-level corrected.

Region	**MNI Coordinate**			***Peak***	***Voxel*** P_{WB}	**Extent**
	X	Y	Z	$t_{(37)}$**-value**		
n-back > Rest						
Posterior medial frontal	6	8	58	13.78	<0.001	4260
Posterior medial frontal	3	17	49	13.45	<0.001	
Insula	33	23	1	12.93	<0.001	
Supramarginal gyrus	42	-40	43	12.93	<0.001	981
Inferior parietal lobule	36	-49	46	12.88	<0.001	
Supramarginal gyrus	54	-43	40	10.12	<0.001	
Inferior occipital gyrus	-42	-67	-8	10.77	<0.001	548
Cerebellum	-36	-61	-32	9.73	<0.001	
Middle occipital gyrus	-39	-85	-5	6.52	0.002	
Inferior parietal lobule	-33	-49	43	10.68	0.000	734
Superior parietal lobule	-15	-70	52	7.41	0.000	
Middle occipital gyrus	-27	-76	28	5.90	0.011	
Inferior temporal gyrus	45	-58	-14	9.58	0.000	503
Cerebellum	33	-58	-32	9.36	0.000	
Inferior occipital gyrus	42	-88	-5	8.27	0.000	
Superior temporal gyrus	-54	-46	16	8.24	0.000	86
Head of caudate	15	2	10	6.76	0.001	63
Cerebellum	-9	-79	-26	6.75	0.001	17
Internal capsule	-18	-1	10	6.56	0.002	74
Superior temporal gyrus	60	-40	16	6.15	0.006	13
Middle occipital gyrus	-33	-94	1	5.32	0.049	1
3-back > 1-back						
Inferior parietal lobule	42	-43	46	10.93	<0.001	1568
Precuneus	12	-70	55	10.70	<0.001	
Inferior parietal lobule	-33	-55	46	8.88	<0.001	
Posterior medial frontal	3	14	49	9.13	<0.001	942
MCC	9	23	37	8.51	<0.001	
Superior frontal gyrus	27	8	52	8.18	<0.001	
DLPFC	39	32	34	8.14	<0.001	269
Cerebellum	-33	-61	-32	8.00	<0.001	106
Insula	-30	20	-2	7.84	<0.001	83
DLPFC	-33	47	22	7.75	<0.001	135
Insula	36	20	-2	7.69	<0.001	108
DLPFC	-39	20	34	7.57	<0.001	463
Precentral gyrus	-45	8	31	7.56	<0.001	
Inferior frontal gyrus	-54	17	34	7.23	<0.001	
Cerebellum	36	-61	-32	6.63	0.001	33
Pons	3	-31	-26	6.54	0.002	13
Inferior frontal gyrus	54	11	19	6.13	0.005	23

Pons	9	-25	-32	5.94	0.007	2
Pallidum	-15	5	-2	5.46	0.026	13
Putamen	-18	2	10	5.42	0.029	

DLPFC: dorsolateral prefrontal cortex; Montreal Neurological Institute (MNI) coordinates indicate the distance (in millimeters) from the stereotaxic origin (anterior commissure), with X representing the lateral distance from the origin (positive numbers to the right), Y representing the anterior-posterior dimension (positive numbers anterior), and Z representing the dorsal-ventral dimension (positive numbers dorsal). Where a cluster survived whole brain family wise error correction ($P < .05$), three local peaks are reported where available.

For the case-control comparison ROI (left DLPFC) analysis, no significant effects of the covariates were found so these were dropped from the model. There were no significant activation differences between the healthy volunteers and depressed patients in the left DLPFC ROI ($F_{(1,36)} = 0.69$, $P = 0.41$; **Figure 5.5A**). Contrasting previous findings, there were no supra threshold voxels more activated in the contrast depressed patients > healthy volunteers for our contrast of interest (3-back > 1-back). However, exploratory whole brain analyses (cluster-forming threshold $P < 0.005$ uncorrected) revealed a trend toward a difference in activation between the two groups in the right superior parietal lobule (SPL; [x = 21, y = -61, z = 52]; $t_{(34)} = 4.22$, $P_{WB} = 0.091$ at the cluster-level; **Figure 5.5B; Table 5.3**), which arose from greater activity in healthy volunteers than depressed patients (**Figure 5.5C**). To further understand this difference, we extracted separate beta values from the peak voxel of this cluster for both 1- and 3-back conditions and conducted a follow-up repeated measures ANOVA in SPSS with difficulty and group entered as within- and between-subjects factors, respectively, and with age and years of education entered as covariates. There were no significant main effects of the covariates, which were dropped from the model. The main effect of group was also not significant ($F_{(1,36)} = 0.13$, $P = 0.72$). However, there was a significant main effect of difficulty ($F_{(1,36)} = 39.81$, $P < 0.001$) and a group by difficulty interaction ($F_{(1,36)} = 9.35$, $P = 0.004$). Post-hoc tests revealed significantly higher SPL activation during the 3-back than the 1-back condition for both depressed patients ($F_{(1,36)} = 41.68$, $P < 0.001$) and healthy volunteers ($F_{(1,36)} = 5.58$, $P = 0.024$. However, no significant between-group differences in SPL activation for 1-back ($F_{(1,36)} = 0.95$, $P = 0.34$) or 3-back ($F_{(1,36)} = 0.09$, $P = 0.77$) were found (**Figure 5.5C**).

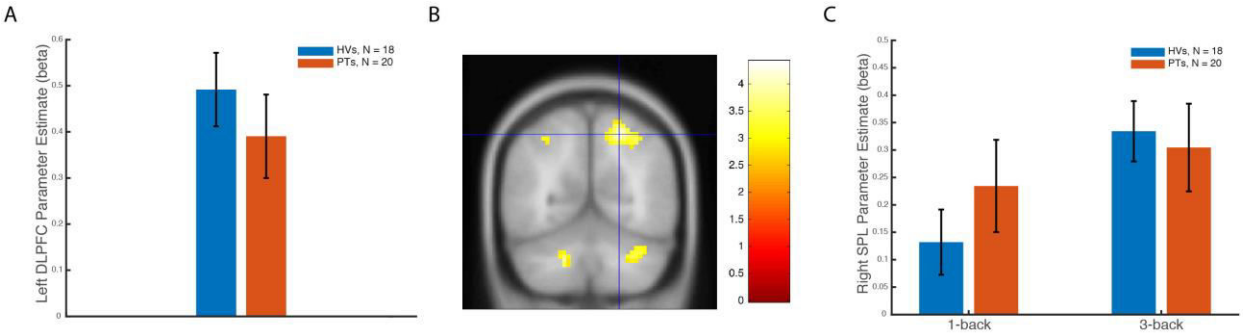

Figure 5.5. Case-control comparison for n-back fMRI activity. (A) Our ROI analysis revealed no group differences in DLPFC activity during our main contrast of interest (3-back vs. 1-back). (B) However, we identified a trend towards a significant group difference in difficulty-related activation in the superior parietal lobule (SPL; cluster-forming threshold $P < 0.005$ uncorrected). (C) The SPL activity difference was driven by greater activity in this region by healthy volunteers than depressed patients. Error bar indicate one standard error of the mean. Colour bar indicates t-value.

Table 5.3. Comparison of healthy volunteer and depressed patient's brain activation to our primary contrast of interest (3-back > 1-back). Threshold at an initial $P < 0.005$ uncorrected. An extent threshold of 5 voxels or more was also applied. Note, there were no supra-threshold voxels in the depressed patients > healthy volunteers contrast.

Region	**MNI Coordinate**			***Peak $t_{(34)}$*-value**	***Cluster P_{WB}***	**Extent**
	X	Y	Z			
HVs > Depressed PTs						
Cerebellum	18	-52	-38	4.41	0.996	16
Mid cingulate cortex	12	5	34	4.33	0.147	202
Superior parietal lobule	21	-61	52	4.22	0.091	239
Angular gyrus	33	-55	46	4.07		
Supramarginal gyrus	45	-34	37	3.81		
Posterior-medial frontal	-9	5	52	3.91	0.695	79
Middle frontal gyrus	-30	20	34	3.84	0.893	49
Precentral gyrus	-30	-7	52	3.79	0.780	67
Insula	-36	2	19	3.75	0.807	63
Cerebellum	-18	-61	-38	3.72	0.997	14
Postcentral gyrus	-54	-1	40	3.67	0.975	29
Cerebellum	36	-64	-32	3.58	0.893	49
Superior temporal gyrus	48	-34	10	3.38	0.996	15
Superior parietal lobule	-27	-67	49	3.38	0.994	18
Precentral gyrus	45	2	37	3.37	0.759	70
Middle temporal gyrus	-63	-46	7	3.34	0.987	23
Insula	48	8	7	3.28	0.999	9
Pons	3	-31	-32	3.27	1.000	7
Precuneus	-6	-70	49	3.07	0.998	11
Superior temporal gyrus	66	-28	22	3.06	1.000	7
Middle temporal gyrus	51	-49	13	3.06	0.999	10

Middle occipital gyrus	-12	-94	1	3.01	0.999	9
Cerebellum	24	-79	-44	2.90	1.000	6

HVs: healthy volunteers; PTs: patients; Montreal Neurological Institute (MNI) coordinates indicate the distance (in millimeters) from the stereotaxic origin (anterior commissure), with X representing the lateral distance from the origin (positive numbers to the right), Y representing the anterior-posterior dimension (positive numbers anterior), and Z representing the dorsal-ventral dimension (positive numbers dorsal). Where a cluster survived whole brain family wise error correction ($P < .05$) or at trend level ($P < 0.1$), three local peaks are reported where available.

5.4.2 Post-Ketamine

Due to attrition (N = 4), hardware difficulties (N = 3), missing functional imaging data (N = 1) and a patient not responding during the task (N = 1), 16 patients had psychometric, 12 behavioural and 11 fMRI analysable data for both 48-hour post-infusion time points.

5.4.2.1 Psychometric data

In comparison to the equivalent time points post-placebo, there was a significant improvement in total depression score (MADRS) at 24- ($t_{(15)} = 2.59$, $P = 0.02$) and 48- ($t_{(15)} = 2.72$, $P = 0.016$) hours post-ketamine. However, only 3 of the 16 (19%) patients who received both infusions reached response criterion (≥ 50% improvement on the MADRS) on days 1 or 2.

Baseline accuracy ($r_{(16)} = 0.30$, $P = 0.26$) and RT ($r_{(13)} = -0.14$, $P = 0.65$) were not significant predictors of the magnitude of the relative antidepressant response to ketamine 24-hours post-infusion. Furthermore, baseline BOLD activity from our left DLPFC ROI ($r_{(16)} = -0.16$, $P = 0.57$) and right SPL (peak voxel, $r_{(16)} = -0.29$, $P = 0.28$) were not significantly associated with the antidepressant response to ketamine 24-hours post-infusion (improvement coded as the relative percentage reduction between ketamine and placebo post-infusion scores and their respective baseline scores). Furthermore, no clusters survived cluster-level correction with our whole brain approach (**Table 5.4**).

Table 5.4. Correlation between pre-treatment brain activity during our primary contrast of interest (3-back > 1-back) and the antidepressant response to ketamine 24-hours post-infusion in depressed patients. Threshold at an initial $P < 0.005$ uncorrected. An extent threshold of 5 voxels or more was also applied. Note, there were no supra threshold voxels for the positive correlation.

Region	**MNI Coordinate**			***Peak $t_{(14)}$*-value**	***Cluster P_{WB}***	**Extent**
	X	Y	Z			
Negative correlation with antidepressant response to ketamine at 24-hours post-infusion						
Superior temporal gyrus	45	-25	-2	4.51	0.907	45
Precuneus	15	-61	46	3.28	1.000	8
Cerebellum	12	-82	-26	3.25	1.000	8

Montreal Neurological Institute (MNI) coordinates indicate the distance (in millimeters) from the stereotaxic origin (anterior commissure), with X representing the lateral distance from the origin (positive numbers to the right), Y representing the anterior-posterior dimension (positive numbers anterior), and Z representing the dorsal-ventral dimension (positive numbers dorsal).

5.4.2.2 Behavioural data

There was no significant interaction between drug administration order and drug (ketamine or placebo) on either accuracy or RT; thus, order was removed from subsequent models examining the effect of drug on task performance. There was no significant effect of drug on post-infusion accuracy ($F_{(1,10)} = 0.01$, $P = 0.91$; **Figure 5.6A**) or RT ($F_{(1,8)} = 1.26$, $P = 0.30$; **Figure 5.6B**). Changes in accuracy ($r_{(12)} = 0.32$, $P = 0.31$) and RT ($r_{(10)} = -0.38$, $P = 0.28$) from placebo to ketamine, relative to baseline levels, did not relate to the magnitude of the antidepressant response to ketamine.

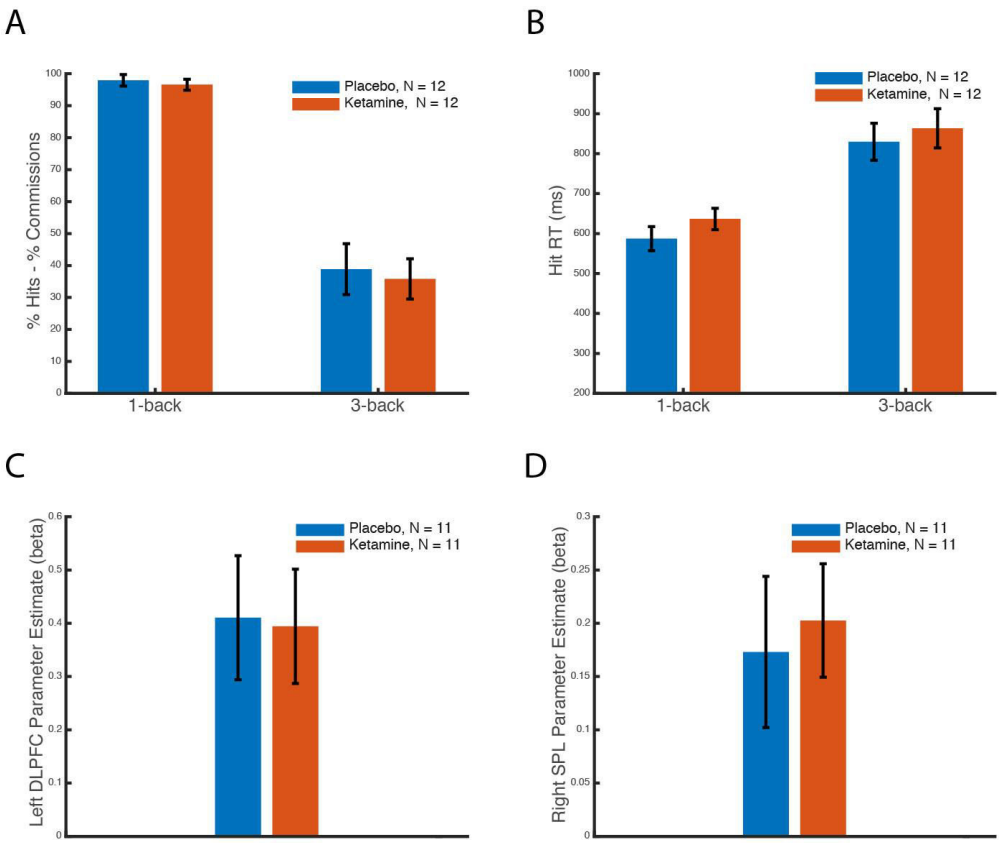

Figure 5.6. The effect of ketamine and placebo on task performance neural activity. There was no difference in accuracy (A) or reaction time (RT; B) between post-placebo (blue) and post-ketamine sessions. There was no difference in neural activity (3-back vs 1-back) in the dorsolateral prefrontal cortex (C; DLFPC) or superior parietal lobule (D; SPL) regions of interest. Error bars indicate one standard error of the mean.

5.4.2.3 fMRI data

There was a significant interaction between drug and order ($F_{(1,8)} = 5.49$, $P = 0.047$) but no significant interaction between baseline and drug ($F_{(1,8)} = 0.41$, $P = 0.54$) or a main effect of drug ($F_{(1,8)} = 0.67$, $P = 0.44$; **Figure 5.6C**) on left DLPFC activity. There was no significant interaction between drug and order ($F_{(1,8)} = 0.24$, $P = 0.64$) or a main effect of drug ($F_{(1,9)} = 0.31$, $P = 0.59$) for our SPL ROI analysis (**Figure 5.6D**). Moreover, no clusters survived cluster correction in our exploratory whole brain analyses and there were no suprathreshold voxels in the post-placebo > post-ketamine contrast (**Table 5.5**). Finally, there was no significant relationship between the antidepressant response to ketamine 48-hours post-infusion and the change in DLPFC ($r_{(11)} = 0.45$, $P = 0.17$) or SPL ($r_{(11)} = -0.21$, $P = 0.54$) ROIs or exploratory whole brain activity (**Table 5.5**).

Table 5.5. Comparison of drug effects on neural activation and the relationship with the antidepressant response 48-hours post-infusion in depressed patients only during our primary contrast of interest (3-back > 1-back). Threshold at an initial $P < 0.005$ uncorrected. An extent threshold of 5 voxels or more was also applied. Note, there were no suprathreshold voxels in the post-placebo > post-ketamine contrast and none in the positive correlation with the antidepressant response to ketamine at 48-hours post-infusion.

Region	**MNI Coordinate**			***Peak*** $t_{(9)}$**-value**	***Cluster*** P_{WB}	**Extent**
	X	Y	Z			
Post-Ketamine > Post-Placebo						
Frontal pole	-3	59	-5	5.71	0.814	54
Precuneus	15	-55	19	4.22	0.994	14
Intraparietal sulcus	-27	-58	28	3.78	0.999	6
Negative correlation with antidepressant response to ketamine at 48-hours post-infusion						
Superior peduncle	-12	-37	-38	5.96	0.967	27
Cerebellum	9	-43	-8	4.78	0.991	17
Hippocampus	-18	-43	7	4.18	0.997	11

Montreal Neurological Institute (MNI) coordinates indicate the distance (in millimeters) from the stereotaxic origin (anterior commissure), with X representing the lateral distance from the origin (positive numbers to the right), Y representing the anterior-posterior dimension (positive numbers anterior), and Z representing the dorsal-ventral dimension (positive numbers dorsal).

5.5 Discussion

The aims of this study were to assess whether treatment with the rapid-acting antidepressant ketamine causes an improvement in working memory, as measured by the n-back, in depressed patients and what neural structures are involved in its mechanism of action. Additional goals included characterising the pre-treatment differences in brain activity and n-back task performance between healthy volunteers and unmedicated, treatment-refractory depressed patients and predicting the antidepressant response to ketamine. Contrary to our predictions, we found no significant changes in working memory or brain activity from placebo to ketamine in depressed patients. Moreover and in contradiction to our predictions and numerous previous reports (Wang *et al.*, 2015), we failed to find any significant baseline differences during our case-control comparison analyses of n-back elicited behavioural performance or brain activity between depressed patients and healthy volunteers. Nevertheless, in comparison to healthy volunteers, we identified a trend toward lower activation in the SPL of depressed patients. Finally, we found no significant association between the antidepressant response to ketamine and either task performance or brain activity at either baseline or post-infusion.

In comparison to placebo, administration of ketamine was associated with a significant improvement in general depression scores 48-hours post-infusion; however, we failed to find a difference in brain activity or behaviour between the post-infusion scans. While these results might at first seem surprising, our findings may fit in the context of the results obtained from our case-control comparisons and some similar reports. Given the treatment refractory nature of the depressed patients tested here, we expected to detect robust pre-treatment between group differences in performance and fMRI measured neural activity. However, contrasting pre-treatment n-back elicited brain activity and behaviour between depressed patients and healthy volunteers revealed no significant differences, suggesting our task may have been sensitive enough to detect trait differences. Moreover and consistent with our null finding, at least one n-back fMRI study failed to detect treatment-related (fluoxetine) linear load response-effects in MDD patients, suggesting that the neural correlates of working memory abnormalities in major depression may be more trait than state-related (Walsh *et al.*, 2007).

While the lack of a significant difference in n-back behaviour may seem surprising, a number of fMRI studies have failed to identify baseline behavioural differences between medication-free depressed patients and healthy volunteers using the n-back task (Matsuo *et al.*, 2007, Walsh *et al.*, 2007), potentially suggesting that fMRI versions of the task may, or perhaps the sample sizes of fMRI studies, not be sensitive enough to detect behavioural differences. Indeed, in a meta-analysis of executive functioning in depression, the behaviourally administered n-back reliably differentiated healthy volunteers and depressed patients with a medium effect size (d = 0.63) across seven studies (Snyder, 2013). A power analysis using the same effect size (d = 0.63) at 80% power to detect a group difference using a two-tailed independent t-test and with an α level of 0.05 revealed that 41 participants would be required per group to detect an effect of this size. Further to this point, Wang and colleagues (2015) note in their working memory fMRI meta-analysis that out of the six studies which utilised the n-back, none of which had an adequate sample size to detect a between groups difference using the aforementioned power parameters, no study detected a performance difference between depressed patients and healthy volunteers, irrespective of medication status.

Wang and colleagues (2015) did however detect evidence for a consistent working memory task-elicited left DLPFC hyper-activation in depressed patients in comparison to healthy controls across 10 studies in their meta-analysis. Unexpectedly, in comparison to healthy volunteers, we failed to detect prefrontal hyperactivity in patients in our ROI analysis using the coordinates identified in the meta-analysis by Wang et al. (2015) or using an exploratory whole brain approach; worryingly, we identified no supra threshold voxels in the contrast depressed patients > controls in our case-control comparison. However, our lack of neural hyperactivity in depressed patients in comparison to healthy volunteers during the n-back is not in isolation; at least one other group has also failed to find this activation pattern during case-control comparisons with depressed patients using the n-back (Barch *et al.*, 2003). To ascertain the robustness of our methodology, we conducted analyses to explore the effects of performing the n-back task and in particular of the increasing working memory load from 1- to 3-back conditions. These analyses showed robust activation of working memory associated networks, including significantly increased activity in our left DLPFC ROI during 3-back in comparison to 1-back blocks. Taken

together, these control analyses suggest the n-back task used here was adequately designed to elicit detectable effects of difficulty in our regions of interest.

Nonetheless, we identified a trend towards a reduction in BOLD activation in the right SPL in depressed patients during the case-control comparison. Although the SPL is a brain region reliably activated by the n-back task during fMRI (Owen *et al.*, 2005) and is critical for the manipulation of information in working memory (Koenigs *et al.*, 2009), we had not anticipated that this region would differ between our groups. However, the SPL region we identified is highly proximal (within 1cm) to the right precuneus, a structure noted by Wang et al. (2015) as showing a very reliable relative hypoactivity in depressed patients during working memory tasks and ipsilateral to the region found here. Moreover, the SPL region we found here is also consistent in direction and location (including laterality) to at least one previous case-control comparison study in MDD patients (Barch *et al.*, 2003). The SPL has consistently been shown via functional neuroimaging and lesion studies to be involved in cognitive tasks that require goal directed attention (Behrmann *et al.*, 2004, Friedrich *et al.*, 1998), such as working memory (Lie *et al.*, 2006), and top-down orienting, such as set-shifting (Shomstein, 2012). One possible interpretation of the trend toward a between group difference in SPL activity seen here is that, relative to healthy volunteers, depressed patients may have had to work harder to maintain similar behavioural performance during the 1-back condition but both groups may have worked equally hard during the 3-back. Thus, the smaller neural difference between 1- and 3-back in depressed patients may reflect more similar levels of attention across task blocks while in healthy volunteers the larger increase may indicate distinct changes in attentional demands from 1- to 3-back conditions. Although the results of our follow up analyses did not confirm this relationship statistically, the main effect of task difficulty on SPL activation was greater in healthy volunteers than depressed patients and beta values between 1- and 3-back were more comparable in patients than controls (**Figure 5C),** lending some credence to this interpretation.

We failed to find an association between pre-treatment working memory performance (RT) and the magnitude of the antidepressant response to ketamine at 24-hours post-infusion. In two independent samples, Murrough and colleagues

(Murrough *et al.*, 2015, Murrough *et al.*, 2013b) found that slower processing speed in depressed patients was predictive of a greater antidepressant response 24-hours post-ketamine. A number of study differences may explain why no significant association was found here. First, Murrough and colleagues (Murrough *et al.*, 2015, Murrough *et al.*, 2013b) used the MATRICS consensus cognitive battery (MCCB), which assesses many cognitive components using distinct sub-tests, including: verbal learning, working memory, visual learning, reasoning/problem solving and processing speed. The n-back task used here may not be a valid measure of the processing speed component assessed by the MCCB. Murrough and colleagues (Murrough *et al.*, 2015, Murrough *et al.*, 2013b) found no association between the MCCB working memory component, which assesses accuracy, and the antidepressant response, a result consistent with our findings. Second, the sample sizes of the two studies by Murrough and colleagues (Murrough *et al.*, 2015, Murrough *et al.*, 2013b) (N = 62 and 25, respectively) are substantially higher than those used here, which may have hindered our ability to detect associations of the magnitude they reported (not enough information was provided in either study to calculate an effect size). Finally, the study designs also differed to ours here, with a midazolam controlled and open label single ketamine infusion design being employed by Murrough and colleagues, respectively (Murrough *et al.*, 2015, Murrough *et al.*, 2013b).

In addition to the failure to replicate an association between pre-treatment behaviour and the antidepressant response to ketamine, we also failed to find a previously found association between pre-treatment medial pregenual activation and response to ketamine in depressed patients. Using MEG, Salvadore and colleagues (2010) found a strong association between pregenual cingulate cortex-localized desynchronization in the beta band and response to ketamine at 230 minutes post-infusion in an open label designed study. However, MEG and fMRI assess distinct neural signals and it is unclear if beta desyncrhonization is comparable to the neurovascular signals detected by fMRI; beta desynchronization is thought to reflect asynchronous firing within cortical networks (Stevenson *et al.*, 2011). Our selection of the 24-hours post-infusion time point, which was based on findings that this point is frequently the time of the maximal antidepressant effect (Zarate *et al.*, 2006) and is completely free of the psychotomimetic effects of ketamine (Luckenbaugh *et al.*,

2014), may also have hindered the possibility of detecting a similar change in neural activity. Finally, pre-treatment and post-treatment activity levels in our ROI, left DLPFC, did not relate to the antidepressant response to ketamine. While a number of studies have demonstrated a change in DLPFC activity in depressed patients following treatment (Brody *et al.*, 2001, Fales *et al.*, 2009, Kennedy *et al.*, 2001), only one study has demonstrated a relationship (positive) between pre-treatment DLPFC activity levels (higher) and treatment response (greater) (Ritchey *et al.*, 2011), suggesting that pre-treatment activity levels in this brain region may be important for treatment response but might not directly relate to the magnitude of post-treatment improvements generally. Moreover, no study has detected a change in DLPFC activity levels that relate to the change in depression scores post-treatment.

A number of limitations of this study merit comment. First, due to resource limitations, the n-back task used here comprised only six blocks of each condition (1-back, 3-back and rest) and lasted six minutes in duration. The brevity of the task and number of blocks, although optimised in duration for BOLD SNR detection, may have hampered the likelihood of detecting a statistically significant result. Future studies would benefit from a greater number of blocks of a similar length. Second and related to the first limitation, the sample size for our case-control comparison was most likely not adequate to detect a medium effect size. Moreover, the sample size for our post-drug analyses was very small (N = 11 for fMRI and N = 12 behaviourally). Third, the differences in demographic variables (age, years of education, IQ) and subsequent required inclusion of these variables (except IQ due to missing data) in our statistical models as covariates may have impaired our ability to detect group differences during fMRI. These three variables may all relate to working memory performance (Alloway and Alloway, 2010, Alloway *et al.*, 2010, Cabeza *et al.*, 2004), thus removing their variance and decreasing the degrees of freedom may have hindered our ability to detect statistically significant between group differences. Fourth, failing to include IQ as a covariate may have missed important and unique variance in our models; IQ could arguably be more sensitive than education years. Fifth, the timing of each non-baseline fMRI scan was 48-hours post-infusion and therefore may have missed the peak antidepressant effects and mechanisms of action of ketamine; however, this is unlikely as the antidepressant effect size at 24- and 48-hours post-infusion was highly comparable. Sixth, as per

Chapter 4, the antidepressant efficacy of ketamine was much poorer than previous investigations (Milak *et al.*, 2015, Zarate *et al.*, 2012, Zarate *et al.*, 2006), with only 19% of patients reaching response criterion; this poorer rate of response may have negatively impacted our ability to detect pre-treatment neural and behavioural predictors of response to ketamine and changes in brain activity and behaviour post-infusion.

In summary, we found no significant brain or behaviour changes between post-placebo and post-ketamine scans in medication-free treatment-refractory depressed patients during a working memory task. Task performance and concomitant brain activity were similar between depressed patients and healthy controls at the baseline pre-treatment study phase with the exception of a trend towards significantly greater brain activity in the SPL in healthy volunteers. Behaviour and brain activity at baseline and changes in these measures were not associated with the antidepressant response to ketamine. Further research in a larger sample of depressed patients and healthy volunteers pre-treatment and post-infusion is required to better understand the cognitive correlates of the antidepressant effects of ketamine.

6 General discussion

This discussion will provide a unifying summary of the experiments presented in Chapters 2-5. Following a brief overview of the aims and results of each experimental Chapter, I will discuss how the novel treatments presented in this thesis relate to antidepressant treatment models mentioned in Chapter 1. I will discuss the implications and limitations of the current research and assess if and how the work demonstrated here advances our knowledge of the cognitive and neural mechanisms underpinnings of tDCS and ketamine as antidepressant treatments. Subsequently, I will chart a path for future studies in the field. Finally, I will conclude the thesis with a summary of the points made during the general discussion.

6.1 Summary of individual chapter investigations and findings

6.1.1 Chapter 2: Does excitatory fronto-extracerebral tDCS lead to improved working memory performance in healthy volunteers?

The aim of this study was to investigate whether the application of excitatory fronto-extracerebral tDCS would enhance performance on a working memory task, the n-back, in healthy volunteers. A number of studies have demonstrated cognitive and mood enhancing capabilities of tDCS in healthy volunteers and depressed patients (Berlim *et al.*, 2013, Clark *et al.*, 2012, Coffman *et al.*, 2012, Meron *et al.*, 2015, Oliveira *et al.*, 2013). However, the optimal stimulation parameters have yet to be explored and a number of methodological criticisms have been voiced (Walsh, 2013). In particular, questions regarding the blinding of tDCS studies, the timing and frequency of stimulation, and the contribution of both stimulation electrodes have been raised (Walsh, 2013). No study yet has employed an excitatory fronto-extracerebral electrode montage to explore the cognitive enhancing effects of tDCS across multiple sessions in a double-blind design. Thus, in Chapter 2 we conducted a double-blind, multiday, between-subjects experiment using a novel fronto-extracerebral electrode montage with the excitatory anodal electrode applied to the left DLPFC and the inhibitory electrode placed on the contralateral cheek. A fronto-extracerebral montage precludes criticisms regarding the contribution of the reference electrode to task or neural activity changes, as only one electrode is located on the scalp. Participants performed the n-back, a working memory task, at baseline,

during stimulation and post-stimulation on day 1 and during and after stimulation on day 2; 24-to-48-hours separated stimulation days 1 and 2. In comparison to sham recipients, participants receiving active stimulation showed a greater improvement in n-back accuracy from baseline to the stimulation period on day 1, only; no other significant between group differences were found. While our results do not support the use of tDCS as an overall working memory performance enhancer in healthy volunteers, our findings are consistent with the idea that tDCS may enhance the speed of learning during early stages of task performance.

6.1.2 Chapter 3: Prefrontal Cortex Glutamate, Glutamine, and Glutathione Signals Using an Adapted Echo Time Optimised PRESS Sequence: A Between- and Within-Sessions Investigation

The goal of Chapter 3 was to evaluate whether an adapted ^{1}H-MRS sequence at 7T was reliable in the measurement of glutamate and its associated metabolites within- and between-sessions in healthy volunteers. For ^{1}H-MRS to be used in the evaluation of clinical treatments and the assessment of underlying pathology, the within- and between-session reliability of each pulse sequence at specific field strengths needs to be established first in healthy volunteers. Our results revealed that within-session measurement of glutamate and glutamine was excellent on average and good for glutathione. Between-session reliability was good-to-acceptable for the measurement of glutamate, glutamine and glutathione. We also noted novel associations between age and glutathione and gender and glutathione and glutamine levels. The results of our assessment support the use of this adapted ^{1}H-MRS sequence at 7T in within- and between-session clinical evaluations of neural glutamatergic metabolite levels.

6.1.3 Chapter 4: Anhedonia, Reward Processing and Medial Prefrontal Glutamate in Major Depression: A 7T ^{1}H-MRS and NMDA Receptor Antagonist Treatment Investigation in Medication Free, Treatment Resistant, Patients

The goals of Chapter 4 were manifold. Firstly, we aimed to replicate and extend recently found improvements in anhedonia levels in MDD and BD patients following a sub-anaesthetic dose of intravenous ketamine (Lally *et al.*, 2014b, Lally *et al.*,

2015b). Specifically, our primary goals were to further understand the nature and underpinning glutamatergic mechanisms of the anti-anhedonic action of ketamine using the adapted ^{1}H-MRS sequence and voxel location (pregenual anterior cingulate cortex) utilized in Chapter 3. Our second goal cantered on replicating a previously found association between pre-treatment glutamine levels and the antidepressant response to ketamine (Salvadore *et al.*, 2012). Our final aim focused on probing the underlying pre-treatment pathophysiology of anhedonia and depression using computerized reward tasks and 7T ^{1}H-MRS. Few studies to date have examined reward processing in medication free, treatment refractory patients and no study has explored the ^{1}H-MRS metabolite profile of such patients at 7T.

As expected, in comparison to healthy volunteers, significantly greater anticipatory and consummatory anhedonia levels were found at baseline in depressed patients. Contrasting previous results, however, no pre-treatment group differences in monetary incentive motivation were found. A significant pre-treatment difference in reward learning was found with patients showing poorer learning to less reliable stimuli than healthy volunteers; however, this difference related to number of years living with depression, but not anhedonia levels. No differences in glutamatergic metabolites were found at baseline. However, baseline levels of glutamine, but not glutamate, were predictive of the antidepressant response to ketamine.

In comparison to placebo, depressed patients showed a robust improvement in anticipatory anhedonia, as measured by the SHAPS, and general depressive symptom levels following ketamine; however, this benefit was not mirrored by changes in consummatory and anticipatory anhedonia, as assessed by the TEPS. Moreover, in comparison to post-placebo, no changes in monetary incentive motivation or reward learning were apparent 24-hours post-ketamine. However, in comparison to post-placebo, a significant change in glutamine, but not glutamate, was found following ketamine but this did not relate to the anti-anhedonic response. Our results suggest that ketamine may be beneficial in targeting anticipatory anhedonia, but do not provide evidence for benefits to monetary incentive motivation, reward learning or consummatory anhedonia; however, the limited sample size limits the interpretation of these null results. Furthermore, our results

suggest that glutamine may play an important role in the mechanisms of action of ketamine, potentially indicating a role for astrocytes.

6.1.4 Chapter 5: Working Memory in Major Depression: An fMRI and NMDA Receptor Antagonist Treatment Investigation in Medication-Free, Treatment-Resistant, Depressed Patients

The primary aims of study four were to evaluate the potential for ketamine to improve cognitive impairment found in depressed patients and to understand its antidepressant mechanisms of action using fMRI; participants performed the n-back, a working memory task used in Chapter 2, at baseline and 48-hours following infusions of ketamine and placebo during fMRI. Additionally, we sought to replicate and extend previous findings by exploring whether baseline task performance (Murrough *et al.*, 2015, Murrough *et al.*, 2013b, Shiroma *et al.*, 2014) and neural activity (Salvadore *et al.*, 2010) during a working memory task were predictive of the antidepressant response to ketamine. Finally, we also conducted case-control comparisons to evaluate pre-treatment differences in behaviour and brain activity. Contrary to our expectations, in comparison to 48-hours post-placebo, a single infusion of sub-anaesthetic intravenous ketamine was not associated with an improvement in n-back performance or a change in neural activity 48-hours later. Moreover, we failed to replicate previous findings of cognitive load-associated prefrontal hyperactivity in depressed patients in comparison to healthy volunteers. However, we did find a trend towards reduced superior parietal cortex activity in depressed patients in comparison to healthy volunteers. Our results do not support the idea that ketamine is a cognitive enhancer for patients with depression.

6.2 How thesis findings relate to models of depression and its treatment

Chapter 1 discussed, amongst other ideas, three theories of standard antidepressant action: the monoamine, cellular plasticity and cognitive neuropsychological models. The conclusion of our review suggested that none of the three models on its own was sufficient to fully explain how standard antidepressants work, but all models have at

least some, albeit tentative in some cases, explanatory power and validity. Furthermore, we suggested that these three models were not mutually exclusive and may in fact all be true to some extent. We then assessed in Chapter 1 how these three models might relate to the novel antidepressant treatments explored in this thesis, namely, brain stimulation and pharmacological treatments, such as ketamine. Again, our review suggested that all three models might have some basis for explaining the efficacy of both ketamine and tDCS. Although our investigations in this thesis were not designed to directly probe these models, our results may shed some light on how tDCS and ketamine fit within the aforementioned frameworks and function as antidepressant treatments.

Our tDCS experiment in Chapter 2 was conducted in healthy volunteers only, so it is speculative to suggest how the results from this chapter may relate to the treatment of depression using this technique. Nevertheless, the significantly better working memory performance found during excitatory fronto-extracerebral tDCS, in comparison to sham stimulation, on day 1 is suggestive of a potential cognitive enhancing capacity of tDCS. Although it should be noted that the groups were not significantly different at any other time point, the results are potentially suggestive of an enhancement in early task learning rates.

The hypothesized mechanism of action of tDCS lends itself cogently to the cellular plasticity model of antidepressant action. As detailed in Chapter 2, tDCS is believed to exert its effects by up-regulating neuronal excitability beneath the anodal (excitatory) electrode, enhancing BDNF secretion (Fritsch *et al.*, 2010) and has been shown to focally increase levels of ^{1}H-MRS measured glutamatergic metabolites (Clark *et al.*, 2011, Hunter *et al.*, 2015). We stimulated the left DLFPC in our study; thus, the neurons in this region may have increased participation in the working memory task-elicited neuronal network, leading to enhanced task performance. Although the evidence pertaining to an antidepressant effect of tDCS in patients or a cognitive enhancing capability in healthy volunteers and depressed patients is mixed (Horvath *et al.*, 2015), methodological issues such as stimulation blinding and reference electrode positioning may have limited the effectiveness of tDCS to date (Horvath *et al.*, 2014). It is possible that an enhancement in neuronal plasticity in the

DLPFC may have cognitive enhancing effects in healthy volunteers and also depressed patients (Oliveira *et al.*, 2013). Moreover, enhancing DLPFC functionality or connectivity may have antidepressant efficacy itself in depressed patients.

Alternatively, enhancing cognitive functioning in depressed patients may have an indirect and beneficial effect on mood. As detailed in Chapter 1, the cognitive neuropsychological model proposes that cognitive impairment may play a causal role in the development and treatment of depression (Roiser *et al.*, 2012). In particular, impairment in cognitive functioning may prevent the deconstruction of negative schemata and the instantiation of modified behaviour via treatments such as CBT. An alternative explanation for our results is that excitatory DLPFC tDCS increased cognitive control or the propensity to ignore distracting stimuli, which may have improved task accuracy. An improvement in cognitive control may also enhance a depressed patient's ability to deconstruct negative schemata. The improvement in working memory effect shown in Chapter 2 may also speak to the monoamine hypothesis. There is a strong link between dopamine and working memory performance (Landau *et al.*, 2009, Sawaguchi and Goldman-Rakic, 1991). Interestingly, one preclinical study has investigated the effects of tDCS on monoaminergic neurotransmitters. Tanaka and colleagues (2013) found that 10 minutes of tDCS resulted in an increase in extracellular dopamine, but not serotonin, levels in the striatum for at least six-hours. Thus, one potential route underlying the cognitive enhancing effects of tDCS may be through dopaminergic neurotransmission enhancement.

As discussed in Chapter 1, the antidepressant effects of ketamine may involve, or even require, monoaminergic transmission. However, as monoamines were not measured in either Chapters 4 or 5, it would be inappropriate to speculate on the relationship between our results in these chapters and the monoaminergic model of antidepressant action. Roiser and colleagues (2012) suggested that ketamine might work as an antidepressant within the cognitive neuropsychological framework by rapidly increasing the plasticity of negative schemata; however, experimental evidence exploring such an effect is thus far lacking. Again, our results from either Chapter 4 or 5 did not directly assess negative schemata or affective biases so it is

difficult to comment on the relevance of our findings regarding the use of ketamine as an antidepressant to the cognitive neuropsychological model of antidepressant action. The rapid acting nature of the antidepressant effect of ketamine (within two-hours) may require an update to the cognitive neuropsychological model, as currently the model does not specify how schemata could possible be broken down so quickly (Pringle *et al.*, 2011, Roiser *et al.*, 2012). One possibility is that a strong dissociative experience is an important component in the rapid acting nature of the antidepressant efficacy of ketamine; unfortunately, our data cannot reflect further on this point.

The ^{1}H-MRS results from Chapter 4 may relate more coherently to the cellular plasticity model of depression and its treatment. We found two potentially important results pertaining to glutamine levels and treatment with ketamine in Chapter 4. Firstly, glutamine levels at baseline were positively correlated with the magnitude of the antidepressant response to ketamine at 24-hours post-infusion, replicating a previous result (Salvadore *et al.*, 2012). Second, medial prefrontal cortex glutamine levels were significantly lower 24-hours post-ketamine than post-placebo. Glutamine is stored in astrocytes and thus these two associations are consistent with their involvement in the antidepressant treatment action of ketamine. Higher levels of glutamine at baseline might suggest greater rates of intracellular glutamate reuptake and conversion via glutamine synthesase. Post-ketamine decreases in glutamine, but not glutamate, may also implicate the rate of glutamate-to-glutamine cycling. Both of these results might suggest that the functionality of astrocytic-related processes connected to the removal and conversion of intrasynaptic glutmatate might be important for the mechanism of action of ketamine. Interestingly, an ex-vivo ^{13}C-MRS study in rodents reported increased glutamate-to-glutamine cycling acutely in the medial prefrontal cortex post-ketamine (Chowdhury *et al.*, 2012). Importantly, glutamatergic excitotoxicity arising from dysfunctional clearance of glutamate is thought to underlie one of the hallmarks of aberrant cellular plasticity, atrophy (Duman, 2009). Interestingly, grey matter volume reductions in the medial prefrontal cortex, the location of our ^{1}H-MRS voxel, have been consistently reported (Bora *et al.*, 2012).

6.3 Implications of the current research

6.3.1 Chapter 2

The results of Chapter 2, our first experimental chapter, have a number of implications. The findings suggest that tDCS could be effective as a cognitive enhancer during early stages of learning, but may not provide noticeable benefit once a certain standard of performance has been attained. Cognitively impaired individuals, such as some depressed patients, may gain substantial benefit from techniques that increase difficult task-learning rates. Hypothetically, tDCS could be paired with CBT or MBSR treatments during early treatment stages and these combinations might then enhance the speed and potentially also the efficacy of the techniques in alleviating depressive symptoms. However, the results of our study suggest that tDCS is limited in its ability to enhance performance in healthy volunteers so there would likely also be efficacy limits in depressed patients. Whether tDCS leads to long lasting improvements once stimulation has ceased also remains an open question. Outside of psychiatric illnesses such as depression, tDCS may also be useful in the enhancement of difficult skills and early task learning stages.

A methodological implication also arose from this study. The excitatory fronto-extracerebral montage used here is atypical but circumnavigates a number of criticisms of previous studies. Typically, tDCS studies employ a dual cerebral montage with both electrodes placed on the scalp. However, as both electrodes are hypothesized to stimulate the underlying neural tissue, this leads to interpretation difficulties in terms of understanding the neural mechanisms mediating changes in brain activity or behaviour. In this study we placed the active electrode on the left DLPFC and the reference electrode on the contralateral cheek, thus removing a potential degree of freedom.

6.3.2 Chapter 3

The accurate measurement of glutamate, glutamine and glutathione using ^{1}H-MRS is important, but also methodologically challenging (Ramadan *et al.*, 2013). These

neural metabolites (glutamate, glutamine and glutathione) have been posited to play a causal role in the pathophysiology of several psychiatric and neurological conditions (Lapidus *et al.*, 2014a, Ramadan *et al.*, 2013). In particular, a strong body of research implicates the glutamatergic system in the pathophysiology and treatment of schizophrenia and depression (Javitt, 2010, Milak *et al.*, 2015, Sanacora *et al.*, 2012). The implications of Chapter 3 add to the earlier work establishing the adapted ^{1}H-MRS pulse sequence used in this chapter in healthy volunteers (An *et al.*, 2015). We demonstrate in Chapter 3 that the within- and between-session reliability of the adapted ^{1}H-MRS sequence at 7T in delineating these metabolites (glutamate, glutamine and glutathione) in the mPFC is on average good. The accurate measurement of these metabolites in a single scan should permit a finer grained classification of the disease states across numerous illnesses. However, our work in Chapter 3 establishing the between- and within-session reliability estimates for each of these metabolites should have particular implications for treatment trials.

The excellent within-session reliability demonstrated in Chapter 3, particularly for the measurement of glutamate and glutamine, should encourage the use of this pulse sequence in the context of intra-scanner treatments and understanding mechanisms of drug action, particularly for treatments that have a rapid onset. For example, as detailed in Chapters 1, 4 and 5, the NMDA receptor antagonist ketamine is known to have strong glutamatergic effects and to rapidly alleviate depressive symptomatology within two-hours. Preclinical evidence suggests that the administration of ketamine is associated with acute increases in glutamate in rodents (Moghaddam *et al.*, 1997), and healthy volunteers (Stone *et al.*, 2012) and depressed patients (Milak *et al.*, 2015) using ^{1}H-MRS at 3T. However, another report using ^{1}H-MRS at 4T suggests that the administration of ketamine is associated with an acute increase in glutamine only. At 7T, the adapted pulse sequence used in Chapters 3 and 4 accurately distinguishes between the glutamate and glutamine ^{1}H-MRS peaks. An intra-scanner ketamine treatment investigation using our sequence at 7T would permit the distinction between these two metabolites and the mechanism of action of this drug.

Although the reliability of our metabolite measurements were not as high between- as within-sessions, our pulse sequence would likely also be highly beneficial in longitudinal multi-scan treatment studies, as in Chapter 4. Glutamate, glutamine and glutathione may serve as biomarkers of disease states and thus accurate between-session measurements of these metabolites may be highly beneficial for understanding the underlying state-dependent changes from response, remission and relapse.

6.3.3 Chapter 4

The results from Chapter 4 have a number of implications, which can be split into the effects of ketamine, the case-control comparison and our brain (^{1}H-MRS metabolites) and behaviour (psychometrics and reward tasks) measures. The case-control comparison revealed a number of anticipated and surprising results. As expected, there were large differences in pre-treatment scores on the anhedonia and general depression psychometric scales. Interestingly however, pre-treatment scores on the TEPS, which splits anhedonia into anticipatory and consummatory components, differed within subjects for depressed patients but not healthy volunteers; depressed patients self-reported significantly greater anticipatory than consummatory anhedonia levels. This result underscores several reports suggesting that anticipatory anhedonia may make a greater contribution to depressive symptoms than consummatory processes (Dichter *et al.*, 2010, Sherdell *et al.*, 2012). The presence of this trend in medication-free treatment refractory patients suggests that it may potentially be a consistent pattern across depressed patients and might inform treatment strategies. Hypothetically, treatments sensitive to improving levels of anticipatory anhedonia may be more important for depressed patients that those targeting subject hedonic experience.

Following the ketamine infusion, there was a significant improvement in general depression and anhedonia scores, as measured by the MADRS and SHAPS, respectively, replicating previous effects (Lally *et al.*, 2014b, Lally *et al.*, 2015b, Zarate *et al.*, 2012, Zarate *et al.*, 2006). Surprisingly, we found no change in TEPS scores post-ketamine, even on the anticipatory sub-component of the TEPS. One

potential interpretation of this null result is that the TEPS is not sensitive to state effects occurring over a short time-scale. We failed to replicate an effect in our mixed (MDD and BD combined) patient sample of an improvement in anhedonia levels post-ketamine once the improvement in depression scores had been accounted in medicated medication-refractory BD patients. One possible interpretation of this null result is that mood stabilizers enhanced the effect of ketamine in the previous study (Lally *et al.*, 2014b); recent evidence has found that lithium, one of the medications used in the previous study, has an enhancing effect on response to ketamine in rodents (Chiu *et al.*, 2015). Another possibility is that the independent anti-anhedonic effect of ketamine is specific to BD patients, whom were in the minority in this investigation. Additionally, the sample size of the current study was approximately half of the previous study (Lally *et al.*, 2014b) so a statistical power may partially explain the null results.

We found no significant pre-treatment performance differences between depressed patients and controls on the EEfRT, a task which examines monetary decisional motivation, failing to replicate a previous effect (Treadway *et al.*, 2012). Considering the treatment-refractory nature and medication free status of the depressed patients tested in this chapter, our results are somewhat surprising; we anticipated substantial levels of amotivation in our depressed patients. Contrastingly, depressed patients in this chapter self-reported a significant level of anhedonia on the SHAPS and TEPS, both of which assess anticipatory anhedonia, a component of motivation. Potential sample differences between the current sample and the previous report showing a significant group difference on the EEfRT between patients with MDD and healthy volunteers (Treadway *et al.*, 2012). In the previous report (Treadway *et al.*, 2012) MDD patients were predominantly medicated (85% or 17/20; either SSRIs or SNRIs) and were excluded from participation if they met criterion for past substance abuse or dependence or any reported use of prescription drugs that acted primarily on the dopaminergic system; past abuse and dependence was permitted in our sample. Given the medication free nature of the patient sample here (89% or 23/26), one interpretation of this null result is that levels of EEfRT-elicited motivation are not aberrant in medication-free depression. Consistent with this interpretation, there were no significant EEfRT performance differences in

depressed patients between placebo and ketamine 24-hours post-infusion; however, the sample size of the post-infusion analysis was small (N = 15). One interpretation for the paradox of self-reported anhedonia yet no behavioural manifestation of anhedonia here may be that anhedonia in depressed patients is more specific to non-monetary decisions, such as social anhedonia (Xie *et al.*, 2014). Interestingly, Sherdell and colleagues (2012) found decreased motivation levels towards expending energy to gain access to humorous stimuli in MDD patients; however, almost 40% of patients were medicated in their study. Further work is needed to understand the relationship between anhedonia, standard antidepressant medication and behaviour.

We found a significant pre-treatment group difference on the stimulus-learning phase of the Scene Choose task; depressed patients were less accurate in learning stimulus associations for stimuli where feedback was less consistent (80% probability) than most consistent (90% probability), while no difference was detected in healthy volunteers. Additionally, depressed patients performed worse than healthy volunteers on the transfer phase of the task during comparisons of frequent winning and frequent losing stimuli. Surprisingly, these between-subjects performance differences were not related to levels of anhedonia levels in depressed patients, suggesting that anhedonia levels were not relevant to task differences. Once more, our results contrast a previous report using a similar version of this task which found no stimulus type by group interaction on stimulus-learning or transfer phase performance levels between medicated (87% or 20/23) MDD patients and healthy volunteers (Chase *et al.*, 2010). Interestingly, Chase and colleagues (2010) noted a significant correlation between anhedonia levels and trial-by-trial learning, with greater anhedonia associated with poorer learning; we did not model learning rate here, however. Again, no significant 24-hour post-infusion performance differences between placebo and ketamine were apparent in depressed patients (N = 15) on the Scene Choose task.

The similar levels of task-related monetary motivation between depressed patients and healthy volunteers and the lack of a relationship between performance differences and anhedonia on the reward-learning task is surprising. Moreover, the changes in anhedonia levels following ketamine were not mirrored by performance

alterations on either task. One interpretation of our task results from this chapter is that neither task measures components of anhedonia that are pertinent to anhedonia in depression. Research on schizophrenia, where anhedonia is a prominent symptom, may also offer an important alternative perspective. Strauss and Gold (2012) document a paradox whereby current and non-current anhedonia-related feelings in patients with schizophrenia are strongly dissonant, with current feelings mirroring healthy volunteers while patients report significantly increased anhedonia retrospectively; they suggest that cognitive impairments and negative schemas may underlie this bias. Interestingly, MDD patients consistently report fewer positive autobiographical memories reported than healthy volunteers (Young *et al.*, 2014). A similar bias may exist in depression, whereby patients report experiencing greater levels of retrospective anhedonia but function at similar levels to healthy volunteers on tasks that assess motivational capacity.

Contrary to our expectations, we found no significant baseline group differences in levels of ^{1}H-MRS measured glutamate or glutamine from the medial pregenual cingulate cortex. A number of ^{1}H-MRS studies have found evidence for reduced prefrontal cortex glutamate levels in MDD patients in comparison to healthy volunteers (Luykx *et al.*, 2012, Yuksel and Ongur, 2010); however, many null results have also been reported (Price *et al.*, 2009c, Walter *et al.*, 2009). As our study was the first to measure neural ^{1}H-MRS metabolites at 7T in depressed patients, we anticipated that significant pre-treatment group differences would be apparent given the increased spectral resolution and the medication-free and treatment resistant nature of our sample; we also hypothesized that glutamine differences may also be apparent.

A number of differences between our study and previous studies may have contributed to the pre-treatment glutamatergic difference null result here, including: medication-free and treatment refractory patient sample, mixed (MDD and BD) patient sample and the difference in ^{1}H-MRS pulse sequence and field strength. However and consistent with our result, Price and colleagues (2009c) compared medication free treatment resistant MDD patients, medication-free non-treatment resistant MDD patients and healthy volunteers using 3T ^{1}H-MRS and found no

differences in medial prefrontal cortex levels of Glx, a proxy for glutamate and glutamine levels combined. As ^{1}H-MRS measures total tissue metabolite levels indiscriminately, it is possible that certain pulse sequences may differ in sensitivity to specific cellular regions. For example, one sequence may be more sensitive to synaptic glutamate or extracellular glutamate levels (Lally *et al.*, 2014a).

Nevertheless, baseline glutamine levels in depressed patients were positively associated with the magnitude of the antidepressant and anti-anhedonic response to ketamine, suggesting that individual differences in pre-treatment levels of glutamine might alter the likelihood of a beneficial antidepressant and anti-anhedonic effect to ketamine. As glutamine is predominantly found in astrocytic glial cells of the brain (Ramadan *et al.*, 2013), this association suggests these cells in particular may play a key role in the beneficial effects of ketamine. Consistent with the proposed importance of glial cells in MDD, post-mortem analyses have repeatedly found reduced astrocyte density in MDD (Cotter *et al.*, 2001, Rajkowska, 2000, Rajkowska *et al.*, 1999). Greater glutamine levels may reflect a higher density of astrocytes in our mPFC region of interest. Alternatively, higher levels of ^{1}H-MRS measured glutamine may indicate greater conversion of glutamate to glutamine, implicating the glial enzyme glutamine synthetase, or enhanced glutamate-to-astrocyte reuptake from the synaptic cleft, indicating excitatory amino acid transporters (EAAT) one and two (Walter *et al.*, 2009). Interestingly, a down-regulation of both EAAT1, EAAT2 and glutamine synthetase has been found in post-mortem analyses in the prefrontal cortex of MDD patients (Choudary *et al.*, 2005). Our results may suggest that greater baseline reuptake of glutamate from the synaptic cleft or conversion of glutamate to glutamine in the pregenual cingulate cortex may enhance the antidepressant and anti-anhedonic effects of ketamine.

Further implicating glutamine levels and astrocytes in the mechanism of action of ketamine, in comparison to placebo, administration of ketamine was associated with a significant decrease in levels of ^{1}H-MRS measured glutamine, but no change in glutamate levels, 24-hours post-infusion. Decreases in glutamine post-ketamine may reflect decreased synaptic cleft glutamate reuptake or astrocytic conversion from glutamate to glutamine. The lack of change in medial pregenual

anterior cingulate cortex glutamate contrasts the acute changes in glutamate seen post-ketamine in preclinical and clinical intra-scanner 3T ^{1}H-MRS studies (Milak *et al.*, 2015, Stone *et al.*, 2012). Given the tight physiological link between glutamate and glutamine levels, one might anticipate changes in glutamate to accompany changes in glutamine. However, ^{1}H-MRS measures total tissue glutamate and may not be sensitive to differing balances of vesicular and extra-synaptic glutamate levels; alternatively, the acute glutamate altering effects of ketamine may occur only during the infusion and thus our scan time, 24-hours post-infusion, may have missed these effects. Consistent with this latter hypothesis, Valentine and colleagues (2011) found no changes in occipital glutamate or glutamine ^{1}H-MRS levels measured at 3T 3- and 48-hours post-ketamine. We found no association between the changes in glutamine levels and the antidepressant or anti-anhedonic response to ketamine, suggesting the relationship may be non-linear and complex if there is an association at all; Milak and colleagues (2015) also found no relationship between the antidepressant response to ketamine and the acute changes in glutamate levels. Taken together, our ^{1}H-MRS results suggest that glutamine and astrocytic processes may be important targets for understanding the mechanisms of action of ketamine but further work is needed to clarify these observations.

6.3.4 Chapter 5

There are a number of implications of Chapter 5. In contrast to previous reports suggesting a cognitive enhancing effect of ketamine (DeWilde *et al.*, 2015, Murrough *et al.*, 2015, Shiroma *et al.*, 2014), we found no evidence for a working memory enhancement post-ketamine. However and surprisingly, depressed patients did not exhibit a decrease in accuracy or RT during pre-treatment performance of the working memory task, the n-back. We also found no association between baseline performance and the antidepressant response to ketamine, again contrasting previous reports (Murrough *et al.*, 2015, Murrough *et al.*, 2013b). Taken together, our results do not support the idea that ketamine may have cognitive enhancing capabilities for depressed patients. However, the lack of a pre-treatment difference between depressed patients and healthy volunteers also raises the possibility that the task might not have been sensitive enough to detect subtle changes in post-infusion

performance. Moreover, the results of our power analyses confirmed that the study was substantially underpowered to detect a baseline group difference. Thus, it is difficult to draw any firm conclusions about the working memory enhancing capacity of ketamine in depression.

The results of our fMRI analyses follow a similar story to our behavioural findings. In comparison to post-placebo, we found no significant differences in brain activation post-ketamine, either at the whole brain level or in our ROI. We failed to find the anticipated prefrontal hyper-activation in depressed patients relative to healthy volunteers as baseline, one of the most robust findings in fMRI studies using the n-back (Wang *et al.*, 2015). Nonetheless, we did detect a trend toward between group pre-treatment difference in parietal cortex activation, with reduced activation in depressed patients, relative to healthy volunteers; however, this region was not sensitive to the effect of ketamine in depressed patients post-infusion.

The time of our MRI scan, 48-hours post-infusion, may however have hindered the detection of group differences as the fMRI detectable neural effects of ketamine may occur more proximally to the infusion. Although we demonstrated robust findings of task-elicited effects across all subjects (N = 38), our failure to detect expected group differences (N= 18 healthy volunteers) at baseline and post-infusion differences (N = 11) might have occurred because our study was not adequately powered to detect subtle fMRI changes. Total scanner time was limited to six minutes, which may not have been enough fMRI data to detect robust differences in such a small sample size. Consistent with this suggestion, we failed to detect a previously found association between baseline n-back elicited brain-activity, using MEG, and the antidepressant response to ketamine (Salvadore *et al.*, 2010). Firm conclusions are difficult to draw from Chapter 5 due to the short amount of scanner time and small sample size used in this study. The results do however suggest the effects of ketamine on working-memory task-elicited brain activation and behaviour may be subtle if present at all, implying larger and longer studies may be important to detect group differences.

6.4 Limitations of studies within this thesis

While the studies mentioned above suggest a potential role for tDCS in modifying cognition in healthy volunteers and ketamine for altering neural metabolite and improving mood, but not cognition, in depressed patients, there are a number of limitations that should be first borne in mind. Importantly, these limitations may strongly influence our ability to draw firm conclusions from the data presented here. We will first discuss the general limitations of the thesis, discussing drawbacks common to more than one chapter and thereafter examine specific limitations within each individual chapter.

Sample sizes throughout the experiments within this thesis were generally small. In particular, the sample sizes for the between-subjects tDCS experiment in Chapter 2, with a mere 10 subjects in one group and 11 in the other, were particularly small. Furthermore, the within-subjects post-infusion comparisons between placebo and ketamine on behaviour and brain imaging variables in Chapters 4 and 5 were also small, with a minimum of 11 subjects and a maximum of 15 for these experiments. Drawing firm conclusions from such small sample sizes is not possible, thus our results require careful extension and replication, especially for the null results. Moreover, the issue of a small sample size may have also have compounded the influence of other limitations.

Another important limitation of the experiments presented in Chapters 4 and 5 is the inclusion of a mixed depressive sample, which included MDD, BD I and BD II patients. Due to the small number of available patients it was decided to combine samples. BD and MDD are distinct psychiatric illnesses with purportedly unique biological profiles (Taylor, 2014) and medication recommendations (Ghaemi *et al.*, 2004), but nonetheless, which share a common ground. While all depressed patients tested during pre-treatment assessments were currently in a major depressive episode, several BD patients (N = 3) in Chapter 4, but none in Chapter 5, were medicated on a mood stabilizer at treatment dosage levels that was ineffective at alleviating the depressive episode. The potential interactions between ketamine and mood stabilizers are unknown; no studies have compared the effects of ketamine on

mood in BD patients on and off a mood stabilizer. Moreover, it is unknown if mood stabilizers influence reward processing, let alone how ketamine and mood stabilizer in combination affect anhedonia.

The inclusion of BD patients in our pre-treatment ^{1}H-MRS analyses may have had a particularly detrimental effect on our ability to detect a between groups glutamate difference between depressed patients and healthy volunteers as BD patients are thought to present with higher glutamate levels than MDD patients and healthy volunteers (Gigante *et al.*, 2012, Taylor, 2014, Yuksel and Ongur, 2010). Moreover, ^{1}H-MRS measured glutamate has even been suggested as a method to differentiate MDD and BD patients (Taylor, 2014) suggesting that combining these two depressive samples may be a major limitation of this section of Chapter 4. No studies have directly compared MDD and BD patients using the fMRI n-back task as in Chapter 5, thus the implications of the mixed sample in this chapter are unknown.

Another potential limitation of the tDCS experiment in Chapter 1 and the ketamine infusion experiments in Chapters 4 and 5 is the issue of condition blinding. Although double-blind methodologies were employed throughout the thesis where possible, the validity of the double-blind claim was not assessed empirically; serious concerns about blinding for both tDCS (Horvath *et al.*, 2014) and ketamine (Murrough *et al.*, 2013a) experiments have been raised. At least one study has attempted to use an active placebo (midazolam) in ketamine depression study (Murrough *et al.*, 2013a). However, as the focus of our experiments here was to understand the mechanisms of action of ketamine, introducing an active placebo agent such as midazolam might have rendered our results difficult to interpret.

Chapters 3 and 4 had one primary limitation in common relating to ^{1}H-MRS acquisition; as spectra in these Chapters were only acquired from one brain region, it is not possible to generalize our results to the rest of the brain. Again, due to time constraints, it was not possible to acquire spectra from other parts of the brain in either study.

6.4.1 Chapter 2

Walsh (2013) documents a series of important measures an ideal tDCS experiment should contain in order to make robust claims about the effects of tDCS, including: a control region, a single scalp electrode, a control task, assessed double blinding, repeated stimulation sessions and real world task validity. While we included a number of these suggestions in our study, we failed to incorporate a control region or a control task in our study, primarily due to time restrictions. However, without these extra measures it is difficult to extrapolate the findings of our experiment; it is possible that effects of stimulating another brain region may have elicited similar enhancement effects on day one. Moreover, it is also important to probe the specificity of improvements in tDCS as it is possible that general attentional enhancement may underlie any task improvements. For example, at least one study has demonstrated that DLPFC tDCS concurrently enhanced performance on one task but also impaired performance on another (Iuculano and Cohen Kadosh, 2013), suggesting that the benefit of tDCS may come at a cost. Additionally, it would have been useful if we had included a second baseline session on day 2 prior to tDCS to examine any post-stimulation effects and allow for greater sensitivity to inter-day variation.

6.4.2 Chapter 3

There are still several limitations associated with the use of ^{1}H-MRS that are important to bear in mind in the context of our results. ^{1}H-MRS measures total tissue metabolite volume so it is unknown what cellular mechanisms underlie a single peak. For example, the glutamate signal may arise predominantly from vesicular glutamate in neurons, extra-synaptic glutamate or astrocytic glutamate or some mix of all three. Some reports suggest that different cellular metabolite locations may provide more ^{1}H-MRS measured signal than others (Kauppinen *et al.*, 1994, Lally *et al.*, 2014a).

6.4.3 Chapter 4

There were a number of limitations of the experiments conducted in Chapter 4. First, aside from the ^{1}H-MRS paradigm (assessed in Chapter 3), the reliability of a number

of our measures has not been assessed for repeated administration; it is unknown if the TEPS, EEfRT or Scene Choose task are suitable for a multiple session or several week long longitudinal study. As there was no association between anhedonia levels, as measured by the SHAPS, and task performance on either tasks, inclusion of other tasks that better related to anhedonia levels reflected by the SHAPS may have offered greater sensitivity to detecting an effect of ketamine on behaviour. Moreover, the inclusion of monetary-based incentive tasks only may have missed possible changes in other forms of hedonic functioning, such as social anhedonia.

6.4.4 Chapter 5

Several potential limitations of Chapter 5 should be borne in mind. Firstly, the duration of the scan was very short, lasting only 6 minutes. Secondly, the timing of the scan, 48-hours post-infusion, may have missed many of the neural changes associated with the infusion; the optimal time to detect neural differences post-ketamine in unknown. Thirdly, the large differences in demographic information between the depressed patients and healthy volunteers may have removed important variance in our covariate analyses. Moreover, we did not have IQ measurements for all participants so could not control for between group differences in this variable.

Additionally, we did not explore the question of the reliability of our n-back fMRI task. However, there are at least two reliability studies that have examined both the repeatability of the n-back behaviour and task-elicited neural network using fMRI. Blokland and colleagues (2011) scanned 40 individuals twice, three months apart during n-back performance using a variant of the version employed in Chapter 5. They reported a highly consistent pattern of behaviour and brain activity during the n-back, with intraclass correlation coefficients between 0.7 and 0.9 for both task-elicited neural-activity and behaviour. A significant improvement in accuracy during session two was also observed. Plichta and colleagues (2012) found a similar pattern of reliability results.

6.5 Directions for future research

Here we will discuss several potential avenues for future research. As previously, we will discuss suggested directions for thesis-related research more generally first, discussing topics that are common to at least two chapters, and thereafter, specific ideas for the extension of experiments in each individual chapter.

Despite the recent suggestions that psychiatry move towards cross diagnostic experiments (Insel *et al.*, 2010, Insel, 2014), the examination of differences between healthy volunteers and psychiatric populations still has much merit and is particularly important for the development of new treatments. Future studies including adequate samples sizes of patients with MDD and BD and healthy volunteers would permit the examination of a number of important variables. There are very few experiments that have examined pre-treatment variables across depressive disorders. For example, there are no known studies that have directly compared ^{1}H-MRS measured glutamatergic metabolites in MDD and BD patients (Taylor, 2014); there is also an absence of ^{1}H-MRS studies comparing subtypes of MDD and BD, such as BDI and BDII. While inclusion of a mixed sample in Chapter 4 and 5 may serve as a limitation here, expansion of the medication free BD sample in particular would allow examination of a number of components thought to differentiate these conditions, including reward processing (Redlich *et al.*, 2015, Whitton *et al.*, 2015) and glutamatergic metabolites (Taylor, 2014).

Inclusion of medication-free BD patients may also be important for understanding the mechanisms of action of ketamine and the exploration of drug interactions. Studies examining the use of ketamine as a treatment for BD suggest that lithium is superior to valproate in the anti-anhedonic effect of ketamine (Lally *et al.*, 2014b) and also in the likelihood of achieving response criterion and study completion (Diazgranados *et al.*, 2010a, Zarate *et al.*, 2012). No studies yet have compared the effect of ketamine in medication free BD patients and medicated BD patients; however, preclinical evidence suggests lithium may enhance the antidepressant effects of ketamine (Chiu *et al.*, 2015). Prolonging the antidepressant effects of ketamine is a topic of strong interest; several clinical studies have explored

the effects of post-ketamine interventions such as the administration of ECT (Abdallah *et al.*, 2012, Jarventausta *et al.*, 2013) and purported glutamatergic antidepressant medication, riluzole (Ibrahim *et al.*, 2012, Mathew *et al.*, 2010), all of which found no enhancement in treatment-refractory MDD patients. A recent preclinical study, however, found that more classical antidepressant medications (fluoxetine and imipramine) administered post-ketamine promoted the antidepressant effect in rats over the use of either ketamine or the classics alone (Melo *et al.*, 2015). Future studies should examine whether ketamine may serve as a priming adjuvant for the treatment of depression in MDD and BD patients, potentially preceding CBT or MBSR, as well as standard antidepressant medication. One distinct possibility is that the time to response would shorten and the time to relapse might lengthen substantially in a ketamine priming study.

A common critique of placebo-controlled ketamine depression treatment studies is the condition blinding. Due to the strong dissociative properties of the medication (Luckenbaugh *et al.*, 2014), blinding is realistically never even single blind in purportedly double-blind investigations. At least one study has attempted to use midazolam as an active placebo to get around this critique (Murrough *et al.*, 2013a); however, the symptom profile difference between midazolam and ketamine is large and informed patients could potentially differentiate between medications (Murrough *et al.*, 2013a); again, in this study blinding was not assessed. A potential alternative to the use of an active placebo is the route of administration. Due to issues of bioavailability, nearly all ketamine depression treatment studies have followed an intravenous administrative protocol. However, other routes such as intranasal (Lapidus *et al.*, 2014b), intramuscular (Chilukuri *et al.*, 2014), subcutaneous (Galvez *et al.*, 2014), and potentially even oral, are possible. Importantly, treatment with intranasal ketamine was associated with a minimal side-effect profile with minimal psychotomimetic effects (Lapidus *et al.*, 2014b), suggesting that blinding may be less of an issue via this route of administration.

6.5.1 Chapter 2

tDCS has received a lot of attention in recent years both as a purported cognitive enhancing technique and a treatment for a plethora of clinical conditions. Unfortunately, the majority of research conducted to date has failed to control for a number of potential issues (Walsh, 2013), which confound the interpretation and extrapolation of many results. These lack of controls may have contributed to recent reports suggesting that tDCS is entirely ineffective as a cognitive enhancer in healthy volunteers (Horvath *et al.*, 2015). Future research should seek to include the necessary experimental controls, including: appropriate and assessed double-blinding, a single stimulation electrode, control stimulation sites, and control tasks, amongst others (Walsh, 2013). If our findings of an early enhancement in working memory performance hold true and are specific to this process and DLPFC stimulation, trialling our protocol in clinical populations such as MDD patients, would be interesting. In particular, tDCS may be appropriate in combination with treatments such as CBT, where cognitive impairment is thought to have a detrimental effect on depression treatment prognosis (Roiser *et al.*, 2012). Given the relative inexpensiveness of the device and the ease of use, future explorations of its cognitive and mood enhancing capabilities are warranted. Finally, intra-scanner tDCS and 7T ^{1}H-MRS investigations, as in Chapters 3 and 4, may help to further understand the neurobiological mechanisms underpinning this exciting form of brain stimulation.

6.5.2 Chapter 3

Reliability studies are vital, particularly for techniques used to measure treatment-related changes. If reliability coefficients are known for a technique or methodology, study designs can factor in the noise estimates and plan investigations accordingly. It would be fruitful to include multiple single voxel brain regions, aside from the pregenual cingulate cortex; estimates from other brain regions shown to be sensitive to treatment effects in depression, such as the occipital cortex (Abdallah *et al.*, 2014b), dorsomedial prefrontal cortex (Lally *et al.*, 2014b, Lally *et al.*, 2015b) and DLPFC (Brody *et al.*, 2001, Fales *et al.*, 2009, Kennedy *et al.*, 2001) would be particularly useful. Additionally, a comparison of different pulse sequences (e.g.

PRESS vs. SPECIAL vs. STEAM) and field strengths (3T vs. 7T) would be extremely beneficial as there is little inter- and intra-scanner comparison research in the ^{1}H-MRS field. Any claims of a particular sequence bettering another should be accompanied by specific comparisons to standard sequences and a between-sessions reliability analysis.

6.5.3 Chapter 4

A reliability analysis of the TEPS and our reward tasks is needed as it is unknown if these measures are reliable enough to be used in a longitudinal design. Given our null results using reward-processing tasks in this chapter, a large-scale assessment of pre-treatment reward processing and anhedonia in medication-free MDD and BD patients is warranted. In particular, a comparison of different forms of anhedonia (e.g. social, monetary, and physical) and the characterization of this profile in depression is needed. These ideas may relate to the all-inclusive clinical definition of anhedonia (comprising anticipatory and consummatory components), which may have hindered research in the area (Treadway and Zald, 2011). Our results suggest that anticipatory anhedonia may be significantly more clinically significant in our sample that consummatory processes, a finding consistent with cognitive task-based evidence (Treadway and Zald, 2011). Appropriate characterization of the precise components of anhedonia and which components are most aberrant in depression is an important point that needs to be addressed.

There are two main limitations to our ^{1}H-MRS investigations reported in Chapter 4, which could be addressed by future studies. Firstly, intra-scanner infusions of ketamine at 7T would be ideal to measure the acute changes occurring in the glutamatergic system and would allow a finer grained assessment of alterations in levels of both glutamate and glutamine and their roles in the treatment of depression. While the non-acute evaluation of changes post-ketamine provides valuable information, the direct mechanisms of action are most likely to be determined at the time of the infusion. Secondly, ^{1}H-MRS does not typically allow the characterization of cycling. However, recent reports suggest that ^{13}C MRS combined with an infusion of [2- ^{13}C] glucose may permit profiling of the glutamate-to-glutamine cycling in the

human cortex (Li *et al.*, 2015a, Shen, 2013). The combination of this latter technique with an intra-scanner infusion of ketamine in depressed patients at 7T offers the best chance of understanding, *in-vivo*, the underpinning antidepressant mechanisms of action of ketamine.

Additionally, considering the positive correlation between pre-treatment levels of glutamine and the anti-anhedonic and antidepressant effects of ketamine, one possibility might be to manipulate levels of glutamine prior to a ketamine infusion in depressed patients via a pharmaceutical lead-in. Interestingly, Brennan and colleagues (2010) found that administration of riluzole for only two days led to an increase in 1.5T ^{1}H-MRS measured glutamine-to-glutamate ratio in BD patients. Although tentative due to the low field strength, their results suggest an alteration in glutamine levels may occur following oral riluzole. This result contrasts our finding in Chapter 4 of reduced glutamine post-ketamine but may explain why riluzole was not shown to be effective as an acute adjunctive treatment to ketamine in MDD patients (Diazgranados *et al.*, 2010a); if ketamine works by decreasing glutamine levels, riluzole may potentially work to counteract these effects. However, a short riluzole lead-in may offer an ideal opportunity to increase glutamine levels and enhance the antidepressant effects of ketamine.

6.5.4 Chapter 5

Future studies exploring the cognitive enhancing capacity of ketamine may benefit from a longer and more sensitive version of the n-back such as the version used in Chapter 2; alternatively, other measures of cognitive functioning or working memory more specifically may be more sensitive than the n-back as it assesses many cognitive components and is not a pure measure of working memory (Miller *et al.*, 2009). Future uses of the n-back might also benefit by reducing the maximum complexity level to the 2-back; anecdotally, a number of participants, particularly depressed patients found the task extremely intimidating.

6.6 Conclusion

In conclusion, this thesis attempted to further understand the neural and cognitive underpinnings of two novel antidepressant treatments, tDCS and ketamine. We found some evidence for a cognitive enhancing capacity of tDCS in healthy volunteers, with specific benefits occurring during the first stimulation session, only. Although we replicated previous reports of a rapid improvement in general depression symptoms and anhedonia post-ketamine, we failed to detect any specific novel components of anhedonia or cognition improved by ketamine. We also failed to replicate an association between baseline working memory task-elicited neural activity and the antidepressant response to ketamine. However, we found an association between pre-treatment ^{1}H-MRS measured glutamine levels and the anti-anhedonic and antidepressant effects of ketamine, replicating a previous finding. We also found a reduction in glutamine levels post-ketamine, but this did not relate to changes in depression or anhedonia.

Altogether, experiments reported in this thesis offer tentative signs of a number of important results. First, there appears to be some basis for the cognitive enhancing capacity of tDCS. Second, ketamine appears to consistently improve reported, but not objective, depressive and anhedonic, but not cognitive, symptomatology. Finally, as tDCS and ketamine are thought to both have glutamatergic effects, our ^{1}H-MRS results are consistent with a glutamatergic or cellular plasticity theory of antidepressant treatment, potentially mediated by astrocytic activity. In sum, tDCS and ketamine are important potential antidepressant treatments, particularly for treatment resistant depressed patients. Further work using 7T ^{1}H-MRS, a technique shown to be reliable, may help to clarify the underpinning neural architecture of depression and its treatment with tDCS and ketamine.

7 References

Abdallah, C. G., Fasula, M., Kelmendi, B., Sanacora, G. & Ostroff, R. (2012). Rapid antidepressant effect of ketamine in the electroconvulsive therapy setting. *J ECT* **28**, 157-61.

Abdallah, C. G., Jiang, L., De Feyter, H. M., Fasula, M., Krystal, J. H., Rothman, D. L., Mason, G. F. & Sanacora, G. (2014a). Glutamate metabolism in major depressive disorder. *Am J Psychiatry* **171**, 1320-7.

Abdallah, C. G., Niciu, M. J., Fenton, L. R., Fasula, M. K., Jiang, L., Black, A., Rothman, D. L., Mason, G. F. & Sanacora, G. (2014b). Decreased occipital cortical glutamate levels in response to successful cognitive-behavioral therapy and pharmacotherapy for major depressive disorder. *Psychother Psychosom* **83**, 298-307.

Alloway, T. P. & Alloway, R. G. (2010). Investigating the predictive roles of working memory and IQ in academic attainment. *J Exp Child Psychol* **106**, 20-9.

Alloway, T. P., Gathercole, S. E. & Elliott, J. (2010). Examining the link between working memory behaviour and academic attainment in children with ADHD. *Dev Med Child Neurol* **52**, 632-6.

American Psychiatric, A. & American Psychiatric Association, D. S. M. T. F. (2013). *Diagnostic and statistical manual of mental disorders : DSM-5*. American Psychiatric Association: Arlington, Va. ; London.

An, L., Li, S., Murdoch, J. B., Araneta, M. F., Johnson, C. & Shen, J. (2015). Detection of glutamate, glutamine, and glutathione by radiofrequency suppression and echo time optimization at 7 tesla. *Magn Reson Med* **73**, 451-8.

An, L., Li, S., Wood, E. T., Reich, D. S. & Shen, J. (2014). N-acetyl-aspartyl-glutamate detection in the human brain at 7 Tesla by echo time optimization and improved Wiener filtering. *Magn Reson Med* **72**, 903-12.

An, L., Willem van der Veen, J., Li, S., Thomasson, D. M. & Shen, J. (2013). Combination of multichannel single-voxel MRS signals using generalized least squares. *J Magn Reson Imaging* **37**, 1445-50.

Anacker, C. (2014). Adult hippocampal neurogenesis in depression: behavioral implications and regulation by the stress system. *Curr Top Behav Neurosci* **18**, 25-43.

Anacker, C., Zunszain, P. A., Cattaneo, A., Carvalho, L. A., Garabedian, M. J., Thuret, S., Price, J. & Pariante, C. M. (2011). Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Mol Psychiatry* **16**, 738-50.

Anand, A., Charney, D. S., Oren, D. A., Berman, R. M., Hu, X. S., Cappiello, A. & Krystal, J. H. (2000). Attenuation of the neuropsychiatric effects of ketamine with lamotrigine: support for hyperglutamatergic effects of N-methyl-D-aspartate receptor antagonists. *Arch Gen Psychiatry* **57**, 270-6.

Andrews, S. C., Hoy, K. E., Enticott, P. G., Daskalakis, Z. J. & Fitzgerald, P. B. (2011). Improving working memory: the effect of combining cognitive activity and anodal transcranial direct current stimulation to the left dorsolateral prefrontal cortex. *Brain Stimul* **4**, 84-9.

Asberg, M., Thoren, P., Traskman, L., Bertilsson, L. & Ringberger, V. (1976). "Serotonin depression"--a biochemical subgroup within the affective disorders? *Science* **191**, 478-80.

Austin, M. P., Mitchell, P. & Goodwin, G. M. (2001). Cognitive deficits in depression: possible implications for functional neuropathology. *Br J Psychiatry* **178**, 200-6.

Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. F., Cheng, P. F., Kavalali, E. T. & Monteggia, L. M. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* **475**, 91-5.

Baardseth, T. P., Goldberg, S. B., Pace, B. T., Wislocki, A. P., Frost, N. D., Siddiqui, J. R., Lindemann, A. M., Kivlighan, D. M., 3rd, Laska, K. M., Del Re, A. C., Minami, T. & Wampold, B. E. (2013). Cognitive-behavioral therapy versus other therapies: redux. *Clin Psychol Rev* **33**, 395-405.

Ballard, E. D., Ionescu, D. F., Vande Voort, J. L., Niciu, M. J., Richards, E. M., Luckenbaugh, D. A., Brutsche, N. E., Ameli, R., Furey, M. L. & Zarate, C. A., Jr. (2014). Improvement in suicidal ideation after ketamine infusion: relationship to reductions in depression and anxiety. *J Psychiatr Res* **58**, 161-6.

Banasr, M., Dwyer, J. M. & Duman, R. S. (2011). Cell atrophy and loss in depression: reversal by antidepressant treatment. *Curr Opin Cell Biol* **23**, 730-7.

Barch, D. M., Sheline, Y. I., Csernansky, J. G. & Snyder, A. Z. (2003). Working memory and prefrontal cortex dysfunction: specificity to schizophrenia compared with major depression. *Biol Psychiatry* **53**, 376-84.

Barker, A. T., Jalinous, R. & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet* **1**, 1106-7.

Bechtholt-Gompf, A. J., Walther, H. V., Adams, M. A., Carlezon, W. A., Jr., Ongur, D. & Cohen, B. M. (2010). Blockade of astrocytic glutamate uptake in rats induces signs of anhedonia and impaired spatial memory. *Neuropsychopharmacology* **35**, 2049-59.

Beck, A. T. (1967). *Depression, clinical, experimental and theoretical aspects*. Harper & Row, Hoeber Medical Division: New York.

Beck, A. T. (1976). *Cognitive therapy and emotional disorders*. Penguin: London.

Behrmann, M., Geng, J. J. & Shomstein, S. (2004). Parietal cortex and attention. *Curr Opin Neurobiol* **14**, 212-7.

Bekinschtein, P., Cammarota, M., Igaz, L. M., Bevilaqua, L. R., Izquierdo, I. & Medina, J. H. (2007). Persistence of long-term memory storage requires a late protein synthesis- and BDNF- dependent phase in the hippocampus. *Neuron* **53**, 261-77.

Belujon, P. & Grace, A. A. (2014). Restoring mood balance in depression: ketamine reverses deficit in dopamine-dependent synaptic plasticity. *Biol Psychiatry* **76**, 927-36.

Berlim, M. T., Van den Eynde, F. & Daskalakis, Z. J. (2013). Clinical utility of transcranial direct current stimulation (tDCS) for treating major depression: a systematic review and meta-analysis of randomized, double-blind and sham-controlled trials. *J Psychiatr Res* **47**, 1-7.

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S. & Krystal, J. H. (2000). Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* **47**, 351-4.

Berryhill, M. E. & Jones, K. T. (2012). tDCS selectively improves working memory in older adults with more education. *Neurosci Lett* **521**, 148-51.

Birtchnell, J. (1970). The relationship between attempted suicide, depression and parent death. *Br J Psychiatry* **116**, 307-13.

Bland, J. M. & Altman, D. G. (1986). Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**, 307-10.
Blier, P., Ward, H. E., Tremblay, P., Laberge, L., Hebert, C. & Bergeron, R. (2010). Combination of antidepressant medications from treatment initiation for major depressive disorder: a double-blind randomized study. *Am J Psychiatry* **167**, 281-8.
Blokland, G. A., McMahon, K. L., Thompson, P. M., Martin, N. G., de Zubicaray, G. I. & Wright, M. J. (2011). Heritability of working memory brain activation. *J Neurosci* **31**, 10882-90.
Boggio, P. S., Zaghi, S. & Fregni, F. (2009). Modulation of emotions associated with images of human pain using anodal transcranial direct current stimulation (tDCS). *Neuropsychologia* **47**, 212-7.
Boldrini, M., Santiago, A. N., Hen, R., Dwork, A. J., Rosoklija, G. B., Tamir, H., Arango, V. & John Mann, J. (2013). Hippocampal granule neuron number and dentate gyrus volume in antidepressant-treated and untreated major depression. *Neuropsychopharmacology* **38**, 1068-77.
Bora, E., Fornito, A., Pantelis, C. & Yucel, M. (2012). Gray matter abnormalities in Major Depressive Disorder: a meta-analysis of voxel based morphometry studies. *J Affect Disord* **138**, 9-18.
Boyer, P., Tassin, J. P., Falissart, B. & Troy, S. (2000). Sequential improvement of anxiety, depression and anhedonia with sertraline treatment in patients with major depression. *J Clin Pharm Ther* **25**, 363-71.
Brainard, D. H. (1997). The Psychophysics Toolbox. *Spat Vis* **10**, 433-6.
Brandt, D. J., Sommer, J., Krach, S., Bedenbender, J., Kircher, T., Paulus, F. M. & Jansen, A. (2013). Test-Retest Reliability of fMRI Brain Activity during Memory Encoding. *Front Psychiatry* **4**, 163.
Bremner, J. D., Vythilingam, M., Ng, C. K., Vermetten, E., Nazeer, A., Oren, D. A., Berman, R. M. & Charney, D. S. (2003). Regional brain metabolic correlates of alpha-methylparatyrosine-induced depressive symptoms: implications for the neural circuitry of depression. *JAMA* **289**, 3125-34.
Brennan, B. P., Hudson, J. I., Jensen, J. E., McCarthy, J., Roberts, J. L., Prescot, A. P., Cohen, B. M., Pope, H. G., Jr., Renshaw, P. F. & Ongur, D. (2010). Rapid enhancement of glutamatergic neurotransmission in bipolar depression following treatment with riluzole. *Neuropsychopharmacology* **35**, 834-46.
Brett, M., Johnsrude, I. S. & Owen, A. M. (2002). The problem of functional localization in the human brain. *Nat Rev Neurosci* **3**, 243-9.
Brody, A. L., Saxena, S., Stoessel, P., Gillies, L. A., Fairbanks, L. A., Alborzian, S., Phelps, M. E., Huang, S. C., Wu, H. M., Ho, M. L., Ho, M. K., Au, S. C., Maidment, K. & Baxter, L. R., Jr. (2001). Regional brain metabolic changes in patients with major depression treated with either paroxetine or interpersonal therapy: preliminary findings. *Arch Gen Psychiatry* **58**, 631-40.
Browning, M., Behrens, T. E., Jocham, G., O'Reilly, J. X. & Bishop, S. J. (2015). Anxious individuals have difficulty learning the causal statistics of aversive environments. *Nat Neurosci* **18**, 590-6.
Brunoni, A. R., Boggio, P. S., De Raedt, R., Bensenor, I. M., Lotufo, P. A., Namur, V., Valiengo, L. C. & Vanderhasselt, M. A. (2014). Cognitive control therapy and transcranial direct current stimulation for depression: a randomized, double-blinded, controlled trial. *J Affect Disord* **162**, 43-9.

Brunoni, A. R., Valiengo, L., Baccaro, A., Zanao, T. A., de Oliveira, J. F., Goulart, A., Boggio, P. S., Lotufo, P. A., Bensenor, I. M. & Fregni, F. (2013a). The Sertraline vs Electrical Current Therapy for Treating Depression Clinical Study: Results From a Factorial, Randomized, Controlled Trial. *JAMA Psychiatry* **70**, 383-91.

Brunoni, A. R., Valiengo, L., Baccaro, A., Zanao, T. A., de Oliveira, J. F., Goulart, A., Boggio, P. S., Lotufo, P. A., Bensenor, I. M. & Fregni, F. (2013b). The Sertraline vs Electrical Current Therapy for Treating Depression Clinical Study: Results From a Factorial, Randomized, Controlled Trial. *JAMA Psychiatry*, 1-9.

Bueno, V. F., Brunoni, A. R., Boggio, P. S., Bensenor, I. M. & Fregni, F. (2011). Mood and cognitive effects of transcranial direct current stimulation in post-stroke depression. *Neurocase* **17**, 318-22.

Cabeza, R., Daselaar, S. M., Dolcos, F., Prince, S. E., Budde, M. & Nyberg, L. (2004). Task-independent and task-specific age effects on brain activity during working memory, visual attention and episodic retrieval. *Cereb Cortex* **14**, 364-75.

Cai, K., Nanga, R. P., Lamprou, L., Schinstine, C., Elliott, M., Hariharan, H., Reddy, R. & Epperson, C. N. (2012). The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T (1)H-MRS study. *Neuropsychopharmacology* **37**, 2764-71.

Cannon, D. M., Ichise, M., Rollis, D., Klaver, J. M., Gandhi, S. K., Charney, D. S., Manji, H. K. & Drevets, W. C. (2007). Elevated serotonin transporter binding in major depressive disorder assessed using positron emission tomography and [11C]DASB; comparison with bipolar disorder. *Biol Psychiatry* **62**, 870-7.

Cavassila, S., Deval, S., Huegen, C., van Ormondt, D. & Graveron-Demilly, D. (2000). Cramer-Rao bound expressions for parametric estimation of overlapping peaks: influence of prior knowledge. *J Magn Reson* **143**, 311-20.

Chase, H. W., Frank, M. J., Michael, A., Bullmore, E. T., Sahakian, B. J. & Robbins, T. W. (2010). Approach and avoidance learning in patients with major depression and healthy controls: relation to anhedonia. *Psychol Med* **40**, 433-40.

Chilukuri, H., Reddy, N. P., Pathapati, R. M., Manu, A. N., Jollu, S. & Shaik, A. B. (2014). Acute antidepressant effects of intramuscular versus intravenous ketamine. *Indian J Psychol Med* **36**, 71-6.

Chiu, C. T., Scheuing, L., Liu, G., Liao, H. M., Linares, G. R., Lin, D. & Chuang, D. M. (2015). The mood stabilizer lithium potentiates the antidepressant-like effects and ameliorates oxidative stress induced by acute ketamine in a mouse model of stress. *Int J Neuropsychopharmacol* **18**.

Choudary, P. V., Molnar, M., Evans, S. J., Tomita, H., Li, J. Z., Vawter, M. P., Myers, R. M., Bunney, W. E., Jr., Akil, H., Watson, S. J. & Jones, E. G. (2005). Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proc Natl Acad Sci U S A* **102**, 15653-8.

Chowdhury, G. M., Behar, K. L., Cho, W., Thomas, M. A., Rothman, D. L. & Sanacora, G. (2012). (1)H-[(1)(3)C]-nuclear magnetic resonance spectroscopy measures of ketamine's effect on amino acid neurotransmitter metabolism. *Biol Psychiatry* **71**, 1022-5.

Christopher, G. & MacDonald, J. (2005). The impact of clinical depression on working memory. *Cogn Neuropsychiatry* **10**, 379-99.

Cipriani, A., Furukawa, T. A., Salanti, G., Geddes, J. R., Higgins, J. P., Churchill, R., Watanabe, N., Nakagawa, A., Omori, I. M., McGuire, H., Tansella, M. & Barbui, C. (2009). Comparative efficacy and acceptability of 12

new-generation antidepressants: a multiple-treatments meta-analysis. *Lancet* **373**, 746-58.

Clark, V. P., Coffman, B. A., Mayer, A. R., Weisend, M. P., Lane, T. D., Calhoun, V. D., Raybourn, E. M., Garcia, C. M. & Wassermann, E. M. (2012). TDCS guided using fMRI significantly accelerates learning to identify concealed objects. *Neuroimage* **59**, 117-28.

Clark, V. P., Coffman, B. A., Trumbo, M. C. & Gasparovic, C. (2011). Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: a (1)H magnetic resonance spectroscopy study. *Neurosci Lett* **500**, 67-71.

Coffman, B. A., Trumbo, M. C., Flores, R. A., Garcia, C. M., van der Merwe, A. J., Wassermann, E. M., Weisend, M. P. & Clark, V. P. (2012). Impact of tDCS on performance and learning of target detection: interaction with stimulus characteristics and experimental design. *Neuropsychologia* **50**, 1594-602.

Cohen Kadosh, R., Soskic, S., Iuculano, T., Kanai, R. & Walsh, V. (2010). Modulating neuronal activity produces specific and long-lasting changes in numerical competence. *Curr Biol* **20**, 2016-20.

Coppen, A. (1967). The biochemistry of affective disorders. *Br J Psychiatry* **113**, 1237-64.

Corlett, P. R., Honey, G. D. & Fletcher, P. C. (2007). From prediction error to psychosis: ketamine as a pharmacological model of delusions. *J Psychopharmacol* **21**, 238-52.

Cotter, D., Mackay, D., Landau, S., Kerwin, R. & Everall, I. (2001). Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. *Arch Gen Psychiatry* **58**, 545-53.

Cowen, P. J. (2008). Serotonin and depression: pathophysiological mechanism or marketing myth? *Trends Pharmacol Sci* **29**, 433-6.

Cowen, P. J. & Browning, M. (2015). What has serotonin to do with depression? *World Psychiatry* **14**, 158-60.

Cuijpers, P., Hollon, S. D., van Straten, A., Bockting, C., Berking, M. & Andersson, G. (2013). Does cognitive behaviour therapy have an enduring effect that is superior to keeping patients on continuation pharmacotherapy? A meta-analysis. *BMJ Open* **3**.

Dakwar, E., Anerella, C., Hart, C. L., Levin, F. R., Mathew, S. J. & Nunes, E. V. (2014a). Therapeutic infusions of ketamine: do the psychoactive effects matter? *Drug Alcohol Depend* **136**, 153-7.

Dakwar, E., Levin, F., Foltin, R. W., Nunes, E. V. & Hart, C. L. (2014b). The effects of subanesthetic ketamine infusions on motivation to quit and cue-induced craving in cocaine-dependent research volunteers. *Biol Psychiatry* **76**, 40-6.

Delgado, P. L. (2000). Depression: the case for a monoamine deficiency. *J Clin Psychiatry* **61 Suppl 6**, 7-11.

Der-Avakian, A. & Markou, A. (2012). The neurobiology of anhedonia and other reward-related deficits. *Trends Neurosci* **35**, 68-77.

DeRubeis, R. J., Hollon, S. D., Amsterdam, J. D., Shelton, R. C., Young, P. R., Salomon, R. M., O'Reardon, J. P., Lovett, M. L., Gladis, M. M., Brown, L. L. & Gallop, R. (2005). Cognitive therapy vs medications in the treatment of moderate to severe depression. *Arch Gen Psychiatry* **62**, 409-16.

DeRubeis, R. J., Siegle, G. J. & Hollon, S. D. (2008). Cognitive therapy versus medication for depression: treatment outcomes and neural mechanisms. *Nat Rev Neurosci* **9**, 788-96.
Devanand, D. P., Dwork, A. J., Hutchinson, E. R., Bolwig, T. G. & Sackeim, H. A. (1994). Does ECT alter brain structure? *Am J Psychiatry* **151**, 957-70.
DeWilde, K. E., Levitch, C. F., Murrough, J. W., Mathew, S. J. & Iosifescu, D. V. (2015). The promise of ketamine for treatment-resistant depression: current evidence and future directions. *Ann N Y Acad Sci* **1345**, 47-58.
Diazgranados, N., Ibrahim, L., Brutsche, N. E., Newberg, A., Kronstein, P., Khalife, S., Kammerer, W. A., Quezado, Z., Luckenbaugh, D. A., Salvadore, G., Machado-Vieira, R., Manji, H. K. & Zarate, C. A., Jr. (2010a). A randomized add-on trial of an N-methyl-D-aspartate antagonist in treatment-resistant bipolar depression. *Arch Gen Psychiatry* **67**, 793-802.
DiazGranados, N., Ibrahim, L. A., Brutsche, N. E., Ameli, R., Henter, I. D., Luckenbaugh, D. A., Machado-Vieira, R. & Zarate, C. A., Jr. (2010b). Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder. *J Clin Psychiatry* **71**, 1605-11.
Dichter, G. S., Smoski, M. J., Kampov-Polevoy, A. B., Gallop, R. & Garbutt, J. C. (2010). Unipolar depression does not moderate responses to the Sweet Taste Test. *Depress Anxiety* **27**, 859-63.
Dierckx, B., Heijnen, W. T., van den Broek, W. W. & Birkenhager, T. K. (2012). Efficacy of electroconvulsive therapy in bipolar versus unipolar major depression: a meta-analysis. *Bipolar Disord* **14**, 146-50.
Dockery, C. A., Hueckel-Weng, R., Birbaumer, N. & Plewnia, C. (2009). Enhancement of planning ability by transcranial direct current stimulation. *J Neurosci* **29**, 7271-7.
Donahue, R. J., Muschamp, J. W., Russo, S. J., Nestler, E. J. & Carlezon, W. A., Jr. (2014). Effects of striatal DeltaFosB overexpression and ketamine on social defeat stress-induced anhedonia in mice. *Biol Psychiatry* **76**, 550-8.
Downar, J., Geraci, J., Salomons, T. V., Dunlop, K., Wheeler, S., McAndrews, M. P., Bakker, N., Blumberger, D. M., Daskalakis, Z. J., Kennedy, S. H., Flint, A. J. & Giacobbe, P. (2014). Anhedonia and reward-circuit connectivity distinguish nonresponders from responders to dorsomedial prefrontal repetitive transcranial magnetic stimulation in major depression. *Biol Psychiatry* **76**, 176-85.
Dranovsky, A. & Hen, R. (2006). Hippocampal neurogenesis: regulation by stress and antidepressants. *Biol Psychiatry* **59**, 1136-43.
Drevets, W. C., Price, J. L. & Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct* **213**, 93-118.
Duman, R. S. (2009). Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. *Dialogues Clin Neurosci* **11**, 239-55.
Duman, R. S. & Aghajanian, G. K. (2012). Synaptic dysfunction in depression: potential therapeutic targets. *Science* **338**, 68-72.
Duman, R. S. & Li, N. (2012). A neurotrophic hypothesis of depression: role of synaptogenesis in the actions of NMDA receptor antagonists. *Philos Trans R Soc Lond B Biol Sci* **367**, 2475-84.

Duman, R. S., Malberg, J. & Thome, J. (1999). Neural plasticity to stress and antidepressant treatment. *Biol Psychiatry* **46**, 1181-91.
Dunkin, J. J., Leuchter, A. F., Cook, I. A., Kasl-Godley, J. E., Abrams, M. & Rosenberg-Thompson, S. (2000). Executive dysfunction predicts nonresponse to fluoxetine in major depression. *J Affect Disord* **60**, 13-23.
Dutta, A., McKie, S. & Deakin, J. F. (2015). Ketamine and other potential glutamate antidepressants. *Psychiatry Res* **225**, 1-13.
Ellison-Wright, I. & Bullmore, E. (2010). Anatomy of bipolar disorder and schizophrenia: a meta-analysis. *Schizophr Res* **117**, 1-12.
Erden-Inal, M., Sunal, E. & Kanbak, G. (2002). Age-related changes in the glutathione redox system. *Cell Biochem Funct* **20**, 61-6.
Eshel, N. & Roiser, J. P. (2010). Reward and punishment processing in depression. *Biol Psychiatry* **68**, 118-24.
Fales, C. L., Barch, D. M., Rundle, M. M., Mintun, M. A., Mathews, J., Snyder, A. Z. & Sheline, Y. I. (2009). Antidepressant treatment normalizes hypoactivity in dorsolateral prefrontal cortex during emotional interference processing in major depression. *J Affect Disord* **112**, 206-11.
Fava, M. (2003). Diagnosis and definition of treatment-resistant depression. *Biol Psychiatry* **53**, 649-59.
Fava, M., Graves, L. M., Benazzi, F., Scalia, M. J., Iosifescu, D. V., Alpert, J. E. & Papakostas, G. I. (2006). A cross-sectional study of the prevalence of cognitive and physical symptoms during long-term antidepressant treatment. *J Clin Psychiatry* **67**, 1754-9.
Fawcett, J., Scheftner, W. A., Fogg, L., Clark, D. C., Young, M. A., Hedeker, D. & Gibbons, R. (1990). Time-related predictors of suicide in major affective disorder. *Am J Psychiatry* **147**, 1189-94.
Fink, M. (1990). The 1990 APA Task Force Report: a quiet revolution. *Convuls Ther* **6**, 75-8.
First, M. B., Spitzer, R. L., Gibbon M., & Williams, J. B. (2002). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition. (SCID-I/P).* Biometrics Research: New York, New York State Psychiatric Institute.
Fountoulakis, K. N. & Moller, H. J. (2011). Efficacy of antidepressants: a re-analysis and re-interpretation of the Kirsch data. *Int J Neuropsychopharmacol* **14**, 405-12.
Fournier, J. C., DeRubeis, R. J., Hollon, S. D., Dimidjian, S., Amsterdam, J. D., Shelton, R. C. & Fawcett, J. (2010). Antidepressant drug effects and depression severity: a patient-level meta-analysis. *JAMA* **303**, 47-53.
Fregni, F., Boggio, P. S., Nitsche, M., Bermpohl, F., Antal, A., Feredoes, E., Marcolin, M. A., Rigonatti, S. P., Silva, M. T., Paulus, W. & Pascual-Leone, A. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Exp Brain Res* **166**, 23-30.
Friedrich, F. J., Egly, R., Rafal, R. D. & Beck, D. (1998). Spatial attention deficits in humans: a comparison of superior parietal and temporal-parietal junction lesions. *Neuropsychology* **12**, 193-207.
Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G. & Lu, B. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* **66**, 198-204.

Frye, M. A., Helleman, G., McElroy, S. L., Altshuler, L. L., Black, D. O., Keck, P. E., Jr., Nolen, W. A., Kupka, R., Leverich, G. S., Grunze, H., Mintz, J., Post, R. M. & Suppes, T. (2009). Correlates of treatment-emergent mania associated with antidepressant treatment in bipolar depression. *Am J Psychiatry* **166**, 164-72.

Frye, M. A., Watzl, J., Banakar, S., O'Neill, J., Mintz, J., Davanzo, P., Fischer, J., Chirichigno, J. W., Ventura, J., Elman, S., Tsuang, J., Walot, I. & Thomas, M. A. (2007). Increased anterior cingulate/medial prefrontal cortical glutamate and creatine in bipolar depression. *Neuropsychopharmacology* **32**, 2490-9.

Galvez, V., O'Keefe, E., Cotiga, L., Leyden, J., Harper, S., Glue, P., Mitchell, P. B., Somogyi, A. A., DeLory, A. & Loo, C. K. (2014). Long-lasting effects of a single subcutaneous dose of ketamine for treating melancholic depression: a case report. *Biol Psychiatry* **76**, e1-2.

Garcia, L. S., Comim, C. M., Valvassori, S. S., Reus, G. Z., Stertz, L., Kapczinski, F., Gavioli, E. C. & Quevedo, J. (2009). Ketamine treatment reverses behavioral and physiological alterations induced by chronic mild stress in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **33**, 450-5.

Gard, D. E., Gard, M. G., Kring, A. M. & Oliver, P. J. (2006). Anticipatory and consummatory components of the experience of pleasure: A scale development study. *Journal of Research in Personality* **40**, 1086-1102.

George, M. S., Lisanby, S. H., Avery, D., McDonald, W. M., Durkalski, V., Pavlicova, M., Anderson, B., Nahas, Z., Bulow, P., Zarkowski, P., Holtzheimer, P. E., 3rd, Schwartz, T. & Sackeim, H. A. (2010). Daily left prefrontal transcranial magnetic stimulation therapy for major depressive disorder: a sham-controlled randomized trial. *Arch Gen Psychiatry* **67**, 507-16.

George, M. S., Wassermann, E. M., Williams, W. A., Callahan, A., Ketter, T. A., Basser, P., Hallett, M. & Post, R. M. (1995). Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. *Neuroreport* **6**, 1853-6.

Ghaemi, S. N., Rosenquist, K. J., Ko, J. Y., Baldassano, C. F., Kontos, N. J. & Baldessarini, R. J. (2004). Antidepressant treatment in bipolar versus unipolar depression. *Am J Psychiatry* **161**, 163-5.

Gibbons, R. D., Hur, K., Brown, C. H., Davis, J. M. & Mann, J. J. (2012). Benefits from antidepressants: synthesis of 6-week patient-level outcomes from double-blind placebo-controlled randomized trials of fluoxetine and venlafaxine. *Arch Gen Psychiatry* **69**, 572-9.

Gigante, A. D., Bond, D. J., Lafer, B., Lam, R. W., Young, L. T. & Yatham, L. N. (2012). Brain glutamate levels measured by magnetic resonance spectroscopy in patients with bipolar disorder: a meta-analysis. *Bipolar Disord* **14**, 478-87.

Gigliucci, V., O'Dowd, G., Casey, S., Egan, D., Gibney, S. & Harkin, A. (2013). Ketamine elicits sustained antidepressant-like activity via a serotonin-dependent mechanism. *Psychopharmacology (Berl)* **228**, 157-66.

Godlewska, B. R., Near, J. & Cowen, P. J. (2015). Neurochemistry of major depression: a study using magnetic resonance spectroscopy. *Psychopharmacology (Berl)* **232**, 501-7.

Goldapple, K., Segal, Z., Garson, C., Lau, M., Bieling, P., Kennedy, S. & Mayberg, H. (2004). Modulation of cortical-limbic pathways in major depression: treatment-specific effects of cognitive behavior therapy. *Arch Gen Psychiatry* **61**, 34-41.

Gotlib, I. H. & Joormann, J. (2010). Cognition and depression: current status and future directions. *Annu Rev Clin Psychol* **6**, 285-312.

Govindaraju, V., Young, K. & Maudsley, A. A. (2000). Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed* **13**, 129-53.
Gregorian, R. S., Golden, K. A., Bahce, A., Goodman, C., Kwong, W. J. & Khan, Z. M. (2002). Antidepressant-induced sexual dysfunction. *Ann Pharmacother* **36**, 1577-89.
Grieve, S. M., Korgaonkar, M. S., Koslow, S. H., Gordon, E. & Williams, L. M. (2013). Widespread reductions in gray matter volume in depression. *Neuroimage Clin* **3**, 332-9.
Group, U. E. R. (2003). Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis. *Lancet* **361**, 799-808.
Gruetter, R. (1993). Automatic, localized in vivo adjustment of all first- and second-order shim coils. *Magn Reson Med* **29**, 804-11.
Haatveit, B. C., Sundet, K., Hugdahl, K., Ueland, T., Melle, I. & Andreassen, O. A. (2010). The validity of d prime as a working memory index: results from the "Bergen n-back task". *J Clin Exp Neuropsychol* **32**, 871-80.
Haile, C. N., Murrough, J. W., Iosifescu, D. V., Chang, L. C., Al Jurdi, R. K., Foulkes, A., Iqbal, S., Mahoney, J. J., 3rd, De La Garza, R., 2nd, Charney, D. S., Newton, T. F. & Mathew, S. J. (2014). Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression. *Int J Neuropsychopharmacol* **17**, 331-6.
Hamilton, M. (1960). A rating scale for depression. *J Neurol Neurosurg Psychiatry* **23**, 56-62.
Hanson, N. D., Owens, M. J. & Nemeroff, C. B. (2011). Depression, antidepressants, and neurogenesis: a critical reappraisal. *Neuropsychopharmacology* **36**, 2589-602.
Harmer, C. J. (2008). Serotonin and emotional processing: does it help explain antidepressant drug action? *Neuropharmacology* **55**, 1023-8.
Harmer, C. J., Bhagwagar, Z., Perrett, D. I., Vollm, B. A., Cowen, P. J. & Goodwin, G. M. (2003a). Acute SSRI administration affects the processing of social cues in healthy volunteers. *Neuropsychopharmacology* **28**, 148-52.
Harmer, C. J., Goodwin, G. M. & Cowen, P. J. (2009a). Why do antidepressants take so long to work? A cognitive neuropsychological model of antidepressant drug action. *Br J Psychiatry* **195**, 102-8.
Harmer, C. J., Hill, S. A., Taylor, M. J., Cowen, P. J. & Goodwin, G. M. (2003b). Toward a neuropsychological theory of antidepressant drug action: increase in positive emotional bias after potentiation of norepinephrine activity. *Am J Psychiatry* **160**, 990-2.
Harmer, C. J., O'Sullivan, U., Favaron, E., Massey-Chase, R., Ayres, R., Reinecke, A., Goodwin, G. M. & Cowen, P. J. (2009b). Effect of acute antidepressant administration on negative affective bias in depressed patients. *Am J Psychiatry* **166**, 1178-84.
Harvey, P. O., Fossati, P., Pochon, J. B., Levy, R., Lebastard, G., Lehericy, S., Allilaire, J. F. & Dubois, B. (2005). Cognitive control and brain resources in major depression: an fMRI study using the n-back task. *Neuroimage* **26**, 860-9.
Hasler, G., Drevets, W. C., Manji, H. K. & Charney, D. S. (2004). Discovering endophenotypes for major depression. *Neuropsychopharmacology* **29**, 1765-81.
Hasler, G., van der Veen, J. W., Tumonis, T., Meyers, N., Shen, J. & Drevets, W. C. (2007). Reduced prefrontal glutamate/glutamine and gamma-aminobutyric

acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry* **64**, 193-200.
Hawton, K., Bergen, H., Simkin, S., Cooper, J., Waters, K., Gunnell, D. & Kapur, N. (2010). Toxicity of antidepressants: rates of suicide relative to prescribing and non-fatal overdose. *Br J Psychiatry* **196**, 354-8.
Hecht, D. (2010). Depression and the hyperactive right-hemisphere. *Neurosci Res* **68**, 77-87.
Hecht, D., Walsh, V. & Lavidor, M. (2010). Transcranial direct current stimulation facilitates decision making in a probabilistic guessing task. *J Neurosci* **30**, 4241-5.
Hidaka, B. H. (2012). Depression as a disease of modernity: explanations for increasing prevalence. *J Affect Disord* **140**, 205-14.
Hindmarch, I. (1998). The behavioural toxicity of antidepressants: effects on cognition and sexual function. *Int Clin Psychopharmacol* **13 Suppl 6**, S5-8.
Hines, D. J., Schmitt, L. I., Hines, R. M., Moss, S. J. & Haydon, P. G. (2013). Antidepressant effects of sleep deprivation require astrocyte-dependent adenosine mediated signaling. *Transl Psychiatry* **3**, e212.
Hollon, S. D., DeRubeis, R. J., Fawcett, J., Amsterdam, J. D., Shelton, R. C., Zajecka, J., Young, P. R. & Gallop, R. (2014). Effect of cognitive therapy with antidepressant medications vs antidepressants alone on the rate of recovery in major depressive disorder: a randomized clinical trial. *JAMA Psychiatry* **71**, 1157-64.
Hollon, S. D., DeRubeis, R. J., Shelton, R. C., Amsterdam, J. D., Salomon, R. M., O'Reardon, J. P., Lovett, M. L., Young, P. R., Haman, K. L., Freeman, B. B. & Gallop, R. (2005). Prevention of relapse following cognitive therapy vs medications in moderate to severe depression. *Arch Gen Psychiatry* **62**, 417-22.
Hollon, S. D. & Ponniah, K. (2010). A review of empirically supported psychological therapies for mood disorders in adults. *Depress Anxiety* **27**, 891-932.
Homan, P., Neumeister, A., Nugent, A. C., Charney, D. S., Drevets, W. C. & Hasler, G. (2015). Serotonin versus catecholamine deficiency: behavioral and neural effects of experimental depletion in remitted depression. *Transl Psychiatry* **5**, e532.
Homayoun, H. & Moghaddam, B. (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J Neurosci* **27**, 11496-500.
Horn, D. I., Yu, C., Steiner, J., Buchmann, J., Kaufmann, J., Osoba, A., Eckert, U., Zierhut, K. C., Schiltz, K., He, H., Biswal, B., Bogerts, B. & Walter, M. (2010). Glutamatergic and resting-state functional connectivity correlates of severity in major depression - the role of pregenual anterior cingulate cortex and anterior insula. *Front Syst Neurosci* **4**.
Horvath, J. C., Carter, O. & Forte, J. D. (2014). Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). *Front Syst Neurosci* **8**, 2.
Horvath, J. C., Forte, J. D. & Carter, O. (2015). Quantitative Review Finds No Evidence of Cognitive Effects in Healthy Populations From Single-session Transcranial Direct Current Stimulation (tDCS). *Brain Stimul* **8**, 535-50.
Hoy, K. E., Emonson, M. R., Arnold, S. L., Thomson, R. H., Daskalakis, Z. J. & Fitzgerald, P. B. (2013). Testing the limits: Investigating the effect of tDCS dose on working memory enhancement in healthy controls. *Neuropsychologia* **51**, 1777-84.
Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P. & Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron* **45**, 201-6.

Hunter, M. A., Coffman, B. A., Gasparovic, C., Calhoun, V. D., Trumbo, M. C. & Clark, V. P. (2015). Baseline effects of transcranial direct current stimulation on glutamatergic neurotransmission and large-scale network connectivity. *Brain Res* **1594**, 92-107.

Husain, M. M., Rush, A. J., Fink, M., Knapp, R., Petrides, G., Rummans, T., Biggs, M. M., O'Connor, K., Rasmussen, K., Litle, M., Zhao, W., Bernstein, H. J., Smith, G., Mueller, M., McClintock, S. M., Bailine, S. H. & Kellner, C. H. (2004). Speed of response and remission in major depressive disorder with acute electroconvulsive therapy (ECT): a Consortium for Research in ECT (CORE) report. *J Clin Psychiatry* **65**, 485-91.

Ibrahim, L., Diazgranados, N., Franco-Chaves, J., Brutsche, N., Henter, I. D., Kronstein, P., Moaddel, R., Wainer, I., Luckenbaugh, D. A., Manji, H. K. & Zarate, C. A., Jr. (2012). Course of improvement in depressive symptoms to a single intravenous infusion of ketamine vs add-on riluzole: results from a 4-week, double-blind, placebo-controlled study. *Neuropsychopharmacology* **37**, 1526-33.

Ibrahim, L., Diazgranados, N., Luckenbaugh, D. A., Machado-Vieira, R., Baumann, J., Mallinger, A. G. & Zarate, C. A., Jr. (2011). Rapid decrease in depressive symptoms with an N-methyl-d-aspartate antagonist in ECT-resistant major depression. *Prog Neuropsychopharmacol Biol Psychiatry* **35**, 1155-9.

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., Sanislow, C. & Wang, P. (2010). Research domain criteria (RDoC): toward a new classification framework for research on mental disorders. *Am J Psychiatry* **167**, 748-51.

Insel, T. R. (2014). The NIMH Research Domain Criteria (RDoC) Project: precision medicine for psychiatry. *Am J Psychiatry* **171**, 395-7.

Inskip, H. M., Harris, E. C. & Barraclough, B. (1998). Lifetime risk of suicide for affective disorder, alcoholism and schizophrenia. *Br J Psychiatry* **172**, 35-7.

Iuculano, T. & Cohen Kadosh, R. (2013). The mental cost of cognitive enhancement. *J Neurosci* **33**, 4482-6.

Jarventausta, K., Chrapek, W., Kampman, O., Tuohimaa, K., Bjorkqvist, M., Hakkinen, H., Yli-Hankala, A. & Leinonen, E. (2013). Effects of S-ketamine as an anesthetic adjuvant to propofol on treatment response to electroconvulsive therapy in treatment-resistant depression: a randomized pilot study. *J ECT* **29**, 158-61.

Javitt, D. C. (2010). Glutamatergic theories of schizophrenia. *Isr J Psychiatry Relat Sci* **47**, 4-16.

Jayatissa, M. N., Henningsen, K., West, M. J. & Wiborg, O. (2009). Decreased cell proliferation in the dentate gyrus does not associate with development of anhedonic-like symptoms in rats. *Brain Res* **1290**, 133-41.

John, C. S., Smith, K. L., Van't Veer, A., Gompf, H. S., Carlezon, W. A., Jr., Cohen, B. M., Ongur, D. & Bechtholt-Gompf, A. J. (2012). Blockade of astrocytic glutamate uptake in the prefrontal cortex induces anhedonia. *Neuropsychopharmacology* **37**, 2467-75.

Joormann, J., Levens, S. M. & Gotlib, I. H. (2011). Sticky thoughts: depression and rumination are associated with difficulties manipulating emotional material in working memory. *Psychol Sci* **22**, 979-83.

Joshi, S. H., Espinoza, R. T., Pirnia, T., Shi, J., Wang, Y., Ayers, B., Leaver, A., Woods, R. P. & Narr, K. L. (2015). Structural Plasticity of the Hippocampus and Amygdala Induced by Electroconvulsive Therapy in Major Depression. *Biol Psychiatry*.

Kalia, L. V., Kalia, S. K. & Salter, M. W. (2008). NMDA receptors in clinical neurology: excitatory times ahead. *Lancet Neurol* **7**, 742-55.
Kalu, U. G., Sexton, C. E., Loo, C. K. & Ebmeier, K. P. (2012). Transcranial direct current stimulation in the treatment of major depression: a meta-analysis. *Psychol Med* **42**, 1791-800.
Kapur, S. & Seeman, P. (2002). NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D(2) and serotonin 5-HT(2)receptors-implications for models of schizophrenia. *Mol Psychiatry* **7**, 837-44.
Karege, F., Widmer, J., Bovier, P. & Gaillard, J. M. (1994). Platelet serotonin and plasma tryptophan in depressed patients: effect of drug treatment and clinical outcome. *Neuropsychopharmacology* **10**, 207-14.
Karpova, N. N., Pickenhagen, A., Lindholm, J., Tiraboschi, E., Kulesskaya, N., Agustsdottir, A., Antila, H., Popova, D., Akamine, Y., Bahi, A., Sullivan, R., Hen, R., Drew, L. J. & Castren, E. (2011). Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science* **334**, 1731-4.
Kaufman, J., Sullivan, G. M., Yang, J., Ogden, R. T., Miller, J. M., Oquendo, M. A., Mann, J. J., Parsey, R. V. & DeLorenzo, C. (2015). Quantification of the Serotonin 1A Receptor Using PET: Identification of a Potential Biomarker of Major Depression in Males. *Neuropsychopharmacology* **40**, 1692-9.
Kauppinen, R. A., Pirttila, T. R., Auriola, S. O. & Williams, S. R. (1994). Compartmentation of cerebral glutamate in situ as detected by 1H/13C n.m.r. *Biochem J* **298 (Pt 1)**, 121-7.
Kennedy, S. H., Evans, K. R., Kruger, S., Mayberg, H. S., Meyer, J. H., McCann, S., Arifuzzman, A. I., Houle, S. & Vaccarino, F. J. (2001). Changes in regional brain glucose metabolism measured with positron emission tomography after paroxetine treatment of major depression. *Am J Psychiatry* **158**, 899-905.
Kessler, R. C., Angermeyer, M., Anthony, J. C., R, D. E. G., Demyttenaere, K., Gasquet, I., G, D. E. G., Gluzman, S., Gureje, O., Haro, J. M., Kawakami, N., Karam, A., Levinson, D., Medina Mora, M. E., Oakley Browne, M. A., Posada-Villa, J., Stein, D. J., Adley Tsang, C. H., Aguilar-Gaxiola, S., Alonso, J., Lee, S., Heeringa, S., Pennell, B. E., Berglund, P., Gruber, M. J., Petukhova, M., Chatterji, S. & Ustun, T. B. (2007). Lifetime prevalence and age-of-onset distributions of mental disorders in the World Health Organization's World Mental Health Survey Initiative. *World Psychiatry* **6**, 168-76.
Khalid, N., Atkins, M., Tredget, J., Giles, M., Champney-Smith, K. & Kirov, G. (2008). The effectiveness of electroconvulsive therapy in treatment-resistant depression: a naturalistic study. *J ECT* **24**, 141-5.
Kirchner, W. K. (1958). Age differences in short-term retention of rapidly changing information. *J Exp Psychol* **55**, 352-8.
Kirsch, I. (2008). Challenging received wisdom: antidepressants and the placebo effect. *Mcgill J Med* **11**, 219-22.
Klein, E., Kreinin, I., Chistyakov, A., Koren, D., Mecz, L., Marmur, S., Ben-Shachar, D. & Feinsod, M. (1999). Therapeutic efficacy of right prefrontal slow repetitive transcranial magnetic stimulation in major depression: a double-blind controlled study. *Arch Gen Psychiatry* **56**, 315-20.
Koenigs, M., Barbey, A. K., Postle, B. R. & Grafman, J. (2009). Superior parietal cortex is critical for the manipulation of information in working memory. *J Neurosci* **29**, 14980-6.

Koga, M., Serritella, A. V., Messmer, M. M., Hayashi-Takagi, A., Hester, L. D., Snyder, S. H., Sawa, A. & Sedlak, T. W. (2011). Glutathione is a physiologic reservoir of neuronal glutamate. *Biochem Biophys Res Commun* **409**, 596-602.
Kupfer, D. J., Frank, E. & Phillips, M. L. (2012). Major depressive disorder: new clinical, neurobiological, and treatment perspectives. *Lancet* **379**, 1045-55.
Lacasse, J. R. & Leo, J. (2005). Serotonin and depression: a disconnect between the advertisements and the scientific literature. *PLoS Med* **2**, e392.
Laje, G., Lally, N., Mathews, D., Brutsche, N., Chemerinski, A., Akula, N., Kelmendi, B., Simen, A., McMahon, F. J., Sanacora, G. & Zarate, C., Jr. (2012). Brain-derived neurotrophic factor Val66Met polymorphism and antidepressant efficacy of ketamine in depressed patients. *Biol Psychiatry* **72**, e27-8.
Lally, N., An, L., Banerjee, D., Niciu, M. J., Luckenbaugh, D. A., Richards, E. M., Roiser, J. P., Shen, J., Zarate, C. A., Jr. & Nugent, A. C. (2015a). Reliability of 7T H-MRS measured human prefrontal cortex glutamate, glutamine, and glutathione signals using an adapted echo time optimized PRESS sequence: A between- and within-sessions investigation. *J Magn Reson Imaging*.
Lally, N., Mullins, P. G., Roberts, M. V., Price, D., Gruber, T. & Haenschel, C. (2014a). Glutamatergic correlates of gamma-band oscillatory activity during cognition: a concurrent ER-MRS and EEG study. *Neuroimage* **85 Pt 2**, 823-33.
Lally, N., Nord, C. L., Walsh, V. & Roiser, J. P. (2013). Does excitatory fronto-extracerebral tDCS lead to improved working memory performance? *F1000Res* **2**, 219.
Lally, N., Nugent, A. C., Luckenbaugh, D. A., Ameli, R., Roiser, J. P. & Zarate, C. A. (2014b). Anti-anhedonic effect of ketamine and its neural correlates in treatment-resistant bipolar depression. *Transl Psychiatry* **4**, e469.
Lally, N., Nugent, A. C., Luckenbaugh, D. A., Niciu, M. J., Roiser, J. P. & Zarate, C. A., Jr. (2015b). Neural correlates of change in major depressive disorder anhedonia following open-label ketamine. *J Psychopharmacol* **29**, 596-607.
Lam, R. W., Kennedy, S. H., McLntyre, R. S. & Khullar, A. (2014). Cognitive dysfunction in major depressive disorder: effects on psychosocial functioning and implications for treatment. *Can J Psychiatry* **59**, 649-54.
Landau, S. M., Lal, R., O'Neil, J. P., Baker, S. & Jagust, W. J. (2009). Striatal dopamine and working memory. *Cereb Cortex* **19**, 445-54.
Lapidus, K. A., Gabbay, V., Mao, X., Johnson, A., Murrough, J. W., Mathew, S. J. & Shungu, D. C. (2014a). In vivo (1)H MRS study of potential associations between glutathione, oxidative stress and anhedonia in major depressive disorder. *Neurosci Lett* **569**, 74-9.
Lapidus, K. A., Levitch, C. F., Perez, A. M., Brallier, J. W., Parides, M. K., Soleimani, L., Feder, A., Iosifescu, D. V., Charney, D. S. & Murrough, J. W. (2014b). A randomized controlled trial of intranasal ketamine in major depressive disorder. *Biol Psychiatry* **76**, 970-6.
Larkin, G. L. & Beautrais, A. L. (2011). A preliminary naturalistic study of low-dose ketamine for depression and suicide ideation in the emergency department. *Int J Neuropsychopharmacol* **14**, 1127-31.
Leal, G., Comprido, D. & Duarte, C. B. (2014). BDNF-induced local protein synthesis and synaptic plasticity. *Neuropharmacology* **76 Pt C**, 639-56.
Leichsenring, F. (2001). Comparative effects of short-term psychodynamic psychotherapy and cognitive-behavioral therapy in depression: a meta-analytic approach. *Clin Psychol Rev* **21**, 401-19.

Leichsenring, F., Leweke, F., Klein, S. & Steinert, C. (2015). The empirical status of psychodynamic psychotherapy - an update: Bambi's alive and kicking. *Psychother Psychosom* **84**, 129-48.

Lepack, A. E., Fuchikami, M., Dwyer, J. M., Banasr, M. & Duman, R. S. (2015). BDNF release is required for the behavioral actions of ketamine. *Int J Neuropsychopharmacol* **18**.

Li, N., Lee, B., Liu, R. J., Banasr, M., Dwyer, J. M., Iwata, M., Li, X. Y., Aghajanian, G. & Duman, R. S. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* **329**, 959-64.

Li, N., Liu, R. J., Dwyer, J. M., Banasr, M., Lee, B., Son, H., Li, X. Y., Aghajanian, G. & Duman, R. S. (2011). Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol Psychiatry* **69**, 754-61.

Li, S., An, L., Yu, S., Ferraris Araneta, M., Johnson, C. S., Wang, S. & Shen, J. (2015a). C MRS of human brain at 7 Tesla using [2- C]glucose infusion and low power broadband stochastic proton decoupling. *Magn Reson Med*.

Li, Y., Zhu, Z. R., Ou, B. C., Wang, Y. Q., Tan, Z. B., Deng, C. M., Gao, Y. Y., Tang, M., So, J. H., Mu, Y. L. & Zhang, L. Q. (2015b). Dopamine D2/D3 but not dopamine D1 receptors are involved in the rapid antidepressant-like effects of ketamine in the forced swim test. *Behav Brain Res* **279**, 100-5.

Liao, Y., Tang, J., Corlett, P. R., Wang, X., Yang, M., Chen, H., Liu, T., Chen, X., Hao, W. & Fletcher, P. C. (2011). Reduced dorsal prefrontal gray matter after chronic ketamine use. *Biol Psychiatry* **69**, 42-8.

Liao, Y., Tang, J., Ma, M., Wu, Z., Yang, M., Wang, X., Liu, T., Chen, X., Fletcher, P. C. & Hao, W. (2010). Frontal white matter abnormalities following chronic ketamine use: a diffusion tensor imaging study. *Brain* **133**, 2115-22.

Lie, C. H., Specht, K., Marshall, J. C. & Fink, G. R. (2006). Using fMRI to decompose the neural processes underlying the Wisconsin Card Sorting Test. *Neuroimage* **30**, 1038-49.

Lisanby, S. H., Maddox, J. H., Prudic, J., Devanand, D. P. & Sackeim, H. A. (2000). The effects of electroconvulsive therapy on memory of autobiographical and public events. *Arch Gen Psychiatry* **57**, 581-90.

Loo, C. K., Alonzo, A., Martin, D., Mitchell, P. B., Galvez, V. & Sachdev, P. (2012). Transcranial direct current stimulation for depression: 3-week, randomised, sham-controlled trial. *Br J Psychiatry* **200**, 52-9.

Lu, B., Nagappan, G., Guan, X., Nathan, P. J. & Wren, P. (2013). BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat Rev Neurosci* **14**, 401-16.

Luckenbaugh, D. A., Niciu, M. J., Ionescu, D. F., Nolan, N. M., Richards, E. M., Brutsche, N. E., Guevara, S. & Zarate, C. A. (2014). Do the dissociative side effects of ketamine mediate its antidepressant effects? *J Affect Disord* **159**, 56-61.

Luykx, J. J., Laban, K. G., van den Heuvel, M. P., Boks, M. P., Mandl, R. C., Kahn, R. S. & Bakker, S. C. (2012). Region and state specific glutamate downregulation in major depressive disorder: a meta-analysis of (1)H-MRS findings. *Neurosci Biobehav Rev* **36**, 198-205.

Lynch, D., Laws, K. R. & McKenna, P. J. (2010). Cognitive behavioural therapy for major psychiatric disorder: does it really work? A meta-analytical review of well-controlled trials. *Psychol Med* **40**, 9-24.

Ma, X. C., Dang, Y. H., Jia, M., Ma, R., Wang, F., Wu, J., Gao, C. G. & Hashimoto, K. (2013). Long-lasting antidepressant action of ketamine, but not glycogen synthase kinase-3 inhibitor SB216763, in the chronic mild stress model of mice. *PLoS One* **8**, e56053.

Maddock, R. J. & Buonocore, M. H. (2012). MR spectroscopic studies of the brain in psychiatric disorders. *Curr Top Behav Neurosci* **11**, 199-251.

Madsen, T. M., Treschow, A., Bengzon, J., Bolwig, T. G., Lindvall, O. & Tingstrom, A. (2000). Increased neurogenesis in a model of electroconvulsive therapy. *Biol Psychiatry* **47**, 1043-9.

Majer, M., Ising, M., Kunzel, H., Binder, E. B., Holsboer, F., Modell, S. & Zihl, J. (2004). Impaired divided attention predicts delayed response and risk to relapse in subjects with depressive disorders. *Psychol Med* **34**, 1453-63.

Malberg, J. E., Eisch, A. J., Nestler, E. J. & Duman, R. S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* **20**, 9104-10.

Marano, C. M., Phatak, P., Vemulapalli, U. R., Sasan, A., Nalbandyan, M. R., Ramanujam, S., Soekadar, S., Demosthenous, M. & Regenold, W. T. (2007). Increased plasma concentration of brain-derived neurotrophic factor with electroconvulsive therapy: a pilot study in patients with major depression. *J Clin Psychiatry* **68**, 512-7.

Marcus, S. C. & Olfson, M. (2010). National trends in the treatment for depression from 1998 to 2007. *Arch Gen Psychiatry* **67**, 1265-73.

Martin, D. M., Liu, R., Alonzo, A., Green, M., Player, M. J., Sachdev, P. & Loo, C. K. (2013). Can transcranial direct current stimulation enhance outcomes from cognitive training? A randomized controlled trial in healthy participants. *Int J Neuropsychopharmacol* **16**, 1927-36.

Massart, R., Mongeau, R. & Lanfumey, L. (2012). Beyond the monoaminergic hypothesis: neuroplasticity and epigenetic changes in a transgenic mouse model of depression. *Philos Trans R Soc Lond B Biol Sci* **367**, 2485-94.

Mathew, S. J., Murrough, J. W., aan het Rot, M., Collins, K. A., Reich, D. L. & Charney, D. S. (2010). Riluzole for relapse prevention following intravenous ketamine in treatment-resistant depression: a pilot randomized, placebo-controlled continuation trial. *Int J Neuropsychopharmacol* **13**, 71-82.

Matsuo, K., Glahn, D. C., Peluso, M. A., Hatch, J. P., Monkul, E. S., Najt, P., Sanches, M., Zamarripa, F., Li, J., Lancaster, J. L., Fox, P. T., Gao, J. H. & Soares, J. C. (2007). Prefrontal hyperactivation during working memory task in untreated individuals with major depressive disorder. *Mol Psychiatry* **12**, 158-66.

Maya Vetencourt, J. F., Sale, A., Viegi, A., Baroncelli, L., De Pasquale, R., O'Leary, O. F., Castren, E. & Maffei, L. (2008). The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science* **320**, 385-8.

McCabe, C., Mishor, Z., Cowen, P. J. & Harmer, C. J. (2010). Diminished neural processing of aversive and rewarding stimuli during selective serotonin reuptake inhibitor treatment. *Biol Psychiatry* **67**, 439-45.

McTavish, S. F., Mannie, Z. N., Harmer, C. J. & Cowen, P. J. (2005). Lack of effect of tyrosine depletion on mood in recovered depressed women. *Neuropsychopharmacology* **30**, 786-91.

Meinzer, M., Jahnigen, S., Copland, D. A., Darkow, R., Grittner, U., Avirame, K., Rodriguez, A. D., Lindenberg, R. & Floel, A. (2013). Transcranial direct

current stimulation over multiple days improves learning and maintenance of a novel vocabulary. *Cortex*.
Melo, A., Kokras, N., Dalla, C., Ferreira, C., Ventura-Silva, A. P., Sousa, N. & Pego, J. M. (2015). The positive effect on ketamine as a priming adjuvant in antidepressant treatment. *Transl Psychiatry* **5**, e573.
Mendels, J., Stinnett, J. L., Burns, D. & Frazer, A. (1975). Amine precursors and depression. *Arch Gen Psychiatry* **32**, 22-30.
Meron, D., Hedger, N., Garner, M. & Baldwin, D. S. (2015). Transcranial direct current stimulation (tDCS) in the treatment of depression: Systematic review and meta-analysis of efficacy and tolerability. *Neurosci Biobehav Rev*.
Miguel-Hidalgo, J. J. & Rajkowska, G. (2002). Morphological brain changes in depression: can antidepressants reverse them? *CNS Drugs* **16**, 361-72.
Milak, M. S., Proper, C. J., Mulhern, S. T., Parter, A. L., Kegeles, L. S., Ogden, R. T., Mao, X., Rodriguez, C. I., Oquendo, M. A., Suckow, R. F., Cooper, T. B., Keilp, J. G., Shungu, D. C. & Mann, J. J. (2015). A pilot in vivo proton magnetic resonance spectroscopy study of amino acid neurotransmitter response to ketamine treatment of major depressive disorder. *Mol Psychiatry*.
Miller, K. M., Price, C. C., Okun, M. S., Montijo, H. & Bowers, D. (2009). Is the n-back task a valid neuropsychological measure for assessing working memory? *Arch Clin Neuropsychol* **24**, 711-7.
Ming, G. L. & Song, H. (2011). Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron* **70**, 687-702.
Miranda, P. C., Lomarev, M. & Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clin Neurophysiol* **117**, 1623-9.
Mitterauer, B. J. (2012). Ketamine may block NMDA receptors in astrocytes causing a rapid antidepressant effect. *Front Synaptic Neurosci* **4**, 8.
Moghaddam, B., Adams, B., Verma, A. & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* **17**, 2921-7.
Moliadze, V., Antal, A. & Paulus, W. (2010). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. *Clin Neurophysiol* **121**, 2165-71.
Moller, H. J., Baldwin, D. S., Goodwin, G., Kasper, S., Okasha, A., Stein, D. J., Tandon, R. & Versiani, M. (2008). Do SSRIs or antidepressants in general increase suicidality? WPA Section on Pharmacopsychiatry: consensus statement. *Eur Arch Psychiatry Clin Neurosci* **258 Suppl 3**, 3-23.
Moncrieff, J. & Kirsch, I. (2015). Empirically derived criteria cast doubt on the clinical significance of antidepressant-placebo differences. *Contemp Clin Trials* **43**, 60-2.
Moncrieff, J., Wessely, S. & Hardy, R. (2004). Active placebos versus antidepressants for depression. *Cochrane Database Syst Rev*, CD003012.
Montgomery, S. A. & Åsberg, M. (1979). A new depression scale designed to be sensitive to change. *Br J Psychiatry* **134**, 382-9.
Montgomery, S. A., Henry, J., McDonald, G., Dinan, T., Lader, M., Hindmarch, I., Clare, A. & Nutt, D. (1994). Selective serotonin reuptake inhibitors: meta-analysis of discontinuation rates. *Int Clin Psychopharmacol* **9**, 47-53.

Morgan, C. J., Dodds, C. M., Furby, H., Pepper, F., Fam, J., Freeman, T. P., Hughes, E., Doeller, C., King, J., Howes, O. & Stone, J. M. (2014). Long-Term Heavy Ketamine Use is Associated with Spatial Memory Impairment and Altered Hippocampal Activation. *Front Psychiatry* **5**, 149.

Mull, B. R. & Seyal, M. (2001). Transcranial magnetic stimulation of left prefrontal cortex impairs working memory. *Clin Neurophysiol* **112**, 1672-5.

Murrough, J. W., Burdick, K. E., Levitch, C. F., Perez, A. M., Brallier, J. W., Chang, L. C., Foulkes, A., Charney, D. S., Mathew, S. J. & Iosifescu, D. V. (2015). Neurocognitive effects of ketamine and association with antidepressant response in individuals with treatment-resistant depression: a randomized controlled trial. *Neuropsychopharmacology* **40**, 1084-90.

Murrough, J. W., Huang, Y., Hu, J., Henry, S., Williams, W., Gallezot, J. D., Bailey, C. R., Krystal, J. H., Carson, R. E. & Neumeister, A. (2011). Reduced amygdala serotonin transporter binding in posttraumatic stress disorder. *Biol Psychiatry* **70**, 1033-8.

Murrough, J. W., Iosifescu, D. V., Chang, L. C., Al Jurdi, R. K., Green, C. E., Perez, A. M., Iqbal, S., Pillemer, S., Foulkes, A., Shah, A., Charney, D. S. & Mathew, S. J. (2013a). Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *Am J Psychiatry* **170**, 1134-42.

Murrough, J. W., Wan, L. B., Iacoviello, B., Collins, K. A., Solon, C., Glicksberg, B., Perez, A. M., Mathew, S. J., Charney, D. S., Iosifescu, D. V. & Burdick, K. E. (2013b). Neurocognitive effects of ketamine in treatment-resistant major depression: association with antidepressant response. *Psychopharmacology (Berl).*

Naughton, M., Clarke, G., O'Leary, O. F., Cryan, J. F. & Dinan, T. G. (2014). A review of ketamine in affective disorders: current evidence of clinical efficacy, limitations of use and pre-clinical evidence on proposed mechanisms of action. *J Affect Disord* **156**, 24-35.

Near, J., Edden, R., Evans, C. J., Paquin, R., Harris, A. & Jezzard, P. (2014). Frequency and phase drift correction of magnetic resonance spectroscopy data by spectral registration in the time domain. *Magn Reson Med.*

Niciu, M. J., Henter, I. D., Luckenbaugh, D. A., Zarate, C. A., Jr. & Charney, D. S. (2014a). Glutamate receptor antagonists as fast-acting therapeutic alternatives for the treatment of depression: ketamine and other compounds. *Annu Rev Pharmacol Toxicol* **54**, 119-39.

Niciu, M. J., Henter, I. D., Sanacora, G. & Zarate, C. A., Jr. (2014b). Glial abnormalities in substance use disorders and depression: does shared glutamatergic dysfunction contribute to comorbidity? *World J Biol Psychiatry* **15**, 2-16.

Nierenberg, A. A., Keefe, B. R., Leslie, V. C., Alpert, J. E., Pava, J. A., Worthington, J. J., 3rd, Rosenbaum, J. F. & Fava, M. (1999). Residual symptoms in depressed patients who respond acutely to fluoxetine. *J Clin Psychiatry* **60**, 221-5.

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., Paulus, W., Hummel, F., Boggio, P. S., Fregni, F. & Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimul* **1**, 206-23.

Nitsche, M. A., Kuo, M. F., Karrasch, R., Wachter, B., Liebetanz, D. & Paulus, W. (2009). Serotonin affects transcranial direct current-induced neuroplasticity in humans. *Biol Psychiatry* **66**, 503-8.

Nitsche, M. A. & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol* **527 Pt 3**, 633-9.
Nock, M. K., Hwang, I., Sampson, N. A. & Kessler, R. C. (2010). Mental disorders, comorbidity and suicidal behavior: results from the National Comorbidity Survey Replication. *Mol Psychiatry* **15**, 868-76.
Nord, C. L., Lally, N. & Charpentier, C. J. (2013). Harnessing electric potential: DLPFC tDCS induces widespread brain perfusion changes. *Frontiers in Systems Neuroscience* **7**.
Normann, C., Schmitz, D., Furmaier, A., Doing, C. & Bach, M. (2007). Long-term plasticity of visually evoked potentials in humans is altered in major depression. *Biol Psychiatry* **62**, 373-80.
Nosyreva, E., Szabla, K., Autry, A. E., Ryazanov, A. G., Monteggia, L. M. & Kavalali, E. T. (2013). Acute suppression of spontaneous neurotransmission drives synaptic potentiation. *J Neurosci* **33**, 6990-7002.
Nugent, A. C., Luckenbaugh, D. A., Wood, S. E., Bogers, W., Zarate, C. A., Jr. & Drevets, W. C. (2013). Automated subcortical segmentation using FIRST: test-retest reliability, interscanner reliability, and comparison to manual segmentation. *Hum Brain Mapp* **34**, 2313-29.
Nutt, D. J. (2008). Relationship of neurotransmitters to the symptoms of major depressive disorder. *J Clin Psychiatry* **69 Suppl E1**, 4-7.
O'Connell, N. E., Cossar, J., Marston, L., Wand, B. M., Bunce, D., Moseley, G. L. & De Souza, L. H. (2012). Rethinking clinical trials of transcranial direct current stimulation: participant and assessor blinding is inadequate at intensities of 2mA. *PLoS One* **7**, e47514.
O'Gorman, R. L., Michels, L., Edden, R. A., Murdoch, J. B. & Martin, E. (2011). In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. *J Magn Reson Imaging* **33**, 1262-7.
O'Reardon, J. P., Solvason, H. B., Janicak, P. G., Sampson, S., Isenberg, K. E., Nahas, Z., McDonald, W. M., Avery, D., Fitzgerald, P. B., Loo, C., Demitrack, M. A., George, M. S. & Sackeim, H. A. (2007). Efficacy and safety of transcranial magnetic stimulation in the acute treatment of major depression: a multisite randomized controlled trial. *Biol Psychiatry* **62**, 1208-16.
Office for National Statistics (2014). Suicides in the United Kingdom, 2012 Registrations.
Ohn, S. H., Park, C. I., Yoo, W. K., Ko, M. H., Choi, K. P., Kim, G. M., Lee, Y. T. & Kim, Y. H. (2008). Time-dependent effect of transcranial direct current stimulation on the enhancement of working memory. *Neuroreport* **19**, 43-7.
Olesen, J., Gustavsson, A., Svensson, M., Wittchen, H. U., Jonsson, B., group, C. s. & European Brain, C. (2012). The economic cost of brain disorders in Europe. *Eur J Neurol* **19**, 155-62.
Oliveira, J. F., Zanao, T. A., Valiengo, L., Lotufo, P. A., Bensenor, I. M., Fregni, F. & Brunoni, A. R. (2013). Acute working memory improvement after tDCS in antidepressant-free patients with major depressive disorder. *Neurosci Lett* **537**, 60-4.
Olver, J. S., Burrows, G. D. & Norman, T. R. (2001). Third-generation antidepressants: do they offer advantages over the SSRIs? *CNS Drugs* **15**, 941-54.
Opbroek, A., Delgado, P. L., Laukes, C., McGahuey, C., Katsanis, J., Moreno, F. A. & Manber, R. (2002). Emotional blunting associated with SSRI-induced

sexual dysfunction. Do SSRIs inhibit emotional responses? *Int J Neuropsychopharmacol* **5**, 147-51.
Owen, A. M., McMillan, K. M., Laird, A. R. & Bullmore, E. (2005). N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. *Hum Brain Mapp* **25**, 46-59.
Pagnin, D., de Queiroz, V., Pini, S. & Cassano, G. B. (2004). Efficacy of ECT in depression: a meta-analytic review. *J ECT* **20**, 13-20.
Palm, U., Reisinger, E., Keeser, D., Kuo, M. F., Pogarell, O., Leicht, G., Mulert, C., Nitsche, M. A. & Padberg, F. (2013). Evaluation of sham transcranial direct current stimulation for randomized, placebo-controlled clinical trials. *Brain Stimul* **6**, 690-5.
Palm, U., Schiller, C., Fintescu, Z., Obermeier, M., Keeser, D., Reisinger, E., Pogarell, O., Nitsche, M. A., Moller, H. J. & Padberg, F. (2012). Transcranial direct current stimulation in treatment resistant depression: a randomized double-blind, placebo-controlled study. *Brain Stimul* **5**, 242-51.
Parsey, R. V., Hastings, R. S., Oquendo, M. A., Huang, Y. Y., Simpson, N., Arcement, J., Huang, Y., Ogden, R. T., Van Heertum, R. L., Arango, V. & Mann, J. J. (2006). Lower serotonin transporter binding potential in the human brain during major depressive episodes. *Am J Psychiatry* **163**, 52-8.
Pascual-Leone, A., Rubio, B., Pallardo, F. & Catala, M. D. (1996). Rapid-rate transcranial magnetic stimulation of left dorsolateral prefrontal cortex in drug-resistant depression. *Lancet* **348**, 233-7.
Paul, I. A. & Skolnick, P. (2003). Glutamate and depression: clinical and preclinical studies. *Ann N Y Acad Sci* **1003**, 250-72.
Paykel, E. S. (2008). Partial remission, residual symptoms, and relapse in depression. *Dialogues Clin Neurosci* **10**, 431-7.
Pelosi, L., Slade, T., Blumhardt, L. D. & Sharma, V. K. (2000). Working memory dysfunction in major depression: an event-related potential study. *Clin Neurophysiol* **111**, 1531-43.
Perera, T. D., Coplan, J. D., Lisanby, S. H., Lipira, C. M., Arif, M., Carpio, C., Spitzer, G., Santarelli, L., Scharf, B., Hen, R., Rosoklija, G., Sackeim, H. A. & Dwork, A. J. (2007). Antidepressant-induced neurogenesis in the hippocampus of adult nonhuman primates. *J Neurosci* **27**, 4894-901.
Perera, T. D., Dwork, A. J., Keegan, K. A., Thirumangalakudi, L., Lipira, C. M., Joyce, N., Lange, C., Higley, J. D., Rosoklija, G., Hen, R., Sackeim, H. A. & Coplan, J. D. (2011). Necessity of hippocampal neurogenesis for the therapeutic action of antidepressants in adult nonhuman primates. *PLoS One* **6**, e17600.
Pittenger, C. & Duman, R. S. (2008). Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology* **33**, 88-109.
Pizzagalli, D. A. (2011). Frontocingulate dysfunction in depression: toward biomarkers of treatment response. *Neuropsychopharmacology* **36**, 183-206.
Plichta, M. M., Schwarz, A. J., Grimm, O., Morgen, K., Mier, D., Haddad, L., Gerdes, A. B., Sauer, C., Tost, H., Esslinger, C., Colman, P., Wilson, F., Kirsch, P. & Meyer-Lindenberg, A. (2012). Test-retest reliability of evoked BOLD signals from a cognitive-emotive fMRI test battery. *Neuroimage* **60**, 1746-58.
Price, J., Cole, V. & Goodwin, G. M. (2009a). Emotional side-effects of selective serotonin reuptake inhibitors: qualitative study. *Br J Psychiatry* **195**, 211-7.

Price, R. B., Nock, M. K., Charney, D. S. & Mathew, S. J. (2009b). Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. *Biol Psychiatry* **66**, 522-6.
Price, R. B., Shungu, D. C., Mao, X., Nestadt, P., Kelly, C., Collins, K. A., Murrough, J. W., Charney, D. S. & Mathew, S. J. (2009c). Amino acid neurotransmitters assessed by proton magnetic resonance spectroscopy: relationship to treatment resistance in major depressive disorder. *Biol Psychiatry* **65**, 792-800.
Pringle, A., Browning, M., Cowen, P. J. & Harmer, C. J. (2011). A cognitive neuropsychological model of antidepressant drug action. *Prog Neuropsychopharmacol Biol Psychiatry* **35**, 1586-92.
Rajkowska, G. (2000). Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biol Psychiatry* **48**, 766-77.
Rajkowska, G., Miguel-Hidalgo, J. J., Wei, J., Dilley, G., Pittman, S. D., Meltzer, H. Y., Overholser, J. C., Roth, B. L. & Stockmeier, C. A. (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry* **45**, 1085-98.
Ramadan, S., Lin, A. & Stanwell, P. (2013). Glutamate and glutamine: a review of in vivo MRS in the human brain. *NMR Biomed* **26**, 1630-46.
Redlich, R., Dohm, K., Grotegerd, D., Opel, N., Zwitserlood, P., Heindel, W., Arolt, V., Kugel, H. & Dannlowski, U. (2015). Reward Processing in Unipolar and Bipolar Depression: A Functional MRI Study. *Neuropsychopharmacology* **40**, 2623-31.
Reis, J., Schambra, H. M., Cohen, L. G., Buch, E. R., Fritsch, B., Zarahn, E., Celnik, P. A. & Krakauer, J. W. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc Natl Acad Sci U S A* **106**, 1590-5.
Ritchey, M., Dolcos, F., Eddington, K. M., Strauman, T. J. & Cabeza, R. (2011). Neural correlates of emotional processing in depression: changes with cognitive behavioral therapy and predictors of treatment response. *J Psychiatr Res* **45**, 577-87.
Robinson, O. J., Cools, R., Carlisi, C. O., Sahakian, B. J. & Drevets, W. C. (2012). Ventral striatum response during reward and punishment reversal learning in unmedicated major depressive disorder. *Am J Psychiatry* **169**, 152-9.
Roiser, J. P., Elliott, R. & Sahakian, B. J. (2012). Cognitive mechanisms of treatment in depression. *Neuropsychopharmacology* **37**, 117-36.
Roiser, J. P., McLean, A., Ogilvie, A. D., Blackwell, A. D., Bamber, D. J., Goodyer, I., Jones, P. B. & Sahakian, B. J. (2005). The subjective and cognitive effects of acute phenylalanine and tyrosine depletion in patients recovered from depression. *Neuropsychopharmacology* **30**, 775-85.
Rose, E. J. & Ebmeier, K. P. (2006). Pattern of impaired working memory during major depression. *J Affect Disord* **90**, 149-61.
Rowland, L. M., Bustillo, J. R., Mullins, P. G., Jung, R. E., Lenroot, R., Landgraf, E., Barrow, R., Yeo, R., Lauriello, J. & Brooks, W. M. (2005). Effects of ketamine on anterior cingulate glutamate metabolism in healthy humans: a 4-T proton MRS study. *Am J Psychiatry* **162**, 394-6.
Ruhe, H. G., Mason, N. S. & Schene, A. H. (2007). Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. *Mol Psychiatry* **12**, 331-59.
Sackeim, H. A. (2001). The definition and meaning of treatment-resistant depression. *J Clin Psychiatry* **62 Suppl 16**, 10-7.

Sackeim, H. A., Haskett, R. F., Mulsant, B. H., Thase, M. E., Mann, J. J., Pettinati, H. M., Greenberg, R. M., Crowe, R. R., Cooper, T. B. & Prudic, J. (2001). Continuation pharmacotherapy in the prevention of relapse following electroconvulsive therapy: a randomized controlled trial. *JAMA* **285**, 1299-307.

Salvadore, G., Cornwell, B. R., Sambataro, F., Latov, D., Colon-Rosario, V., Carver, F., Holroyd, T., DiazGranados, N., Machado-Vieira, R., Grillon, C., Drevets, W. C. & Zarate, C. A., Jr. (2010). Anterior cingulate desynchronization and functional connectivity with the amygdala during a working memory task predict rapid antidepressant response to ketamine. *Neuropsychopharmacology* **35**, 1415-22.

Salvadore, G., van der Veen, J. W., Zhang, Y., Marenco, S., Machado-Vieira, R., Baumann, J., Ibrahim, L. A., Luckenbaugh, D. A., Shen, J., Drevets, W. C. & Zarate, C. A., Jr. (2012). An investigation of amino-acid neurotransmitters as potential predictors of clinical improvement to ketamine in depression. *Int J Neuropsychopharmacol* **15**, 1063-72.

Sanacora, G. & Schatzberg, A. F. (2015). Ketamine: promising path or false prophecy in the development of novel therapeutics for mood disorders? *Neuropsychopharmacology* **40**, 259-67.

Sanacora, G., Treccani, G. & Popoli, M. (2012). Towards a glutamate hypothesis of depression: an emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology* **62**, 63-77.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C. & Hen, R. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805-9.

Sawaguchi, T. & Goldman-Rakic, P. S. (1991). D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* **251**, 947-50.

Schatzberg, A. F. (2014). A word to the wise about ketamine. *Am J Psychiatry* **171**, 262-4.

Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Am J Psychiatry* **122**, 509-22.

Schildkraut, J. J., Gordon, E. K. & Durell, J. (1965). Catecholamine metabolism in affective disorders. I. Normetanephrine and VMA excretion in depressed patients treated with imipramine. *J Psychiatr Res* **3**, 213-28.

Schmaal, L., Veltman, D. J., van Erp, T. G., Samann, P. G., Frodl, T., Jahanshad, N., Loehrer, E., Tiemeier, H., Hofman, A., Niessen, W. J., Vernooij, M. W., Ikram, M. A., Wittfeld, K., Grabe, H. J., Block, A., Hegenscheid, K., Volzke, H., Hoehn, D., Czisch, M., Lagopoulos, J., Hatton, S. N., Hickie, I. B., Goya-Maldonado, R., Kramer, B., Gruber, O., Couvy-Duchesne, B., Renteria, M. E., Strike, L. T., Mills, N. T., de Zubicaray, G. I., McMahon, K. L., Medland, S. E., Martin, N. G., Gillespie, N. A., Wright, M. J., Hall, G. B., MacQueen, G. M., Frey, E. M., Carballedo, A., van Velzen, L. S., van Tol, M. J., van der Wee, N. J., Veer, I. M., Walter, H., Schnell, K., Schramm, E., Normann, C., Schoepf, D., Konrad, C., Zurowski, B., Nickson, T., McIntosh, A. M., Papmeyer, M., Whalley, H. C., Sussmann, J. E., Godlewska, B. R., Cowen, P. J., Fischer, F. H., Rose, M., Penninx, B. W., Thompson, P. M. & Hibar, D. P. (2015). Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. *Mol Psychiatry*.

Schoenfeld, T. J. & Cameron, H. A. (2015). Adult neurogenesis and mental illness. *Neuropsychopharmacology* **40**, 113-28.

Schramm, E., van Calker, D., Dykierek, P., Lieb, K., Kech, S., Zobel, I., Leonhart, R. & Berger, M. (2007). An intensive treatment program of interpersonal psychotherapy plus pharmacotherapy for depressed inpatients: acute and long-term results. *Am J Psychiatry* **164**, 768-77.

Schubert, F., Gallinat, J., Seifert, F. & Rinneberg, H. (2004). Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 Tesla. *Neuroimage* **21**, 1762-71.

Scott, B. W., Wojtowicz, J. M. & Burnham, W. M. (2000). Neurogenesis in the dentate gyrus of the rat following electroconvulsive shock seizures. *Exp Neurol* **165**, 231-6.

Selikoff, I. J., Robitzek, E. H. & Ornstein, G. G. (1952). Treatment of pulmonary tuberculosis with hydrazide derivatives of isonicotinic acid. *J Am Med Assoc* **150**, 973-80.

Sheline, Y. I., Gado, M. H. & Kraemer, H. C. (2003). Untreated depression and hippocampal volume loss. *Am J Psychiatry* **160**, 1516-8.

Shen, J. (2013). Modeling the glutamate-glutamine neurotransmitter cycle. *Front Neuroenergetics* **5**, 1.

Sherdell, L., Waugh, C. E. & Gotlib, I. H. (2012). Anticipatory pleasure predicts motivation for reward in major depression. *J Abnorm Psychol* **121**, 51-60.

Shields, D. C., Asaad, W., Eskandar, E. N., Jain, F. A., Cosgrove, G. R., Flaherty, A. W., Cassem, E. H., Price, B. H., Rauch, S. L. & Dougherty, D. D. (2008). Prospective assessment of stereotactic ablative surgery for intractable major depression. *Biol Psychiatry* **64**, 449-54.

Shiroma, P. R., Albott, C. S., Johns, B., Thuras, P., Wels, J. & Lim, K. O. (2014). Neurocognitive performance and serial intravenous subanesthetic ketamine in treatment-resistant depression. *Int J Neuropsychopharmacol* **17**, 1805-13.

Shomstein, S. (2012). Cognitive functions of the posterior parietal cortex: top-down and bottom-up attentional control. *Front Integr Neurosci* **6**, 38.

Shungu, D. C., Weiduschat, N., Murrough, J. W., Mao, X., Pillemer, S., Dyke, J. P., Medow, M. S., Natelson, B. H., Stewart, J. M. & Mathew, S. J. (2012). Increased ventricular lactate in chronic fatigue syndrome. III. Relationships to cortical glutathione and clinical symptoms implicate oxidative stress in disorder pathophysiology. *NMR Biomed* **25**, 1073-87.

Smagula, S. F., Butters, M. A., Anderson, S. J., Lenze, E. J., Dew, M. A., Mulsant, B. H., Lotrich, F. E., Aizenstein, H. & Reynolds, C. F., 3rd (2015). Antidepressant Response Trajectories and Associated Clinical Prognostic Factors Among Older Adults. *JAMA Psychiatry*.

Snaith, R. P., Hamilton, M., Morley, S., Humayan, A., Hargreaves, D. & Trigwell, P. (1995). A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *Br J Psychiatry* **167**, 99-103.

Snyder, H. R. (2013). Major depressive disorder is associated with broad impairments on neuropsychological measures of executive function: a meta-analysis and review. *Psychol Bull* **139**, 81-132.

Snyder, J. S., Soumier, A., Brewer, M., Pickel, J. & Cameron, H. A. (2011). Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* **476**, 458-61.

Sos, P., Klirova, M., Novak, T., Kohutova, B., Horacek, J. & Palenicek, T. (2013). Relationship of ketamine's antidepressant and psychotomimetic effects in unipolar depression. *Neuro Endocrinol Lett* **34**, 287-93.

Speer, A. M., Wassermann, E. M., Benson, B. E., Herscovitch, P. & Post, R. M. (2014). Antidepressant efficacy of high and low frequency rTMS at 110% of motor threshold versus sham stimulation over left prefrontal cortex. *Brain Stimul* **7**, 36-41.
Spielmans, G. I., Berman, M. I., Linardatos, E., Rosenlicht, N. Z., Perry, A. & Tsai, A. C. (2013). Adjunctive atypical antipsychotic treatment for major depressive disorder: a meta-analysis of depression, quality of life, and safety outcomes. *PLoS Med* **10**, e1001403.
Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev* **99**, 195-231.
Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., Morris, P. G., Matthews, P. M. & Johansen-Berg, H. (2009). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J Neurosci* **29**, 5202-6.
Stagg, C. J., Lin, R. L., Mezue, M., Segerdahl, A., Kong, Y., Xie, J. & Tracey, I. (2013). Widespread modulation of cerebral perfusion induced during and after transcranial direct current stimulation applied to the left dorsolateral prefrontal cortex. *J Neurosci* **33**, 11425-31.
Steinert, C., Hofmann, M., Kruse, J. & Leichsenring, F. (2014). Relapse rates after psychotherapy for depression - stable long-term effects? A meta-analysis. *J Affect Disord* **168**, 107-18.
Stephenson, M. C., Gunner, F., Napolitano, A., Greenhaff, P. L., Macdonald, I. A., Saeed, N., Vennart, W., Francis, S. T. & Morris, P. G. (2011). Applications of multi-nuclear magnetic resonance spectroscopy at 7T. *World J Radiol* **3**, 105-13.
Stevens, A., Fischer, A., Bartels, M. & Buchkremer, G. (1996). Electroconvulsive therapy: a review on indications, methods, risks and medication. *Eur Psychiatry* **11**, 165-74.
Stevenson, C. M., Brookes, M. J. & Morris, P. G. (2011). beta-Band correlates of the fMRI BOLD response. *Hum Brain Mapp* **32**, 182-97.
Stone, J. M., Dietrich, C., Edden, R., Mehta, M. A., De Simoni, S., Reed, L. J., Krystal, J. H., Nutt, D. & Barker, G. J. (2012). Ketamine effects on brain GABA and glutamate levels with 1H-MRS: relationship to ketamine-induced psychopathology. *Mol Psychiatry* **17**, 664-5.
Strauss, G. P. & Gold, J. M. (2012). A new perspective on anhedonia in schizophrenia. *Am J Psychiatry* **169**, 364-73.
Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., Hen, R. & Belzung, C. (2008). Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biol Psychiatry* **64**, 293-301.
Tanaka, T., Takano, Y., Tanaka, S., Hironaka, N., Kobayashi, K., Hanakawa, T., Watanabe, K. & Honda, M. (2013). Transcranial direct-current stimulation increases extracellular dopamine levels in the rat striatum. *Front Syst Neurosci* **7**, 6.
Taylor, M. J. (2014). Could glutamate spectroscopy differentiate bipolar depression from unipolar? *J Affect Disord* **167**, 80-4.
Taylor, M. J., Freemantle, N., Geddes, J. R. & Bhagwagar, Z. (2006). Early onset of selective serotonin reuptake inhibitor antidepressant action: systematic review and meta-analysis. *Arch Gen Psychiatry* **63**, 1217-23.
Taylor, M. J., Tiangga, E. R., Mhuircheartaigh, R. N. & Cowen, P. J. (2012). Lack of effect of ketamine on cortical glutamate and glutamine in healthy volunteers: a proton magnetic resonance spectroscopy study. *J Psychopharmacol* **26**, 733-7.

Tharyan, P. & Adams, C. E. (2005). Electroconvulsive therapy for schizophrenia. *Cochrane Database Syst Rev*, CD000076.
Thase, M. E., Clayton, A. H., Haight, B. R., Thompson, A. H., Modell, J. G. & Johnston, J. A. (2006). A double-blind comparison between bupropion XL and venlafaxine XR: sexual functioning, antidepressant efficacy, and tolerability. *J Clin Psychopharmacol* **26**, 482-8.
Thase, M. E., Entsuah, A. R. & Rudolph, R. L. (2001). Remission rates during treatment with venlafaxine or selective serotonin reuptake inhibitors. *Br J Psychiatry* **178**, 234-41.
Tortella, G., Selingardi, P. M., Moreno, M. L., Veronezi, B. P. & Brunoni, A. R. (2014). Does non-invasive brain stimulation improve cognition in major depressive disorder? A systematic review. *CNS Neurol Disord Drug Targets* **13**, 1759-69.
Tranter, R., Bell, D., Gutting, P., Harmer, C., Healy, D. & Anderson, I. M. (2009). The effect of serotonergic and noradrenergic antidepressants on face emotion processing in depressed patients. *J Affect Disord* **118**, 87-93.
Treadway, M. T., Bossaller, N. A., Shelton, R. C. & Zald, D. H. (2012). Effort-based decision-making in major depressive disorder: a translational model of motivational anhedonia. *J Abnorm Psychol* **121**, 553-8.
Treadway, M. T., Buckholtz, J. W., Schwartzman, A. N., Lambert, W. E. & Zald, D. H. (2009). Worth the 'EEfRT'? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. *PLoS One* **4**, e6598.
Treadway, M. T. & Zald, D. H. (2011). Reconsidering anhedonia in depression: lessons from translational neuroscience. *Neurosci Biobehav Rev* **35**, 537-55.
Trivedi, M. H. & Greer, T. L. (2014). Cognitive dysfunction in unipolar depression: implications for treatment. *J Affect Disord* **152-154**, 19-27.
Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., Norquist, G., Howland, R. H., Lebowitz, B., McGrath, P. J., Shores-Wilson, K., Biggs, M. M., Balasubramani, G. K. & Fava, M. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry* **163**, 28-40.
Tseng, P., Hsu, T. Y., Chang, C. F., Tzeng, O. J., Hung, D. L., Muggleton, N. G., Walsh, V., Liang, W. K., Cheng, S. K. & Juan, C. H. (2012). Unleashing potential: transcranial direct current stimulation over the right posterior parietal cortex improves change detection in low-performing individuals. *J Neurosci* **32**, 10554-61.
Uher, R., Perlis, R. H., Henigsberg, N., Zobel, A., Rietschel, M., Mors, O., Hauser, J., Dernovsek, M. Z., Souery, D., Bajs, M., Maier, W., Aitchison, K. J., Farmer, A. & McGuffin, P. (2012). Depression symptom dimensions as predictors of antidepressant treatment outcome: replicable evidence for interest-activity symptoms. *Psychol Med* **42**, 967-80.
Valentine, G. W., Mason, G. F., Gomez, R., Fasula, M., Watzl, J., Pittman, B., Krystal, J. H. & Sanacora, G. (2011). The antidepressant effect of ketamine is not associated with changes in occipital amino acid neurotransmitter content as measured by [(1)H]-MRS. *Psychiatry Res* **191**, 122-7.
Vollenweider, F. X., Vontobel, P., Oye, I., Hell, D. & Leenders, K. L. (2000). Effects of (S)-ketamine on striatal dopamine: a [11C]raclopride PET study of a model psychosis in humans. *J Psychiatr Res* **34**, 35-43.
Walker, A. K., Budac, D. P., Bisulco, S., Lee, A. W., Smith, R. A., Beenders, B., Kelley, K. W. & Dantzer, R. (2013). NMDA receptor blockade by ketamine

abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J mice. *Neuropsychopharmacology* **38**, 1609-16.
Walsh, N. D., Williams, S. C., Brammer, M. J., Bullmore, E. T., Kim, J., Suckling, J., Mitterschiffthaler, M. T., Cleare, A. J., Pich, E. M., Mehta, M. A. & Fu, C. H. (2007). A longitudinal functional magnetic resonance imaging study of verbal working memory in depression after antidepressant therapy. *Biol Psychiatry* **62**, 1236-43.
Walsh, V. Q. (2013). Ethics and social risks in brain stimulation. *Brain Stimul* **6**, 715-7.
Walter, M., Henning, A., Grimm, S., Schulte, R. F., Beck, J., Dydak, U., Schnepf, B., Boeker, H., Boesiger, P. & Northoff, G. (2009). The relationship between aberrant neuronal activation in the pregenual anterior cingulate, altered glutamatergic metabolism, and anhedonia in major depression. *Arch Gen Psychiatry* **66**, 478-86.
Wang, J., Goffer, Y., Xu, D., Tukey, D. S., Shamir, D. B., Eberle, S. E., Zou, A. H., Blanck, T. J. & Ziff, E. B. (2011). A single subanesthetic dose of ketamine relieves depression-like behaviors induced by neuropathic pain in rats. *Anesthesiology* **115**, 812-21.
Wang, X. L., Du, M. Y., Chen, T. L., Chen, Z. Q., Huang, X. Q., Luo, Y., Zhao, Y. J., Kumar, P. & Gong, Q. Y. (2015). Neural correlates during working memory processing in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry* **56**, 101-8.
Warren, M. B., Pringle, A. & Harmer, C. J. (2015). A neurocognitive model for understanding treatment action in depression. *Philos Trans R Soc Lond B Biol Sci* **370**.
Weiduschat, N., Mao, X., Hupf, J., Armstrong, N., Kang, G., Lange, D. J., Mitsumoto, H. & Shungu, D. C. (2014). Motor cortex glutathione deficit in ALS measured in vivo with the J-editing technique. *Neurosci Lett* **570**, 102-7.
Whitton, A. E., Treadway, M. T. & Pizzagalli, D. A. (2015). Reward processing dysfunction in major depression, bipolar disorder and schizophrenia. *Curr Opin Psychiatry* **28**, 7-12.
Wijtenburg, S. A., Rowland, L. M., Edden, R. A. & Barker, P. B. (2013). Reproducibility of brain spectroscopy at 7T using conventional localization and spectral editing techniques. *J Magn Reson Imaging* **38**, 460-7.
Winer, E. S., Nadorff, M. R., Ellis, T. E., Allen, J. G., Herrera, S. & Salem, T. (2014). Anhedonia predicts suicidal ideation in a large psychiatric inpatient sample. *Psychiatry Res* **218**, 124-8.
Wolkenstein, L. & Plewnia, C. (2013). Amelioration of cognitive control in depression by transcranial direct current stimulation. *Biol Psychiatry* **73**, 646-51.
Wolkowitz, O. M., Wolf, J., Shelly, W., Rosser, R., Burke, H. M., Lerner, G. K., Reus, V. I., Nelson, J. C., Epel, E. S. & Mellon, S. H. (2011). Serum BDNF levels before treatment predict SSRI response in depression. *Prog Neuropsychopharmacol Biol Psychiatry* **35**, 1623-30.
World Health Organization (2012). Depression: A Global Public Health Concern. WHO.
Xie, W. Z., Yan, C., Ying, X. Y., Zhu, S. Y., Shi, H. S., Wang, Y., Cheung, E. F. & Chan, R. C. (2014). Domain-specific hedonic deficits towards social affective but not monetary incentives in social anhedonia. *Sci Rep* **4**, 4056.

Yamamoto, S., Ohba, H., Nishiyama, S., Harada, N., Kakiuchi, T., Tsukada, H. & Domino, E. F. (2013). Subanesthetic doses of ketamine transiently decrease serotonin transporter activity: a PET study in conscious monkeys. *Neuropsychopharmacology* **38**, 2666-74.

Yamanaka, H., Yokoyama, C., Mizuma, H., Kurai, S., Finnema, S. J., Halldin, C., Doi, H. & Onoe, H. (2014). A possible mechanism of the nucleus accumbens and ventral pallidum 5-HT1B receptors underlying the antidepressant action of ketamine: a PET study with macaques. *Transl Psychiatry* **4**, e342.

Young, K. D., Bellgowan, P. S., Bodurka, J. & Drevets, W. C. (2014). Neurophysiological correlates of autobiographical memory deficits in currently and formerly depressed subjects. *Psychol Med* **44**, 2951-63.

Yuksel, C. & Ongur, D. (2010). Magnetic resonance spectroscopy studies of glutamate-related abnormalities in mood disorders. *Biol Psychiatry* **68**, 785-94.

Yun, R. J., Krystal, J. H. & Mathalon, D. H. (2010). Working memory overload: fronto-limbic interactions and effects on subsequent working memory function. *Brain Imaging Behav* **4**, 96-108.

Zaehle, T., Sandmann, P., Thorne, J. D., Jancke, L. & Herrmann, C. S. (2011). Transcranial direct current stimulation of the prefrontal cortex modulates working memory performance: combined behavioural and electrophysiological evidence. *BMC Neurosci* **12**, 2.

Zakzanis, K. K., Leach, L. & Kaplan, E. (1998). On the nature and pattern of neurocognitive function in major depressive disorder. *Neuropsychiatry Neuropsychol Behav Neurol* **11**, 111-9.

Zarate, C., Jr., Machado-Vieira, R., Henter, I., Ibrahim, L., Diazgranados, N. & Salvadore, G. (2010). Glutamatergic modulators: the future of treating mood disorders? *Harv Rev Psychiatry* **18**, 293-303.

Zarate, C. A., Jr., Brutsche, N. E., Ibrahim, L., Franco-Chaves, J., Diazgranados, N., Cravchik, A., Selter, J., Marquardt, C. A., Liberty, V. & Luckenbaugh, D. A. (2012). Replication of ketamine's antidepressant efficacy in bipolar depression: a randomized controlled add-on trial. *Biol Psychiatry* **71**, 939-46.

Zarate, C. A., Jr., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., Charney, D. S. & Manji, H. K. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch Gen Psychiatry* **63**, 856-64.

Zhou, X., Ravindran, A. V., Qin, B., Del Giovane, C., Li, Q., Bauer, M., Liu, Y., Fang, Y., da Silva, T., Zhang, Y., Fang, L., Wang, X. & Xie, P. (2015). Comparative efficacy, acceptability, and tolerability of augmentation agents in treatment-resistant depression: systematic review and network meta-analysis. *J Clin Psychiatry* **76**, e487-98.