Does N-methyl-D-aspartate (NMDA) receptor modulation affect emotional processing? Implications for novel antidepressant treatments

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Abstract

Conventional antidepressants have a relatively slow onset of action, requiring a number of weeks before clinical improvement in depressive symptoms is apparent. The delay in onset of action of antidepressants may result in patients suffering increased morbidity due to the persistence of depressed mood and associated symptomatology. More recently, animal and human studies suggest that N-methyl-D-aspartate (NMDA) receptors may participate in the pathophysiology of depression and play an important role in effective pharmacological treatment. Indeed, several NMDA receptors antagonists, such as ketamine, lanicemine and nitrous oxide (N_2O) have been shown to produce an antidepressant effect within hours in some clinical trials. Another group of drugs, acting at the glycine site of the NMDA receptor, have also shown potential as antidepressant agents.

The cognitive neuropsychological model of antidepressant drug action proposes that in the early stage of conventional antidepressant treatments the primary effect of antidepressants is to modify negative biases in the processing of emotional information. However, there have been no systematic studies to identify how NMDA-targeting antidepressants may change emotional processing in humans. Thus, this thesis aimed to investigate the effects of different NMDA receptor ligands on emotional processing in both depressed patients and healthy participants using a combination of behavioral and neuroimaging approaches. The first study assessed the effects of single doses of lanicemine and ketamine on neural and behavioural measures of emotional processing in depressed patients, 24 hours post infusion. It was found that ketamine did not show the broad range of effects on emotional processing seen with conventional antidepressants but

instead significantly increased the error rate for recognizing facial expression as positive, and also produced higher false positive memory intrusions in the emotional recall task. Lanicemine only showed a trend for better recognition of facial expression across all emotions and did not produce positive shifts in any emotional processing tasks (Chapter 2). Furthermore, a single dose of ketamine increased brain activation in the right dorsolateral prefrontal cortex region during negative self-referential processing (Chapter 3).

The second study assessed the effects of a single dose of N_2O 24 hours post infusion using behavioural measures of emotional processing in treatment-resistant depressed (TRD) patients. It was found that N_2O did not change the processing of emotions in TRD patients compared to those in the placebo group (Chapter 4).

The third study was designed to assess the effects of an acute dose of an NMDA receptor glycine site, partial agonist D-cycloserine (DCS), on autobiographical memory, emotional processing and stress response in healthy volunteers. Relative to placebo, DCS enhanced autobiographical memory specificity at 3 and at 24 hours post treatment. DCS also increased positive bias in emotional word categorisation and subsequent free recall at 3 hours; however, this effect did not extend to other emotional processing tasks (chapter 5). In addition, further investigation on the effects of DCS on stress responses (chapter 6) found no effect of DCS on psychophysiological changes and self-reported mood and anxiety in an acute psychosocial stress paradigm.

These findings suggested that NMDA receptor targeting antidepressants showed early effects on emotional processing in the absence of subjective improvement in mood symptoms, though the

changes seen differ from those observed with conventional antidepressants. These findings are important for understanding the therapeutic action of NMDA receptor targeting antidepressants in the treatment of depression and stress-related disorders and also for the ability of DCS to augment psychological treatment in patients with anxiety and depression. This thesis also outlines the challenges and future research directions for better understanding the cognitive neuropsychological mechanisms underlying the effects of NMDA receptor drugs in depression. This could have implications for the development of antidepressants with an early onset of clinical action.

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Data collection for the studies reported in Chapters 2 and 3 was conducted by colleagues from Manchester University and Oxford University. Data collection for the study reported in Chapter 4 was conducted by colleagues from the Department of Psychiatry, Second Xiangya Hospital, Central South University, Changsha, China. None of the data used in this thesis have been published previously.

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List of abbreviations

5HT	5-hydroxytryptamine
AAL	Automated anatomical labelling
ACC	Anterior cingulate cortex
ACG	Anterior cingulate gyrus
ANOVA	One-way analyses of variance
AMT	Autobiographical Memory Task
BDNF	Brain-derived neurotrophic factor
BDI	Beck Depression Inventory
BFS	Befindlichkeits scale
BMI	Body mass index
BMP	Beats per minute
BOLD	Blood oxygenation level dependent
CADSS	Clinician Administered Dissociative Status Scale
CBT	Cognitive Behavioural Therapy
CNS	Central nervous system
CO_2	Carbon dioxide
CSF	Cerebrospinal fluid
DBS	Deep brain stimulation
DCS	D-cycloserine
DLPFC	Dorsal lateral prefrontal cortex
DMPFC	Dorsal medial prefrontal cortex
DSM	Diagnostic and Statistical Manual of Mental Disorders
DTD	Difficult to treat depression
ECAT	Emotional Categorisation Task
ECT	Electroconvulsive therapy
EMEM	Emotional Recognition Memory Task
EPQ	Eysenck Personality Questionnaire
EREC	Emotion Recall Task
ETB	Emotional Test Battery
FDOT	Facial Dot-Probe Task
FERT	Facial Expression Recognition Task
fMRI	Functional magnetic resonance imaging
FWHM	Full width at half maximum
GluN1	Glutamate receptor ionotropic, NMDA 1
GluN2	Glutamate receptor ionotropic, NMDA 2
GM	Grey matter
HAMD	Hamilton Rating Scale for Depression
HF	High frequency
ICD	International Statistical Classification of Diseases and Related Health Problems
IQ	Intelligence quotient
ITI	Intertrial interval
LTP	Long-term potentiation
MADRS	Montgomery–Åsberg Depression Rating Scale
MDD	Major depressive disorder

Mg2	Magnesium ions
MIST	Montreal Imaging Stress Task
MPFC	Medial Prefrontal cortex
NE	Noradrenaline
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
N_2O	Nitrous oxide
NRI	Noradrenaline reuptake inhibitor
OCD	Obsessive-compulsive disorder
OCST	Oxford Cognition Stress Task
PANAS	Positive and Negative Affect Schedule
PFC	Prefrontal cortex
ph	Pharmacological
phfMRI	Pharmacological fMRI
QIDS-SR	Quick Inventory of Depressive Symptomatology Self Report
ROI	Region of interest
rTMS	repetitive transcranial magnetic stimulation
SCID	Structured clinical interview
SCR	Skin conductance response
SD	Standard deviation
SNRI	Serotonin noradrenaline reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
STAI-S	Spielberger State Anxiety Inventory
STAI-T	Trait Anxiety Scale
STW	Sport-The-Word
TCA	Tricyclic antidepressant
TRD	Treatment resistant depression
TSST	Trier Social Stress Test
VAS	Visual analogue scales
VNS	Vagal nerve stimulation
WHO	World health organization
WM	White matter

Chapter 1: Research Background

1.1 Part A: Major Depressive Disorders (MDD) & Treatment Resistant Depression (TRD)

1.1.1 MDD: Diagnosis, Epidemiology & Treatment

Major depressive disorder (MDD) is an affective disorder, which is characterised by persistent low mood, loss of energy and anhedonia. MDD is accompanied by a variety of other cognitive, vegetative and behavioural changes, such as difficulties in concentration, reduced engagement in pleasurable activities and difficulties with sleep (Belmaker and Agam, 2008). Patients with MDD often present with suicidal thinking and behaviours, and MDD carries a significant mortality worldwide (Belmaker and Agam, 2008). Without treatment, a single episode of untreated depression will last between 12 and 16 weeks (although the duration is not normally distributed), and 50% of cases of depression are recurrent (Kessler et al., 2003, Eaton et al., 2008).

The most widely used tools for diagnosing MDD are the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (American Psychiatric Association, 2013) and the global health agency World Health Organization's International Statistical Classification of Diseases and Related Health Problems, Eleventh Edition (ICD-11) (WHO, 2018).

According to the DSM-5 diagnostic criteria for major depression, five or more of the following symptoms should be present within the same two-week period, and at least one of the symptoms must be either 1）depressed mood or 2）loss of interest or pleasure.

1. Depressed mood most of the day, nearly every day;

2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day;
3. Significant weight changes or change in appetite;
4. Sleep habit changes (insomnia or hypersomnia);
5. Psychomotor agitation or retardation;
6. Loss of energy or feelings of fatigue;
7. Feelings of worthlessness or excessive or inappropriate guilt;
8. Diminished ability to think or concentrate or indecisiveness;
9. Recurrent thoughts of death, suicide plans, or suicide attempts.

For a diagnosis to be established, these symptoms need to cause clinically significant distress or impairment of important areas of functioning, such as social, educational, and occupational. Furthermore, these symptoms are not to be attributable to the physiological effects of a substance, or to a general medical condition.

The diagnostic criteria for depression in ICD-11 are very similar to those of DSM-5, although the symptom of 'hopelessness' is given a significant weight in ICD-11. Also, in ICD-11, bereavement, while not being an exclusion for the diagnosis of major depression, requires a higher threshold of symptoms for the diagnosis of depression to be made. Like DSM-5, ICD-11 classifies depression as being either a single episode or part of a recurrent illness (Stein *et al.*, 2020). Also, like the DSM-5, ICD-11 has 'qualifiers' (specifiers in DSM-5) which allow the rather heterogeneous picture of major depression to be further classified according to the severity of the episode and other features such as the presence of psychotic symptoms or prominent anxiety symptoms.

Because of its recurrence throughout the lifespan, high relapse rate, social and occupational disability, and increased risk of suicide, MDD has become a global public health problem. According to a World Health Organization (WHO) World Health Survey, the 12-month prevalence of depression episodes (based on the ICD-10 criteria) averaged 3.2% among 245404 participants from 60 countries (Moussavi *et al.*, 2007). According to the latest WHO data from 2017, there were over 300 million people suffering from depression worldwide in 2015, which represented 4.4% of the global population. Moreover, there were more female than male patients (5.1% vs 3.6% of the total world population, respectively) (WHO, 2017). Rates of depression are higher in the unemployed and divorced.

Depression was projected to be the second leading cause of disability across all age groups by 2020 (Kessler and Bromet, 2013). For example, in the United States, the economic burden of depression was approximately $210.5 billion in 2010, which represented an increase of 21.5% compared to approximately $173.2 billion in 2005 (Greenberg *et al.*, 2015). Moreover, MDD increases susceptibility to other somatic conditions, such as cardiovascular, metabolic and neurological diseases (Hare *et al.*, 2014, Rickards, 2005, Van der Kooy *et al.*, 2007). These significant co-morbidities lead to higher overall morbidity and mortality rates (Rovner *et al.*, 1991).

MDD is generally regarded as a condition with a multifactorial causation. These causes include biological factors (genetic susceptibility, neurochemical changes, alterations in gut microbiota, inflammation, and cytokines) and psychosocial factors (personality traits such as neuroticism, poor stress tolerance, exposure to trauma, increased life stress, poor coping strategies, interpersonal difficulties, etc.) (Belmaker and Agam, 2008, Hames *et al.*, 2013, Klein *et al.*, 2011, Morris *et al.*, 2012, Young *et al.*, 2014).

When treating depression, for patients with mild to moderate depression, psychotherapy, particularly cognitive-behavioural therapy (CBT), is regarded as the first-line treatment (Parikh *et al.*, 2016). For patients with more severe conditions or where psychotherapy has not been effective, antidepressant medication is appropriate. The mainstay of antidepressant treatment is treatment with drugs that potentiate the activity of monoamines, particularly serotonin (5-hydroxytryptamine) [5-HT] and noradrenaline (NE). Most antidepressant drugs achieve this effect by blockade of the reuptake of NE and 5-HT into the presynaptic nerve ending.

The original agents with this effect were tricyclic antidepressants (TCAs), such as amitriptyline and imipramine. While these agents are effective, their clinical utility is limited by adverse effects and danger in overdose. These problems are largely due to the actions of TCAs at other neurotransmitter receptors, for example, muscarinic cholinergic and histaminic H1 receptors while cardiotoxicity is attributable to an action on membrane excitability (Anderson, 2001). These problems led to the development of more selective reuptake blockers such as selective serotonin reuptake inhibitors (SSRIs), and serotonin NE reuptake inhibitors (SNRIs) whose acute pharmacological actions are essentially confined to blockade of 5-HT and NE reuptake (Spina *et al.*, 2008). These agents are better tolerated and safer than TCAs and have thereby come to have a predominant role in the drug treatment of depression.

It is worth noting that the newer antidepressants are not more effective than TCAs in the treatment of MDD. A recent large meta-analysis included 522 trials comprising 116,477 participants and aimed to compare the efficacy of 21 antidepressants in the acute treatment of MDD. The results showed that the most effective antidepressant was amitriptyline and that reboxetine was the least

effective (Cipriani et al., 2018). The British Association for Psychopharmacology guidelines for the treatment of MDD with antidepressant suggests that TCAs should be reserved for patients in whom first-line antidepressant treatment has not been effective (Cleare et al., 2015).

In addition, conventional antidepressants have a relatively slow onset of action requiring a number of weeks before clear clinical improvement in depressive symptoms is apparent. This delayed onset of antidepressant action may lead to patients being at a prolonged risk of suicide due to the persistence of depressed mood (Gourion, 2008). There is an urgent need to develop antidepressants with a more rapid onset of action. A meta-analysis indicated that early improvement during the first 2 weeks of treatment with antidepressant medication can predict treatment outcome (stable response and remission) at weeks 4 or later with continued treatment in patients with MDD (Arjen et al., 2009). These results suggest that after the first 2 weeks of antidepressant treatment, clinicians should consider changing the treatment regimen in non-responders.

1.1.2 TRD: Definition, Epidemiology & Treatment

Despite the significant developments regarding antidepressants in recent years, the rate of response and remission in MDD patients is still only modest. Currently, about 40% to 50% of patients are unresponsive to first-line antidepressant therapy; moreover, only about 35%-40% patients have relatively complete symptom remission during first line treatment (Connolly and Thase, 2011, Fava, 2003). A recent systematic review noted that 50% of patients require at least one second-line antidepressant treatment to achieve remission (MacQueen *et al.*, 2017).

An important pragmatic study, the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study by the US National Institute of Mental Health, took seven years to complete, included 4,041 patients in total, and had four designed stages of treatment (Rush *et al.*, 2006). Patients who failed to recover at one stage moved on to the next stage of treatment. STAR*D used depressive symptom remission as a treatment goal, and the results showed that after four adequate courses of antidepressant treatment over about a year, about one third of patients still failed to achieve complete remission. Moreover, the more the number of treatment steps, the higher was the subsequent relapse rate in patients who did eventually remit. In fact, patients who failed to remit following the first two courses of antidepressant medication, faired relatively poorly subsequently.

Patients who fail to respond adequately to two courses of antidepressant treatment are conventionally defined as having treatment resistant depression (TRD). These patients are at a significantly increased risk of suicide, and even if they ultimately recover, their professional, social, and family life can be persistently impaired. Furthermore, TRD patients have a higher frequency and length of hospitalization, and are at increased risk of experiencing co-morbid medical diseases such as hypertension and diabetes, which results in higher morbidity and mortality rates (Mrazek *et al.*, 2014, Olchanski *et al.*, 2013).

In general, as noted above, TRD refers to inadequate response of a depressed patient who has been treated with at least two courses of antidepressant medication of adequate dosage and duration (4-8 weeks) (Nemeroff, 2007). This definition of TRD has been criticised because it does not include response to psychological treatment and describes a rather heterogenous group of depressed patients. The concept of ‘Difficult to Treat Depression‘ (DTD) may be superior because it allows

a more holistic assessment of the patient, their illness and its treatment (McAllister-Williams *et al.*, 2020). However, in this thesis the usual definition of TRD will be used as it is generally employed in trials of drug treatment for this patient group. Generally patients with TRD are thought to represent about10% to 30% of all patients with MDD (Al-Harbi, 2012).

Currently, there are a number of pharmacological and other 'biological' strategies for depressed patient who have failed to recover after two courses of antidepressant medication.

1) Combining two antidepressants with different mechanisms of action (Lam *et al.*, 2002). Most commonly, an SSRI or SNRI is combined with mirtazapine, an antidepressant which increases NE release though alpha$_2$-adrenoceptor antagonism. Alternatively, particularly in the US, SSRIs might be combined with bupropion, an NE and dopamine re-uptake inhibitor. Rush et al (2011) compared the efficacy of these drug combinations with that of SSRI monotherapy to see whether combination strategies have higher remission rates in the acute phase (12 weeks) and long-term treatment (7 months) of over 600 depressed outpatients. The results showed that the remission rates were moderate (37.8–38.9%), and there were no significant differences between treatment groups at 12 weeks or 7 months. Also, in the combination antidepressant group, the patients experienced more adverse events (Rush *et al.*, 2011).

   However, results in TRD patients with this form of combination treatment are also not particularly encouraging. A large placebo-controlled UK study in TRD patients in primary care showed no clear clinical benefit of adding mirtazapine to SSRI or SNRI treatment (Kessler *et al.*, 2018). In STAR*D, which was not placebo-controlled, bupropion

augmentation of ineffective citalopram was superior on some secondary outcome measures to augmentation with the anxiolytic drug buspirone (Bech *et al.*, 2012).

2) Another general pharmacological approach to TRD is to use what is called augmentation therapy. In augmentation, a drug which is usually not itself an antidepressant is added to an existing antidepressant medication, with the aim of achieving an improved clinical response. The best evidence for a particular augmentation strategy comes from the addition of atypical antipsychotic drugs such as quetiapine and aripiprazole. At low doses (below the antipsychotic range) these medications can produce useful antidepressant effects in patients with suboptimal responses to SSRIs and SNRIs.

Spielmans *et al.* (2016) carried out a meta-analysis of randomised, placebo-controlled trials of atypical antipsychotic addition to primary antidepressant treatment in about 3,500 patients with TRD. Useful antidepressant effects were found with quetiapine, aripiprazole and risperidone. Olanzapine produced somewhat lesser benefit. However, atypical antipsychotic addition in depression can cause problematic side effects including sedation, weight gain and extrapyramidal movement disorders.

Lithium augmentation of antidepressant medication is also regarded as a useful therapeutic strategy. However, the total numbers of patients studied in randomised studies is very small (n=237) (Nelson *et al.*, 2014). The take up of lithium augmentation treatment in clinical practice is low, probably because of the need for blood tests and perceived poor tolerance and renal toxicity.

Zhou *et al.* (2015) carried out a network meta-analysis of 48 randomised antidepressant augmentation studies involving over 6500 participants with TRD. The augmentation strategies studied included atypical antipsychotics, lithium and T3 (thyroid hormone) but also methylphenidate, pindolol, lamotrigine and buspirone. This meta-analysis found that quetiapine, aripiprazole, T3 and lithium were significantly more effective than placebo, with the most robust efficacy estimates for aripiprazole and quetiapine.

Carter et al. (2020) conducted a meta-analysis aiming to examine the efficacy of augmentation treatment (antipsychotic, mood stabilizers, NMDA antagonists targeting medications, other mechanisms) relative to placebo in TRD patients; treatment with NMDA antagonists was superior to placebo and had the highest efficacy relative to other pharmacological classes.

3) Another treatment approach for TRD involves various direct forms of neural stimulation (Vieta and Colom, 2011), of which electroconvulsive therapy (ECT) has been in clinical use for several decades. A recent meta-analysis indicated that bitemporal ECT and high dose right-sided unilateral ECT were associated with increased treatment response as an alternative or add-on therapy for patients with MDD (Mutz et al., 2019). The main adverse effect of ECT is memory impairment, particularly long term episodic memory or autobiographical memory (Semkovska and McLoughlin, 2010). ECT is effective in TRD but the relapse rate is high making its use problematic in the management of chronic refractory depression (Cowen and Anderson, 2015).

Studies have confirmed that a proportion of TRD patients experience short-term relief after repetitive transcranial magnetic stimulation (rTMS), which uses a powerful magnetic field to produce current flow in neural tissue (Miniussi *et al.*, 2005). However, a widely accepted 'standard' for rTMS has not been established. Furthermore, important factors including stimulation site, frequency, intensity, pulse count, safety, etc., have not been clarified. Allan *et al.* (2011) carried out a meta-analysis of 1300 depressed patients randomised to either TMS or sham treatment. There was a greater clinical response for active treatment (36% versus 15%) and the presence of antidepressant treatment resistance was not a predictor of outcome.

Vagal nerve stimulation (VNS) is used in the treatment of refractory epilepsy and has also been employed in patients with severe and longstanding TRD. A number of observational studies have assessed the efficacy of VNS in TRD patients at 5-year follow-up. Overall, compared to treatment as usual, VNS in combination with conventional medication significantly increased response and remission rates for resistant depression patients (response rate was 67% versus 40%) (Aaronson *et al.*, 2017). However, its application is limited by the requirement for surgery and potential side effects. It is also important to note that these neurostimulation methods are only effective in a subset of TRD patients. Most patients still fail to meet the criteria of effectiveness or remission at long-term follow up assessments (Ontario, 2016).

1.2 Part B: Glutamate and N-methyl-D-aspartate (NMDA) receptors: relevance to depression

Glutamate is the most important excitatory neurotransmitter in the mammalian and is unevenly distributed in the central nervous system. Glutamate neurons are found in cortical and subcortical structures such as the hippocampus, striatum, and cerebellum. These are involved in critical physiopathological processes including cognition, learning, and memory, as well as in neurodegenerative diseases (Krystal *et al.*, 1999, Krystal *et al.*, 2010). Glutamate receptors can be divided into ionotropic and metabotropic glutamate receptors.

The NMDA receptor (NMDAR), an important subset of ionotropic glutamate receptors, is at the core of central nervous system development. In pathological states, overactivate NMDARs produce excitotoxicity, which is associated with a variety of central nervous system (CNS) disorders such as Parkinson's disease, Alzheimer's disease and mood disorders (Blandini *et al.*, 1996, Butterfield and Pocernich, 2003, Sanacora *et al.*, 2008). Schizophrenia is also proposed to be a disorder of NMDA hypofunction (Tsai and Coyle, 2002). Given the important role of NMDA excitotoxicity in many neurological diseases, the NMDAR has become a key therapeutic target to protect against neuronal damage by antagonizing NMDA overactivation to attenuate excitotoxicity.

The NMDAR is composed of four subunits, two GluN1 and two GluN2 surrounding an ion channel that is blocked by magnesium ions (Mg^{2+}). Depolarisation removes this Mg^{2+} block, which allows channel blockers such as ketamine and lanicemine to enter the channel. GluN1 subunits also contain strychnine insensitive glycine binding sites. Glycine is a co-agonist at the NMDAR and both the NMDAR and the glycine receptor need to be occupied by the relevant neurotransmitter

for the NMDAR ion channel to open (Gerhard *et al.*, 2016). The amino acid, D-serine, also functions as a co-transmitter at the glycine site.

NMDARs have dual effects on learning and memory (Li and Tsien, 2009, Lüscher and Malenka, 2012). When glutamate binds to NMDARs, postsynaptic neurons produce the physiological effect of long-term potentiation (LTP), which promotes learning and memory. NMDARs in the hippocampus have an important role in mediating the generation and maintenance of LTP during the period of learning and memory. Thus, in rats, NMDAR antagonism impairs learning and memory (Butelman, 1989, Morris *et al.*, 1986). On the other hand, NMDAR-mediated excitotoxicity and the over-release of glutamate could cause pathological LTP in the hippocampus; this could impede the information transfer and also cause impairment of learning and memory (Castellano *et al.*, 2001).

Animal experimental studies have suggested a role for the NMDAR in the treatment of depression. For example, Trullas and Skolnick (1990) found that NMDAR antagonists can reverse behavioural despair in an animal model of depression. Upon analysing the effects of different NMDAR antagonists (MK801 [non-competitive NMDA antagonist] and AP7 [competitive NMDA antagonist]) after 15 minutes administration in mice, significant reductions in the duration of immobility in the forced swimming test and tail suspension test were observed. In addition, other competitive NMDARs antagonists (CGP 40116, CGP37849) and MK-801 also showed positive effects in a chronic mild stress model of depression in rats (Papp and Moryl, 1994). Garcia *et al.* (2008) reported that, compared with saline, acute doses (10 and 15 mg/kg) of the NMDAR non-competitive antagonist ketamine reduced the immobility time in the forced swimming test. Finally,

Skolnick et al (1996) found that repeated administration of different types of conventional antidepressant in mice produced adaptive changes in NMDARs. These authors suggested that the NMDAR may be a key mechanism in mediating the therapeutic effects of antidepressant treatment (Skolnick *et al.*, 1996).

1.2.1 Discovery of the antidepressant effects of ketamine

Ketamine is a US FDA-approved anaesthetic agent. It is a high affinity, non-competitive NMDAR antagonist which binds in the open ion channel of the NMDAR. In 2000, the results of a small, double-blind, placebo-controlled, cross-over study showed that intravenous infusion of a low (subanaesthetic) dose of ketamine (0.5 mg/kg) produced a rapid antidepressant effect within 4 hours (Berman *et al.*, 2000). Intriguingly this effect persisted for at least 3 days after the ketamine infusion, showing a clear separation from the acute subjective effects of ketamine. In a subsequent randomised double-blind crossover controlled trial (Zarate *et al.*, 2006), TRD patients who received ketamine (0.5 mg/kg) showed significant improvement in depressive symptoms within 110 min post injection; furthermore, depressive symptom improvement was maintained for up to one week. More than 70% of patients achieved responses the day following ketamine treatment.

Control infusions in the latter studies were with saline which can be easily distinguished from the subjective effects of ketamine. Murrough *et al.* (2013) conducted a large sample clinical trial using the benzodiazepine, midazolam, instead of saline as a control for ketamine and showed that ketamine was more effective than midazolam after 24 hours of treatment, with the response rate being 64% and 28% for the ketamine and midazolam groups, respectively. However, after 7 days, there was no statistically significant difference between the two groups. Compared to

conventional antidepressants, the most important advantage of ketamine is its rapid onset of action. As noted above, the delayed onset of action of conventional antidepressants can increase morbidity as well as the risk of suicide of depressive patients in the early stages of treatment.

Many clinical randomised controlled trials have replicated and extended our understanding of the rapid antidepressant effects of ketamine. A systematic review reported that ketamine has a rapid and significant antidepressant effect on TRD patients and that depressive symptoms are significantly improved within hours of ketamine treatment (Serafini *et al.*, 2014). Furthermore, a meta-analysis summarizing the data from 21 clinical studies, revealed that a single administration of ketamine has significant and rapid antidepressant effects on depressive symptoms from 4 hours to 7 days in MDD and bipolar disorder. However, these effects are no longer significant after 12–14 days of injection (Coyle and Laws, 2015). Another review found that a single dose of ketamine could rapidly and significantly relieve suicidal ideation in depressed patients within 24 hours. These studies reaffirmed the important role of ketamine in rapidly reducing suicide risk for depressed patients (Wilkinson *et al.*, 2018).

Besides the intravenous and intramuscular administration routes, ketamine can also be administered orally and nasally. Case reports by De Gioannis and De Leo (2014) demonstrated the effectiveness of oral ketamine administration for TRD patients who have suicidal ideation. Daly *et al.* (2018) conducted a randomised controlled study that evaluated the efficacy and safety of intranasal esketamine for TRD patients. Sixty-seven patients were screened and randomly assigned to the esketamine and placebo groups in a 1:1 ratio. The esketamine group was divided into three different dose groups (28 mg, 56 mg, and 84 mg) and patients were treated twice weekly. The

results showed that patients in all three esketamine dose groups showed effective improvements in depression after one week of treatment. Moreover, it was also found that antidepressant efficacy was dose dependent, and TRD patients who received higher doses had greater improvement and durable efficacy. Esketamine nasal spray medication was approved by the US Food and Drug Administration and European Medicines Agency for the treatment of TRD in 2019 (Cristea & Naudet, 2019; Mahase, 2019).

Regarding the safety of intravenous ketamine use, a recent systematic review by Short *et al.* (2018), which included 60 studies in total, comprehensively summarized the side effects of ketamine. The results showed that side effects are frequently reported after acute administration of ketamine when compared with placebo treatment, although these usually resolved within a short period of time. Anxiety, irritability and euphoria are often described. Dissociative effects are also common as are transient psychotic experiences. Cognitive impairment is frequently apparent during the infusion but quickly resolves. Similarly, increased heart rate and hypertension are common but transient.

Ketamine is misused for recreational purposes and there have been concerns about dependence and bladder problems during long-term use. Currently, there is limited evidence on the addictive nature of ketamine during the treatment of depression. Another review by Sanacora *et al.* (2017) indicated that frequent and long-term use of ketamine could trigger the risk of dependence and could possibly lead to enduring cognitive deficits. Importantly, a study by Williams *et al.* (2018) found that pre-treatment with naltrexone (opioid receptor antagonist) can attenuate the antidepressant effects of ketamine on TRD patients, hence indicating that these effects might require opioid system activation. This increases concerns about the risk of drug dependence when

using ketamine repeatedly. Therefore, developing an antidepressant which has similar antidepressant effects to ketamine, while lacking addictive potential and psychomimetic effects, has become an important goal for drug development.

1.2.2 Lanicemine (AZD6765) and memantine

Lanicemine is a low-trapping NMDAR antagonist. 'Trapping' refers to the extent to which a drug is liable to remain in the NMDA ion channel after the removal and re-application of glutamate. Due to their high-trapping and slow dissociation rates, high-trapping NMDAR antagonists such as ketamine and MK-801, can cause prolonged blockade of normal glutamate physiological function and result in serious side effects. Therefore low-trapping NMDAR antagonists are predicted to have fewer side effects because of their high dissociation rates—the accumulation of the drug in the channel would be avoided, thus reducing the inhibition of normal synaptic activity (A Jaso *et al.*, 2017, Mealing *et al.*, 1999).

Lanicemine shows 54% of trapping compared to ketamine (86% trapping) (Becker *et al.*, 2019). This is the theoretical hypothesis for the ability of lanicemine to retain its antidepressant effects while having fewer psychomimetic dissociative effects. Zarate *et al.* (2013) found that TRD patients showed a significant but short-lived reduction in depressive symptom 80 min after single administration of lanicemine compared to that of placebo. Furthermore, Sanacora et al. (2014) using quantitative electroencephalography techniques, found that lanicemine showed antidepressant effects in TRD patients consistent with NMDAR channel blockade, without producing psychomimetic and dissociative effects.

However, a large randomised, placebo-controlled, trial of lanicemine (15 intravenous infusions over twelve weeks) in TRD showed no significant antidepressant effect. In this phase 3 clinical trial (Sanacora *et al.*, 2017), 302 patients between 18 to 70 years of age from four countries were included. The inclusion criteria were a single episode or recurrence of depression and an inadequate patient response to antidepressant medication. Four weeks prior to screening, patients were allowed to receive at least 4 weeks of a different antidepressant medication and then were randomly assigned to receive 50 mg or 100 mg lanicemine or saline intravenously for 12 weeks without discontinuation of their previous antidepressant medication. The study found that 80% of the patients enrolled eventually completed the full course of treatment, and although lanicemine was well tolerated overall, it was not superior to placebo in reducing depressive symptoms.

Although clinical development of lanicemine was discontinued, greater understanding of the neurobiological effects of different NMDA antagonists in the treatment of depression may be helpful in identify promising biomarkers for the future development of novel rapid-acting drugs without psychomimetic and dissociative effects. For instance, a recent study (Becker et al., 2019) aimed to understand how the network effect induced by different NMDA antagonists is associated with antidepressant or dissociative effects. The results showed that lanicemine showed a lack of significant effects on network topology and hippocampal-prefrontal coupling compared to those of other NMDA antagonists, such as ketamine. The absence of intra-frontal cortex hyperconnectivity in response to lanicemine could help explain the lack of dissociative side effects and lower antidepressant effects (Becker, 2019).

Another NMDAR antagonist, memantine, is also an NMDAR ion channel blocker with low trapping ability relative to ketamine (Parsons *et al.,* 1999). Memantine is an oral drug licensed for the treatment of Alzheimer's disease. Generally, it is well tolerated and does not cause psychotomimetic effects or drug dependence.

Zarate *et al.* (2006) conducted a double-blind controlled trial study of memantine in 30 patients with MDD. Compared to placebo, memantine did not produce an antidepressant effect after 8 weeks of treatment. Smith *et al.* (2013) also found no effect of memantine to relieve depression in a placebo-controlled trial in 31 patients with TRD. A meta-analysis by Kishi *et al.* (2017) confirmed that memantine appears to lack significant antidepressant properties in either MDD or bipolar depression.

A number of animal experimental studies have explored why memantine may lack clinical antidepressant properties despite being an NMDAR channel blocker. These studies have shown that ketamine but not memantine can promote the rapid expression of brain-derived neurotrophic factor (BDNF) in the hippocampus. This is consistent with the view that the antidepressant effect of ketamine relates to mechanisms of action involving synaptic plasticity and neurotrophic signaling (Autry *et al.,* 2011, Gideons *et al.,* 2014). One can certainly conclude that not all NMDAR antagonists have a rapid antidepressant effect in TRD patients.

1.2.3 Nitrous oxide

Nitrous oxide (N_2O), also known as laughing gas, is used as an analgesic and anaesthetic in dentistry, childbirth, etc. and also to supplement general anaesthesia in surgical operations. It was

found in animal studies that N_2O, at routine clinical doses, could protect against glutamate excitotoxicity, similarly to other non-competitive NMDAR antagonists (Zorumski *et al.*, 2015). Jevtović-Todorović *et al.* (1998) also found in vitro that N_2O blocked ionic currents produced by NMDAR activation. The precise site of action of N_2O at the NMDAR is not established but unlike ketamine it does not involve open channel blockade (Zorumski *et al.*, 2015).

There is only limited evidence on the potential utility of nitrous oxide as a treatment for depression. Nagele *et al.* (2015) conducted a first proof-of-concept clinical trial of 20 patients with TRD who were randomised to receive 1 hour of inhalation treatment with either a 1:1 mixture of N_2O and oxygen or a 1:1 mixture of nitrogen and oxygen (as placebo) at an inhalation concentration of 44%. Fifteen patients completed the study; two patients discontinued N_2O treatment due to side effects of anxiety and nausea. Compared to the placebo group, the patients who were treated with N_2O had significant improvement in depression symptoms at 2 hours and 24 hours. Nevertheless, replication studies are needed in the future to explore the efficacy of N_2O in the treatment of TRD.

N_2O has some advantages over ketamine for the treatment of depression. The method of usage and safety of this gas is well understood, and it has been used clinically as an anaesthetic agent for over 150 years. Once N_2O stops being inhaled, the gas is quickly cleared from the body through the lungs; thus, the blood concentration of the drug drops quickly. It is important to note that the psychotomimetic effects of N_2O are of lesser extent than those of ketamine and is relatively safe to use (Zorumski *et al.*, 2015). N_2O is, in fact, widely used as a recreational agent in order to produce a brief feeling of euphoria and mild feelings of dissociation. Adverse effects are transient and include nausea, fainting, confusion and occasionally hallucinations.

Clinical studies also describe the relative safety of N_2O. For instance, a large sample study of 35828 hospitalized adult and pediatric patients (Onody *et al.*, 2006) investigating the side effects of the inhalation of 50% concentration of laughing gas and oxygen found that 4.4% of patients experienced adverse events, and only 0.08% of patients experienced serious adverse events. However, long-term inhalation of laughing gas might result in nerve damage through vitamin B12 deficiency (Garakani *et al.*, 2016).

1.2.4 Other NMDA receptor antagonists

The striking therapeutic effect of ketamine in patients with TRD has led to a burgeoning of research interest in other drugs acting at the NMDAR complex. As noted above, the NMDAR is a tetramer composed of four subunits. The GluN2 subunit is generated by four separate genes (GluN2A, GlunN2B, GluN2C, GluN2D) (Hansen *et al.*, 2014). The GluN2B-containing NMDA receptor has attracted particular interest because it is located at extrasynaptic as well as synaptic sites and appears to play a role in glutamate-induced excitoxicity. A class of drugs called GluN2B antagonists bind to an extracellular allosteric site on the GluN2B subunit of the NMDAR.

Preclinical studies have shown that GluN2B antagonists can ameliorate stress and depressive symptom in animals (Li *et al.*, 2011, Wang *et al.*, 2006). Ibrahim *et al.* (2012) conducted a small, randomised, double-blind clinical trial of the orally available, selective GluN2B antagonist, MK-0657, where patients with TRD were randomly assigned to an active treatment group (4-8 mg/day) and a placebo group. Patients in the active treatment group demonstrated significant antidepressant effects on day five after treatment, without experiencing psychotomimetic and dissociative effects.

Another selective GluN2B antagonist, traxopodril (CP101,606), has been studied by Preskorn *et al.* (2008) who conducted a double-blind placebo-controlled clinical study in 30 TRD patients. Over five days a significant antidepressant response was seen in 60% of traxopodril patients versus 20% of the placebo-treated group. Furthermore, compared to ketamine, the psychotomimetic effects of traxopodril appear small. However, these interesting findings do not appear to have been followed up probably because the drug was found to cause abnormalities in cardiac conduction.

1.2.5 NMDAR co-agonists

It was noted above that for the NMDAR to be activated, the glycine site on the NMDAR needs to be occupied by a neurotransmitter, either glycine or D-serine. Hence pharmacological modulation of the glycine site is another means to alter NMDAR function in patients with TRD (Chan *et al.*, 2017).

Animal studies found that the NMDA co-agonist, D-serine, reduced immobility in the forced swim test and led to improvements in the learned hopelessness paradigm (Malkesman *et al.*, 2012). Huang *et al.* (2013) also found that the amino acid, sarcosine, which prevents re-uptake of glycine, decreased immobility in the forced swim test and tail suspension test. These findings, similarly to those regarding ketamine antidepressant effects, suggested that NMDA co-agonists may have potential antidepressant/anxiolytic effects. In addition, an acute dose of D-serine was found to be effective in reducing anxiety and sadness and in enhancing cognitive parameters in healthy subjects (Levin *et al.*, 2015).

In another clinical study conducted by Huang *et al.* (2013), 40 patients with MDD were randomly assigned to receive sarcosine or citalopram for 6 weeks. The authors found that, compared with citalopram, sarcosine led to significantly greater and faster depressive symptom improvements and a higher remission rate. Based on previous findings, both NMDA antagonists and co-agonists have antidepressant effects, and the pathophysiology of depression may involve both upregulation (enhancement) and downregulation (inhibition) of NMDAR function in distinct neural networks through specific signalling pathways (Chan et al., 2017).

1.2.6 NMDAR glycine receptor partial agonists

There has also been interest in the activity of partial agonists at the glycine site. GLYX-13 (rapastinel), with 25% of the activity of a glycine site full agonist, acts like an NMDAR glycine site partial function agonist. GLYX-13 is a cognitive enhancer and can facilitate learning and memory in animal and human studies (Moskal *et al.*, 2014). Furthermore, like ketamine, GLYX-13, produced rapid antidepressant effects in an animal study (Burgdorf *et al.*, 2013). GLYX-13 acts as a partial agonist at low levels of NMDAR activity, but as an antagonist at higher levels of NMDAR activity, which may explain why GLYX-13 may have ketamine-like antidepressant effects without psychotomimetic side effects.

Preskorn *et al.* (2015) reported that GLYX-13 effectively reduced depressive symptoms in depressed patients nonresponsive to previous antidepressant treatment in a short period of time (2 h) and that the antidepressant effect lasted for 7 days. Importantly, GLYX-13 did not show psychotomimetic side effects in this clinical trial. However, in a series of phase III trials in TRD,

GLYX-13 apparently failed to meet its primary endpoint, though these data have not yet been fully reported.

D-cycloserine (DCS) is a natural product of species of *Streptomyces* and has long been used in the antibiotic treatment of tuberculosis. In addition, studies have suggested that DCS is a partial agonist of the NMDAR, behaving as an agonist at low doses (up to 250mg daily) but as an NMDAR antagonist at higher doses, blocking the co-agonist function of glycine and reducing the activity of the NMDAR (Schade and Paulus, 2016).

When acting as a glycine site partial agonist, DCS has been shown to have cognition-enhancing properties. Previous studies have also reported that DCS as an adjunctive therapy could significantly improve cognitive function and clinical symptoms in patients with schizophrenia (Evins *et al.*, 2002, Goff *et al.*, 1999). For depression treatment, the first study in clinical patients was conducted by Heresco-Levy *et al.* (2006); this study recruited 22 TRD patients in a 6 weeks crossover trial where 250 mg/day DCS was administered in combination with ongoing antidepressant medication. The results indicated that although DCS treatment apparently reduced the severity of depressive symptoms, no significant differences were found compared to the cross-over period when patients were taking placebo.

A subsequent, controlled clinical trial was conducted by the same study group, again in TRD patients (Heresco-Levy *et al.*, 2013); 26 participants continuing their ongoing antidepressant treatments were randomly allocated to receive either a gradually titrated high dose of DCS (1000 mg/day) or placebo for 6 weeks. On this occasion, DCS was superior to placebo with 54% of DCS-

treated patients showing a clinical response versus 15% in the placebo group. Furthermore, Kantrowitz *et al.* (2015) found that daily DCS administration (dose increasing to 1000mg/d) prolonged the antidepressant response to ketamine in patients with treatment-resistant bipolar depression over 3 weeks. The study was not placebo controlled but is consistent with the notion that high doses of DCS may have the same antidepressant-like effects as NMDA antagonists like ketamine.

Low dose DCS has been extensively studied as an adjunct to psychological treatments. Kim *et al.* (2016) used low dose DCS to enhance extinction learning during exposure treatment for obsessive-compulsive disorder (OCD). However, unexpectedly, this study documented a large improvement in depressive symptoms in the patients treated with DCS compared to those receiving placebo. This finding was consistent with that of a study by Wilhelm *et al.* (2008), where DCS was used to enhance the efficacy of behaviour therapy for OCD; results indicated that there was a significant improvement in depressive symptoms which were independent of improvement in OCD symptoms. A recent review indicated that the antidepressant effect of DCS is most apparent when used as an adjuvant in combination with other antidepressants, while the effects of DCS as an independent antidepressant remain largely unexplored but probably require a high dose treatment (Brainch *et al.*, 2018). Therefore, further studies are required to evaluate how DCS may function as an antidepressant in combination with both conventional antidepressant medicines and with psychotherapies.

In addition, stress-related disorders are also associated with abnormalities in the central glutamatergic nervous system, particularly with dysfunctional NMDARs (Bergink *et al.*, 2004,

Popoli *et al.*, 2012). In animal models, different NMDAR antagonists, such as the non-competitive NMDA antagonist (MK-801) and the competitive NMDA antagonist (CGP37849, AP-7), were shown to have anxiolytic-like effects (Plaznik *et al.*, 1994, Xie and Commissaris, 1992). Additionally, stimulation of the amygdala leads to LTP of neurotransmission at amygdala synapses, and the induction of LTP involves NMDARs (Walker and Davis, 2002).

Evidence shows that NMDARs in the amygdala play an important role in the formation of fear memory and acquisition of fear learning (Campeau *et al.*, 1992, Miserendino *et al.*, 1990, Walker and Davis, 2000). Meanwhile, the GluN2B subunit is critical for the plasticity mediating acquisition of conditioned fear. Previous studies found that injecting ifenprodil, a selective GluN2B receptor antagonist, in the amygdala before training can impair the acquisition of fear conditioning (Day *et al.*, 2011, Rodrigues *et al.*, 2001).

Walker and Davis (2008) found that the infusion of the GluN2B antagonist CP101,6060 in the amygdala before training disrupted fear conditioning in the fear-potentiated startle test. Furthermore, studies also examined the role of the NMDAR in the extinction of fear conditioning. For example, Falls *et al.* (1992) found that infusing the NMDA antagonist AP5 into the amygdala blocked the extinction of conditioned fear in the fear-potentiated startle. Several studies also suggested that MK-801 can block the extinction of conditioned responses in rats in a dose-dependent manner (Baker and Azorlosa, 1996, Cox and Westbrook, 1994, Langton *et al.*, 2007). Previous research also showed that hippocampal NMDARs are involved in the mechanism of extinction of conditioned fear (Matsumoto *et al.*, 2013, Szapiro *et al.*, 2003). Therefore, the above

studies indicate that both fear extinction and acquisition of Pavlovian conditioning are modulated by NMDARs.

Walker et al (2002) showed that systematic administration of DCS into the amygdala before extinction training significantly enhanced fear extinction but did not affect fear-potentiated startle in rats not receiving extinction training. This effect of DCS was blocked by a selective antagonist at the glycine NMDA site (HA-966) suggesting that in this case DCS was acting as an agonist or partial agonist. Follow-up preclinical studies have confirmed that DCS could increase fear extinction in rats (Ledgerwood *et al.*, 2003, 2004, Parnas *et al.*, 2005, Weber *et al.*, 2007). These results suggested facilitation of NMDAR function can promote the extinction of conditioned fear. Clinically, there is evidence suggesting that DCS is able to augment the response to CBT for anxiety and related disorders (Otto *et al.*, 2007, Otto *et al.*, 2010). A recent meta-analysis further confirmed that DCS is superior to placebo in promoting the therapeutic effects of CBT in patients with anxiety disorders, both at post-treatment and at follow-up (Mataix-Cols *et al.*, 2017). Taken together, these findings suggest that DCS enhances the effects of CBT by improving extinction learning, which is a critical element of CBT treatment for anxiety.

On the other hand, animal studies have found that low dose of DCS tends to increase anxiety responses in the plus-maze test, for example, by leading to a reduction in the open arm time, open arm entry, and open arm activity (Ho *et al.*, 2005, Moraes Ferreira and Morato, 1997). However, Ho *et al.* (2005) found that this effect was apparent only in rats with low trait anxiety. Further investigation into the effects of DCS on emotional and stress responses in humans would be of great clinical interest for the treatment of clinical depressive and anxiety disorders.

1.3 Part C: The neuropsychological model of antidepressant drug action

1.3.1 Negative information and memory bias in depression

Depressed individuals have a negative emotional processing biases. They are therefore more likely to process negative cues or decrease attention to positive information (Deldin *et al.*, 2001, Gotlib and Joormann, 2010, Gotlib *et al.*, 2004). Beck (1979) proposed that cognitive processing bias towards negative information is a core factor in the development and maintenance of depression. Particularly, if depressed patients are biased towards noticing and processing negative emotional information that is consistent with their state of mind, this would be expected to maintain the presence of the depressed state (Wright and Beck, 1983).

In order to confirm the presence of negative attentional bias in depressed patients, many classical experimental paradigms have been developed, such as the facial expression recognition and the attentional dot-probe tasks. The facial expression recognition task generally uses pictures of positive (e.g., happy, surprise), neutral, and negative (e.g., sad, anger, fear, disgust) emotional faces as stimulus material to record subject accuracy, response time, and response bias. A large number of studies have found that depressed patients have a selective attention bias toward negative facial expressions and away from positive (Bourke *et al.*, 2010). For example, compared to healthy subjects, depressed individuals show a higher accuracy and response bias for negative facial expressions (sadness) (Milders *et al.*, 2010) and reduced recognition of positive facial expressions (happiness and surprise) (Harmer *et al.*, 2009b). Douglas and Porter (2010) found that, compared to healthy participants, depressed patients tended to interpret neutral faces more often

as sad and less often as happy. In addition, Anderson et al. (2011) found that currently depressed patients had a slower reaction time when recognizing happy expressions than healthy controls. This indicates that depressed patients are relatively insensitive to positive emotional facial expressions.

In the dot-probe paradigm, Joormann and Gotlib (2007) showed that both currently depressed patients and previously depressed patients in remission have an attentional bias toward sad faces relative to control (never-depressed) subjects. The control group showed a selective attentional bias towards happy faces, but this positive bias was not apparent in either currently depressed or previously depressed patients in remission. Importantly, this study showed that an attentional bias towards negative information was also found in patients following recovery from depression. Keshavan *et al.* (2014) investigated the attentional bias towards threatening (angry faces) and happy facial stimuli in patients with mood dysregulation and found that this patient group had a significant attentional bias towards angry facial expressions. Additionally, compared to the control group, the degree of attentional bias was positively related to the severity of depressive symptoms.

Moreover, previous studies also showed that, compared to healthy controls, depressed patients have a better memory for negative words that have been self-referentially encoded than for positive words (Wisco, 2009). Tests of the self-reference effect (i.e. encoding of words in reference to self) in memory generally use the self-referential encoding task (Derry and Kuiper, 1981), which presents different positive and negative personality words to the subject and asks the subject to categorise whether these personality words describe them or not, and the participant's accuracy

and response time are recorded. After that, subjects are required to freely recall as many words as they remember from the words presented in the encoding task.

Studies have found that, compared to healthy controls, depressed subjects in the self-referential encoding task endorse more negative words, reject more positive words, and respond faster to negative words (Davis, 1979, Gilboa *et al.*, 1997), and healthy controls recall more positive words than negative words compared to depressed patients in the following recall task (Gilboa *et al.*, 1997). Harmer *et al.* (2009b) found that, compared to healthy controls, depressed individuals took longer to respond to positive self-referent words. In the subsequent recall task, healthy controls recalled a higher number of positive personality words than depressed patients. In addition, a meta-analysis showed that negative self-referential processing is significantly associated with depression (Phillips *et al.*, 2010). In a longitudinal cohort study (Lewis *et al.*, 2017) which included 534 depressed patients who completed self-referential encoding, memory recall task, and depressive symptoms were assessed every two weeks (using Beck Depression Inventory BDI). The study found that positive words recalled at baseline significantly predicted patient depressive symptoms; the fewer the positive words recalled, the greater the depression symptom score.

Roiser et al. (2012) propose a cognitive neuropsychological model of depression that suggests that negative processing bias plays an important causal role in the development of depression. Some evidence supports this notion. First, negative information processing bias was seen in individuals at high-risk of developing depression. For instance, Miskowiak *et al.* (2015) found that high-risk twins (co-twin history of hospital admission for depression) had higher attentional bias towards fearful faces than low-risk twins (no co-twin or first-degree family history of depression) in the

face dot-probe task. Chan (2007) found that individuals with a high neuroticism index without a history of depression showed increased negative processing bias in facial expression recognition, self-referential encoding, and memory. This finding suggests that a negative information processing bias may be a marker of vulnerability to developing depression. Second, a negative information processing bias can successfully predict symptom relapse in a longitudinal study. Bouhuys et al. (1999) reported that remitted depressed patients who perceived more facial emotions as negative at baseline demonstrated a significant association with a relapse of depression at the 6-month assessment. Third, both psychological treatments and antidepressant medications alter the negative processing bias (Fu et al., 2008; Clark et al., 2009), which may represent a common mechanism in the treatment of depression.

In addition, patients with depression experience memory deficits, of which the most common is autobiographical memory impairment (Gotlib and Joormann, 2010, Williams and Scott, 1988). Autobiographical memory impairment refers to the inability of individuals to extract specific events or episodes over a specific period of time and occurring at a particular place. Instead, depressed patients tend to employ extended memory (which refers to an event lasting more than a day) or categoric memory (refers to an event that occurred repeatedly); this phenomenon is known as overgeneralised autobiographical memory (Gibbs and Rude, 2004). Overall the literature suggests that patients with depression have significant difficulties in recalling specific personal memories and instead display overgeneralised autobiographical memory (Köhler *et al.*, 2015).

Previous studies suggest that difficulties with autobiographical memory specificity could contribute to the maintenance of depression through pathways that involve perceiving negative

social encounters, lack of social problem solving skills, lack of self-efficacy, and feelings of hopelessness (Hermans *et al.*, 2005, Young *et al.*, 2016). Mackinger *et al.* (2000) found that female patients in remission from depression also significantly retrieved more overgeneralized memory than healthy controls. A meta-analysis (Sumner *et al.*, 2010) including 15 studies revealed overgeneralised memory as a negative predictor of the course of depression. Thus, deficits in the specificity of autobiographical memory may serve as a cognitive marker for depression.

1.3.2 Theories of antidepressant action

The traditional theory of antidepressant drug action suggests that the delayed onset of antidepressants is due to slowly evolving downstream neuroadaptive changes, such as downregulation of post-synaptic 5-HT or noradrenergic (NA) receptors and desensitisation of 5-HT and NA autoreceptors (Frazer and Benmansour, 2002). More recently, neurobiological theories of antidepressant action (derived from animal experimental studies) have focused on the delayed ability of antidepressants to increase BDNF, neurogenesis and synaptic plasticity (Harmer *et al.*, 2017). This process is thought to underpin the mood elevating properties of antidepressants. Rapid acting antidepressants such as ketamine are believed to improve mood quickly because they promote production of BDNF and synaptic plasticity right away, without the delay seen with conventional antidepressants (Harmer *et al.*, 2017).

A new theory of antidepressant drug action - the cognitive neuropsychological model - suggests that in fact antidepressants do not act primarily on mood but rather on the processing of emotion. Experimental studies show that antidepressants produce positive biases in emotional processing from the start of treatment. This is an automatic effect which does not affect subjective mood state

immediately (Harmer *et al.*, 2009a). However, this early change in emotional bias is suggested to result in patients starting to pay more attention to the positive aspects of social stimuli/reinforcement and processing less negative information. Over time, as patients learn these new contingencies, subjective mood gradually improves. This theory provides a psychological explanation for the delayed onset of antidepressant action. Interestingly the changes in synaptic plasticity observed in neurobiological studies would be expected to facilitate the new emotional learning required by this neuropsychological theory. Methodologically this change in emotional bias is measured by the set of cognitive tasks described above (e.g., facial expression recognition, attentional dot-probe paradigm, self-referential processing, and memory tasks).

1.3.3 Cognitive effects of antidepressants in psychological tasks

Studies on both healthy populations and depressed patients support the effect of conventional antidepressant treatments to bias emotional processing in a positive direction. Harmer *et al.* (2009b) showed that a single dose of 4mg dose of the selective NE re-uptake blocker, reboxetine, significantly reversed negative emotional bias in depressed patients 3h after administration. For example, in a facial expression recognition task, patients taking reboxetine showed enhanced recognition of happy facial expressions compared to depressed patients taking placebo, and in a self-referential encoding task, patients taking reboxetine had an enhanced memory recall of positive words compared to depressed patients taking placebo. However, despite these changes in emotional processing, there were no significant differences in subjective ratings of mood between treatment groups after a single dose of reboxetine.

Tranter *et al.* (2009) found that two types of antidepressants (citalopram and reboxetine) significantly increased the recognition accuracy of positive facial expressions after 2 weeks of treatment. In addition, the results showed that there was significant correlation between increased recognition of happy faces after 2 weeks of treatment and the clinical symptom improvement after 6 weeks of treatment. These findings suggest that early changes in emotional processing due to antidepressants can predict future clinical outcomes, and the changes in emotional processing might also serve as early biomarkers to determine the efficacy of drugs in depressed patients.

As an example of a study in healthy, non-depressed, participants, Harmer *et al.* (2004) randomly assigned 42 healthy volunteers to take an SSRI (citalopram), reboxetine, or placebo for seven days. Compared to placebo, participants in both antidepressant groups showed reduced recognition of negative facial expressions (fear and anger). Single doses of antidepressants can also produce positive biases in the measures of emotional processing in healthy volunteers. Harmer *et al.* (2003c) found that, compared to participants taking placebo, healthy volunteers receiving a single dose of reboxetine had significantly increased recognition of happy facial expressions and a faster response for positive self-referential processing. A single dose of mirtazapine (a selective NE alpha2-adrenoceptor antagonist and serotonin antagonist) also resulted in a significantly faster response in the self-referential encoding task and increased positive bias in the subsequent memory recall test (Arnone *et al.*, 2009). A recent study found that, compared to placebo, a single dose of bupropion (a dual dopamine and noradrenaline reuptake inhibitor) increased the recognition of happy faces and also reduced negative bias for fearful faces in the face dot-probe task in healthy volunteers (Walsh *et al.*, 2018). The emotional processing changes in these studies were not associated with any significant changes in subjective mood following a single dose of an

antidepressant, suggesting that these shifts in emotional processing precede subsequent changes in mood.

In addition, several studies reported that an acute dose of the SSRI citalopram enhances fearful face recognition and startle response (Browning *et al.*, 2007, Grillon *et al.*, 2007, Harmer *et al.*, 2003), which has been suggested to contribute to symptoms of increased anxiety and agitation in the early phase of antidepressant treatment seen in some patients (Kent *et al.*, 1998). Recent research has found that a serotonin-driven anxiety neural circuit can explain the exacerbated expression of anxiety and fear in the early phase of antidepressant treatment (Marcinkiewcz *et al.*, 2016).

Neuroimaging studies also show abnormal neural responses to positive and negative emotional stimuli (perception, appraisal, and identification) in depressed patients associated with brain regions such as the ventrolateral and medial prefrontal cortex, amygdala, insula, ventral striatum, ventral anterior cingulate gyrus (ACG), and orbitofrontal cortex. In addition, depressed patients show decreased neural responses within regions important for emotional regulation such as the dorsolateral prefrontal cortex (DLPFC), hippocampus, and dorsal ACC (Ma, 2015).

These neural changes in depressed patients would be expected to underpin to the negative bias that is characteristic of depression and to impair effortful regulation of emotional behaviour during cognitive emotional processing tasks (Phillips *et al.*, 2003a, b), as depressed patients have difficulties in negative emotion regulation where there are increased executive control demands (Garnefski and Kraaij, 2006).

Victor *et al.* (2010) used functional magnetic resonance imaging (fMRI) to investigate neural responses in three groups of subjects (currently depressed patients, depressed patients in full remission, and healthy controls) to different emotional faces. The authors found that compared to healthy controls, currently depressed patients and remitted depressed patients showed exaggerated activation of the amygdala in response to negative stimuli (sad faces). Siegle *et al.* (2007) found that, compared to healthy controls, depressed patients showed increased amygdala activity and decreased DLPFC activity in response to negative words during the self-referential encoding task. Epstein *et al.* (2006) also found that when presented with self-referential positive words, the dorsomedial frontal region in depressed patients showed a deactivation relative to that in healthy controls.

Antidepressants are also associated with changes in brain activity underlying emotional processing, leading to the normalization of abnormal neural responses in depressed patients, such as increasing brain activity to positive stimuli, decreasing activity to negative stimuli, and enhancing activity in brain regions associated with emotion regulation (Ma, 2015). For instance, Godlewska *et al.* (2012) found that, compared to placebo administration, seven days of SSRI (escitalopram) treatment normalised neural responses in depressed patients by reducing the response of the amygdala to negative stimuli (fearful facial expressions). Komulainen *et al.* (2018) found that after seven days of SSRI (escitalopram) treatment, the neural response to positive self-referential processing was normalised, as depressed patients showed increased blood oxygenation level dependent (BOLD) response in the medial PFC and ACC, compared to the placebo group. Interestingly, these studies found that the neural changes seen during the early stages of antidepressant treatment were also

independent of subjective mood changes, that is, there were no significant differences in depressive symptoms ratings between treatment and placebo groups at this point in time, which is consistent with the hypothesis suggested by the cognitive neuropsychological model of antidepressant drug action that emotional changes following conventional antidepressant treatments precede subjective changes in mood.

In addition, in a study including 35 untreated depressed patients and healthy control volunteers (Godlewska *et al.*, 2016), depressed patients, prior to treatment, showed significantly increased activation of the insula and dorsal anterior cingulate in response to fearful facial expressions compared to healthy controls. Subsequently, the patients received SSRI (escitalopram) treatment for six weeks. Based on a 50% reduction in depressive symptoms on the HAM-D scale, 22 patients were classified as responders and 13 as non-responders. The results revealed that the early changes in neural response to fearful faces following seven days of treatment were significantly predictive of the later clinical response in week six. The treatment responders showed decreasing response to fearful facial expressions versus happy faces in the left amygdala, insula, anterior and posterior cingulate, bilateral supramarginal gyri and thalamus. This study suggests that early changes in the neural activity of brain regions supporting emotional processing can predict clinical response in depression.

Similar neural effects were seen in healthy populations. Norbury *et al.* (2007) reported that, compared to participants in the placebo group, healthy participants receiving reboxetine for seven days had significantly reduced amygdala responses to fearful faces and increased responses to happy facial expressions. Similar results were obtained in another study (Norbury *et al.*, 2009)

which found that, compared to placebo, 7–10 days of citalopram treatment increased amygdala activation in response to happy faces. These effects occurred without any changes in subjective mood ratings. Murphy *et al.* (2009a) reported that, compared to placebo, a single dose of citalopram in healthy volunteers significantly reduced amygdala response to fearful facial expressions. In a study by Di Simplicio *et al.* (2012), results showed that after seven days citalopram treatment, responses to negative words in the medial prefrontal cortex (MPFC) and ACC during self-referential processing were decreased in participants at high risk of depression.

1.3.4 Study implications

Overall, the cognitive neuropsychological model of antidepressant drug action suggests that conventional antidepressants modify negative biases early on in treatment, and these changes are thought to precede and contribute to subsequent changes in mood. fMRI can also be employed to study changes in the brain associated with positive and negative biases in emotional processing that occur when patients and healthy volunteers undergo antidepressant treatment.

The cognitive neuropsychological model can provide a useful biomarker for screening and understanding the therapeutic mechanisms of novel compounds that are being considered for the treatment of depression and are in the early stages of clinical development. However, it is currently unclear how NMDAR targeting antidepressants might affect emotional processing in depressed patients and healthy populations. The effects of such compounds on the perception and processing of emotional stimuli and the relevant neural circuitry have not been much studied in the current literature. This is particularly important, as a better understanding of the neuropsychological mechanisms underlying the rapid effects of NMDAR antagonists and agonists in depression will

have key implications for future drug development focused on agents with early clinical onset of action.

1.4 Aims and hypotheses

The thesis includes three studies. Study 1 assessed the effects of a single dose of lanicemine, ketamine or placebo on neural and behavioural measures of emotional processing in depressed patients, 24 hours post infusion. The hypotheses tested here were that compared to placebo, single doses of ketamine and lanicemine would reduce negative vs positive bias in emotional processing, similar to the findings seen with conventional antidepressants. It was also hypothesised that compared with treatment with placebo, treatment with lanicemine and ketamine would reduce brain activity of MPFC and ACC, and increase right DLPFC activation during negative self-referential processing.

Earlier in this chapter I reviewed the evidence that the anaesthetic N_2O is an NMDAR antagonist and has rapid antidepressant effects in patients with TRD. Study 2 assessed the effects of a single dose of N_2O using behavioural measures of emotional processing in TRD patients. It was hypothesised that an acute session of N_2O would reduce negative vs positive bias in emotional processing in TRD patients 24 hours after inhalation.

Importantly, by exploring the antidepressant action of these NMDAR antagonists (ketamine, lanicemine, and N_2O) 24 hours post infusion, we aimed to avoid the potential confound of acute or short-lasting changes in subjective experience, such as dissociative effects induced by the drug treatments. Thus, we could examine the neuropsychological mechanisms. In addition, although

previous studies found that some patients showed antidepressant effects within a few hours of ketamine treatment, the more common pattern of antidepressant effect is a gradual increase after repeated dose of ketamine greater than at the beginning (Diamond et al., 2014).

Agents acting at the glycine site of the NMDAR, particularly partial agonists, continue to show potential as antidepressant agents, lacking psychotomimetic effects. It is therefore important to understand how NMDAR glycine site partial agonists may function as antidepressants. The present thesis investigated the effects of DCS, a drug that has been shown to have antidepressant and pro-cognitive effects in some studies, as well as showing potential to reduce anxiety, by promoting extinction learning in patients receiving CBT for anxiety disorders. Also, given the important role of NMDA glutamate receptor function in memory, it is possible that the NMDAR-targeting antidepressants work differently from conventional antidepressants, and therefore, study 3 focused specifically on memory.

Study 3 was designed to assess the effects of an acute low dose of DCS (250 mg) on autobiographical memory, emotional processing and stress response in healthy volunteers. We hypothesised that, compared to placebo, an acute dose of DCS would enhance autobiographical memory specificity and positive bias in emotional processing (specifically increase the processing of positive vs. negative stimuli in tasks measuring facial expression recognition and emotional memory). Given the finding that a single dose of the NMDAR antagonist ketamine and the glycine site partial agonist GLYX-13 has antidepressant effects lasting for at least 24 hours, we also explored the potential for long lasting emotional and memory effects of a single dose of DCS 24 hours after administration.

I also investigated the effects of low dose of DCS (250 mg) on mood, anxiety, and physiological responses to a novel psychosocial stress task in healthy volunteers. Such studies can provide valuable information on the impact of NMDAR modulating agents on stress responsiveness, and may provide insights into the mechanisms underlying the benefit of such drugs in stress and anxiety disorders. It was hypothesized that, compared to those receiving placebo, participants who received DCS would show less physiological responses and subjective anxiety ratings to the novel stress procedure.

Chapter 2: Effect of the NMDA antagonist ketamine and the low-trapping NMDA channel blocker lanicemine on emotional processing in major depressive disorder

2.1 Introduction

As discussed in Chapter 1, conventional antidepressants need to be administered several weeks before a therapeutic response can be achieved, perhaps due to slowly evolving downstream neuroadaptive changes that increase synaptic levels of monoamine neurotransmitters. However, this delayed onset can put patients at an increased risk of suicide (Gourion, 2008). Therefore, there is significant clinical interest in developing antidepressants with a more rapid onset of action.

Recent clinical studies have established that ketamine, an NMDA antagonist, shows rapid antidepressant effects in patients with depression (Serafini *et al.*, 2014, Wilkinson *et al.*, 2018). The first double-blind, placebo-controlled study showed that a low dose of ketamine (0.5 mg/kg) produced rapid antidepressant effects within 4 hours, and these effects persisted for at least 3 days after infusion (Berman *et al.*, 2000). Since then, several randomised controlled clinical trials have replicated these findings in depressed patients and extended our understanding of the rapid antidepressant effects of ketamine (Murrough *et al.*, 2013, Zarate *et al.*, 2006).

Clinically, however, ketamine can induce psychosis-like symptoms as well as significant dissociation. Ketamine binds to the NMDA ion channel and is considered a "high-trapping" blocker (Becker *et al.*, 2019). This means that it is slow to dissociate from its binding site and causes a prolonged NMDAR blockade. This property is thought to underlie ketamine's propensity to cause troublesome psychotomimetic effects. Therefore, developing an antidepressant that has

similar antidepressant effects to ketamine by modulating the NMDAR receptor complex without psychotomimetic effects has become a priority for drug development.

In contrast to ketamine, lanicemine (AZD6765) is a 'low-trapping', NMDA channel blocker that has little propensity to cause psychotomimetic effects, and is generally well tolerated. A double-blind placebo-controlled study by Zarate Jr *et al.* (2013) revealed that acute administration of 150 mg of lanicemine induced a rapid antidepressant response in patients with treatment-resistant depression without showing psychotomimetic or dissociative side-effects. This was confirmed in a further study by Sanacora *et al.* (2014). However, a recent large clinical trial of lanicemine failed to show a significant antidepressant effect in patients who did not adequately respond to previous antidepressant treatment (Sanacora *et al.*, 2017).

As described in Chapter 1, a novel model of antidepressant drug action has been proposed previously by Harmer and colleagues (Harmer *et al.*, 2009a, Harmer, 2010). This theory suggests that antidepressants may work by reversing the negative emotional biases characteristic of depression, which are crucial in the maintenance of this disorder. This effect emerges rapidly in the absence of subjective changes in mood after conventional antidepressant administration in both depressed and healthy participants. However, the neuropsychological effects of novel, fast acting antidepressants such as ketamine and lanicemine have been little investigated.

It is important to understand the neuropsychological mechanisms underlying the rapid effects of NMDAR antagonists in depression, as emotional processing measurements can provide an effective biomarker for the investigation of the therapeutic mechanisms of novel compounds early

in development. Given the ambiguous results from clinical trials, in this study, we compared the effects of a single administration of ketamine, lanicemine, and placebo on emotional processing in patients with major depressive disorder, 24 hours after a single intravenous infusion. We hypothesised that both lanicemine and ketamine would have effects consistent with the neuropsychological model of antidepressant drug action by reducing negative versus positive biases in emotional processing as compared with a placebo, similar to the pattern seen with other conventional antidepressant agents.

2.2 Methods

The study used a multicentre, randomized, double-blind, placebo-controlled parallel group design. Participants were recruited from two centres, one at Manchester University and one at Oxford University via website advertisement. The current study was approved by the Capenhurst Independent Research Ethics Committee (CIREC) (REF:1029). All participants gave written consent prior to participation in the study. The study was registered on ClinicalTrials.gov (Identifier: NCT01046630).

2.2.1 Participants

Between December 2009 and March 2011, 60 participants (male and female) aged 18–45 years were enrolled in this study. The inclusion criteria were as follows: 1) meeting the DSM-IV criteria for a single episode or recurrent MDD, as determined using a structured clinical interview (SCID) (First *et al.*, 2002); 2) outpatient status during screening and at randomization; 3) currently drug-free, and abstinence from any psychoactive and CNS medications (such as antidepressants,

antipsychotic, mood stabilizers); 3) no lifetime history of bipolar disorder, psychosis, schizophrenia, and psychotic depression; 4) no current or previous substance dependence; 5) severe physical illness; 6) right-handedness; 7) without contraindications for MRI; 8) non- or light smoker (< 10 cigarettes a day of tobacco or any equivalent); 9) low caffeine use (< 8 cups a day); 10) negative urine drug screen; 11) female participants not pregnant or lactating; 12) fluent in English.

2.2.2 Study procedure

Participants with a self-rated Beck Depression Inventory (BDI) score ≥ 12 were then contacted by the study team and invited to screening by telephone, followed by completing the consent form and formal screening at the local research unit (visit 1) (see Table 1).

On day 1 (visit 2), eligible participants arrived at the research unit in the morning. After completing the questionnaires (BDI, Montgomery–Åsberg Depression Rating Scale [MADRS), and Clinician Administered Dissociative Status Scale [CADSS]), participants were randomly allocated to one of three groups: a single dose of ketamine, lanicemine, or placebo (saline). Each participant received an intravenous infusion of the allocated agent over 60 minutes via a syringe pump. Participants in the lanicemine group were given a dose of 100 mg, whereas participants in the ketamine group were given a sub-anaesthetic dose of 0.5 mg/kg. The placebo group received 0.9% saline. Pharmacological functional magnetic resonance imaging (phfMRI) data were collected during the drug infusion (phfMRI data reported in Downey et al., 2016). At each site, the randomization codes were generated by research pharmacists who were not involved in the study. Additionally, the research pharmacists dispensed the drug infusions labelled with a numerical code. Participants

again completed the CADSS immediately after infusion, and the MADRS and CADSS were repeated again at 4 hours after the infusion.

At 24 hours after the infusion (visit 3), participants returned to the research unit for completing the MADRS and BDI questionnaires, and behavioural and fMRI measures of emotional processing (data presented in Chapter 2 and Chapter 3).

At 8–11 days after the infusion (visit 4), participants returned to the research unit for completing the MADRS and BDI questionnaires and a safety assessment. The maximum study duration was 42 days.

Table 1. Study assessment

Study	**Screening**	**Day 1**	**Day 2**	**Follow-up**
visit	**1**	**2**	**3**	**4**
SCID	X			
MADRS	X	Before infusion At 4 hours after start of infusion	X	X
BDI	X	X Before infusion	X	X
CADSS		Before infusion Immediately after infusion At 4 hours after start of infusion		
phfMRI			X	
fMRI emotional processing			X	

SCID: Structured Clinical Interview for DSM Disorders; MADRS: Montgomery-Åsberg Depression Rating Scale; BDI: Beck Depression Inventory; CADSS: Clinician Administered Dissociative Status Scale; ph: Pharmacological; fMRI: functional magnetic resonance imaging

2.2.3 Questionnaire measures

Beck Depression Inventory (BDI-II)

The BDI-II is a 21-question self-report inventory. This questionnaire measures symptoms of depression, such as low mood, hopelessness, suicidal thinking, irritability, pessimistic thinking, low self-esteem, guilt, poor concentration and impaired sleep and appetite (Beck *et al.*, 1996). Each item is rated from 0 to 3. A total score is calculated as the sum of the 21 items and ranges from 0 to 63. Higher scores indicate higher levels of depressive symptoms.

Montgomery–Åsberg Depression Rating Scale (MADRS)

The MADRS is an observer rated 10-item scale that assesses depressive symptoms (Davidson *et al.*, 1986). Symptoms assessed include low mood, loss of interest and pleasure, impaired sleep and appetite, poor concentration and inner tension. The MADRS was administered by the study clinicians. Each item is rated on a 0–6-point scale. Higher MADRS scores indicate higher levels of depressive symptoms. The total score is calculated as the sum of the 10 items and ranges from 0 to 60.

Clinician Administered Dissociative Status Scale (CADSS)

The CADSS is a valid and reliable instrument for evaluating dissociative symptoms; it has 5 subscales: altered environmental perception, time perception, body perception, feelings of unreality, and memory impairment (Bremner *et al.*, 1998). In total, the CADSS has 27 items, of which 19 are self-rated and 8 are observer-rated, ranging from 0 (not at all) to 4 (extremely). Higher CADSS scores indicate higher levels of dissociative symptoms.

2.2.4 Psychological tasks

The Emotional Test Battery (ETB) includes several validated measures of emotional processing (Pringle *et al.*, 2011a).

2.2.4.1 Emotional Categorisation Task

The Emotional Categorisation Task (ECAT) involves the presentation of 90 personality words that participants need to categorize as either extremely disagreeable (e.g., domineering, untidy, hostile) or agreeable (e.g., cheerful, honest, optimistic) (Anderson, 1968). These text stimuli are presented on a computer screen, and each trial lasts for 1000 ms. These words were matched for word length, frequency rating, and meaningfulness. Additionally, 20 presentations of 'left' and 'right' words were also included, as a sensorimotor control in the task. The task included 110 words in total, which were presented in randomised order across participants. In each trial, a fixation cross was presented for 500 ms, which was immediately followed by the personality word (500 ms). The intertrial interval (ITI) ranged from 4000 to 8000 ms, with a mean value of 5000 ms. Participants were asked to imagine themselves overhearing someone describing themselves using each of these words and to judge as quickly and accurately as possible whether they would like or dislike to be described by each of the words. Accuracy of response and reaction times for correct identifications were analysed separately, according to the two affective tones. The fMRI results of the self-referential encoding task are presented in Chapter 3.

Following the fMRI session, participants were asked to complete the other ETB tasks. The tasks are listed below (in order):

2.2.4.2 Emotional Memory Tasks

The emotional recall task (EREC) was conducted approximately 40 minutes after completion of the ECAT, outside of the MRI scanner. Participants were asked to recall (write down) as many words of the personality traits they saw in the previous task as possible, within 2 minutes. The number and valence of correctly recalled words and intrusions were recorded. For the emotional recognition memory task (EMEM), participants were presented with another set of positive and negative personality trait words, and asked to indicate whether they recognised each word from the ECAT that they had finished earlier. All personality words that appeared in the ECAT, as well as 60 new distractor words, were presented in random order on a computer screen with a trial length of 500 ms. Participants were required to respond as quickly and accurately as possible to indicate whether they recognised each stimulus word from the self-referential encoding task or not. The accuracy of responses and reaction times for correct identifications were analysed separately for positive and negative words.

2.2.4.3 Facial Expression Recognition Task

The facial expression recognition task (FERT) presents photographs of faces showing one of six different emotions (fear, anger, sadness, disgust, happiness, and surprise) taken from the Ekman and Friesen Pictures of Affect series (Ekman and Friesen, 1971). These had been morphed between each prototype and neutral using techniques by Young *et al.* (1997). Each emotional expression was conveyed with varying levels of intensity (from 10% to 100%, in 10% increments). There were four examples of each emotion for each intensity (from 10 individuals in total). Thus, there were 40 face stimuli of various intensity for 6 emotions, including 10 neutral face stimuli, yielding 250 face stimuli in total. These facial expressions were presented in a random order on a screen.

Each picture was shown for 500 ms, followed by a blank screen before the next trial. Participants were asked to identify which emotion the picture represents, as quickly and as accurately as possible. The numbers of correct responses, reaction times for correct identifications, and misclassification (responses to each emotional expression incorrectly classified as each of the other facial expression categories) were analysed separately for each emotional expression.

2.2.4.4 Attentional Dot-Probe

In the attentional dot-probe task, two emotional words (out of 60 socially relevant/positive and 60 socially threatening/negative) were presented. Each emotional word was paired with a neutral word that started with the same letter and was matched for length. Neutral–neutral pairs were also included. Each trial started with a fixation cross for 500 ms, followed by a word pair. In each trial, one word was presented above and the other below the central fixation position. The emotional words were equally likely to appear above and below. In the unmasked condition, the word pair was presented for 500 ms. Then, a probe appeared in the location where one of the preceding words had previously appeared. The probe was either one or two stars, and participants were asked to press one of two labelled buttons to indicate the number of stars presented on the screen. The participant's response terminated the probe display, and participants were asked to respond as quickly and as accurately as possible. The probe was equally likely to appear at the top and bottom of the screen. Thus, on half of the emotion–neutral word pair trials, the probe appeared in the same position as the emotional word, and on the other half, the probe appeared in the same position as the neutral word. The same sequence of events was used in the masked condition, except that the duration of the word pair was 14 ms, and the display of the word pair was immediately followed by a mask that was displayed for 186 ms. The mask included digits, letters, and other non-letter

symbols (e.g., @B%2#), and was matched for word position and length. There were 180 trials in total (masked: 30 positive–neutral, 30 negative–neutral, 30 neutral–neutral; unmasked: 30 positive–neutral, 30 negative– neutral, 30 neutral–neutral), and masked and unmasked trials were presented in a random order. Reaction time and accuracy were recorded, and vigilance scores were computed from the reaction time when the probe was congruent as compared to when it was incongruent with the location of the emotional stimulus for each condition. Higher scores thus indicate greater vigilance to that emotion.

2.2.5 Statistical analysis

All the data were analysed using IBM SPSS V23. Participants' baseline characteristics were analysed using one-way analyses of variance (ANOVA) or chi-square (for gender). For MADRS and BDI data, mixed-design ANOVAs were applied using treatment group (lanicemine, ketamine, and placebo) as between-subject factor and the time sequence of five levels for MADRS and four for BDI as the within-subject/repeated-measure factor. All ETB tasks were analysed by mixed-design ANOVAs, with treatment (lanicemine, ketamine, and placebo) and centre (Oxford and Manchester) as the between-subject factors. For the ECAT, EREC, EMEM and FERT, emotion was the within-subject factor. For the Attentional Dot-Probe, within-subject factors were masking and valence. Where sphericity was violated, Greenhouse-Geisser corrected p values were used. Significant interactions were followed up with Bonferroni-corrected pairwise comparisons, adjusted for the two main comparisons of interest (ketamine vs placebo and lanicemine vs placebo), where a $p < 0.05$ denoted statistical significance. Effect size was estimated suing ηp2. Marginal differences with a p-value < 0.10 were also reported. In addition, the MADRS baseline scores were significantly different between groups; thus, the MADRS baseline scores were added as a covariate

in the ETB analysis. One lanicemine participant was excluded from the ECAT, EREC, and EMEM analyses due to falling asleep during the scan in the ECAT, and one ketamine participant was excluded from the ECAT analysis due to missing data. One placebo participant was excluded from the BDI analysis due to missing data.

2.3 Results

2.3.1 Baseline characteristics

There were no significant differences between groups in terms of age, gender and BDI scores at baseline. However, groups differed in terms of the baseline MADRS, with the placebo group displaying significantly lower scores (p=0.011) (see Table 2); there were no significant group differences between ketamine and lanicemine treatment in the post-hoc analysis (p=0.084).

Table 2. Baseline Demographic and Mood Scores

	Lanicemine (n=20)	Ketamine (n=21)	Placebo (n=19)	*p value*
Male	8	8	8	NS
Female	12	13	11	NS
Mean age (years)	26.7	27.1	25.7	NS
BDI	33.4 (SD 9.46)	31.38 (SD 6.34)	29.56 (SD7.92)	0.339
MADRS	28.1 (SD 6.9)	24.48 (SD 6.18)	21.74 (SD 6.10)	0.011

ANOVA: analysis of variance; NS: represents non-significant differences; MADRS: Montgomery–Åsberg Depression Rating Scale; BDI: Beck Depression Inventory; SD: standard deviation. Values of p were assessed by one-way ANOVA (mean age, BDI and MADRS) or χ2-test (male, female) as appropriate.

2.3.2 Mood and subjective effects

2.3.2.1 Mood ratings

Table 3 shows a significant main effect of time for BDI scores [$F(2.19, 159)=40.69$, $p<0.001$, $\eta_{p2}=0.43$] and MADRS [$F(3.12, 216) = 26.34$, $p<0.001$, $\eta_{p2}=0.33$], indicating a significant decrease over time from baseline to subsequent ratings across all conditions in depression scores. However, no significant time × treatment interaction was found, indicating that neither drug improved mood more than placebo (Figure 1 and Figure 2). There was a significant main effect of treatment on BDI [$F(2, 53)=3.69$, $p=0.032$, $\eta_{p2}=0.12$] and MADRS scores [$F(2, 54)=5.59$, $p=0.006$, $\eta_{p2}=0.17$], indicating that the active drug groups (lanicemine and ketamine) had higher scores than the placebo group across all timepoints (lanicemine > ketamine > placebo). In addition, there was a significant main effect of centre on MADRS scores [$F(1, 54)=4.87$, $p=0.032$], indicating that MADRS scores were higher in the Manchester centre than in the Oxford centre ($p=0.032$).

Table 3. MADRS and BDI Ratings

Effect	MADRS (*p*)	BDI (*p*)
Treatment group	0.006	0.032
Time	0.000	0.000
Treatment group × Time	0.526	0.123
Centre	0.032	0.871
Treatment group × Centre	0.983	0.636
Treatment group × Time × Centre	0.185	0.344

MADRS Montgomery-Åsberg Depression Rating Scale; BDI: Beck Depression Inventory

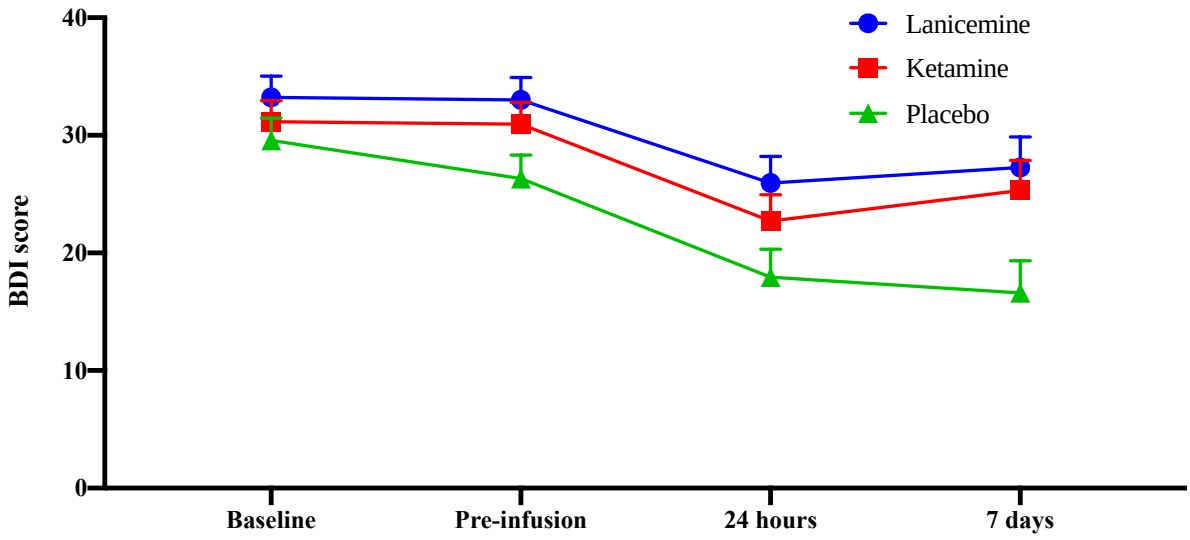


Figure 1. The effect of treatment on BDI scores. *BDI: Beck Depression Inventory; Error bars represent standard error of the mean.*

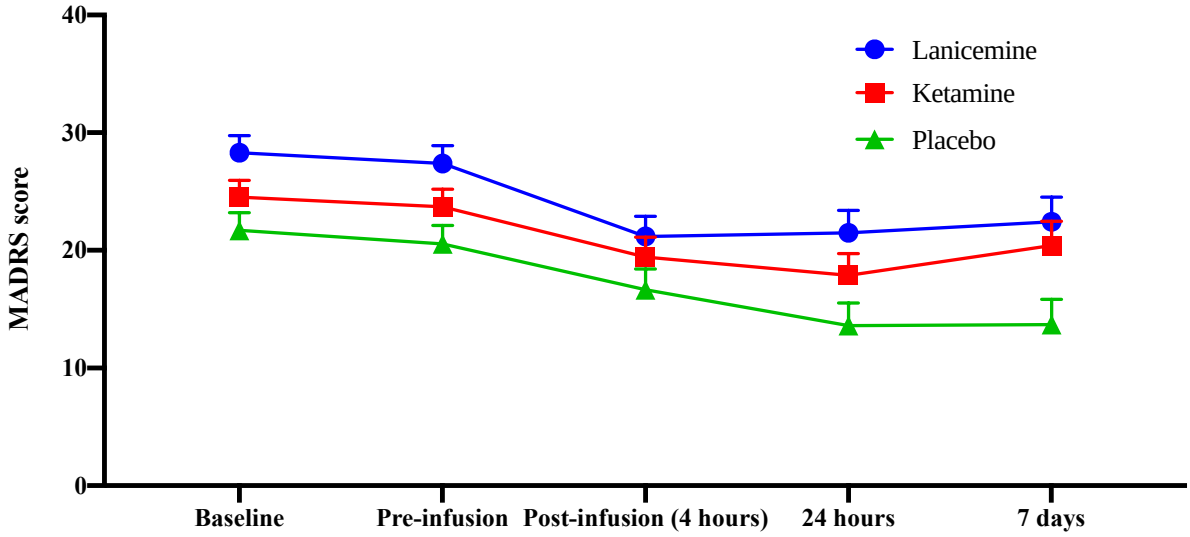


Figure 2. The effect of treatment on MADRS scores. *MADRS: Montgomery–Åsberg Depression Rating Scale; Error bars represent standard error of the mean.*

2.3.2.2 Dissociation Effects

A significant main effect of time was found, indicating a significant increase over time from baseline to immediately post-infusion ratings across all conditions in the CADSS [$F(1.43, 108)=37.62$, $p<0.001$, $\eta_{p2}=0.40$]. There was no significant main effect of treatment group on

CADSS ratings [F(2, 54)=1.984, p=0.147]. However, there was a significant interaction between time and treatment group for CADSS [F(2.87, 108)=4.98, p=0.004, η_{p2}=0.16]. Follow-up pairwise comparisons indicated that there was a significant difference between the ketamine and placebo groups in terms of CADSS ratings at immediately post-infusion (p=0.007), with ketamine displaying higher ratings, but no such statistically significant effect was observed between the lanicemine and placebo groups (p=0.419) (Figure 3).

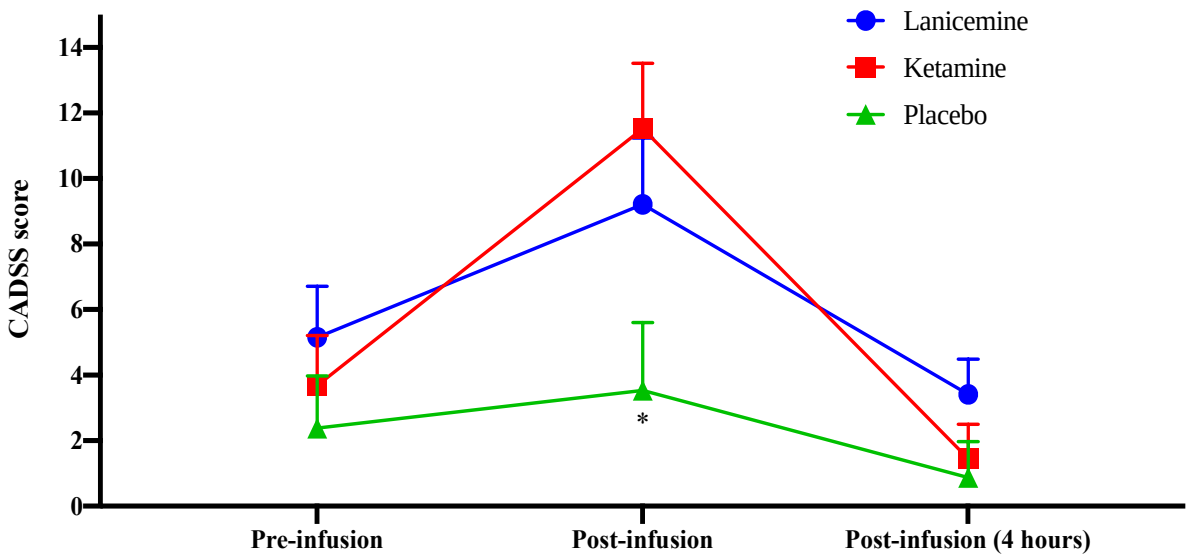


Figure 3. The effects of treatment on dissociation. *CADSS: Clinician Administered Dissociative Status Scale; *p<0.05 [ketamine vs placebo]; Error bars represent standard error of the mean.*

2.3.3 Psychological tasks

2.3.3.1 Emotional Categorisation Task (ECAT)

Accuracy

There was no significant main effect of word valence on the number of words correctly categorized [F(1, 51)=0.013, p=0.911]. There was neither a significant main effect of treatment [F(2, 51)=0.462, p=0.632], nor a significant treatment × word valence interaction [F(2, 51)=0.63,

p=0.537]. There was no statistically significant effect of centre, or centre × group or emotional expression interaction (all *p* values>0.2).

Reaction Time

There was a significant main effect of word valence [F(1, 51)=4.503, p=0.039, η_{p2}=0.081]. In all groups, reaction times were lowest for positive self-referential processing. There was no significant main effect of group condition [F(2, 51)=0.264, p=0.769] and no significant group × word valence interaction [F(2, 51)=2.128, p=0.13]. There was a significant main effect of centre [F(1, 51)=9.622, p=0.003, η_{p2}=0.159], indicating that higher reactions times were seen in the Manchester centre than in the Oxford centre, but there was no statistically significant effect of centre × treatment group or word valence interaction (all *p* values>0.4).

2.3.3.2 Emotional memory

The EREC and EMEM assess the memory of positive and negative words from the ECAT. The EREC is a 2-minute free-recall task (measured for accuracy), and the EMEM requires participants to distinguish familiar ECAT words from novel distractor words (measured for accuracy and reaction times).

2.3.3.3 Emotional recall task (EREC)

Accuracy

There was no significant main effect of word valence on the number of words correctly recalled in the EREC [F(1, 52)=1.957, p=0.168]. There was neither a significant main effect of treatment [F(2, 52)=0.292, p=0.748] nor a significant treatment × word valence interaction [F(2, 52)=0.803,

p=0.453]. There was a significant main effect of centre [F(1, 52)=5.609, p=0.022, η_{p2}=0.097], indicating that a higher number of words were correctly recalled in the Manchester centre than in the Oxford centre (p=0.022), but there was no statistically significant effect of centre × group or emotional expression interaction (all p values>0.3).

Number of intrusions

There was no significant main effect of word valence when considering the number of intrusions (false recall) [F(1, 52)=2.713, p=0.106]. However, there was a significant main effect of treatment [F(2, 52)=6.069, p=0.004, η_{p2}=0.189] and a trend for a significant treatment × word valence interaction [F(2, 52)=2.532, p=0.089, η_{p2}=0.089] on the number of intrusions (see Figure 4). This effect was driven by an increase in positive versus negative words falsely recalled after ketamine administration, as compared to placebo (p=0.054).

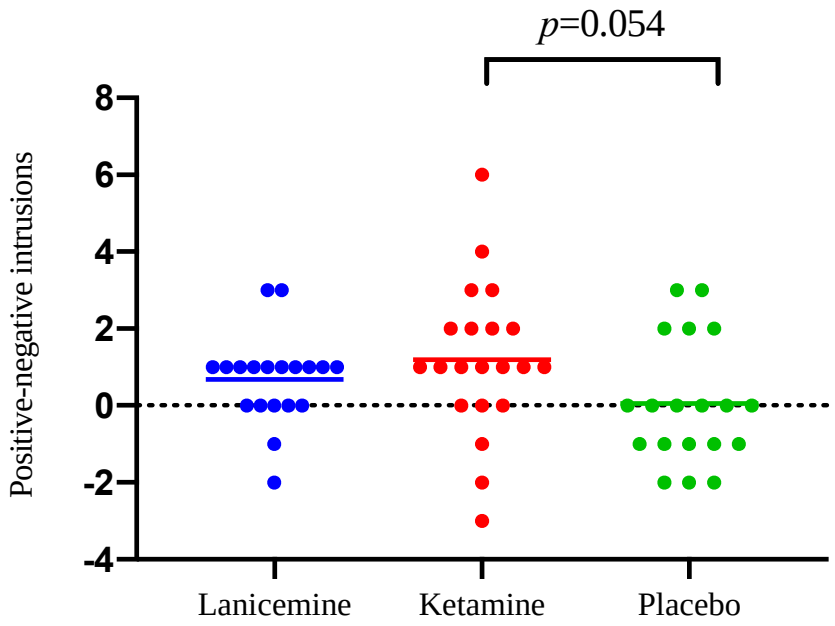


Figure 4. Comparison for positive versus negative intrusions (mean number of items falsely recalled) in the lanicemine, ketamine, and placebo groups.

2.3.3.4 Emotional recognition memory task (EMEM)

Accuracy

There was no significant main effect of word valence in the EMEM [$F(1, 52)=1.947$, $p=0.169$]. Additionally, there was no significant main effect of group [$F(2, 52)=0.915$, $p=0.407$] on words accurately remembered, and no significant interaction between group and emotion [$F(2, 52)=1.119$, $p=0.334$]. There was no significant main effect of centre, or centre × group interaction (all *p* values>0.2).

Misclassification rate

There was no significant main effect of word valence [$F(1, 52)=1.778$, $p=0.188$]. There was neither a significant main effect of treatment [$F(2, 52)=1.091$, $p=0.343$], nor a significant treatment × word valence interaction [$F(2, 52)=0.15$, $p=0.861$]. There was no significant main effect of centre, or centre × group interaction (all *p* values>0.8).

2.3.3.5 Facial Expression Recognition Task (FERT)

Accuracy

There was a significant main effect of emotional expression [$F(3.64, 312)=5.96$, $p<0.001$, $\eta_{p2}=0.103$]. In all groups, accuracy was highest for neutral and happy expressions, and lowest for fearful expressions (Figure 5). There was a trend for a main effect of group on overall accuracy [$F(2, 52)=2.974$, $p=0.065$, $\eta_{p2}=0.103$]; however, follow-up pairwise comparisons revealed only a trend for patients receiving lanicemine to be better at recognizing facial expressions than the placebo group ($p=0.066$). There was no significant interaction with treatment group or emotion

[F(7.28, 312)=0.588, p=0.771]. There was no significant main effect of centre, or centre × treatment group interaction (all p values>0.1).

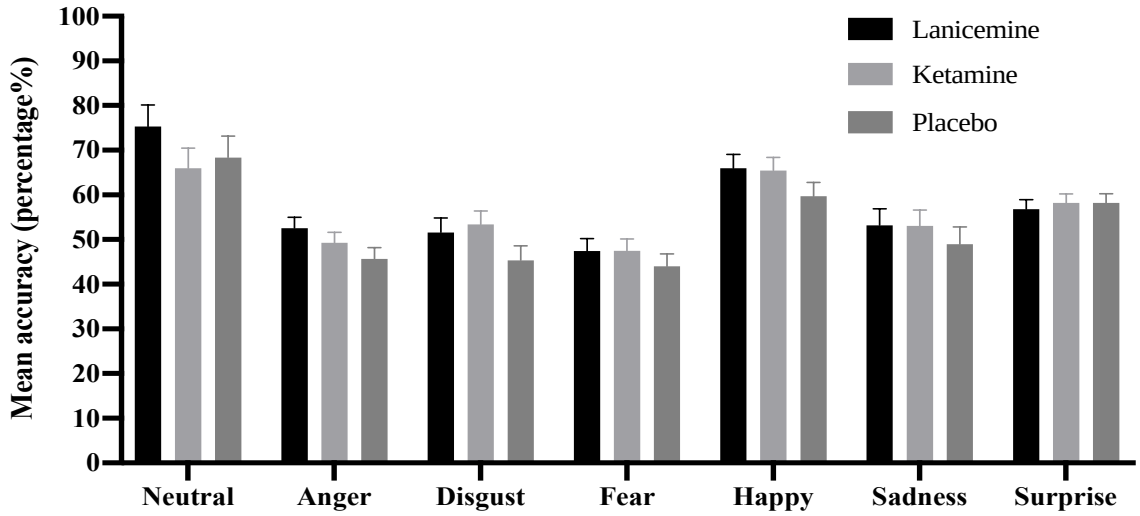


Figure 5. Accuracy of facial expression recognition in the lanicemine, ketamine, and placebo groups**.** *Values represent the mean accuracy rates for each of the 7 facial expressions. Error bars represent standard error of the mean.*

Reaction times

There was a significant main effect of emotional expression [F(4.55, 288)=5.905, p<0.001, η_{p2}=0.11]. In all groups, reaction times were shortest for happy and longest for fearful emotional expressions (see Figure 6). There was no significant main effect of treatment [F(2, 48)=0.044, p=0.957] and no significant group × emotional expression interaction [F(9.09, 288)=1.079, p=0.379]. There was a significant main effect of centre [F(1, 48)=1.111, p=0.001, η_{p2}=0.206] indicating that reactions times were longer in the Manchester centre than in the Oxford centre (*p*=0.001), but there was no statistically significant interaction between centre × treatment group and between centre x emotional expression (all *p* values>0.1).

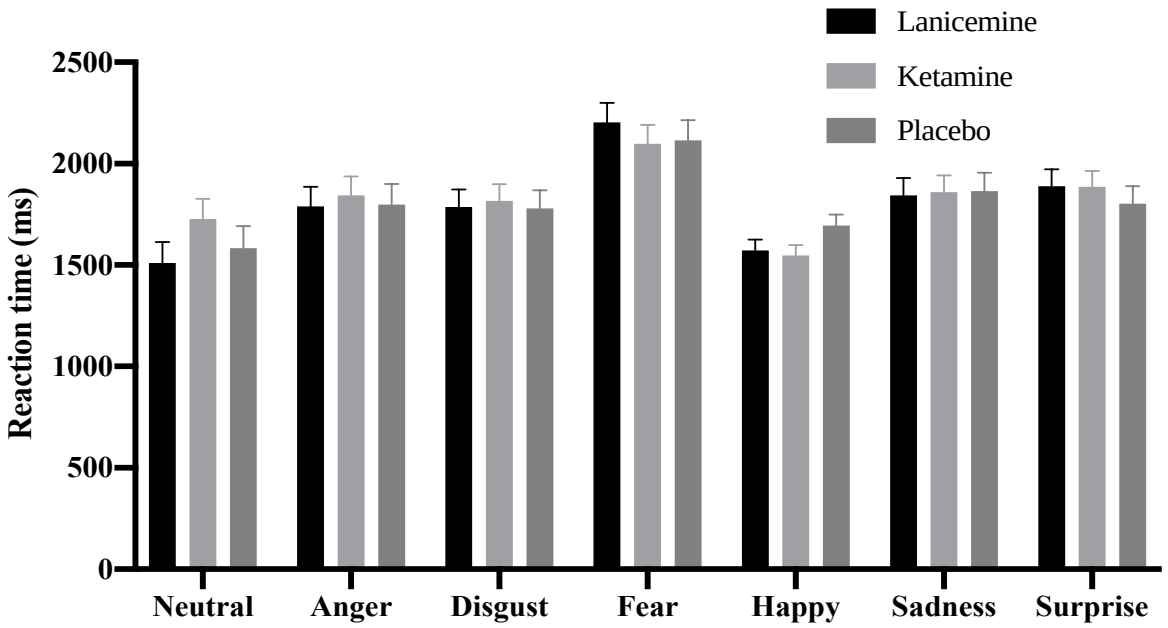


Figure 6. Average reactions times for facial expression recognition in the lanicemine, ketamine, and placebo groups. *Values represent the mean reaction times for each of the seven facial expressions. Error bars represent standard error of the mean.*

Misclassification rates

There was a significant main effect of emotional expression [F(1.83, 288)=23.86, p< 0.001]. In all groups, the misclassification rate was highest for angry expressions, and lowest for happy expressions (see Figure 7). There was no significant main effect of treatment [F(2, 48)=1.658, p=0.201] and no significant treatment × emotional expression interaction [F(3.65, 288)=0.613, p=0.64]. There was a significant main effect of centre [F(1, 48)=4.992, p=0.03, η_{p2}=0.094] indicating that the overall number of misclassifications was higher in the Oxford centre than in the Manchester centre (*p*=0.03), but there was no interaction between centre × treatment group and between centre x emotional expression (all *p* values>0.3).

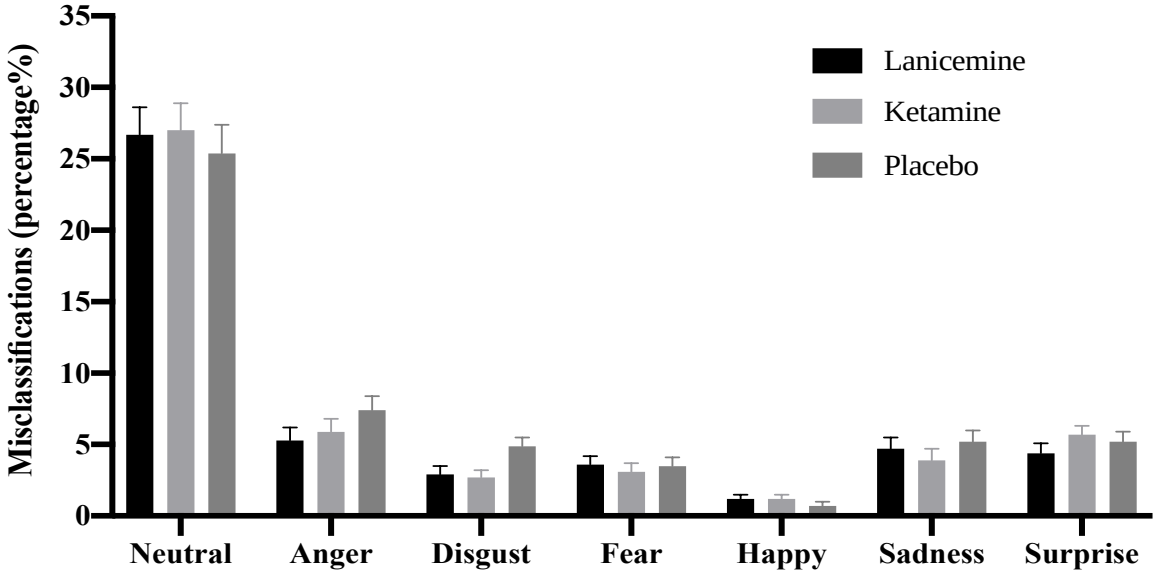


Figure 7. Percentage of misclassifications for each facial expression in the lanicemine, ketamine and placebo groups. *Values represent the mean misclassifications rates for each of the seven facial expressions. Errors bars represent standard error of the mean.*

In a subsequent exploratory analysis, we combined the number of misclassifications for positive [happy, surprise] vs negative [anger, disgust, fear, sad] facial expressions to test whether there was any interaction between treatment (lanicemine, ketamine, and placebo) and positive vs negative misclassifications. There was no significant main effect of emotion [$F(1, 49)=0.371$, $p=0.545$] and no significant main effect of treatment group [$F(2, 49)=0.984$, $p=0.381$]. However, there was a significant interaction between emotion and treatment group [$F(2, 49)=3.694$, $p=0.032$, $\eta_{p2}=0.131$], driven by a higher proportion of positive versus negative facial expressions misclassified in the ketamine vs placebo group ($p=0.008$) (Figure 8). There was no significant main effect of centre, nor was there was a centre × treatment group interaction (all p values>0.2).

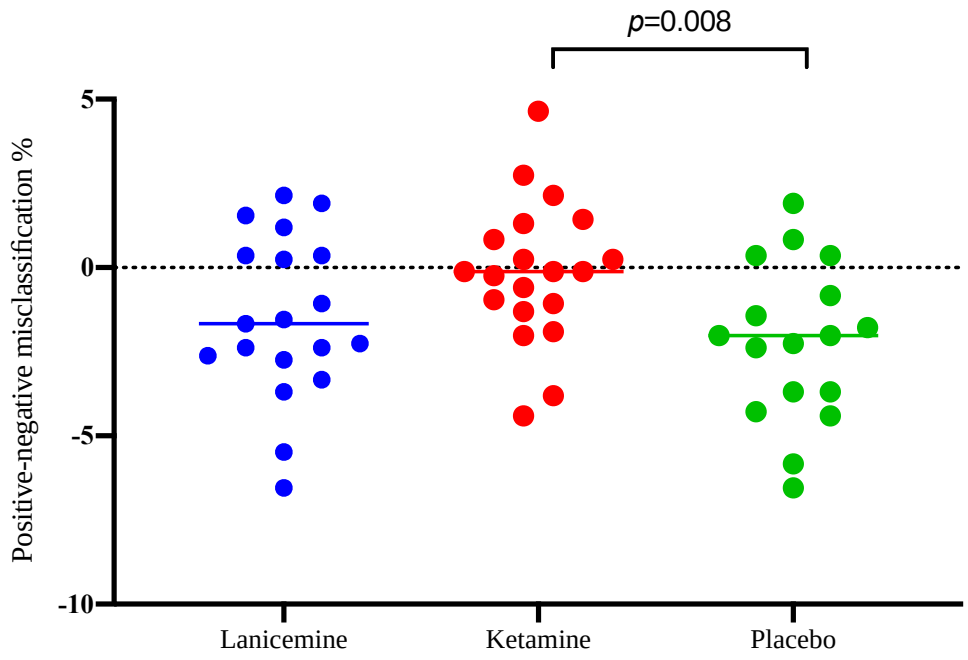


Figure 8. Comparison for positive stimuli versus negative stimuli (mean number of emotion facial expression misclassified) in the lanicemine, ketamine, and placebo groups.

2.3.3.6 Attentional Dot-Probe

In the Attentional Dot-Probe, the attentional vigilance to positive vs negative words can be evaluated from participants' response latency to indicate the alignment of a dot probe appearing in the place of one of the emotional words. A mixed-design ANOVA with treatment (placebo, ketamine, and lanicemine) × masking (mask, unmasked) × valence (positive, negative) on vigilance scores yielded no significant main effect of valence, masking, or treatment and no significant interactions for attentional vigilance (all p values>0.3). Additionally, there was no main effect of treatment group [F(1, 51)=1.856, p=0.967] or a significant effect of centre (all *p* values>0.2).

2.4 Discussion

This chapter aimed to explore the effects of a single intravenous dose of two NMDAR antagonists, ketamine and lanicemine, as compared to that of a placebo on mood and emotional processing in patients with MDD 24 hours post-infusion. There were no significant group differences in mood ratings (both MADRS and BDI), indicating that neither ketamine nor lanicemine produced a greater antidepressant effect than placebo. This current study also aimed to examine whether NMDAR antagonists had similar effects on emotional processing to the effects of conventional antidepressants, such as SSRIs and SNRIs. The results found that there was a trend for patients receiving lanicemine to be better at recognising facial expressions overall as compared to those who received placebo in the FERT, but only ketamine significantly increased the error rate for recognising facial expressions as positive rather than negative. Moreover, there was a trend for ketamine to produce more false positive memory intrusions as compared to placebo.

Conventional antidepressants have well replicated effects on emotional processing tasks. For example, the SSRI citalopram (20 mg) was shown to increase the recognition of happy faces and reduce identification of negative facial expressions (Harmer *et al.*, 2003, Harmer *et al.*, 2004). The noradrenaline-reuptake inhibitor (NRI), reboxetine, increased the recognition of positive facial expressions and the speed of reaction time in responding to self-referential descriptors (Harmer *et al.*, 2003c). However, the present study revealed no significant effects of ketamine similar to those identified in the above studies on facial recognition accuracy, speed of reaction time, and self-referential processing. Thus, the current study suggests that an acute dose of ketamine does not show the broad range of effects on emotional processing seen with other conventional

antidepressants. Nevertheless, this direct comparison should be received with some caution. The findings with conventional antidepressants were obtained soon after drug administration, while the effects of ketamine and lanicemine were studied 24 hours after administration. Hence it cannot be excluded that the NMDAR antagonists might have produced effects on emotional processing for a relatively short period after infusion, which may not have been detected due to the current study design.

The antidepressant effects of ketamine are thought to persist for a number of days and the current data suggests that ketamine increased the number of misclassifications of ambiguous facial expressions as positive rather than negative in the FERT and showed a trend to increase false positive memory intrusions in the emotional recall task 24 hours after administration. Indeed it may be relevant that ketamine particularly appeared to increase false positive memories in the recall task rather than increasing the accuracy of positive vs negative retrieval. Stuart *et al.* (2015) investigated how the SNRI venlafaxine and ketamine acted to change emotional biases associated with learning in a rodent model. They found that ketamine, but not venlafaxine, reversed a previously encoded negative memory (allowing more positive retrieval), but ketamine did not have this effect when administered before learning. However, venlafaxine, but not ketamine, increased positive memory encoding when administered before learning. These effects illustrate interesting differences between rapid-onset NMDAR antagonist antidepressants and delayed-onset conventional antidepressants in terms of how they may modulate emotional biases in depression. It also suggests that it will be important to repeat the present study with ketamine being administered after emotional learning rather than before.

The neuropsychological model of antidepressant drug action (Harmer *et al.*, 2009a, Zghoul *et al.*, 2019) suggests that conventional, delayed acting antidepressants, require the inflow of social information to change the way in which this material is encoded, such as increasing the patient's motivation to participate in socially rewarding activities. This interaction between the way the patient perceives the environment and the social experiences that they experience is thought to contribute to later improvements in mood and subjective experience. This provides an explanation for the delayed onset of effects of conventional antidepressants. In contrast, we can hypothesise that the more positive retrieval of already encoded memories after treatment with fast-acting antidepressants such as ketamine would not be dependent on this environmental enrichment interaction. In the present study, it was found that ketamine seemed to increase the recall of false positive stimuli, indicating a potentially similar psychological effect, which could be of benefit in the treatment of depression.

It is worth noting that, unlike conventional antidepressants, ketamine may lack the ability to modify learning associated with new experiences in a positive direction. Indeed, Stuart *et al.* (2015) showed that unlike SSRI treatment, ketamine lacked the ability to change learning associated with new experiences. A similar effect in depressed patients may explain why the striking effects of ketamine to alleviate depressed mood diminish over a few days (Coyle and Laws, 2015).

Previous animal and human studies have suggested that ketamine has a detrimental effect on cognitive performance and memory when administered at the time of encoding. For instance, Pitsikas *et al.* (2008) reported that ketamine produces a dose-related impairment of non-spatial and spatial recognition memory in rodents, using object recognition and an object location task. Danion

et al. (2000) found that, when human participants learned a word list before the ketamine infusion, their recognition and free recall performances were not impaired during ketamine administration; however, when participants learned the words during ketamine administration, their memory performances were substantially impaired.

In the current study, we did not observe any significant treatment group differences in terms of memory performance, specifically the number of words correctly recalled in the EREC task and EREM task. This is likely to be because memory was tested 24 hours post-infusion, and not at encoding during ketamine or lanicemine administration. Thus a previous study by Moretti *et al.* (1984) reported that ketamine impaired psychological measurements only immediately after infusion, and that no effects on cognitive performance and subjective symptoms were shown at 24 hours, 2 weeks, and 4 months after infusion.

No significant effect of ketamine on the accuracy of facial emotion recognition was found in the current study. Ebert *et al.* (2012) investigated whether ketamine administration (0.5mg/kg intravenously) acutely impaired the recognition of facial expressions in 18 healthy male participants in a placebo-controlled crossover design. Ketamine significantly impaired global facial expression recognition, particularly for the emotion of sadness. Ketamine in the later study was being used to test the NMDA hypofunction hypothesis of schizophrenia and facial expression recognition impairment is indeed seen in patients with schizophrenia. However, direct comparisons between our study and the study by Ebert *et al.* (2012) are difficult due to differences in the study sample and design and in particular differences in the time of testing (acute vs 24

hours later) in relation to ketamine administration. It will be important to carry out further studies on the acute effect of ketamine on emotional processing in depressed patients.

Pringle et al (2012) investigated the effect of a single oral 10mg dose of another NMDAR channel blocker, memantine, on emotional processing and the results confirmed the lack of typical antidepressant effects of memantine on facial expression recognition and attentional performance. However, memantine treatment did produce fewer negative false alarms in the emotional memory task. This has an interesting parallel with the effect of ketamine seen in the present study to increase false positive memories and does suggest common effects of NMDAR antagonism on key aspects of emotional recall.

In the current study, although fewer dissociative symptoms were observed in the lanicemine treatment group, lanicemine only showed a trend towards improving facial expression across all emotions when compared to the placebo group. It did not produce positive bias in emotion processing. In fact, although preliminary data on the efficacy of lanicemine in depression have been promising, a recent large, placebo-controlled study in patients with MDD and a history of inadequate response to antidepressants failed to show a therapeutic benefit for lanicemine over the placebo group (Sanacora *et al.*, 2017). Godlewska (2019) suggested that antidepressant lacking an ability to produce positive bias in emotional processing in human studies are more likely to fail as effective antidepressants. However, the lack of an effect on positive emotional processing could have also emerged if we happened to study a patient group who did not show a specific antidepressant response to lanicemine.

2.4.1 Limitation

This study had several limitations. Neither ketamine nor lanicemine produced an antidepressant effect as compared to placebo. As suggested by Downey et al. (2016), the lack of an antidepressant effect by drug treatments may be attributable to a strong placebo response across all timepoints and the main effect of centre on MADRS scores. Most previous ketamine studies recruited TRD patients, who might be expected to demonstrate a relatively low placebo response.

In addition, the lack of an antidepressant effect by ketamine in this study may be due to less of dissociative symptoms being observed, the average score of the CADSS in the current study was 11.5 and 1.4 immediately post-infusion and 4 hours post-infusion, respectively. Previous ketamine studies showed higher dissociative symptoms compared to the current study. For instance, Luckenbaugh *et al.* (2014) found that the average score of the CADSS was 27.5 in TRD patients who received a single subanesthetic ketamine (0.5mg/kg) at 40 minutes post-infusion; Lai *et al.* (2014) showed that the average scores of the CADSS was 33 in TRD patients after receiving 0.4mg/kg intravenous ketamine at 4 hours post-infusion. Importantly, evidence indicated an association between increased CADSS score and treatment response to ketamine in terms of the Hamilton Depression Rating scale (Luckenbaugh *et al.,* 2014, Niciu *et al.,* 2018).

Other limitations include the fact that the placebo group had lower baseline depression scores than did the active treatment groups, and that the dose of lanicemine (100 mg) used was relatively low. Moreover, the current study did not measure dissociation effects 24 hours post-infusion. It is possible that patients in the ketamine group had persisting dissociative effects that may have influenced performance on the emotional processing test. However, the results also indicated that

ketamine only produced dissociative effects immediately post-infusion, gradually decreasing at the 4 hours assessment, where it reached non-significant differences as compared to the placebo group. Further studies repeatedly assessing dissociative effects are needed in order to clarify this issue.

2.4.2 Conclusion

In conclusion, this study found that ketamine increases behavioural measures of positive bias in facial expression classification, and produced a similar trend in an aspect of emotional memory recall. These effects would be expected to attenuate negative biases in emotional processing seen in depression and could play a role in the therapeutic effects of this NMDA antagonist in the treatment of depression. Neuroimaging studies can add more mechanistic information and help elucidate the early effect of NMDA antagonists on the neural substrates of emotional processing. Therefore, in Chapter 3, I use fMRI to examine the neural effects of NMDA antagonists (ketamine and lanicemine) on emotional processing in this group of depressed participants.

Chapter 3: The effects of NMDA receptor antagonists on self-referential processing in depressed patients: An fMRI pilot study

3.1 Introduction

In Chapter 2, it was found that ketamine produced a positive bias in the way in which depressed patients responded to emotional information. In the current chapter, I present the fMRI data collected using this same sample of depressed patients. fMRI is a useful tool that complements behavioural analysis, by allowing us to study the neural mechanisms underlying the effects of pharmacological agents. This will help advance our understanding of how NMDA antagonists influence neural activity, an area of research that has been little investigated.

There is evidence suggesting that patients with depression have decreased glutamate levels in different prefrontal regions, such as the medial prefrontal cortex (MPFC), anterior cingulate cortex (ACC), and dorsolateral prefrontal cortex (DLPFC) (Arnone *et al.*, 2015, Hasler *et al.*, 2007, Luykx *et al.*, 2012). Those brain regions are part of a limbic-cortical-striato-pallido-thalamic circuit associated with the processing of emotion, such as perception, evaluation of emotion, and emotion regulation (Phillips *et al.*, 2003a, b). NMDA antagonists with antidepressant action have been shown to normalise glutamate levels in the brain, consistent with the hypothesis that altered glutamate levels may contribute to the pathophysiology of depression (Ionescu *et al.*, 2018).

Depression is associated with negative cognitions, specifically increased rumination and self-processing/focus in negative emotions, which contribute to self-blame and guilt in individuals with major depression. A meta-analysis has found that negative self-referential cognition significantly

predicted past, current, and future depression, as well as treatment efficacy (Phillips *et al.*, 2010). In neural circuitry studies, self-referential processing is shown to be mediated by cortical midline structures, and particularly activates the MPFC and ACC brain regions (Northoff *et al.*, 2006). For example, compared with healthy volunteers, depressed patients demonstrate hyperactivity in the MPFC and ACC during self-referential processing of negative words (Yoshimura *et al.*, 2010).

As discussed in Chapter 1, the early changes in neural responses to emotional processing are a biomarker of antidepressant drug action. Studies have shown that antidepressants can rapidly modulate self-referential processing in healthy populations and depressed patients. For instance, when healthy volunteers received a single dose of mirtazapine, neural responses to self-referential processing in the MPFC and ACC regions were attenuated (Komulainen *et al.*, 2016). In addition, seven days' administration of escitalopram treatment has been shown to normalize self-referential processing in depressed patients, and specifically increase responses to positive words as compared to negative words in the MPFC and ACC (Komulainen *et al.*, 2018). Di Simplicio *et al.* (2012) found that, after 7 days of citalopram treatment, responses in the MPFC and ACC to negative words during self-referential processing were decreased in participants at a high risk of depression. However, it remains unclear whether and how NMDA antagonists exert antidepressant effects during self-referential processing.

Phillips *et al.* (2003a) proposed that DLPFC is an important brain region for the regulation of emotional responses. Emotion regulation includes suppression, inhibition, and reappraisal functions towards reducing the duration and intensity of emotional expressions (Laeger *et al.*, 2012). For example, Mayberg *et al.* (1999) reported that right DLPFC brain activity decreased

when healthy volunteers experienced a sad mood, which was induced via autobiographical scripts. Phan *et al.* (2005) found that the inhibition via cognitive reappraisal of negative emotional experience (evoked by highly arousing aversive pictures) was significantly associated with DLPFC activation in healthy subjects. In addition, DLFPC helps to maintain cognitive and attentional control during emotional information processing (De Raedt *et al.*, 2010, Leyman *et al.*, 2009).

Depressed patients tend to show decreased cortical engagement with aversive or negative stimuli owing to greater difficulty regulating negative emotions (Beck, 2008, Ma, 2015). For example, Fales *et al.* (2008) found that healthy volunteers showed increased brain activity in the right DLPFC when responding to fearful versus neutral stimuli. However, depressed patients did not show this same pattern, suggesting that depressed patients may have impairments in emotion regulation and cognitive control over emotional interference. After 8 weeks of SSRI (escitalopram) treatment, however, depressed patients showed significantly increased right DLPFC activity to fearful stimuli (Fales *et al.*, 2009). Moreover, a meta-analysis further supported the idea that antidepressant treatment can normalise emotional regulation responses to negative stimuli in depressive patients by increasing brain activity in the DLPFC (Ma, 2015).

Moreover, studies have also demonstrated that self-referential processing in depressed patients involves cognitive control, allocation of attention, and emotion regulation, which are functions associated with the DLPFC (Lemogne *et al.*, 2009, Lemogne *et al.*, 2010, Siegle *et al.*, 2007). For instance, Lemogne *et al.* (2009) reported that DLPFC activation during self (positive or negative personality words) versus general (non-human neutral attributes) categorisation was unique to depressed patients. However, after 9 weeks of antidepressant treatment, patients showed more

balanced left DLPFC activation (Lemogne *et al.*, 2010). Depressed patients need to exercise cognitive control during self-referential processing in order to reduce adverse emotional responses for either self-focused inhibition or cognitive re-appraisal (Lemogne *et al.*, 2010). Siegle *et al.* (2007) found that, compared with healthy controls, depressed patients showed sustained decreased DLPFC activity during a personal relevance rating task (participants are required to categorise whether personality words are relevant to them). Deactivation of DLPFC may have indicated that depressed patients had insufficient resources for concurrent emotion regulation.

Importantly, Scheidegger *et al.* (2016) recently investigated the effects of single dose of ketamine on cognition-emotion interaction by incorporating stimuli with emotional content in to the context of a working memory task, and the results found that ketamine decreased BOLD reactivity to negative emotional words in the right DLPFC, but no significant effects were seen on the left DLPFC in healthy volunteers. Also, Carlson *et al.* (2013) reported that treatment-resistant depressed patients had reduced metabolism in the right DLPFC region, and this region was associated with a rapid antidepressant response to ketamine treatment, but there was no effect on the left DLPFC. Combined with the above findings, this suggests that the right DLPFC plays an important role in ketamine treatment.

Given the importance of negative self-referential processing as an underlying cognitive mechanism of depression, the current pilot study aimed to examine the effects of a single dose of NMDAR antagonists (ketamine and lanicemine) on self-referential processing in patients with depression. Based on previous fMRI studies (Di Simplicio *et al.*, 2012, Scheidegger *et al.*, 2016), and the behavioural findings from Chapter 2 indicating that ketamine can quickly reverse previously

encoded negative information and memories, it was hypothesised that compared with a placebo, treatment with NMDAR antagonists would reduce brain activity of MPFC and ACC, and increase right DLPFC activation during the negative self-referential processing.

3.2 Methods

3.2.1 Design, procedure, and participants

The study design and study participants have been described in detail in Chapter 2. Briefly, 60 treatment-naïve patients with depression, meeting DSM IV criteria, were randomized to receive a single dose of ketamine (0.5 mg/kg), lanicemine (100 mg), or saline placebo as intravenous infusion. Data were collected from centres based at Oxford University and Manchester University. The phfMRI data were collected during drug infusion on day 1. At 24 hours after the drug infusion, participants returned to the research unit for fMRI measurements of emotional processing. Baseline and follow-up (24 hours and 7 days) MADRS and BDI scores were also collected.

We excluded all imaging data from Manchester University due to a gantry tilt setting error during acquisition. In addition, four participants were excluded from the Oxford University centre (three participants had brain abnormalities and one participant fell asleep during the task). The final analysis therefore consisted of 28 right-handed depressed patients (age range 19–44 years, 16 females). Nine patients received lanicemine, ten patients received ketamine, and nine patients received placebo.

3.2.2 Task design

The fMRI self-referential encoding task design has been fully described in Chapter 2. Briefly, 90 personality words were randomly presented, of which half were positive and half negative (Anderson, 1968). These words were controlled for word length, frequency rating, and meaningfulness. Participants were asked to categorise whether they would like or dislike to be described to as each characteristic by pressing a button with the right and left index or middle finger, respectively (Di Simplicio *et al.*, 2012). Additionally, 20 presentations of 'left' and 'right' were also included, as a sensorimotor baseline control in the task.

3.2.3 fMRI acquisition and activation paradigms

The fMRI data were acquired on a 3-T Siemens TIM TRIP (Munich, Germany) in Oxford, with a voxel resolution of 3 × 3 × 3 mm, TR = 3 s, and TE = 30 ms, with a 64 × 64 × 40 grid. T1-weighted structural images were acquired for participant alignment using a Turbo FLASH sequence with the following parameters: voxel resolution 1 × 1 × 1 mm with a TE/TR = 4.7/2040 ms, 192 slices, and 8° flip angle. Stimuli were presented on a personal computer using E-Prime (version 1.0; Psychology Software Tools Inc., Sharpsburg, PA, USA) and a cloned projection was displayed to participants on an opaque screen located at the head of the scanner bore, which they viewed using angled mirrors. Stimulus presentation/participant button presses were registered and time-locked to fMRI data using E-Prime.

3.2.4 Data analysis

3.2.4.1 Statistical analysis of subjective ratings

For MADRS and BDI data, mixed-design ANOVAs were used, with the treatment group (lanicemine, ketamine, and placebo) as between-subject factor and the time-sequence of 4 levels

for MADRS (baseline, before infusion, after infusion, 24-h post-infusion) and 3 levels for BDI (baseline, before infusion, 24-h post-infusion) as within-subject factors.

3.2.4.2 Statistical analysis of behavioural data

The behavioural data from the emotional categorization task (accuracy of correct words and mean reaction times) were analysed with mixed-design ANOVAs. Treatment group (lanicemine, ketamine, and placebo) was used as the between-subject factor, and word valence (positive and negative) as the within-subjects variable. Significant interactions were followed up with Bonferroni-corrected pairwise comparisons, adjusted for the two main comparisons of interest (ketamine vs placebo and lanicemine vs placebo), where a $p<0.05$ denoted statistical significance.

3.2.4.3 Statistical analysis of fMRI data

Functional images were preprocessed using SPM12 software (http://www.fil.ion.ucl.ac.uk/spm) and DPABI (Yan *et al.*, 2016). The fMRI images were realigned to the middle EPI image. The individual T1-weighted structural image was co-registered to the mean functional image after motion correction using linear transformation. The transformed structural images were then segmented into grey matter (GM), white matter (WM), and cerebrospinal fluid (CSF) by using a unified segmentation algorithm (Ashburner and Friston, 2005). The motion-corrected (realigned) functional images were spatially normalized to the Montreal Neurological Institute (MNI) space using the normalization parameters estimated during unified segmentation. The functional data were then resampled to 3-mm isotropic voxels and smoothed with a 9-mm full-width at half-maximum (FWHM) Gaussian kernel to conform to the assumptions of random field theory and improve sensitivity for group analysis. Data were analysed using general linear models as

described below, with a high-pass filter cut-off of 128 s, and autoregressive AR(1)-model correction for autocorrelation.

In the first-level analysis, explanatory variables were modelled separately for 'lanicemine', 'ketamine', and 'placebo' groups: correct 'negative', correct 'positive', correct 'neutral/control', and incorrect words categorisation. Only trials responses to correct words valence were included in the final analysis. All variables were modelled by convolving the onset of each stimulus with a canonical hemodynamic response function. To identify brain activity during the processing of emotional information, and three separate contrasts were performed: negative vs control words, positive vs control words, and positive vs negative words.

The contrast maps from the first-level model of parametric analyses were taken for second-level group analyses and entered into a full factorial ANOVA. The statistical threshold was set at $p<0.05$ (FEW-corrected) at cluster level and $p<0.001$ at an uncorrected peak level according to the SPM12 standard procedure. The activation cluster locations were indicated by the peak voxels on the normalized structural images and were labelled using the nomenclature of Talairach (1988).

It was also performed a region-of-interest (ROI) analysis with different regions defined a-priori. Based on peak voxels reported in a previous ketamine study (Scheidegger *et al.*, 2016), spherical ROIs were generated with a 10-mm radius for the right DLPFC (x=42, y=30, z=28) and left DLPFC (x=-46, y=26, z=30). The mask for the MPFC (0, 40, 16) was based from a previous fMRI study using the same self-referential processing task (Di Simplicio *et al.*, 2012). The whole

anatomical mask of the ACC was taken from the automated anatomical labelling (AAL) template using the MarsBar version 0.44 software (http://marsbar.sourceforge.net/).

3.3 Results

3.3.1 Baseline characteristics and subjective mood ratings

There were no significant differences between groups in terms of gender, age, and baseline subjective mood scores (BDI and MADRS) (see Table 4). In addition, there was no significant treatment × time interaction for any variable (BDI, MADRS and CADSS) (all p values>0.1), although there was a significant main effect of treatment for the BDI [$F(2, 24)=3.562$, $p=0.044$] and MADRS [$F(2, 25)=4.297$, $p=0.025$], reflecting the lower baseline clinical ratings in the placebo group. The effect of time was significant (all p values<0.001), with all groups improving over time (from baseline to 24 hours after infusion).

Table 4. Baseline Demographic and Baseline Mood Scores

	Lanicemine (N=9)	Ketamine (N=10)	Placebo (N=9)
Male (N)	4	4	4
Female (N)	5	6	5
Age (mean; SD)	29.67 (2.83)	29.8 (2.95)	24.22 (6.98)
BDI (mean; SD)	35.22 (7.24)	33.8 (4.73)	29.78 (5.63)
MADRS (mean; SD)	27.22 (7.36)	23.8 (4.08)	21.22 (4.63)

BDI: Beck Depression Inventory; MADRS: Montgomery–Åsberg Depression Rating Scale; Values represent mean and standard deviation (in parenthesis)

3.3.2 Behavioural results

There was no significant main effect of emotional valence ($F(1, 25)=0.90$, $p=0.35$), treatment group ($F(2, 25)=0.602$, $p=0.555$) or emotional valence × treatment group interaction ($F(2,25)=0.90$, $p=0.419$) in the accuracy to categorise self-referential personality words. There was a significant main effect of reaction time ($F(1, 25)=83.875$, $p<0.001$), indicating that, in all groups, reaction times were lowest for positive personality word categories; however, there was no significant effect of treatment group ($F(2, 25)=0.471$, $p=0.664$) or any significant interaction between reaction time and treatment group ($F(2, 25)=1.647$, $p=0.213$).

3.3.3 fMRI results

3.3.3.1 Differences between ketamine, lanicemine, and placebo: ROI analysis

There was a marginal significant difference in BOLD activity between the ketamine and placebo groups in the right DLPFC ($t(25)=2.33$, $p=0.028$, Holm correction $p=0.084$), but not in the left DLPFC ($t(25)=1.10$, $p=0.281$, Holm correction $p=0.84$) specifically for negative stimuli relative to neutral stimuli (see Figure 9). There were no significant differences between ketamine vs placebo in either the right or left DLPFC regions, specifically for negative stimuli relative to positive stimuli. Additionally, no significant differences were found between the lanicemine and placebo groups in either the right or left DLPFC regions for negative stimuli relative to neutral or positive stimuli.

There were no significant differences in the MPFC and ACC between treatment groups for negative stimuli relative to neutral or positive stimuli. Additionally, no significant differences were

observed between treatment groups in any brain regions for positive stimuli relative to neutral stimuli or negative stimuli.

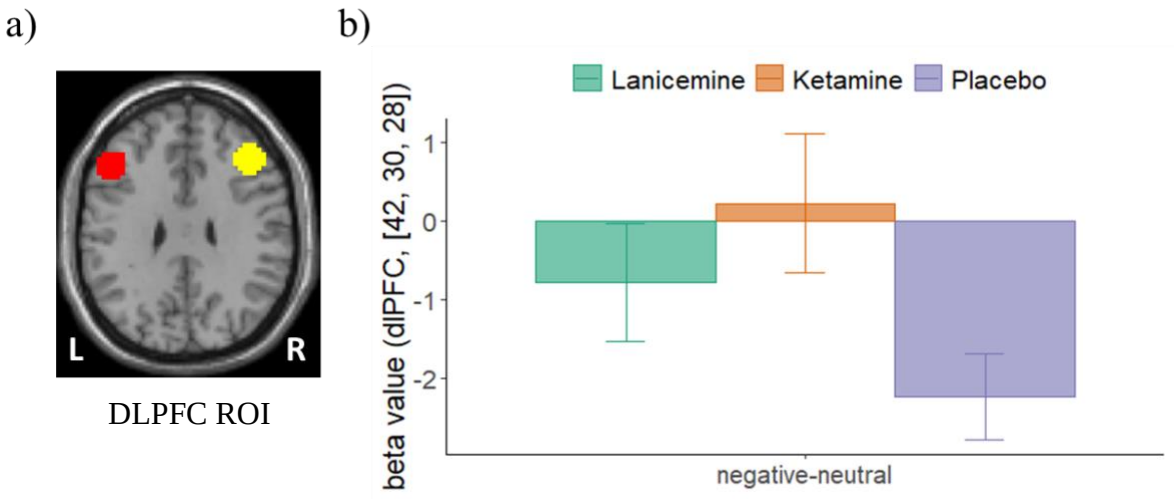


Figure 9. a) spherical masks of the left and right dorsolateral prefrontal cortex (DLPFC); b) BOLD activity in the right dorsolateral prefrontal cortex region-of-interest (DLPFC ROI) (MNI x, y, z = 42, 30, 28) in the lanicemine, ketamine and placebo groups, for negative vs neutral stimuli. *Error bars denote the standard error of the mean.*

3.3.3.2 Differences between ketamine, lanicemine, and placebo: explorative whole-brain analysis

In the whole-brain analysis, we conducted an ANOVA to test the effect of drug treatment on emotion – separately for positive-neutral and negative-neutral stimuli – across the three groups. However, there were no significant differences between treatment groups in any brain region.

3.4 Discussion

This pilot study suggests that a single dose of ketamine may mediate the neural processing of emotional information in patients with depression. Specifically, during negative self-referential processing, there was a trend for ketamine to increase activation in the right DLPFC versus placebo. These neural effects occurred in the absence of effects on subjective mood ratings. However, no significant differences were found between the lanicemine and placebo groups in the right DLPFC regions for negative stimuli relative to neutral or positive stimuli. In contrast, no significant differences were observed between groups in the neural activity of the MPFC and ACC during the categorization of negative self-referential terms relative to neutral or positive stimuli.

Unexpectedly, no neural activity differences on the MPFC and ACC between the groups were observed in the present study. It is possible that the activation of the MPFC and ACC may require longer antidepressant treatment than the DLPFC to normalize, as previous antidepressant studies used a long-term drug-administration study design. For example, after 7 days' administration of escitalopram, self-referential processing normalised in depressed patients, and specifically, there were increased responses to positive self-referential processing in the MPFC and ACC (Komulainen *et al.*, 2018). In a separate study, 6 to 7 weeks of treatment with agomelatine increased activation in the ACC during self-referential processing in depressed patients (Delaveau *et al.*, 2016). Di Simplicio *et al.* (2012) found that, after 7 days' citalopram treatment, responses in the MPFC and ACC to negative self-referential processing decreased in participants at high risk of depression. Therefore, future studies should examine self-referential processing with repeated doses of ketamine. Additionally, the small sample size limited the stability of our report findings, and larger sample sizes are required to capture significant differences.

As predicted, a single dose of ketamine increased right DLPFC activity during negative self-referential processing in depressed patients, further supporting previous studies indicating that antidepressants can normalise emotion regulation responses via increasing engagement with negative or aversive stimuli and emotions (Ma, 2015). Deficits in regulation of negative stimuli are associated with impairments in the ability to inhibit negative attentional processing of emotional information. For instance, Leyman *et al.* (2009) found that a single session of high-frequency repetitive transcranial magnetic stimulation (HF-rTMS) over the right DLPFC produces an impairment in the inhibition of negative information in healthy volunteers. De Raedt *et al.* (2010) used HF-rTMS to manipulate activity within the DLPFC in order to explore changes in attentional processing of emotional information among healthy volunteers. The authors found that right prefrontal HF-rTMS impaired disengagement from negative stimuli (angry faces) and was associated with decreased right DLPFC activity in healthy volunteers. Altogether, these findings suggest a specific and important role for the right DLPFC in emotional regulation (emotional attention processes).

Furthermore, previous studies indicated that different effects on the left DLPFC and right DLPFC on emotional information processing may represent distinct contributions of the two hemispheres. Specifically, Dolcos *et al.* (2004) used an fMRI paradigm to differentiate neural activity associated with emotional evaluation and memory in healthy volunteers; they found that the left DLPFC was activated during the evaluation of positive pictures, while the right DLPFC showed greater activation in response to negative picture. Balconi and Ferrari (2012) reported that rTMS stimulation to the left DLPFC facilitated positive vs negative emotional cue retrieval in healthy volunteers. Similarly, in the study by Grimm *et al.* (2006), it was shown that positive valence

showed a significantly correlation with activation in the left DLPFC, and negative valence significantly correlated with the right DLPFC. Viinikainen *et al.* (2010) found a positive correlation between the activation of right DLPFC and negative emotion pictures. Together, these results further support the notion that the DLPFC is parametrically dependent on emotional valence, indicating the distinct role for the left DLPFC hemisphere in positive emotional processing and the right DLPFC hemisphere in negative emotional processing (Davidson and Irwin, 1999).

We found that ketamine increased activation in the right DLPFC as compared to a placebo during negative self-referential processing. However, these findings contrast with a previous ketamine study in a healthy population. Specifically, Scheidegger *et al.* (2016) reported that ketamine reduced activity in the right DLPFC in response to negative emotional words. It should be noted that, in Scheidegger *et al.* (2016) study, the aim was to investigate the interaction between cognition and emotion by incorporating stimuli with emotional content into a cognitive task. Thus, the task design was not the same as in our study. On the other hand, the authors suggested that ketamine-induced changes in neural activity during processing of emotional information may differ between healthy populations and depressed patients (Scheidegger *et al.*, 2016). Recent fMRI studies have also found that ketamine had opposite effects in depressed patients as compared to healthy controls in several brain regions during emotional processing (Reed *et al.*, 2018, 2019). Thus, future work should further investigate whether changes in neural activity following a single dose of ketamine is similar in healthy volunteers and depressed patients using the same fMRI task paradigm (e.g., a self-referential processing task).

We did not observe any significant effects on the left DLPFC, but the right DLPFC plays a dominant role in negative emotion processing, implying that a single dose of ketamine may quickly

reverse previously encoded negative information and memory (as also seen in the behavioural findings in Chapter 2). This may explain the therapeutic effects of this NMDA antagonist in the treatment of depression (Carlson *et al.*, 2013, Scheidegger *et al.*, 2016). Recently, Gärtner *et al.* (2019) found that increased functional connectivity 24 hours after ketamine administration between right lateral prefrontal cortex and subgenual ACC are positively associated with treatment response in depressed patients.

In addition, we did not observe either behavioural or neural effects with lanicemine, which also failed to show antidepressant effects in a phase III clinical trial (Sanacora *et al.*, 2017). Thus, these biomarker models of emotional processing combining both behavioural and neural measures may be useful when characterising novel drugs being developed for the treatment of depression.

3.4.1 Limitation

There are several limitations in the current study. First, although there were no statistically significant differences in the BDI and MADRS score between the treatment groups, the placebo group had lower depression scores, which may have affected the current results. Second, we did not find any significant neural differences when comparing negative and positive words, but only when comparing negative and neutral words. This investigation should be replicated in the future to examine the specific emotional valence effects underlying the antidepressant action of ketamine. However, consistent with this pattern, a previous study examined the effects of 7-day citalopram treatment on a self-referential processing task, and only found differences in the contrast between negative and control words (not when comparing positive and negative self-descriptors) (Di Simplicio *et al.*, 2012). Third, this study was limited by the small sample size, which may

potentially influence the reliability and generalizability of the current findings. Future studies should use larger sample sizes.

3.4.2 Conclusion

In conclusion, increased right DLPFC activation during negative self-referential processing may represent a biomarker for the therapeutic effect of ketamine treatment on depression. This effect was not confounded by subjective effects and was seen within 24 hours after drug infusion, in line with sustained clinical actions. However, due to the small sample size and the fact that this effect only emerged as a trend, this study should be treated as preliminary, and these findings need to be replicated in a larger clinical sample. If a future study confirms our findings, this would provide compelling evidence for the rapid effects of a single dose of ketamine on neural measures of emotional processing involved in the pathophysiology of depression.

Chapter 4: The effect of nitrous oxide on emotional processing in treatment-resistant patients with major depression: a preliminary analysis

4.1 Introduction

In previous chapters, the effects of the NMDAR antagonists ketamine and lanicemine on behavioural and neural measures of emotional processing in patients with depression were discussed. These results suggested that a single administration of ketamine displayed some possible positive effects on emotional processing 24 hours after infusion. However, the effect of NMDAR antagonists on neural activity and cognition in patients with TRD has been less studied. In chapter 1 it was indicated that N_2O is an NMDAR antagonist and may have rapid antidepressant effects in patients with TRD. Therefore, in this chapter, I assessed the effects of N_2O on behavioural measures of emotional processing in patients with TRD, 24 hours after a one hour session of N_2O inhalation.

N_2O, like other NMDAR antagonists, blocks glutamate-mediated excitotoxicity. In addition, N_2O can produce a distinctive neuropathological reaction in pyramidal neurons with intracellular vacuole formation, similarly to other NMDAR antagonists such as ketamine (Zorumski *et al.*, 2015). Also, administration of N_2O to humans produces dissociative, mood elevating and amnestic effects, again in line with the effects of ketamine (Kaar *et al.*, 2016). However, the psychological effects of N_2O reverse more quickly than ketamine, presumably because N_2O is rapidly expelled by the lungs from the body. While N_2O is an NMDAR antagonist, its precise site of action has not been clearly identified. It does not appear to involve the NMDAR ion channel and N_2O probably

acts as an allosteric modulator of NMDAR activity at a site distinct from that of ethanol and xenon, another dissociative anaesthetic (Mennerick *et al.*, 1998, Ogata *et al.*, 2006).

Nagele *et al.* (2015) conducted a double-blind, placebo-controlled cross-over trial in which 20 patients with TRD were randomly allocated to either a one hour single session of 50% N_2O with 50% oxygen inhalation (treatment group) or 50% nitrogen and oxygen (control group). They found that patients in the N_2O treatment group had a significant improvement in their depressive symptoms (as assessed using the HAMD-21) at 2 hours and 24 hours compared with the control group. Additionally, four patients had a clinically significant treatment response (Hamilton Depression Rating Scale [HAMD] score reduction ≥50%), and a further three experienced a full remission (HAMD-21 ≤ 7 points). The most common side effects of N_2O (relative to placebo) were nausea and vomiting. Anxiety and claustrophobia were also reported in a minority of patients but dissociative effects and psychotic symptoms were not apparent in this study. Importantly, this pilot study demonstrated that N_2O has a rapid onset of action as an antidepressant but does not result in the typical psychotomimetic reactions seen with ketamine. This may be due to N_2O having more favourable pharmacokinetics or perhaps because it acts at a different NMDAR site. However, this study highlighted the need to replicate these findings in other patient groups with TRD.

Compared to healthy volunteers, patients with depression have been reported to have significantly impaired recognition of happy facial expressions but significantly enhanced recognition of sad facial expressions (Mikhailova *et al.*, 1996). Additionally, patients with depression take longer to recognise happy facial expressions compared with sad facial expressions (Sloan *et al.*, 2001). Previous studies have also demonstrated that patients with depression endorse more negative

words and reject more positive words in a self-referential encoding task, and respond faster to negative words compared to healthy controls (Davis, 1979, Gilboa *et al.*, 1997). Several studies have examined the effects of psychopharmacological treatments and neural stimulation on emotional processing in patients with TRD. In a study by Stroud *et al.* (2018), 17 patients with TRD completed an emotion expression recognition task at baseline and after 1 month on psilocybin (two doses) combined with psychological support. Sixteen healthy volunteers did not receive treatment but completed the same task. The results suggested that TRD patients who received psilocybin and psychological support had significantly faster reaction times for all emotions after 1 month of treatment compared with the baseline. However, this effect was not seen in the healthy controls.

In a study by Miskowiak *et al.* (2017), 29 patients with TRD randomly received either a single session of active or sham electroconvulsive therapy (ECT) treatment. After 24 hours, participants completed a self-referential encoding task. It was found that there were no significant differences on accuracy and reaction time in the categorisation of positive vs negative words between the active ECT treatment and sham groups. Hilimire *et al.* (2015) reported that patients with TRD (n=7) endorsed fewer negative words as self-descriptive in the self-referential encoding task after both one and six months of deep brain stimulation (DBS) compared to baseline. It would therefore be important to investigate the effects of NMDAR antagonists on a self-referential encoding task, to investigate whether these agents have similar effects to those seen with pharmacological and stimulation treatments.

Previous studies have mainly focused on the neural effect of ketamine on emotional processing in patients with TRD (Murrough *et al.*, 2015, Reed *et al.*, 2019). In Murrough *et al.* (2015), 20 patients with TRD completed a facial expression recognition (happy and sad faces) fMRI task 24 hours after a single dose of ketamine. They found that patients with TRD showed increased responses in the right caudate to happy faces. A positive correlation was also found between the connectivity of the right caudate and improvement in depressive symptoms. However, there were no significant effects in relation to sad faces. To the best of my knowledge, there have been no studies that have examined the effect of N_2O on emotional processing in patients with TRD.

Therefore, this study aimed to examine the effects of a single dose of N_2O using behavioural measures of emotional processing in patients with TRD. It was hypothesised that 24 hours after a single N_2O inhalation session, patients with TRD would have reduced negative vs positive bias in emotional processing. In particular, it was predicted that the N_2O group would show improved accuracy and reaction times in recognising happy faces and endorse more positive self-descriptive personality words compared to the placebo group.

4.2 Methods

4.2.1 Participants

Patients were recruited from the outpatient clinic of the Department of Psychiatry, Second Xiangya Hospital of Central South University, Changsha, China. Data collection was undertaken by our collaborators in China. The inclusion criteria were: 1) 18-60 years old; 2) met the DSM-5 criteria for major depressive disorder, which was assessed using the Mini-International Neuropsychiatric Interview (Chinese version); 3) a pre-treatment score >17 on the 17-item HAMD; 4) met the

criteria for TRD, defined as at least two adequate dose-duration (6-8 weeks) antidepressant failures in the current depressive episode. Exclusion criteria were: 1) current or past history of bipolar disorder, psychosis, schizophrenia, schizoaffective disorder, obsessive-compulsive disorder and personality disorders; 2) substance abuse or dependence; 3) acute or significant medical condition (such as pulmonary disease); 4) severe suicidal ideation; 5) received ECT treatment in the last 6 months; 6) history of NMDA-antagonists (ketamine) intake; 7) any contraindications to the use of N_2O (such as pneumothorax, chronic cobalamin or folate deficiency treated with folic acid or vitamin B_{12}); 8) pregnant, breastfeeding or planning to become pregnant.

Ethical approval for this study was obtained from the Second Xiangya Hospital, Central South University Ethics Committee and all participants gave their written informed consent.

4.2.2 Study procedure

The study used a randomised, double-blind, placebo-controlled parallel-group design. The physician providing the N_2O or placebo-control treatment and the research team who conducted the psychiatric and behavioural assessments were completely separate. All the equipment used was identical between the N_2O and placebo control groups. The randomisation code of active treatment or placebo (with an 1:1 allocation ratio) was generated by a member of the research group who was not involved in the study. All patients were required to attend an initial screening session and complete baseline assessments including HAMD-17, Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR) scale, Clinical Administered Dissociative States Scale (CADSS) and the Facial Expression Recognition Task (FERT).

On day 1, participants were randomised to receive either a mixture of 50% oxygen and 50% N_2O (treatment group) or 50% oxygen with nitrogen (control group) for 1 hour. The concentration of N_2O (50%) was based on a previous clinical trial, which employed the concentration used clinically for rapid analgesia (Nagele *et al.*, 2015). The gas mix was administered using an anaesthesia facemask via tubing connected to the anaesthetic machine. Patients were monitored using a three-lead electrocardiogram, pulse oximetry, non-invasive blood pressure and end-tidal carbon dioxide during and after the treatment by an anaesthesiologist. After around one hour of gas administration, patients were transferred to the recovery room and monitored for two hours by the study team physicians. The HAMD, QIDS-SR, and CADSS assessments were repeated two hours after the end of the inhalation.

On day 2 (24 hours after treatment administration), participants returned to the lab and completed the HAMD, QIDS-SR, CADSS assessment, FERT and ECAT.

4.2.3 Questionnaire measures

Hamilton Depression Scale (HAMD)

The Chinese-validated version of the 17 item HAMD scale for the assessment of depressive symptoms (Williams, 1988, Zheng *et al.*, 1988) was administered by the practice clinicians. The total score is calculated as the sum of the 17 items and ranges from 0 to 52. Higher HAMD scores indicate higher levels of depressive symptoms.

Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR)

The Chinese-validated version of the 16 item QIDS-self-report inventory (Liu *et al.*, 2013, Rush *et al.*, 2003) is used to evaluate the severity of depressive symptoms, including low mood, concentration, self-criticism, suicidal ideation, interest, energy, sleep, and appetitive and psychomotor agitation or retardation. The total score ranges from 0 to 27. Higher QIDS-SR scores indicate higher levels of depressive symptoms.

Clinician-Administered Dissociative Status Scale (CADSS)

The Chinese-validated version of the CADSS is used for evaluating dissociative symptoms. It has 5 subscales, including altered environmental perception, time perception, body perception, feelings of unreality, and memory impairment (Bremner *et al.*, 1998, Hu *et al.*, 2016). Higher CADSS scores indicate higher levels of dissociative symptoms.

Safety outcomes were also recorded using clinical observation and a series of questions regarding side effects and their severity.

4.2.4 Psychological tasks

Facial Expression Recognition Task (FERT)

Patients suffering from severe depression may not feel well during the study and may have difficulty completing psychological tasks. Therefore, this study lessened the difficulty of the FERT task. The current study used only happy and sad faces as emotional expressions in this task, as these emotional faces are likely to be of particular relevance to the patient's negative schemata (Leppänen *et al.*, 2004, Surguladze *et al.*, 2004). This paradigm has been employed in a previous study in social anxiety (Silvia *et al.*, 2006). The facial expression recognition task uses facial

photographs from individuals (10 male and 10 female) displaying two emotions (sad and happy). These facial pictures were obtained from the Chinese Facial Affective Picture System (Gong *et al.*, 2011). There were 40 presentations of each emotion (100% intensity), with a total of 80 facial presentations during the task. The facial expressions were presented in a random order on a screen. Each trial started with a blank screen for 1000 ms, followed by a face for 500 ms. After the faces were presented, the subjects were given unlimited time to identify the emotion, and participants were instructed to identify each facial expression as happy or sad as quickly and as accurately as possible.

Emotional Categorisation Task (ECAT)

The ECAT presents the participants with eighty personality characteristics that are categorised as either extremely disagreeable (e.g. domineering, untidy, hostile) or agreeable (e.g. cheerful, honest, optimistic) (taken from Anderson (1968)). These personality characteristics were translated from English to Chinese, as the ECAT has not yet been validated in Chinese cultural settings. Participants were asked to imagine that they had overheard someone describing them using each of the words and to judge as quickly and accurately as possible whether they would like or dislike to be so described. The subjects were required to press a key (like or dislike).

4.2.5 Statistical analysis

Data were analysed using IBM SPSS V23. For all tasks, any scores observed to be greater than 3 standard deviations from the group mean were considered extreme outliers and were excluded from the analysis (Thomas *et al.*, 2016). It was only necessary to exclude two participants from the placebo group in the FERT. Participants' baseline characteristics were analysed using a t-test

and Fisher's exact test as appropriate between the active treatment and placebo groups. Mixed ANOVAs were conducted using the group (N_2O or placebo) as the between-subject factor and the time sequence of the three levels for HAMD, QIDS-SR and CADSS as the within-subject factor. Accuracy and reaction time data on the FERT were analysed using a mixed ANOVAs with time (pre and post) and emotion type (happy and sad) as the within-subject factors and the group (N_2O and placebo) as the between-subject factor. The ECAT was analysed using mixed ANOVAs, with the group (N_2O and placebo) as the between-subject factor, and emotion (positive and negative personality characteristics) as the within-subject factor. Where sphericity was violated, Greenhouse-Geisser corrected *p* values were used. Missing data were analysed as the last-observation-carried-forward. A *p*-value of <0.05 was considered to be statistically significant. The effect size was estimated suing ηp2.

4.3 Results

Between April and December 2019, 33 patients were screened to participate in this study. After excluding 5 patients that did not meet the inclusion criteria, 28 eligible patients participated in this study. The 28 patients were randomly assigned to the N_2O treatment (n=16) or placebo group (n=12). Three patients withdrew from the study after the screening session, leaving 25 patients who received treatment and completed the assessments. All results reported include only these 25 patients. The initial aim was to recruit 40 participants; however, the study had to be suspended due to the COVID-19 pandemic.

4.3.1 Baseline demographic and clinical characteristics

There were no significant differences between the groups in terms of age, gender, current medication use, depression history and the duration of the current episode, or the HAMD and QIDS scores. At the time of the study, 88% of the patients were taking SSRIs. Significantly more patients in the placebo group had received psychotherapy than patients in active treatment (N_2O) group (p=0.06). The mean HAMD and QIDS-SR scores were 21.2 ± 4.4 and15 ± 4.9 points, respectively (see Table 5).

Table 5. Baseline demographic and clinical characteristics of the participants

	Total		N_2O (N=14)		Placebo (N=11)		Statistics	
	N	%	N	%	N	%	$\chi 2$	*p*-value
Gender							0.17	0.24
Male	13	52	9	64.3	4	36.4		
Female	12	48	5	35.7	7	63.6		
History of ECT	6	24	5	35.7	1	9.1	0.12	0.14
History of TMS Treatment	5	20	3	21.4	2	18.2	0.84	0.62
History of Psychotherapy	6	24	1	7.1	5	45.5	0.03	0.06
Number of Current antidepressants								
SSRI	22	88	13	92.9	9	81.8	0.40	0.57
SNRI	9	36	4	28.6	5	45.5	0.38	0.43
Other antidepressants	3	12	1	7.1	2	18.2	0.40	0.57
Current antipsychotics	3	12	1	7.1	2	18.2		
Quetiapine	1	4	1	7.1	0	0	0.40	0.57
Aripiprazole	1	4	1	7.1	0	0	0.37	1.0
Olanzapine	2	8	2	14.3	0	0	0.19	0.49
Other antipsychotics	2	8	0	0	2	18.2	0.10	0.18
	Mean	SD	Mean	SD	Mean	SD	F	*p*
Mean age (years)	35.5	11.8	35.6	10.8	35.4	13.4	0.002	0.97
Education (years)	13.2	2.9	13.6	3.1	12.7	2.8	0.58	0.45
BMI	24.03	3.88	24.97	3.96	22.83	3.61	1.96	0.18
Depression history (years)	8.1	7.5	9.0	8.7	7.0	6.2	0.38	0.54
Duration of current episode (months)	5.5	4.8	6.8	5.9	3.8	1.8	2.5	0.13
QIDS-SR	15.0	4.9	14.5	5.2	15.5	4.7	0.27	0.61
HAMD	21.2	4.4	21	4.4	21.4	4.6	0.04	0.84

ECT: electroconvulsive therapy; TMS: transcranial magnetic stimulation; QIDS-SR: Quick Inventory of Depressive Symptomatology-Self Report; HAMD: Hamilton Depression Scale; SSRI: Selective serotonin reuptake inhibitor; SNRI: Serotonin-norepinephrine reuptake inhibitor; BMI: Body mass index

4.3.2 Effect on mood and subjective symptoms

4.3.2.1 Mood ratings

For the HAMD [$F(2, 46)=27.39$, $p<0.001$, $\eta_{p2}=0.54$] and QIDS-SR [$F(2, 46)=7.38$, $p=0.002$, $\eta_{p2}=0.24$] scores, there was a significant decrease over time from baseline to subsequent ratings across all groups (see Figures 10 and 11). However, there was no significant interaction between time and group or a significant main effect of group, indicating that N_2O did not improve mood more than the placebo ($p>0.2$).

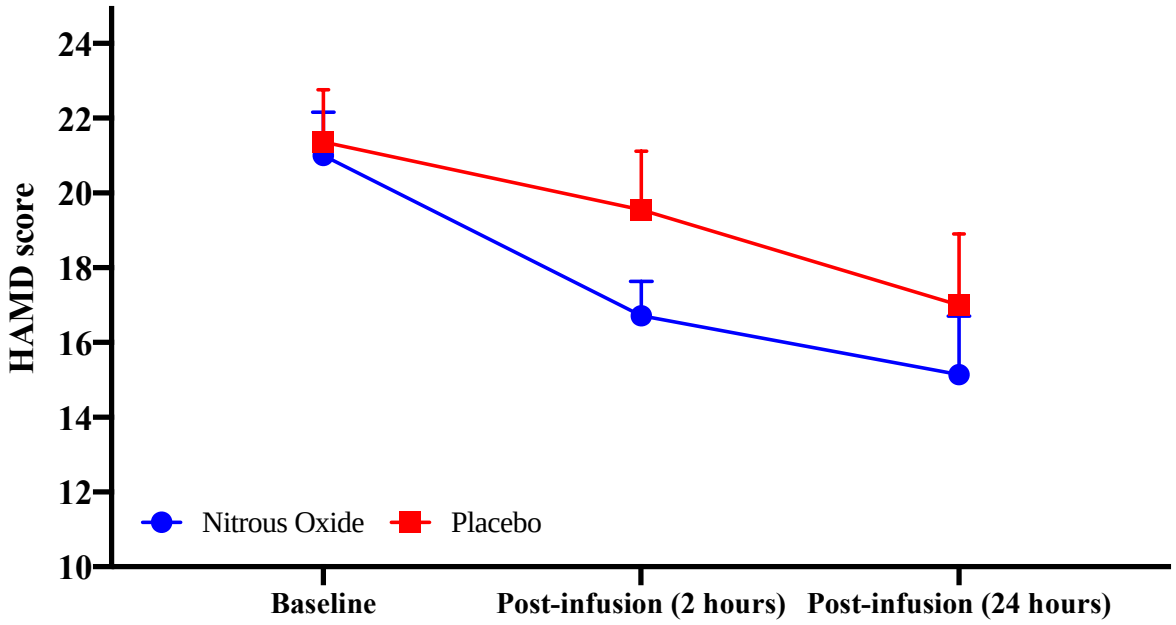


Figure 10. The effect of N_2O vs placebo on the HAMD scores. HAMD: Hamilton Depression Scale; *Error bars represent the standard errors of the mean.*

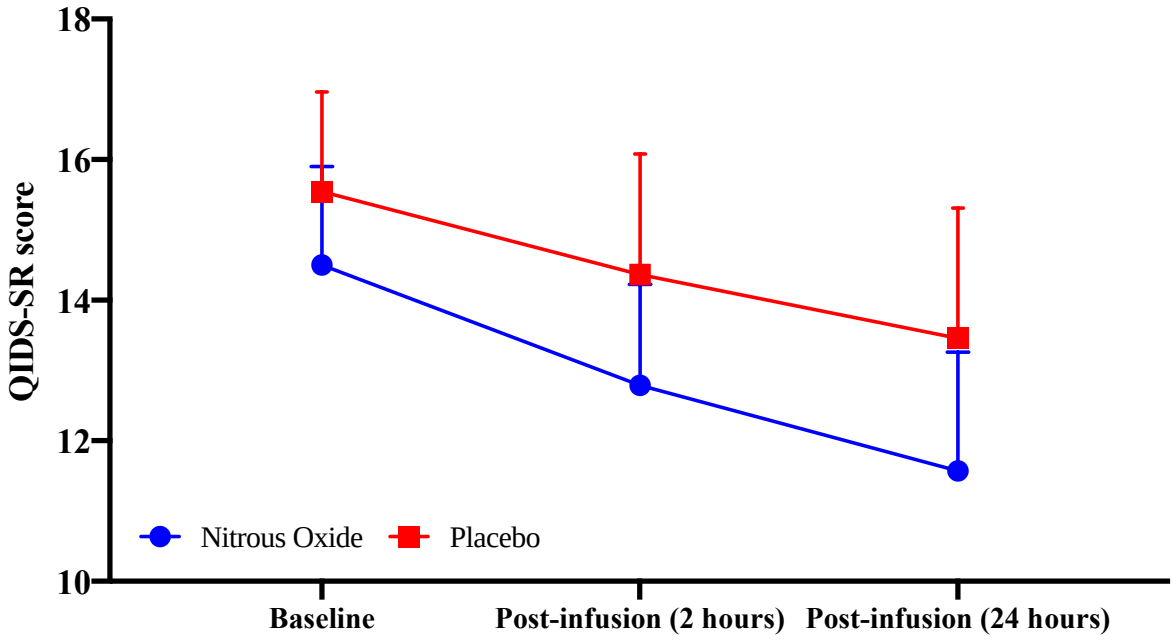


Figure 11. The effect of N_2O vs placebo on the QIDS-SR scores. QIDS-SR: Quick Inventory of Depressive Symptomatology-Self Report; *Error bars represent the standard errors of the mean.*

4.3.2.2 Dissociation effects and adverse events

In the CADSS ratings, there was no significant main effect of time [F(1.59, 46)=0.70, p=0.47] or group [F(1, 23)=0.12, p=0.73], and no significant interaction between time and group [F(1.59, 46)=0.15, p=0.81] (see Figure 12), indicating that the active treatment group (N_2O) did not have an elevated dissociative effect compared to the placebo group. No serious adverse events were reported. However, two patients in the N_2O group suffered from nausea and vomiting after the inhalation.

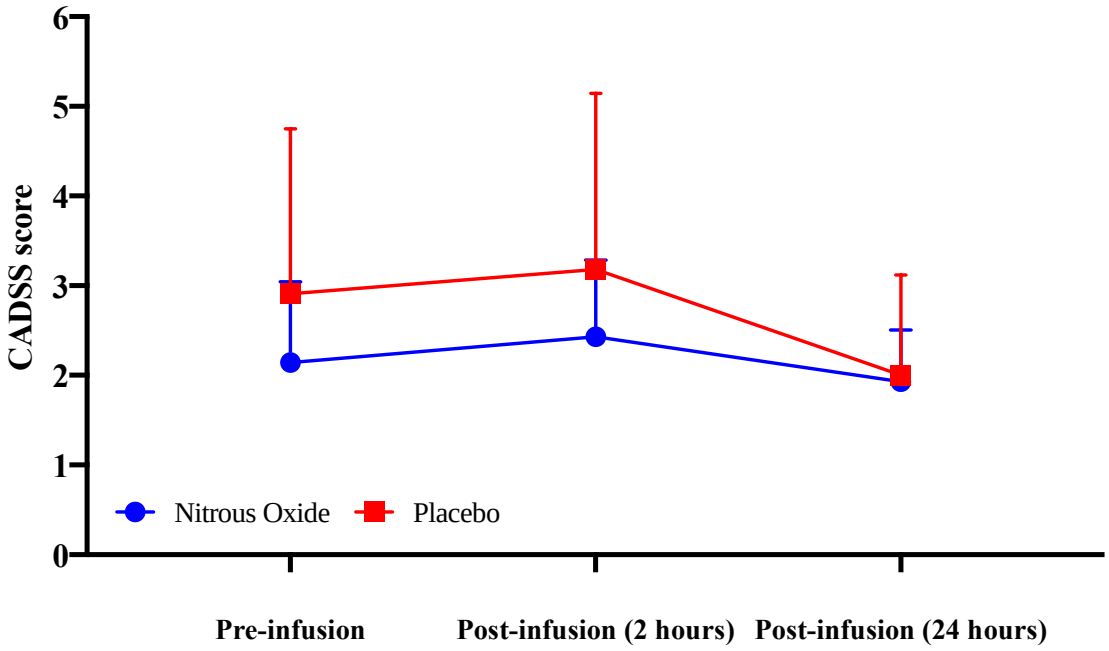


Figure 12. The effects of N_2O vs placebo on dissociation (CADSS scores). CADSS: Clinician-Administered Dissociative Status Scale; *Error bars represent the standard errors of the mean*.

4.3.3 Psychological tasks

4.3.3.1 Facial Expression Recognition Task (FERT)

Accuracy

There was no significant main effect of time [F(1, 21)=0.006, p=0.94] or emotional valence [F(1, 21)=2.56, p=0.12] and no main effect of group [F(1, 21)=0.3, p=0.87]. There were also no significant interaction between emotion and treatment group [F(1, 21)=0.31, p=0.58], time and treatment group [F(1, 21)=0.004, p=0.95], time and emotion [F(1, 21)=2.71, p=0.12] or emotion, time and treatment group [F(1, 21)=1.576, p=0.23] (see Figure 13).

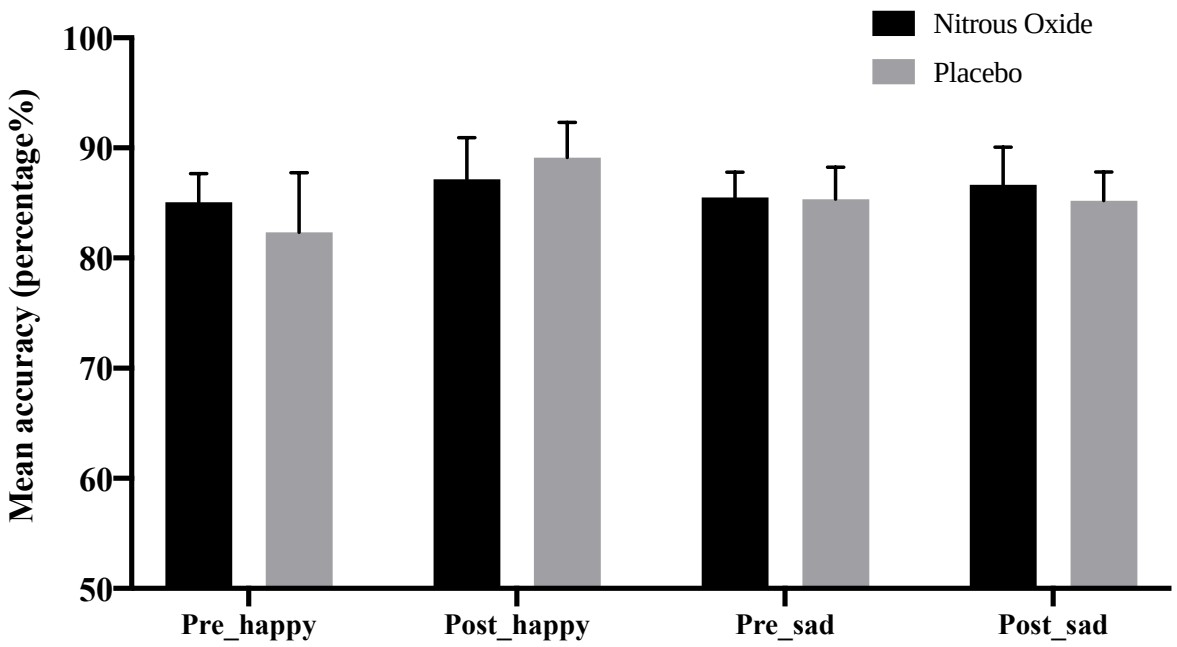


Figure 13. Accuracy of facial expression recognition in the N_2O and placebo groups. *Values represent the mean accuracy rates for each of the two facial expressions; Error bars represent the standard errors of the mean.*

Reaction Times

Participant reaction times were measured to see if there were any significant differences in recognising sad vs happy faces between the N_2O and placebo groups. There was a significant main effect of time [$F(1, 21)=6.27$, $p=0.021$, $\eta_{p2}=0.23$], and emotional valence [$F(1, 21)=12.65$, $p=0.002$, $\eta_{p2}=0.38$]. In all groups, reaction times were slower for the sad facial expression. Also, across all emotions, the reaction times were decreased with repeat testing. However, there was no significant interaction between emotion and treatment group [$F(1, 21)=0.02$, $p=0.90$], or between time and treatment [$F(1, 21)=0.11$, $p=0.74$], time and emotion [$F(1, 21)=0.11$, $p=0.75$] or emotion, time and treatment group [$F(1, 21)=0.429$, $p=0.52$] (see Figure 14).

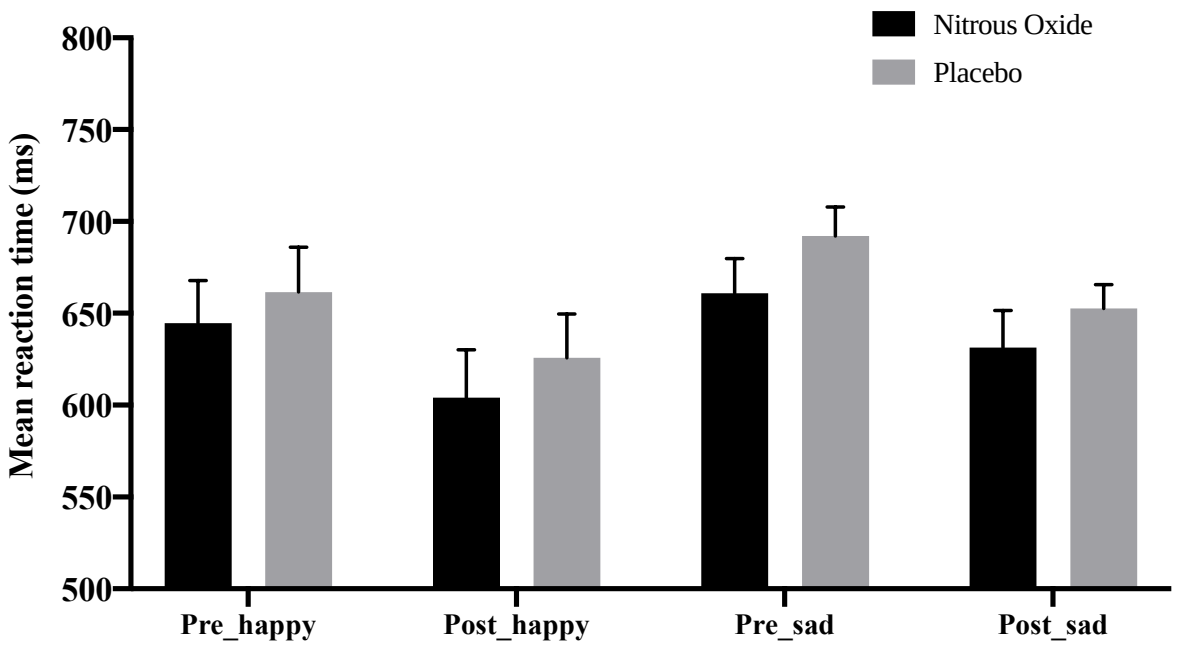


Figure 14. Average reaction times of facial expression recognition in the N_2O and placebo groups. *Values represent the mean accuracy rates for each of the two facial expressions; Error bars represent the standard errors of the mean.*

4.3.3.2 Emotional Categorisation Task (ECAT)

There were no significant differences between the treatment groups in task performance during the emotional categorisation task (all $p>0.1$). The average correct performance rate on the positive and negative self-descriptors was 57.88% and 48.13%, respectively (see Figure 15). The analysis of reaction times was not performed due to the low accuracy performance rates on the task. Therefore, this chapter will not interpret the findings from the self-referential encoding task.

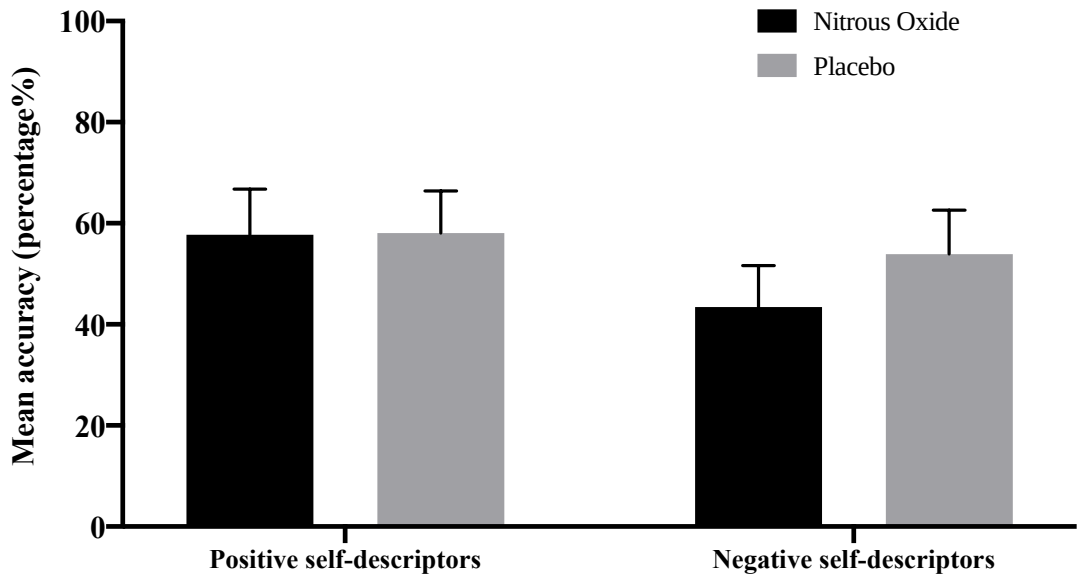


Figure 15. Accuracy in the emotional categorisation task. *Values represent the mean number of correctly recalled positive and negative words. Error bars represent the standard errors of the mean.*

4.4 Discussion

This is the first study to examine the effect of N_2O in patients with TRD on a facial emotion recognition task pre- and post-treatment and an emotional categorisation task post-treatment. In this preliminary analysis, the results found that a single dose of N_2O did not affect emotional processing in patients with TRD. Specifically, there were no effects of the drug on the recognition of facial expressions, both in terms of accuracy and reaction time.

Although both groups showed a significant decrease over time from baseline to 24 hours in terms of the depressive symptoms ratings, patients receiving N_2O did not have statistically different HAMD and QIDS-SR scores compared to the placebo, which contradicts a previous pilot trial (Nagele *et al.*, 2015). However, direct comparisons between the two studies are difficult due to the

different study sample and study designs. First, Nagele *et al.* (2015) used a cross-over design and the patients with TRD underwent two treatment sessions (N_2O and placebo) one week apart. In the current study, we used a parallel group design and patients only received either N_2O or the placebo. Second, the sample characteristics were different between the two studies. Nagele *et al.* (2015) included a highly treatment-resistant cohort (e.g. the average depression history was 19 years, with higher baseline depressive symptom scores and an average age of 48 years) as compared to the current study (the average depression history was 8 years, with lower baseline depressive scores and an average age of 35 years). Previous findings suggest that patient characteristics (such as BMI, previous treatment history, anhedonia) are associated with treatment response to ketamine treatment (Niciu *et al.*, 2014, Schwartz *et al.*, 2016, Thomas *et al.*, 2018). Also, the small sample size may limit the detection of statistical differences between the two groups. In fact, the size of the difference in HAM-D scores between N_2O and placebo groups in our study at 2 hours was similar to that reported by Nagele *et al.* (2015). In the future, a study with a larger cohort should be conducted to examine the efficacy of N_2O in treating resistant depression.

Furthermore, in the current study, a lack of dissociative effects on the CADSS ratings in the N_2O group was found. This may be because the psychological effects of N_2O are quick to reverse once the treatment is stopped. While N_2O can certainly cause psychotomimetic and dissociative effects in healthy volunteers (Kaar *et al.*, 2016), it is possible that depressed patients are less susceptible to this reaction. However, we did not assess the CADSS score immediately afterwards. Nagele *et al.* (2015) also did not find psychotomimetic effects in their patient group following N_2O administration. Future studies might examine whether if acute dissociation symptoms occur during N_2O inhalation in TRD, they can predict the antidepressant treatment response. Dissociative

symptoms have been suggested to predict the antidepressant response to ketamine (Luckenbaugh *et al.*, 2014, Niciu *et al.*, 2018). However, Williams *et al.* (2018) found that while naltrexone abolished the antidepressant effect of ketamine, it did not alter the dissociative responses.

The facial expression recognition task (of happy vs sad) has also been examined in patients with depression using neuroimaging. Murrough *et al.* (2015) included 20 patients with TRD (defined as nonresponse to at least two previous antidepressant medications) who were free of concomitant antidepressants and 20 healthy volunteers as the control group. At baseline, the patients with TRD showed a reduced response in the right caudate to the happy faces. However, 24 hours after a single dose of ketamine, the patients with TRD displayed an increased right caudate response to the happy faces, but no neural changes were observed with the sad faces. In addition, there were no significant differences between the groups in terms of accuracy and reaction times, which is similar to the findings of the current study.

Furthermore, Reed *et al.* (2019) recently examined the effects of a single dose of ketamine on neural responses during the facial emotion recognition task (with sad, angry and happy emotions) in patients with TRD and healthy controls. The results showed a significant group-by-drug interaction in certain brain regions such as the anterior cingulate cortex and insula. However, the behavioural results did not show a significant group and emotion (positive vs negative faces) interaction in terms of accuracy and reaction time. Behavioural findings may differ from the neuroimaging results in these studies because fMRI BOLD activation is likely to be a more sensitive measurement of brain function than behavioural measures. To the best of my knowledge, the effects of N_2O on emotional stimuli and the relevant neural circuity in patients with depression

have not been studied. A future study could consider exploring the neural effects of N_2O during the facial expression recognition task.

Unexpectedly, patients in both the treatment and placebo groups had a low number of correct responses on the positive and negative self-descriptors in the ECAT (43-58%) compared to previous studies (80-90% accuracy rates) (Di Simplicio *et al.*, 2012, Miskowiak *et al.*, 2017). The ECAT has not been previously adapted to Chinese cultural settings, so we translated the personality words (Anderson, 1968) from English to Chinese in order to design this task. However, this translation process was not validated given the lack of norms available and different personality characteristics may also be viewed differently between western and Chinese cultures. For example, "shame" in western cultures is a negative emotion and is associated with a sense of worthlessness and powerlessness. In contrast, in the Chinese society, an individual without shame would be perceived as lacking moral sensibility (Zhong *et al.*, 2008). Other key differences in the perception of words include introversion-extraversion, altruism, compliance, modesty, straightforwardness, and individualism-collectivism (Cheung *et al.*, 2001). Unfortunately, these cultural differences were not taken into account in the current ECAT design, which was not empirically cross-culturally validated. Future studies should first validate the ECAT in the Chinese population. On the other hand, it is possible that patients were not sufficiently engaged with completing the task or found it difficult to focus due to their depression.

4.2.1 Limitations

There are several limitations to the current study. First, N_2O induces sedation and euphoric effects during inhalation, which may mean that some patients were able to guess whether they were receiving active treatment or a placebo. However, we did not ask patients whether they could guess

which treatment group they were assigned to. Second, patients with TRD did not go through a wash-out period, and most patients were taking concomitant psychotropic drugs. This was because an ethical committee advised that a wash-out period would be unethical and could result in patients feeling more depressed and presenting a higher suicide risk. The use of concomitant psychotropic medications could have therefore added confounding factors, which limits the current findings. Future studies should consider using drug-free patients with TRD to investigate emotional processing during N_2O treatment, although this may be difficult to achieve from a practical point of view as most TRD patients are on some form of medication. Third, a single N_2O treatment may be insufficient to modulate the measurement of behavioural tasks in a heterogeneous cohort. Future studies should conduct a long-term RCT with repeated N_2O to explore the effects of N_2O on emotional processing. It would also be important to examine behavioural responses closer in time to the end of N_2O inhalation. Fourth, as mentioned, the ECAT has not undergone cross-cultural validation which may have affected the findings in the current study. Fifth, previous exposure to the same face at two timepoints may have reduced the amount of change observable in the FERT due to learning effects and habituation.

Previous research has indicated that fMRI is likely to be a more sensitive method to use than behavioural measures. Future studies should explore the neural effects of N_2O during the facial expression recognition task. Also, the simplified version of the FERT used here did not assess potential differences between all negative emotions, such as anger and fear, which are also central to emotional face processing. Finally, the sample size was small, which may mean that potential group differences in mood symptoms and FERT performance could not be detected. However, the small sample size is common in studies that have focused on emotional processing in treatment-

resistant depression, given the challenges associated with recruiting such population (Salvadore *et al.*, 2009, Stroud *et al.*, 2018).

4.2.2 Conclusion

In conclusion, this randomised, placebo-controlled, double-blind study showed that single-dose N_2O treatment did not change the processing of emotions in patients with treatment-resistant depression compared to placebo. However, the current findings must be cautiously interpreted, as these results are preliminary due to the small sample size. It is therefore important that future studies are conducted with larger groups of TRD patients.

Chapter 5 Effect of the NMDA receptor partial agonist, d-cycloserine, on emotional processing and autobiographical memory

5.1 Introduction

Previous chapters have examined the effect of NMDAR antagonists on emotional processing in depressed patients. The results suggested that NMDAR antagonists did not show positive effects on emotional processing such as those seen with conventional antidepressants. The role of NMDAR targeting antidepressants in emotional processing is complex, and therefore, looking at an NMDAR glycine site partial agonist is of interest, especially because these have been shown to have antidepressant effects in some studies. As noted previously, there is now substantial evidence that conventional antidepressant treatments produce positive biases in emotional processing in both healthy populations and depressed patients. Therefore, the aim of this chapter was to explore the effects of the NMDAR partial agonist d-cycloserine (DCS) on emotional processing in healthy volunteers.

As discussed in Chapter 1, NMDAR hyperfunction is associated with excitotoxicity and psychotomimetic effects, but modulation of NMDAR activity by actions at the glycine site of the NMDAR could produce antidepressant effects without psychotomimetic reactions (Ragguett *et al.*, 2019). For example, a single oral dose of D-serine (a glycine site agonist) was found to be effective in reducing anxiety and sadness, and in enhancing cognitive parameters in human studies (Levin *et al.*, 2015). Preskorn *et al.* (2015) found that GLYX-13 (a glycine site partial agonist) significantly improved depressive symptoms in depressed patients who were nonresponsive to a previous antidepressant, according to an assessment performed within 2 hours post-treatment.

DCS is an antibiotic which is used to treat tuberculosis and urinary tract infections. DCS appears to be a partial NMDAR agonist at low doses, but it acts as an NMDAR antagonist at higher doses (Schade and Paulus, 2016). DCS has been studied as an 'add-on' treatment in two placebo-controlled trials in patients with treatment-resistant depression. Positive results were obtained with a higher dose of DCS (1000 mg/day), but not with a lower dose (250 mg/day) (Heresco-Levy et al, 2006; 2013). However, Kim *et al.* (2016) found that when low-dose DCS was used to enhance extinction learning in exposure treatment for OCD, a marked improvement in depressive symptoms was unexpectedly noted. This result was consistent with the study of Wilhelm *et al.* (2008) who used DCS to enhance the efficacy of behaviour therapy for OCD; they found that there was significant improvement in depressive symptoms, which was independent of improvement in OCD symptoms. Therefore, further studies are required to evaluate how DCS may function as an antidepressant, in combination with both conventional antidepressant medicines and with psychotherapies.

Importantly, the previous chapter showed that NMDAR antagonists operate differently from conventional antidepressants in their effects on emotional processing. Here we propose to investigate the effects of an NMDAR partial agonist, d-cycloserine, which may also show effects that are different from standard antidepressant drugs. Given the important role of NMDA glutamate receptor function in memory and learning (Castellano *et al.*, 2001, Riedel *et al.*, 2003), this chapter focused specifically on memory.

Deficits in the specificity of autobiographical memory are a cognitive marker of depression. Patients with depression have significant difficulty recalling specific personal memories and also display an overgeneralised autobiographical memory (Köhler *et al.*, 2015). Previous studies have suggested that difficulties in autobiographical memory specificity contribute to the maintenance of depressive illness, through pathways such as perceiving negative social encounters, lack of social problem-solving skills, lack of self-efficacy, and feelings of hopelessness (Hermans *et al.*, 2005, Young *et al.*, 2016). Moreover, evidence also suggests that increases in memory-specificity are associated with improvement in depressive symptoms, rumination, and cognitive avoidance in depressive patients (Neshat-Doost *et al.*, 2013, Raes *et al.*, 2009).

The effect of conventional antidepressants on autobiographical memory has been little studied, but an investigation in healthy volunteers found that neither sertraline nor bupropion affected cued recall of autobiographical memory (Carvalho *et al.*, 2006). DCS has not previously been tested in this respect, but animal studies have suggested that it facilitates long-term synaptic plasticity, presumably through its effects on NMDARs. A number of studies have also explored the potential of DCS to enhance retention and extinction learning when combined with CBT for anxiety disorders (Bontempo *et al.*, 2012, Norberg *et al.*, 2008), although the results have been mixed. As autobiographical memory is a novel therapeutic target for depression (Köhler *et al.*, 2015), there is a need to investigate whether the psychological treatment-enhancing effects of DCS could improve autobiographical memory for example, by increasing retrieval of specific autobiographical memories.

Also, the effects of low dose of DCS on measures of emotional processing have not been investigated to date. Given the contradictory results of different trials in the current literature, it is unclear whether DCS has antidepressant effects similar to conventional re-uptake blockers in acting to increase positive biases or reduce negative biases in emotional processing.

The current study was therefore designed to assess the effects of an acute low dose DCS (250 mg) on autobiographical memory tasks and emotional processing in healthy volunteers. Given the finding that a single dose of the NMDAR antagonist ketamine and the partial agonist GLYX-13 has antidepressant effects lasting for at least 24 hours, it was also explored the potential for long-lasting emotional and memory effects of a single dose of DCS. Hence, all participants performed autobiographical memory tasks and emotional processing at 3 and 24 hours following active drug/placebo administration. This study hypothesized that DCS would enhance memory specificity and induce a positive bias in emotional processing (specifically increase the processing of positive vs. negative stimuli in tasks measuring facial expression recognition and emotional memory) as compared to placebo.

5.2 Methods

5.2.1 Participants

Between August and December 2018, 40 healthy volunteers aged 18–40 years enrolled in this study. Inclusion criteria were: 1) BMI within the range 19–30 kg/m^2; 2) no current or a history of any psychiatric disorders, as assessed using the SCID for DSM-5; 3) no lifetime history of significant physical disease; 4) sufficiently fluent in English; 5) non- or light smoker (< 5 cigarettes a day); 6) low caffeine use (< 5 cups a day); 7) low alcohol use (< 30 units per week); 8) abstinence

from any CNS-active medication during the last 6 weeks; 9) female participants must not be pregnant, breastfeeding, or planning pregnancy; 10) no current or past drug- or alcohol-dependency.

Ethical approval for this study was obtained from the Central University Research Ethics Committee (CUREC) (Oxford University) and all participants gave written and verbal consent prior to participation.

5.2.2 Study procedure

This was a double-blind, placebo-controlled, randomised study. All participants were required to complete baseline assessments, including the Eysenck Personality Questionnaire (EPQ) (Eysenck and Eysenck, 1975), the Spot-the-Word (STW) test to assess verbal intelligence (Baddeley *et al.*, 1993), the BDI (Beck *et al.*, 1961), and the Trait Anxiety Scale (STAI-T) (Speilberger *et al.*, 1970).

Eligible participants arrived at the laboratory in the morning and were randomised to receive either a single dose of 250 mg DCS or placebo. Participants were required to abstain from alcohol and caffeine from 12 hours before testing and fast for 2 hours in the morning of the testing day. A randomisation code (1:1 allocation ratio) was generated by a member of our group who was not otherwise involved in the study. DCS is thought to have a half-life of 8 h to 15 h (Patel *et al.*, 2011). Given that plasma peak levels of DCS are reached within 3–4 hours after administration (Scholl *et al.*, 2014), all participants were tested at 3 hours after drug administration. During the 3 hours between dosing and testing, participants were asked to stay in the testing room, but they were free to engage in an activity of their choice, e.g., reading a book, working, or using their laptop.

Before taking the active drug or placebo, participants filled out the Spielberger State Anxiety Inventory (STAI-S) (Spielberger and Gorsuch, 1983), Positive and Negative Affect Schedule (PANAS) (Watson *et al.*, 1988), the Befindlichkeits Scale (BFS) (Von Zerssen and Bf-SR, 2011), as well as the Bond–Lader visual analogue scales (VAS) (Bond and Lader, 1974) to assess side-effects and subjective mood ratings. These questionnaires were repeated at 3 hours after drug administration and again at the end of the third study visit (24 hours after drug administration). At 3 hours after drug administration, participants completed the following tasks from the ETB: FERT, ECAT, Facial Dot-Probe Task [FDOT], EREC and EMEM, as well as an autobiographical memory task (AMT) (see below) and a cognitive stress task. The whole process (study visit 2) occurred over approximately 5 hours and the tasks were performed in the above order. The results of the stress task will be reported in Chapter 6. In order to examine longer-lasting emotional effects, participants returned to the laboratory after 24 hours and completed the FERT, EREC and AMT tasks once again.

5.2.3 Psychological tasks

5.2.3.1 Emotional test battery (ETB)

The details of each task involved in the ETB have been described in previous chapters. Briefly, the FERT presents individual facial expression reflecting seven different emotions (neutral, fear, anger, sadness, disgust, happiness, and surprise). Each emotional expression is conveyed with varying levels of intensity and participants are required to recognise these different emotions in the images of the stimulus faces. The ECAT involves the presentation of 60 personality characteristics which are categorised as either extremely disagreeable (e.g., bossy, crude, and

scornful) or agreeable (e.g., enthusiastic, honest, and thoughtful). Participants are required to imagine overhearing someone describing themselves using each of the words and to judge as quickly and accurately as possible whether they would like or dislike to be described by each of these personality descriptors. The FDOT tests attention to positive versus negative emotions using happy and fearful faces. The accuracy and the time needed to identify the probe orientation correctly were recorded. Vigilance scores were calculated for emotional pairs by subtracting reaction times in incongruent trials (i.e., the probe appears behind the neutral expression) from congruent trials (i.e., the probe appears behind the emotional expression) as an index of attentional bias to emotional information. The EREC was tested 15 minutes after completion of the ECAT. Participants were asked to recall as many words as they could recall from the ECAT task, regardless of valence, within 4 minutes. The EMEM was administered after a 19-minute long distraction period after the ECAT; participants were presented with personality words from the ECAT and also with another set of positive and negative personality words (which had not been seen before) and were asked to classify each word from the ECAT as familiar or unfamiliar.

5.2.3.2 Autobiographical Memory Task (AMT)

In the AMT (Williams and Broadbent, 1986, Williams *et al.*, 1996), memory cues were presented visually on a computer screen in a fixed order, using positive, negative, and neutral words. Participants were exposed twice to the AMT, using a parallel version of the cues, on day 1 and day 2. Each word was presented only once. For each cue word, participants were required to recall an important or trivial event which occurred recently or a long time ago, within 1 minute, but the event should be something which happened at a particular time/day and in a particular place, and the event must have lasted less than 1 day. Participants were told that they should not recall the

same event for more than one cue word. Additionally, on day 2, participants were told that the memory they recalled could not be the same as any of the responses from day 1. Correct and incorrect examples were provided. Three practice cues words (tree, chair, and car) and feedback were given. Responses were tape-recorded for subsequent coding. The memories were coded (Haddad *et al.*, 2009) as (1) specific memories: an event which occurred on a particular time/day and in a particular place; (2) extended memories: an event which lasted more than a day; (3) categorical memories: an event which occurred repeatedly; (4) semantic memories: something associated with the cue word, rather than a memory; (5) no response (omission); (6) repeated responses: the same response given for different cue words. Extended and categorical memories were combined to form the overgeneralised memory type. A second independent rater coded 20% of the responses to assess reliability of coding; the mean kappa coefficient of reliability on specific memories was 0.97.

5.2.4 Statistical Analyses

All analyses were performed using IBM SPSS V23. For all tasks, any scores observed to be greater than 3 standard deviations from the group mean were considered extreme outliers and were excluded from the analysis (Thomas *et al.*, 2016). It was only necessary to exclude one participant, in the ECAT. The baseline measurements (age, education years, BMI, EPQ, Verbal IQ, and STAI-T) as well as the mood and anxiety scales administered on day 2 (BFS, STAI-S, PANAS, and VAS) were analysed using independent sample *t*-tests. Gender differences were assessed using the chi square test. Mood, anxiety, and side-effects scales (BFS, STAI-S, PANAS, and VAS) on day 1 were analysed using repeated measures analysis of variance (ANOVA), with time (pre-post drug/placebo) and group condition (DCS/placebo) as within- and between-subjects factors,

respectively. The data from the AMT and each ETB task were analysed using mixed design ANOVA with treatment group (DCS and placebo) as the between-subject factor and different within-subject factors, depending on the task (FERT: facial expression; ECAT/EREC/EMEM/AMT: word valence; FDOT: facial expression and masking). Where sphericity was violated, Greenhouse-Geisser corrected *p* values were used. The effect size was estimated using ηp2. Significant interactions between time and group were further examined using between-group independent sample *t*-tests.

5.3 Results

5.3.1 Baseline characteristics

The groups were well matched in terms of age, gender, BMI, Verbal IQ, STAI-T (trait anxiety), EPQ, and depression symptoms (Table 6). The groups were also comparable in their scores on the STAI-S (state anxiety), PANAS and VAS (mood levels and side-effects).

Table 6. Baseline demographic and baseline mood scores

	DCS (n = 20)	Placebo (n = 20)	T	Significance *p*
Age (years)	23.45 (4.09)	22.7 (3.3)	0.637	0.528
Gender, n				
Male	10	9		
Female	10	11		
Years of education	16.75 (2.47)	16.7 (1.92)	0.071	0.943
EPQ neuroticism	7.45 (5.12)	6.65 (4.25)	0.537	0.594
EPQ psychoticism	4.35 (3.34)	3.6 (2.11)	0.848	0.402
EPQ extraversion	13.25 (4.84)	14.9 (3.74)	-1.206	0.235
BMI	22.59 (2.20)	22.81 (2.64)	-0.321	0.772
BDI	3.15 (3.05)	2.7 (3.48)	20 (7.4)	0.666
Verbal IQ	62.9 (12.77)	66.75 (12.23)	-0.974	0.336
STAI-T	34.8 (7.73)	33.55 (8.94)	0.473	0.639

EPQ, Eysenck Personality Questionnaire; BFS, Befindlichskeit Scale; BDI, Beck Depression Inventory; STAI-T, Spielberg Trait Anxiety Inventory; BMI, body mass index; IQ, intelligence quotient.

5.3.2 Changes in subjective mood

There were no significant main effect of group or time by group interaction on any of the questionnaires measuring subjective mood and anxiety, such as the BFS, STAI-S, and PANAS (all $p>0.5$). There were also no significant main effect of group or time × group interactions on subjective symptoms (assessed by VAS) (all $p>0.05$). A few side-effects were reported, but there were no significant differences between the DCS and placebo groups (all $p>0.05$) (Table 7).

Table 7. Mood, anxiety, and side-effects ratings for day 1

	DCS		Placebo	
	Pre	Post	Pre	Post
BFS	12.3 (11.2)	14.95 (12.63)	11.9 (9.18)	13.3 (11.93)
State Anxiety (STAI-S)	29 (7.03)	29 (7.28)	28.3 (6.48)	27.95 (5.70)
VAS Happy	70.4 (14.7)	65.95 (18.96)	67.35 (12.25)	64.85 (17.18)
VAS Sad	8.2 (9.34)	8.7 (13.19)	8.35 (12.39)	6.25 (9.79)
VAS Interested	72.25 (17.80)	64.4 (24.05)	71.05 (15.24)	61.55 (18.44)
VAS Anxious	13.4 (15.76)	11.15 (15.23)	11.7 (14.27)	4.6 (6.85)
VAS Stressed	16 (13.35)	7.65 (8.79)	13.55 (12.58)	11.1 (16.14)
VAS Hostile	5.25 (9.80)	5.3 (10.98)	6.4 (8.32)	4.85 (7.60)
PANASPOS	30.45 (6.39)	29.20 (7.19)	30.45 (7.29)	28.95 (7.70)
PANASNEG	12.25 (4.84)	11.75 (2.90)	10.60 (0.75)	10.55 (0.89)
VAS Nausea	5.35 (8.04)	4.45 (5.01)	4.35 (6.80)	6.65 (8.46)
VAS Dizziness	4.55 (4.77)	11.90 (16.13)	5.35 (8.91)	7.20 (9.66)
VAS Dry mouth	11.35 (14.73)	8.90 (12.23)	11.05 (15.32)	7.80 (13.88)
VAS Headache	5.05 (8.53)	15.55 (23.32)	3.70 (5.25)	5.55 (7.13)
VAS Alert	46.55 (33.77)	44.30 (28.68)	56.35 (30.33)	50.15 (29.75)
VAS Agitation	9.75 (9.05)	7.80 (9.40)	9.45 (15.80)	8.60 (17.69)

BFS, Befindlichskeit Scale; VAS, Visual Analogue Scale; STAI-S, Spielberg State Trait Anxiety Inventory-State; PANAS: Positive and Negative Affect Schedule. Values represent mean and standard deviation.

5.3.3 Psychological tasks

5.3.3.1 Facial Expression Recognition Task (FERT)

On the FERT, there were no significant effects of DCS on facial expression recognition in terms of accuracy, misclassifications, and reaction times (all $p>0.2$).

5.3.3.2 Emotional Categorisation Task (ECAT)

On the ECAT, one participant was excluded from this analysis due to extreme reaction times (above 3 SD from the group mean). There was no significant main effect of group in the reaction times to categorise self-referent personality words ($F(1, 37)=0.012$, $p=0.913$) or emotional valence × group interaction ($F(1, 37)=3.818$, $p=0.913$). However, there was a trend for a significant interaction between treatment and valence, driven by a higher proportion of positive versus negative words which were accurately categorised in the DCS compared to the placebo group ($F(1, 37)=3.818$, $p=0.058$, $\eta_{p2}=0.094$).

5.3.3.3 Emotional Recall Task

The EREC is a free recall task in which participants are required to remember as many of the positive and negative personality words from the ECAT as they can within 4 minutes. DCS increased the proportion of positive versus negative words accurately recalled, as compared to the placebo group ($F(1, 38)=5.391$, $p=0.026$, $\eta_{p2}=0.124$) (see Figure 16).

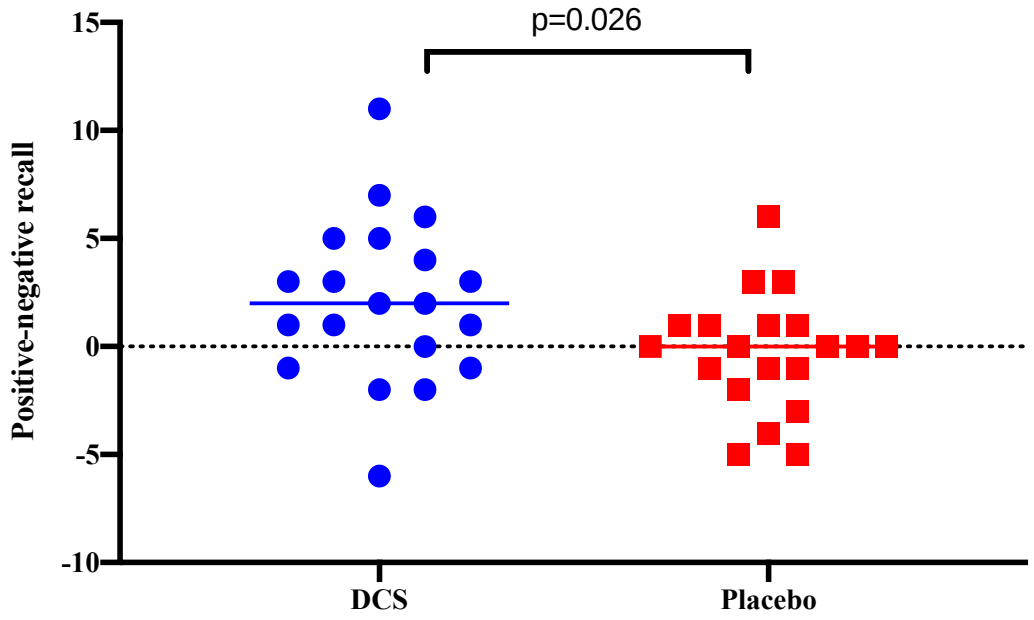


Figure 16. Mean number of positive versus negative words recalled in participants receiving DCS and placebo.

5.3.3.4 Emotional Recognition Memory Task

The EMEM includes the personality words from the ECAT as well as previously unseen words which participants are required to classify as familiar or unfamiliar. No significant treatment effects on word recognition accuracy or reaction times were found for this task (all $p>0.1$).

5.3.3.5 Facial Dot-Probe Task

The analysis with treatment (DCS and placebo) × masking (mask, unmasked) × valence (happy, fear) on vigilance scores yielded no significant effect of valence, masking, or treatment and no significant interactions ($p>0.2$).

5.3.3.6 Autobiographical Memory Task

When considering specific autobiographic events in the AMT, there was no significant interaction between the treatment condition and the three emotional categories ($F(2, 38)=0.852$, $p=0.431$). However, participants receiving DCS recalled more specific events overall, regardless of the condition ($F(1, 38)=15.948$, $p<0.001$) (see Figure 17).

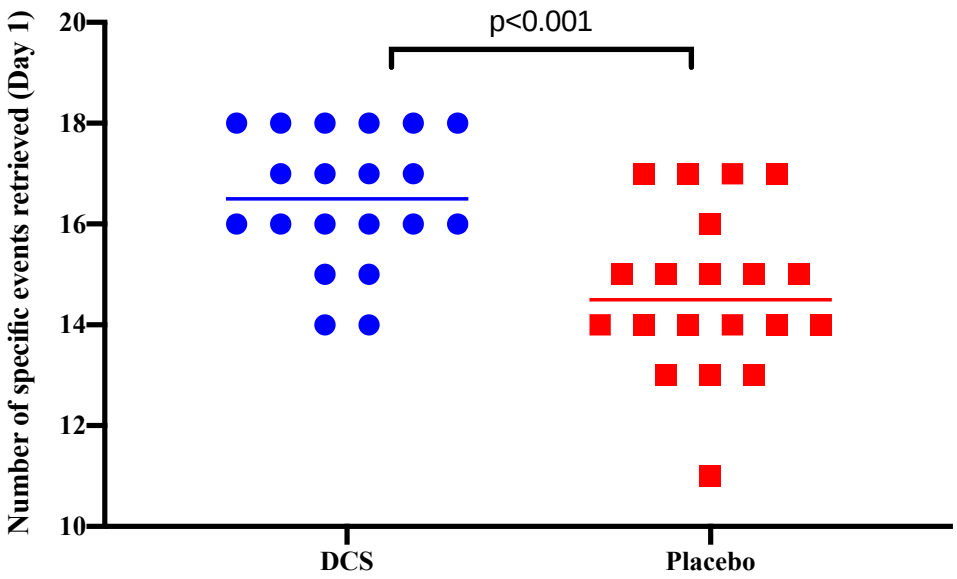


Figure 17. Mean number of total specific memories retrieved at 3 hours (Day 1) after DCS or placebo administration.

5.3.4 Day 2 Changes in subjective mood

There were no significant effects of group on any of the questionnaires measuring subjective mood and anxiety (BFS, STAI-S, and Positive and Negative PANAS) on day 2 (all $p>0.05$). There were also no significant effects of group on mood levels and side-effects ratings as assessed by VAS (all $p>0.05$) (Table 8).

Table 8. Mood, anxiety, and side-effects ratings for day 2

	DCS	Placebo
BFS	12.95 (12.22)	14.9 (16.42)
State Anxiety (STAI-S)	29.25 (6.73)	30.15 (5.75)
VAS Happy	70 (18.83)	63.9 (16.20)
VAS Sad	6.35 (8.63)	13.8 (21.32)
VAS Interested	62.35 (24.14)	58.65 (21.44)
VAS Anxious	7.10 (12.19)	7.75 (8.82)
VAS Stressed	13.30 (18.44)	13.50 (16.10)
VAS Hostile	4.80 (10.39)	7.20 (13.16)
PANASPOS	29.50 (7.94)	28.25 (8.41)
PANASNEG	11.40 (2.28)	11.10 (1.41)
VAS Nausea	3.25 (3.57)	3.7 (6.60)
VAS Dizziness	3.45 (3.02)	3.95 (6.55)
VAS Dry mouth	7.90 (14.89)	5.90 (10.20)
VAS Headache	5.10 (6.68)	3.40 (5.47)
VAS Alert	38.55 (30.19)	43.25 (29.77)
VAS Agitation	5.20 (8.36)	7.00 (8.30)

BFS, Befindlichskeit Scale; VAS, Visual Analogue Scale; STAI-S, Spielberg State Anxiety Inventory-State; PANAS: Positive and Negative Affect Schedule. Values represent mean and standard deviation.

5.3.5 Day 2 Psychological task

5.3.5.1 Facial Expression Recognition Task (FERT)

There were no significant effects of DCS on facial expression recognition in terms of accuracy, misclassification, and reaction times in the FERT on day 2 (all $p>0.3$).

5.3.5.2 Emotional Recall Task (EREC)

There were no significant effects of DCS on the proportion of words correctly recalled ($p>0.1$).

5.3.5.3 Autobiographical Memory Task

Similar to day 1, when considering specific autobiographical events, there was no significant interaction between the treatment condition and the three emotional categories ($F(1, 38)=0.223$, $p=0.80$). However, there was a significant main effect of treatment ($F(1, 38)=5.779$, $p=0.021$, $\eta_{p2}=0.132$), with participants receiving DCS recalling more specific events than those receiving placebo, regardless of the category (see Figure 18).

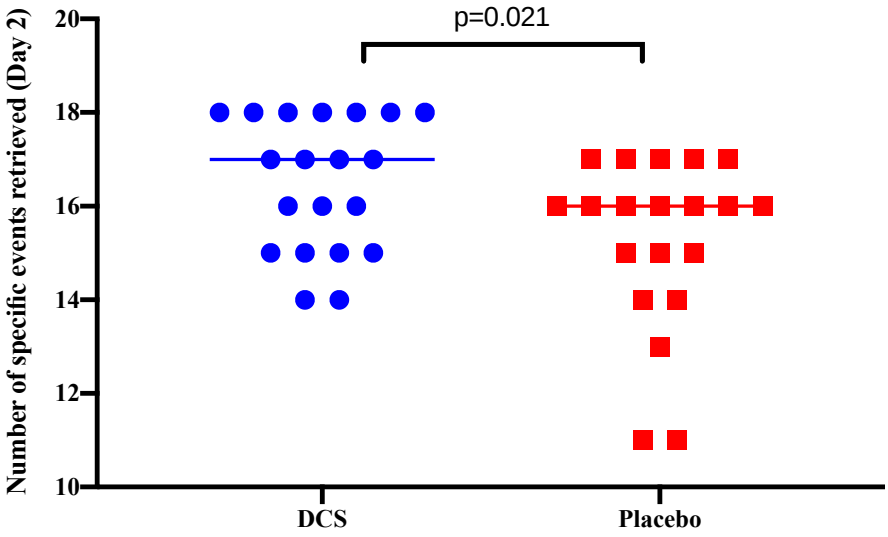


Figure 18. Number of specific memories (number of items retrieved) at 24 hours (Day 2) after DCS or placebo administration.

5.4 Discussion

DCS significantly enhanced autobiographical memory specificity when compared to placebo at 3 and at 24 hours after treatment. DCS also increased positive bias in emotional word categorisation and subsequent free recall at 3 hours; however, this effect did not extend to other tasks measuring emotional recognition, facial expression recognition, or attentional vigilance.

Our results indicated that DCS, when acting as an NMDAR glycine site agonist (at the low dose used here), increases the retrieval of specific autobiographical memories. Depression is associated with deficits in the retrieval of autobiographic memories, particularly of specific events (Köhler *et al.*, 2015). This has been regarded as an aberrant coping style, serving to maintain the disorder (Hermans *et al.*, 2005, Young *et al.*, 2016). There is also evidence suggesting that increases in memory specificity are associated with improvement in depressive symptoms, rumination, and cognitive avoidance in depressed patients (Neshat-Doost *et al.*, 2013, Raes *et al.*, 2009).

Importantly, the effect on autobiographical memory observed in this study was consistent with pre-clinical evidence suggesting that agents acting to facilitate NMDAR activity enhance memory. The NMDA glutamate receptor is an important component of memory formation, which is modelled by long-term potentiation in the hippocampus and amygdala (Rezvani, 2006). Animal studies have shown that NMDARs play an important role in different types of learning and memory, including spatial working memory and long-term (reference) memory (Shapiro and Caramanos, 1990). Importantly, previous rodent studies have shown that acute administration of DCS facilitates acquisition and subsequent memory retrieval (Flood *et al.*, 1992, Quartermain *et al.*, 1994).

In humans, the effects of NMDA modulation on memory have been poorly investigated, but there is emerging evidence corroborating this association. The glycine-blinding site of NMDAR is a target for modulating NMDA-mediated neural transmission to enhance memory (Roesler *et al.*, 1998). For example, Schwartz *et al.* (1991) used a word-retrieval task to test the effect of a glycine-

site agonist on memory retrieval in healthy individuals. They found that a glycine pro-drug significantly increased memory retrieval and decreased the latency in both young and older adults.

Clinically, there is evidence suggesting that DCS augments the response to CBT for anxiety and related disorders (Otto *et al.*, 2007, Otto *et al.*, 2010). For instance, a recent meta-analysis found that DCS is superior to placebo in augmenting the effects of CBT in patients with anxiety disorders, both at post-treatment and at follow-up (Mataix-Cols *et al.*, 2017). DCS has been found to enhance extinction learning in both human and animal studies (Richardson *et al.*, 2004); therefore, it is possible that DCS enhances CBT effects through the enhancement of extinction learning, which is a critical element of CBT for anxiety. There is limited evidence on the effects of DCS as an adjunctive treatment when combined with CBT for depression. However, Wilhelm *et al.* (2008) found that treatment with low-dose DCS (200–250 mg) unexpectedly produced large improvements in depression ratings in patients undergoing exposure therapy for OCD, and these effects were seen independently of improvement in OCD symptoms. A study with a similar design by Kim *et al.* (2016) also found an unexpected improvement in depressive symptoms in patients with OCD. As noted by Kim and colleagues, these findings raise the intriguing possibility that low-dose DCS may have antidepressant properties. It would therefore be important for future studies to test the effects of DCS as an adjunctive drug therapy when combined with CBT for depression. There are CBT approaches specifically designed to alleviate difficulties in retrieving specific autobiographical memories shown by patients with depression (McBride *et al.*, 2007, Raes *et al.*, 2009), and it would be worth investigating whether DCS could enhance these effects.

In the present study, DCS also affected self-referential processing. DCS appears to significantly increase the categorisation and subsequent recall of positive versus negative personality words. This effect is also seen with traditional antidepressant drugs. For instance, the commonly prescribed drugs, citalopram and reboxetine, have both been shown to increase the relative recall of positive (versus negative) personality words, using the same task as used in the present study (Harmer *et al.*, 2003c, Harmer *et al.*, 2009b, Harmer *et al.*, 2004). In addition, venlafaxine has been shown to enhance positive bias during social and emotional evaluations when administered before learning. In contrast, the NMDAR antagonist ketamine can abolish memory of previously learnt negative biases, but does not produce positive affective information biases in newly-learnt information (Stuart *et al.*, 2015). It is noteworthy that recurrent negative thoughts (such as self-blame and feeling hopeless/worthless) are important cognitive markers in depression and suicidal behaviour (Grunebaum *et al.*, 2005, Northoff, 2007). Increasing positive self-referential processing (use of positive information for self-description) is another core therapeutic component of CBT for depression (Segal and Gemar, 1997, Tarrier, 2010) and might be enhanced by DCS treatment.

Our study also revealed that the autobiographical memory-enhancing effect of DCS persisted for 24 hours after drug administration. A number of animal experimental studies demonstrated that DCS improves memory consolidation for new learning, typically within 24 hours after training (Goff, 2012). For instance, a rodent study found that a single dose of DCS given within 30 minutes of extinction training increased the retention of fear extinction 24 hours later by approximately threefold (Parnas *et al.*, 2005). Additionally, a rodent fear extinction model found that DCS improves memory consolidation for new learning, assessed at 24 hours after training, but did not

show any effect on learning performance during the training itself (Santini *et al.*, 2001). These findings are interesting in light of a study in patients with panic disorder, in which DCS produced effects on emotional neural processing 24 hours after administration (Reinecke *et al.*, 2018). A recent review article proposed that the reconsolidation of autobiographical memories in patients with depression may represent a novel therapeutic target, and the authors suggested that future research should explore the modulation of reconsolidation of distressing autobiographical memory following antidepressant treatment (Köhler *et al.*, 2015). DCS might be a pharmacological candidate for this approach.

It is important to mention that this memory-enhancing effect 24 hours after DCS administration was only seen with the AMT and not with the memory word recall. It is possible that the personal and specific elements of the autobiographical task may have enhanced the effects of DCS (as these effects were also only seen with specific, but not with general memories); further studies with different paradigms are needed to identify the key memory elements which are associated with the effects of DCS (e.g., personal relevance, specificity of the memory, etc.).

In addition, it was previously found that an acute dose of SSRI (citalopram) (20 mg) increased facial recognition of happy faces. Additionally, in the short-term, citalopram increased the recognition of fearful faces and increased the startle response (Browning *et al.*, 2007, Harmer *et al.*, 2003), which is consistent with clinical findings suggesting that SSRI treatment may increase anxiety in the early stages (Kent *et al.*, 1998). Noradrenaline reuptake inhibitors, such as reboxetine, also increased the recognition of positive facial expressions and reduced the reaction time in responding to positive personality words; however, there was no significant effect on fear

processing (Harmer *et al.*, 2003c). Similar findings emerged using bupropion, which is an inhibitor of dopamine and noradrenaline reuptake (Walsh *et al.*, 2018). However, the present study revealed no significant effects of DCS similar to those in the above studies on facial recognition, fear processing changes, or speed of reaction time in self-referential processing. Thus, the current study suggested that low doses of DCS, presumably acting as a glycine site partial agonist, did not show the broad range of positive effects on emotional processing which are seen with conventional antidepressants.

It is possible that the positive bias effect seen with DCS is specific to the encoding and retrieval of memory. Such an effect would be consistent with the modulation of glutamate receptors by DCS. It should be noted, however, that no significant effects were seen in the memory recognition task, although this is consistent with previous antidepressant drug studies which showed that the free memory recall is more sensitive to antidepressant medications (Harmer *et al.*, 2011). Indeed, the lower level of difficulty of the memory recognition paradigm vs. free recall may allow for less variability in performance, which would be important for detecting more subtle drug effects. Further investigation is needed to delineate the effects of DCS on cognitive functions relevant to depression, including different types of memory.

5.4.1 Limitations

There are several limitations to the present study. First, the autobiographical memory task was not tested at baseline, before drug administration, and it is possible that baseline differences in memory performance may have existed between the groups, independent of the effects of DCS. However, the baseline demographic information revealed that the treatment and control groups were well

matched, which should minimise this confounding effect. Second, the significant findings within the AMT cannot exclude the possibility of a ceiling effect, as all groups recalled a high percentage of specific autobiographical memories. Third, this study did not examine other memory tasks, such as working and verbal memory, which could have provided a more comprehensive exploration of the memory-enhancing effects of DCS. Future studies should extend the current findings to patients with depression. It would also be helpful to examine the neural substrates of the effect of DCS on memory using functional neuroimaging.

5.4.2 Conclusion

To the best of our knowledge, no previous studies have provided evidence that low-dose DCS improves retrieval of specific autobiographic memories. The current study also suggests that DCS increases the recall of positive vs. negative words. DCS may have particular utility when combined with psychological therapies aimed at increasing specific personal memories and positive self-referential processing. The potential antidepressant-like properties of DCS are therefore worth exploring in future studies.

Chapter 6: The effects of NMDA receptor partial agonist d-cycloserine: responses to psychosocial stress

6.1 Introduction

In chapter 5, the findings supported the suggestion that d-cycloserine (DCS) increases specific autobiographical memory retrieval and positive emotional memory. Such effects make DCS an intriguing agent for further investigation in clinical depression, which is characterized by decreased autobiographical memory and increased negative bias in memory recall. It also underscores the potential role of DCS as an adjunct to CBT in depression. Further investigation on the effects of DCS on stress responses in humans would be of great clinical interest for the treatment of stress-related depression disorders. Therefore, in this chapter, I also investigated the effects of DCS on mood, anxiety, and physiological responses to a novel psychosocial stress task in healthy volunteers.

Stress refers to physical or psychological events which disrupt and threaten homeostasis. Stress could result in dysregulation of the stress system, and in immune and psychological dysfunction (Glaser and Kiecolt-Glaser, 2005, Holsboer, 2001), and such dysregulation has been suggested to be a robust risk factor for developing neuropsychiatric disorders, such as major depressive disorder (MDD) (Hammen, 2004). High levels of psychosocial stress are strongly associated with depression onset (Kenneth S. Kendler *et al.*, 1999). For instance, Hammen *et al.* (2009) found that increased acute life stress was significantly associated with depressive episode onset in a sample of 816 community-living women. This study also revealed that depressed patients experienced more stressors. A systematic review found that psychosocial stress at work, measured by a demand–control and effort–reward imbalance model, was significantly associated with depression

in prospective studies (Siegrist, 2008). Previous animal studies also demonstrated that stress (such as inescapable stress and early separation stress) can induce 'depressive' behaviour, for example, anhedonia-like behaviour measured by decreased sensitivity to reward (Anisman and Matheson, 2005). Furthermore, Bogdan and Pizzagalli (2006) found that acute stress (negative performance feedback or threat-of-shock) in healthy volunteers increased negative affect and anxiety and reduced reward responsiveness.

Antidepressant treatment can modify the stress response in animal studies, and specifically attenuate external stress-induced depressive-like behaviours (Kubera *et al.*, 2011). Furthermore, stress-related disorders are also associated with abnormalities in the central glutamatergic nervous system, particularly dysfunctional NMDAR activity (Bergink *et al.*, 2004, Popoli *et al.*, 2012). Previous studies have found that the NMDAR system (both the high-affinity glutamate and the glycine-binding sites) is involved in anxiety and depression in animal models. For example, the NMDAR antagonist ketamine can alleviate stress-induced depressive-like behaviours and can prevent stress-induced cognitive dysfunction (Miserendino *et al.*, 1990). A channel blocking NMDAR antagonist (MK-801) and a competitive NMDAR antagonist (CGP37849, AP-7) were also shown to have anxiolytic-like effects in animal studies (Plaznik *et al.*, 1994, Xie and Commissaris, 1992).

In addition, a partial agonist acting at the glycine-binding site, DCS, has been shown to facilitate the extinction of fear responses in animal and human studies (Bryant, 2005). A previous study showed that a high dose of DCS (200 and 300 mg/kg) showed an anxiolytic-like effect by reducing the fear-potentiated startle response (Anthony and Nevins, 1993). Kłodzińska and Chojnacka-

Wójcik (2000) reported that DCS (200 and 300 mg/kg) showed anxiolytic effects by increasing the number of shocks accepted using the Vogel conflict test in rats.

On the other hand, a low dose of DCS (12 mg/kg) blocked the anxiolytic effect of ethanol in the elevated plus-maze test in rats (Moraes Ferreira and Morato, 1997) where entries and time spent in the open arms are used to assess avoidance behaviours, and measure anxiety levels. Also, Ho *et al.* (2005) found that a low dose of DCS (at doses of 10 and 30 mg/kg) had anxiogenic effects in low-anxiety rats, by decreasing the open arm time, open arm activity, and increasing the enclosed arm time and risk assessment in the elevated plus-maze test. Furthermore, Bailey *et al.* (2007) investigated the effects of DCS (50 mg) on a learning task (challenging visuospatial cognitive task) in healthy volunteers under either a high-level anxiety condition (inhalation of CO_2 7.5%) or a low-level anxiety condition (inhalation of medical air). The results showed that DCS improves the performance on the learning task at lower anxiety levels, but this effect was not seen in the high-level anxiety group. Thus, further investigation of the effects of DCS on stress responses in humans will have important implications for the treatment of stress-related depression disorders.

There have been advances in research investigating whether pharmacotherapy can modify the stress response in humans undertaking a psychosocial stress task, as most stressors are psychosocial in nature. The acute stress protocol widely used in a laboratory setting is the Trier Social Stress Test (TSST), which is a verbal task based on anxiety induced through public speaking with critical feedback. For example, Makatsori *et al.* (2004) carried out a study examining the stress response using the TSST in healthy volunteers with pre-treatment with a single dose of lamotrigine (300 mg) or placebo. The results showed that lamotrigine, a putative inhibitor of

glutamate release, significantly inhibited growth hormone and cortisol release and blood pressure increase during psychosocial stress, as compared to placebo. Heinrichs *et al.* (2003) found that healthy volunteers receiving oxytocin showed decreased cortisol concentrations and altered subjective mood ratings (increased calmness) when responding to acute stress (TSST) as compared to the placebo group.

However, no human studies have addressed the effect of NMDA glutamatergic signalling on psychosocial stress and anxiety within a laboratory setting. Given the important role of glutamate in the stress response (Bergink *et al.*, 2004), the present study was designed to investigate the effects of DCS (250 mg) on stress responses to a psychosocial stress task in healthy volunteers. Understanding and being able to modify the mechanisms underlying the stress response may facilitate development of treatments for depression and other stress-related disorders.

In neuroimaging settings, a reliable psychosocial stress protocol is the Montreal Imaging Stress Task (MIST), which is a non-verbal task derived from the Trier mental challenge test and is used in fMRI studies (Dedovic *et al.*, 2005). Participants are asked to solve arithmetic operations under time constraints and social pressure. The time constraint makes the task above the mental capacity of the participants. Wheelock *et al.* (2016) reported that the MIST is associated with activity in the ventromedial prefrontal cortex (VMPFC) and posterior cingulate, and that self-reported stress is associated with activity in the dorsal medial PFC (DMPFC) and VMPFC. A large stress study (n = 239) also confirmed that increased activity in the VMPFC and DMPFC was associated with changes in the skin conductance response (SCR) and self-reported stress (Orem *et al.*, 2019). To the best of my knowledge, there have been no human studies which have examined the effect of

antidepressant administration on the stress response using the MIST. In addition, while the MIST was developed for use during fMRI, a novel psychosocial stress task (Oxford Cognition Stress Task, OCST) was adapted from the MIST for use outside of the MRI scanner in a laboratory setting. The OCST is an acute psychosocial stress-induction paradigm comprised of a series of computerised cognitive tasks measuring mathematical, verbal and visuospatial processes. This paradigm is easier to implement than paradigms such as the TSST, as it is computer-based and does not require the use of confederates.

Therefore, the current study used psychophysiological (SCR, blood pressure, and pulse rate) and self-reported mood and anxiety questionnaire measurements to assess the effect of DCS on the stress response induced by the OCST. The data reported here was collected as part of the larger study described in Chapter 5. It was hypothesised that, compared to healthy volunteers receiving placebo, participants who received DCS would show less physiological responses and subjective anxiety ratings to the novel stress procedure.

6.2 Methods

6.2.1 Participants

Forty healthy volunteers, aged 18–40 years, participated in this study, which were recruited as part of the study reported in Chapter 5. Only one participant did not complete the stress task and was excluded from the analysis. The groups were well matched in terms of age, gender, BMI, Verbal IQ, STAI-T (trait anxiety), EPQ, and depression symptoms (Table 9). The groups were also not different in their scores on the PANAS and VAS (mood levels and side-effects).

Table 9. Baseline demographic characteristics and baseline mood scores

	DCS (n = 19)	Placebo (n = 20)	T	Significance *p*
Age	23.68 (4.07)	22.7 (3.3)	0.83	0.412
Gender, n				
Male	9	9		
Female	10	11		
Year of education	16.89 (2.45)	16.7 (1.92)	0.27	0.78
EPQ neuroticism	7.21 (5.15)	6.65 (4.25)	0.37	0.71
EPQ psychoticism	4.42 (3.42)	3.6 (2.11)	0.91	0.37
EPQ extraversion	13.0 (4.84)	14.9 (3.74)	-1.38	0.18
BMI	22.61 (2.30)	22.81 (2.64)	-0.26	0.80
BDI	3.16 (3.13)	2.7 (3.48)	0.43	0.67
Verbal IQ	62.21 (12.73)	66.75 (12.23)	-1.14	0.26
STAI-T	34.63 (7.90)	33.55 (8.94)	0.40	0.69

EPQ, Eysenck Personality Questionnaire; BFS, Befindlichskeit Scale; BDI, Beck Depression Inventory; STAI-T, Spielberg Trait Anxiety Inventory; BMI, body mass index; Verbal IQ was assessed by Spot-the-Word test; Values represent mean and standard deviation.

6.2.2 Study procedure

Participants were randomly assigned to receive 250 mg DCS or placebo in a double-blind design. Participants were tested on emotional processing, the AMT, and psychosocial stress tasks 3 hours after drug administration. The results of emotional processing and the AMT were reported in Chapter 5.

6.2.3 Questionnaire measurement

Participants filled out the following questionnaires before commencing the stress task: the STAI-S (Spielberger and Gorsuch, 1983), PANAS (Watson *et al.*, 1988), as well as the Bond–Lader VAS (Bond and Lader, 1974) to assess subjective anxious, stressed, and hostile feelings. These questionnaires were repeated again immediately after completing the stress task. Blood pressure and heart rate were recorded immediately before and after the stress task. The SCR was also measured continuously throughout the task. The whole process required approximately 35-40 minutes.

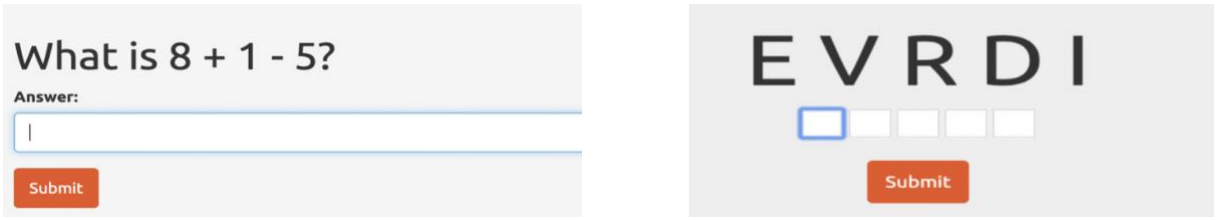
6.2.4 Psychosocial stress task design

All subjects were told that the purpose of the task was to examine whether the cognitive task was associated with mood state, blood pressure, heart rate, and skin sweating. The OCST was adapted from the MIST (Dedovic *et al.*, 2005, Pruessner *et al.*, 2004) and is a web-based paradigm. This task presents a series of mental arithmetic, verbal anagram, and visuospatial search challenges for participants to solve. In the mental arithmetic task (Figure 19a), participants were asked to complete the arithmetic exercise as a measure of their mathematical ability. The mental arithmetic algorithm generates mathematical challenges at five difficulty levels. The question at levels 1 and 2 involved 2 or 3 single-digit integers, respectively, and only addition and subtraction operators. Levels 3 and 4 involved multiplication of two single-digit integers, addition or subtraction of a two-digit integer or of two single-digit integers. Level 5 included multiplication, addition, or subtraction of two single-digit integers from which the product of two further single-digit integers and 2 two-digit integers are added or subtracted. In the verbal anagram (Figure 19b), participants were presented with a jumbled letter string which needs to be re-arranged to produce a meaningful word in English. All of the letters were to be utilized. In the visuospatial search (Figure 19c),

participants were asked to locate a blue T which could appear in any of four orientations and is hidden in an array of distractor letters (non-blue coloured Ls and Ts in different orientations). The visual search algorithm generated questions of four difficulty levels corresponding to distractor arrays of increasing size: 5 × 5, 6 × 6, 7 × 7, and 8 × 7. The task lasted approximately 25 minutes, and it included five blocks, comprised of three blocks of mental arithmetic, and one block each of anagrams and visual search.

a. Mental arithmetic

b. Verbal anagram



c. Visual search

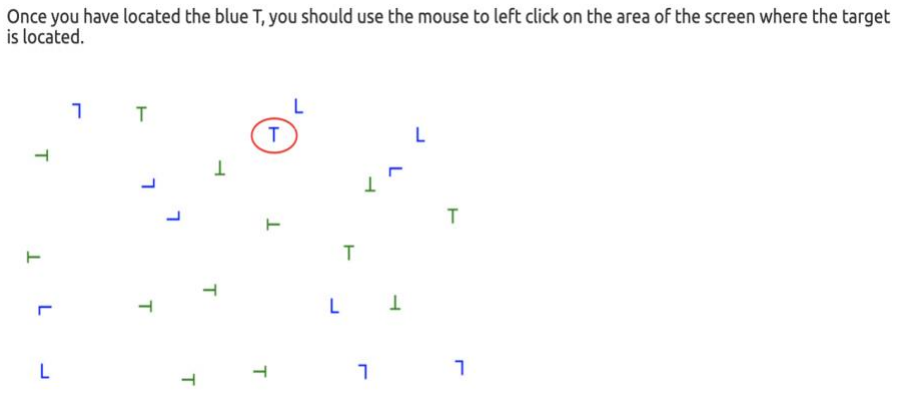


Figure 19. Screenshot of the stress task. *Examples of a) mental arithmetic, b) verbal anagram and c) visual search challenges.*

In order to build the social evaluative threat, a 2-minute video, delivering task instructions, was presented to participants before starting practical questions, which led to participants to believe

that the task difficulty was tailored to their ability based on their answers to a pre-test question, and they were also told that most people could achieve 70–90% correct answers throughout the whole task (Figure 20).

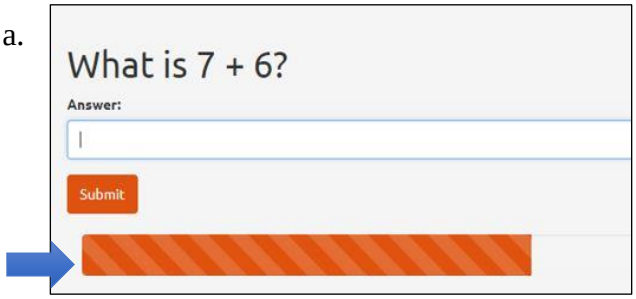


Figure 20. Screenshot of Introductory video.

This task was used to induce a state of stress and uncontrollability. Participants were informed that they were being filmed during the task for later analysis of facial expressions, although no actual video was recorded. A time-limit bar for completing each challenge, with a ticking clock sound, was displayed on the screen (Figure 21a). To induce a high failure rate, the timing for each challenge would be reduced by 10% according to the time the participant spent on the previous challenge. The difficulty of the challenges was also automatically adjusted to ensure that each participant's success rate was less than 50% accuracy within the time limit. Some of the verbal anagrams were impossible to solve. Feedback for each challenge was provided on the screen and comprised an angry face (when a wrong answer was given or when no answer was given within the time limit) and aversive sound (Figure 21b), so that participants were aware of the fact that they were performing poorly. A continuous coloured bar [higher than average (green), average

(yellow), low (orange/red)] was given in order to indicate the participant's performance (Figure 21c). Moreover, participants were given their results and their score shown in comparison to the high accuracy of performance in the general population at the end of each trial (Figure 21d). At the end of the test session, participants were fully debriefed and informed of the true aim of the task. During the debriefing session, participants were told that the task was specifically designed to be out of reach for their ability, and comparisons between their performance and the general population were false. They were also told that no actual video was recorded.

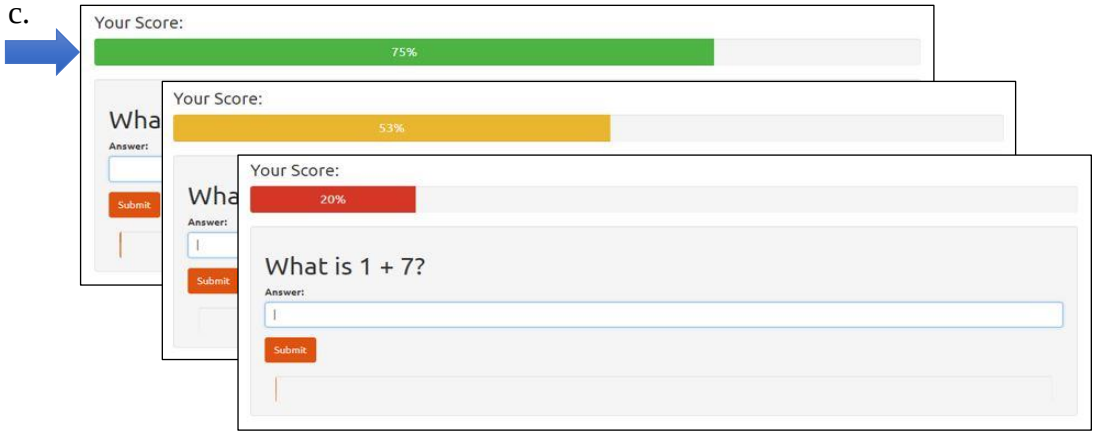
a.



b.



c.



d.

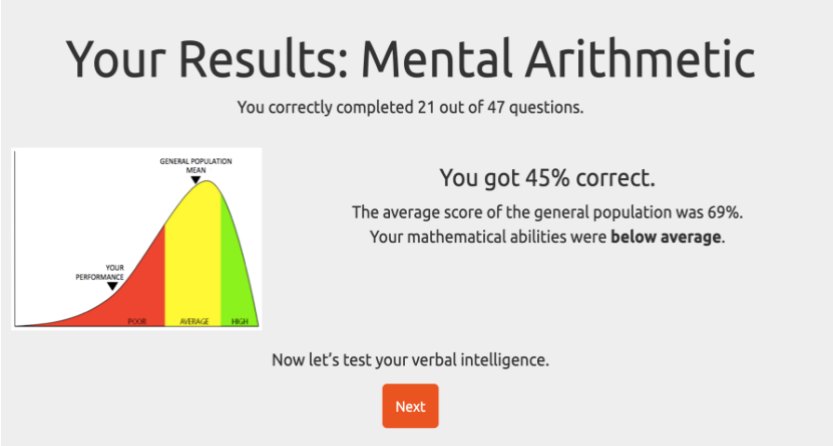


Figure 21. Screenshot of the stress task. *Examples of a) the time-limit bar for completing each challenge, with a ticking clock sound; b) feedback for each challenge, which included an angry face (when an incorrect answer was given or when no answer was given within the time limit) and an aversive sound; c) a continuous coloured bar to indicate the participant's performance; d) participants were given their results and their score was shown in comparison to the high-accuracy performance of the general population at the end of each trial.*

6.2.5 Physiological measures of stress and arousal

The SCR was recorded using constant-voltage (0.5V) at 500 Hz using the Contact Precision Instruments PsychLab SC-EKG system. Skin conductance was measured using Ag/Ag-C1 electrodes with a diameter of 8 mm, which were filled with isotonic electrolyte gel, attached to the participants' middle and index fingers on their non-dominant hand. Skin conductance was expressed as the mean conductance level. Baseline measurements of the SCR were taken during the 2-minute task introduction video, in order to allow stress and arousal comparisons between baseline and during the presentation of the stress task. Skin conductance was calculated as the mean value of six skin conductance values obtained in the first 2-minute period of each task

[baseline (introduction video), mental arithmetic, verbal anagram, mental arithmetic, and visuospatial search challenges and mental arithmetic].

Blood pressure was recorded immediately before and after the stress task from the upper arm using an electronic sphygmomanometer.

6.2.6 Statistical analysis

All analyses were performed using the IBM SPSS V23. The baseline measurements (age, education years, BMI, EPQ, Verbal IQ, and STAI-T) and task performance (behavioural data) were analysed using independent sample t-tests. Gender differences were assessed using the chi square test. The mood and anxiety ratings (STAI, PANAS and VAS), blood pressure, pulse rate, and SCR were analysed using a mixed ANOVA analysis with time (pre- vs. post-stress task) and group condition (DCS vs. placebo) as within- and between-subjects factors, respectively. Where sphericity was violated, Greenhouse-Geisser corrected *p* values were used. The effect size was estimated suing ηp2. Significant interactions between time and group were further examined using between-group independent sample *t*-tests. Two participants' skin conductance response data were lost due to technical problems. Additionally, the last-observation-carried-forward was used to replace skin conductance response data for one participant at the sixth time point (mental arithmetic), which was missing in the recording.

6.3 Results

6.3.1 Task performance

Across all participants, the mean correct performance for the task was low, with a high failure rate, as expected. The average correct performance rate for the entire task was 40.1% (Table 10). There were no significant effects of group in the overall task performance ($F(1, 37)=2.22$, $p=0.15$) or in any of the specific domains ($p>0.1$).

Table 10. Task performance in participants receiving placebo or DCS

	DCS (N = 19)		Placebo (N = 20)	
Task Phase	Mean	SD	Mean	SD
Mental arithmetic	67.43	9.28	65.06	8.22
Anagram	28.07	17.61	23.33	14.71
Mental arithmetic	36.35	8.25	36.62	5.95
Visual search	39.46	9.07	36.22	8.64
Mental arithmetic	35.24	7.89	33.70	5.07
Overall performance	41.31	5.23	38.98	4.53

SD: Standard deviation

6.3.2 Changes in subjective mood and anxiety

Irrespective of whether participants received DCS or placebo, self-reported STAI-S ($F(1, 37)=72.55$, $p<0.001$, $\eta_{p2}=0.66$), VAS anxious ($F(1, 37)=11.0$, $p=0.002$, $\eta_{p2}=0.23$), VAS stressed ($F(1, 37)=14.43$, $p=0.001$, $\eta_{p2}=0.28$), VAS hostile ($F(1, 37)=3.88$, $p=0.056$, $\eta_{p2}=0.1$), PANAS-positive ($F(1, 37)=20.78$, $p<0.001$, $\eta_{p2}=0.36$), and PANAS-negative ($F(1, 37)=35.89$, $p<0.001$, $\eta_{p2}=0.49$) values significantly increased over time. However, there were no significant effects of group or time × group interaction on any of the questionnaires (all $p>0.2$).

6.3.3 Changes in physiological measures of stress and arousal

Before the start of the task, the pulse rate (beats per minute, bpm) and blood pressure (mmHg) were greater in the DCS group than in the placebo. However, these differences did not reach

statistical significance (all $p>0.1$). As expected, participants who received either DCS or placebo showed increases in systolic blood pressure ($F(1, 37)=3.49$, $p=0.07$, $\eta_{p2}=0.09$), diastolic blood pressure ($F(1, 37)=7.35$, $p=0.01$, $\eta p2=0.17$), heart rate ($F(1, 37)=10.21$, $p=0.003$, $\eta p2=0.22$) after the stress task as compared to baseline (Figure 22). However, there were no significant effects of group or a time × group interaction on the measurements of heart rate and blood pressure (all $p>0.2$).

There was also a significant effect of time when considering skin conductance levels, indicating that the task induced a robust increase in these levels ($F(1.50, 175)=27.18$, $p<0.001$, $\eta p2=0.44$). However, there were no significant effect of group ($F(1, 35)=2.34$, $p=0.14$) or time × group interaction ($F(1.50, 175)=0.44$, $p=0.59$) (Figure 23).

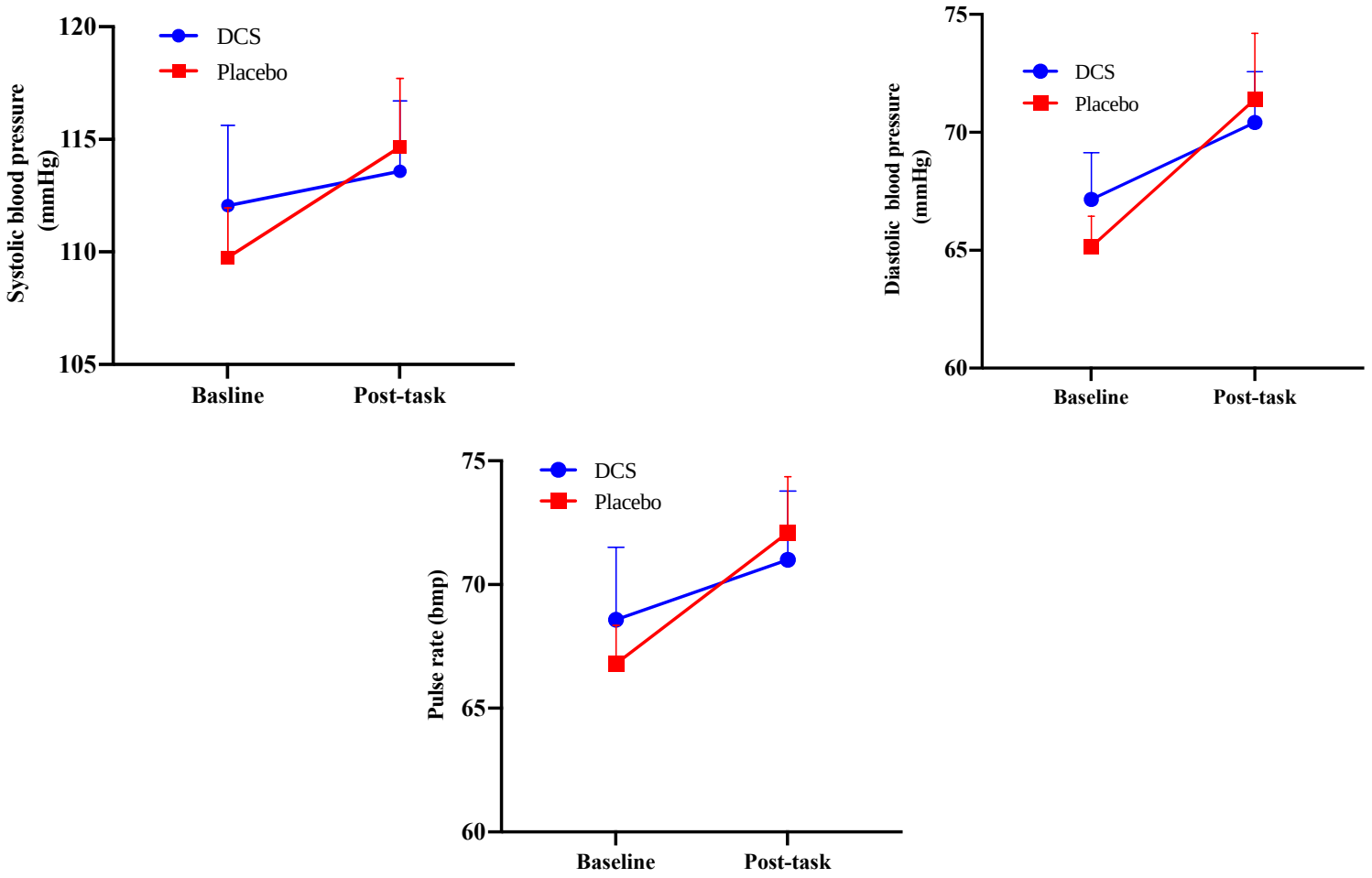


Figure 22. The effect of DCS and placebo on changes in systolic blood pressure, diastolic blood pressure, and heart rate in response to the stress task. *Error bars represent the standard errors of the mean.*

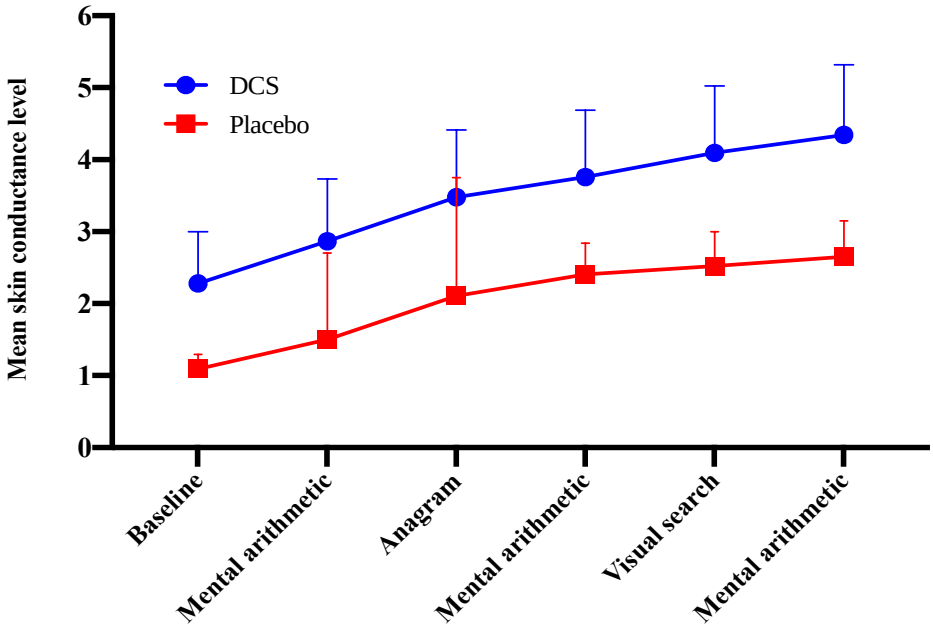


Figure 23. Time indices of skin conductance levels during the stress task. *Error bars represent the standard errors of the mean.*

6.4 Discussion

This study aimed to investigate the effects of the glycine site partial agonist, DCS, on physiological, mood, and anxiety responses to a psychosocial stress in humans. As expected, participants who received either DCS or placebo showed increases in blood pressure, pulse rate, SCR, and subjective anxiety and altered mood ratings after the stress task as compared to baseline, which indicated that this novel psychosocial task was successful in inducing stress in participants. However, compared to placebo, DCS did not modify any of the measured objective or subjective responses to the stress paradigm.

Previous animal studies found that high doses of DCS (200 and 300 mg/kg) exhibited an anxiolytic-like effect, such as increasing the number of shocks accepted and reducing the fear potentiated startle response (Anthony and Nevins, 1993, Kłodzińska and Chojnacka-Wójcik,

2000). On the other hand, in animal studies, low doses of DCS may be anxiogenic (Ho *et al.*, 2005, Moraes Ferreira and Morato, 1997). As the effects of DCS were dose-dependent according to human and animal studies (Lanthorn, 1994), DCS has been investigated as an adjunctive treatment in two placebo-controlled trials in patients with treatment-resistant depression. Positive results were reported with a higher dose of DCS, which acts as an NMDAR antagonist (1000 mg), but not with a lower dose of DCS (250 mg) (Heresco-Levy *et al.*, 2013, Heresco-Levy *et al.*, 2006). Thus, it is possible that the low dose of DCS may have been insufficient to reduce the stress response in the current study. On the other hand, this study did not detect an anxiogenic effect in the stress task with the low dose of DCS. Future studies should investigate the effect of a higher dose of DCS in an acute stress paradigm.

Also, previous studies have examined the effects of different doses of DCS to augment cognitive training in different stress paradigms in laboratory settings, and the findings are inconsistent. Behar *et al.* (2010) found that DCS (50 mg) augmentation with attentional training away from threat, using the dot-probe task, significantly reduced attentional bias from day 1 to day 2 in individuals with high trait anxiety as compared to participants who received placebo. However, this study did not find any significant effect of DCS on emotional reactivity (STAI-S and PANAS measurements) in two stress tasks (anagram stress task and mirror-tracing persistence task) in day 2.

Woud *et al.* (2018) recently examined whether a single dose of DCS (250 mg) could enhance modification of appraisal using an emotional learning paradigm examined in the context of analogue posttraumatic stress symptoms in healthy volunteers. They found that, compared to the placebo group, participants in the DCS group had reduced distress when participants were

asked to recall a negative life event, and also showed lowered distress and intrusions after receiving positive cognitive bias modification-appraisal training.

However, to the best of our knowledge, no study has yet examined the effect of DCS augmentation of cognitive training in response to an acute psychosocial stress paradigm. Gaab *et al.* (2003) found that healthy volunteers trained in cognitive-behavioural stress management had significantly decreased stress appraisal and higher control expectancies during the TSST than a control group. Therefore, future studies could examine whether DCS treatment results in greater decreases in the stress response (i.e., reduced emotional reactivity and physiological measurement) after cognitive training.

It is also possible that the effect of DCS on stress may be more apparent during other psychosocial stress paradigms, such as the TSST, rather than the paradigm used here. The TSST involves a more naturalistic design where participants are required to give a speech in front of confederates, which may evoke stronger stress responses that may be more easily modulated by drug agents. For instance, a single dose of the glutamate release-inhibiting drug, lamotrigine, inhibited diastolic blood pressure and growth hormone release, and increased salivary cortisol release during the TSST (Makatsori *et al.*, 2004). A single administration of oxytocin also reduced cortisol concentrations and subjective anxiety ratings during the TSST relative to the placebo group (Heinrichs *et al.*, 2003). Additionally, previous studies found that the TSST increased objective measurements (cortisol levels) about 200–400% compared to baseline in 70–80% of participants (Martens, 2019). Therefore, in future, it may be worthwhile testing the effect of DCS on the stress response during the TSST.

6.4.1 Limitation

There are some limitations to the current study. First, the study design may limit the size of apparent differences between groups, as baseline measures were taken during task instructions, which itself might have been stressful, thereby limiting the increase in the subsequent stress response. The video informed the participants that all tasks would be very simple, and that most participants would be able to answer correctly 90% of the time. This information may have already increased participants' stress/arousal. Future study designs should include actual baseline measurements, taken before presenting the video or starting any task. In addition, at 3 hours after drug administration, the participants performed emotional processing and autobiographical tasks first, for about 1 hour; thus, the psychosocial stress task was performed around 4 hours after drug treatment. It is possible that participants' fatigue may have affected their performance in the psychosocial stress task. Finally, we did not measure participant's cortisol in the current study; however, cortisol is one of the most objective psychophysiological parameters.

6.4.2 Conclusion

This study investigated the effect of DCS (250 mg) in healthy volunteers during a novel psychosocial stress task. In contrast to the study's predictions, DCS appeared to have no effect on stress responsiveness. Testing DCS in a different study design, such as using other stress paradigms and at different doses, in the future is warranted. Nevertheless, this study highlighted the need for future research to address the acute stress response in order to evaluate the use of DCS for treating depression and other stress-related disorders. Such studies can provide valuable information on the impact of antidepressants on stress responsiveness and may provide insight on the mechanisms underlying the benefit of antidepressants used to treat depression.

Chapter 7: General discussion

7.1 Summary of findings

A significant proportion of patients with MDD do not respond fully to two courses of antidepressant medications; these patients are conventionally defined as having TRD. This, together with the delayed onset of conventional antidepressant action, increases the morbidity of MDD patients because of the persistence of depressive symptomatology, functional impairment and thoughts of self-harm.

There has been much interest in NMDARs as potential targets for rapid antidepressant effects in the treatment of depression. However, the neuropsychological effects of NMDAR-targeting antidepressants remain poorly understood. To address this issue, this thesis used a combination of behavioural and neuroimaging measures to explore the effects of different NMDAR-targeting antidepressants on emotional processing in both depressed patients and healthy participants. This thesis included three experimental studies: 1) a double-blind, placebo-controlled randomised trial in which depressed patients received a single dose of ketamine, lanicemine, or placebo, with emotional processing being measured 24 hours post infusion via behavioural and fMRI techniques (Chapters 2–3); 2) a double-blind, placebo-controlled randomised trial in which TRD patients received either N_2O or placebo, and emotional processing was assessed at baseline and 24 hours post inhalation via behavioural methods (Chapter 4); 3) a double-blind, placebo-controlled, randomised design in which healthy volunteers received either a single dose of DCS or a placebo, and emotional processing was assessed at 3 hours and 24 hours following drug administration via behavioural methods (Chapter 5), and the acute effects of DCS on mood, anxiety, and physiological responses to a novel psychosocial stress task were also examined (Chapter 6).

Importantly, by exploring the antidepressant action of these NMDAR antagonists (ketamine, lanicemine, and N_2O) 24 hours post infusion, we aimed to avoid the confound of any acute or short-lasting changes in subjective experience (such as dissociation or cognitive impairment) induced by drug treatments. We thereby hoped to investigate the relevant neuropsychological mechanisms unconfounded by the acute subjective effects of the drug.

However, it needs to be considered that a postulated advantage of drugs such as ketamine is their rapid onset of action in terms of subjective clinical benefit. According to well-controlled trials, clinically important antidepressant effects may be apparent in some patients within a few hours of ketamine administration. Therefore, it is not clear that changes in emotional processing necessarily underpin the rapid antidepressant response seen in such patients. However, a more common pattern of antidepressant response is a gradually increasing benefit from repeated ketamine treatment, so that response rates at the end of a series of infusions is greater than at the beginning (see for example, Diamond et al 2014). In this situation there may be an important role for positive changes in emotion processing to consolidate the antidepressant response.

In the first experimental study (Chapter 2), it was found that ketamine increased the error rate for recognising facial expressions as positive rather than negative, and lanicemine showed a trend towards improving facial expression recognition generally as compared to the placebo group in the facial expression recognition task. Further, ketamine showed a trend towards higher false positive memory intrusions as compared to placebo in the emotional recall task. Neither drug showed attentional or recognition effects on either in the facial expression recognition task or the dot-probe task. Moreover, a lack of significant group differences in

mood ratings (MADRS and HAMD) indicated that the effects of ketamine on emotional processing were independent of mood changes.

The effects of NMDAR antagonists on emotional stimuli and the relevant neural circuitry in depressed patients have not been sufficiently studied. To address this, Chapter 3 describes fMRI data collected using the same sample reported in Chapter 2 to explore the neural effects of a NMDAR antagonist on emotional processing. To the best of our knowledge, this is the first study to report that depressed participants on ketamine showed a trend for an increased activation in the right DLPFC when compared to a placebo during negative self-referential processing. This data suggests that ketamine can normalize emotional regulation responses by increasing engagement with negative or aversive stimuli and emotions. This may represent a biomarker for the effect of ketamine treatment on depression. These neural effects also occurred in the absence of any effects of ketamine on subjective mood ratings or behavioural performance in terms of accuracy and reaction times in the self-referential processing task. Furthermore, no significant differences were observed between lanicemine and placebo in terms of neural effects. However, these results should be interpreted with caution and considered as preliminary due to the small sample size. It would be interesting to replicate these findings in a larger sample in the future.

A single dose of NMDAR antagonist-targeting antidepressants seems to modulate both behavioural and neural processing of emotional information in depressed patients, but the effect of NMDAR antagonists on emotional processing in patients with TRD has not been studied. Thus, a further exploratory study was conducted in Chapter 4 with the aim of examining whether a single administration of an NMDA antagonist (N_2O) would reduce

negative as compared to positive bias in emotional processing in both a facial expression recognition task and a self-referential processing task 24 hours following drug administration.

It was found that a single administration of N_2O did not modulate emotional processing in TRD patients, and specifically, N_2O did not affect facial expression recognition for either accuracy or reaction time relative to patients in the placebo group. These findings are consistent with those of previous fMRI studies using the same task in TRD patients, which found that ketamine modulates the neural effects on brain regions when responding to the positive or negative faces but a lack of significant effects were seen in behavioural measures (accuracy and reaction time) (Murrough *et al.*, 2015, Reed *et al.*, 2019). Given these findings, fMRI BOLD activation may a more sensitive measure to determine the neural effect of N_2O in future studies. However, due to the small sample size, these findings should be treated as preliminary. Further studies with larger samples are needed to explore the effect of NMDAR antagonists on TRD patients. In addition, patients in both the treatment and the placebo group had low accuracy on the self-referential processing task, resulting in considerable data loss and limiting the interpretative capacity of this study.

In addition to NMDAR antagonists, NMDAR partial agonists have also shown potential antidepressant action without psychotomimetic effects. Thus, it is important to explore how NMDAR partial agonists may function as antidepressants. Previous findings suggested that DCS may have antidepressant potential through its interaction with the glycine site of NMDARs (Kim *et al.*, 2016); however, the clinical evidence for the efficacy of DCS as a treatment for depression is limited. Importantly, the previous chapter showed that the NMDAR antagonist ketamine works differently from conventional antidepressants in its effect on emotional processing, and given the important role of NMDA glutamate receptor function in

learning and memory (Castellano *et al.*, 2001, Riedel *et al.*, 2003), Chapter 5 aimed to explore the acute and short-term effects (3 hours and 24 hours post administration) of a low dose of DCS (250 mg) on autobiographical memory and emotional processing in healthy volunteers.

To the best of our knowledge, this is the first study to show that DCS enhanced the retrieval of more specific autobiographical memories. This effect persisted at 24 hours. DCS also significantly increased emotional memory and classification of positive as compared to negative words in a self-referential processing task. However, DCS did not affect the recognition of positive facial expressions or the speed to identify positive self-referential words. Moreover, there was no significant effect in the attention dot-probe faces task. These findings suggest that DCS may have particular utility when combined with psychological therapies aimed at increasing specific personal memories and positive self-referential processing. These potential antidepressant-like properties of DCS are therefore worth exploring in future studies.

Previous studies found that a low dose of DCS (12 mg/kg) showed anxiogenic effects in animals (Ho *et al.*, 2005, Moraes Ferreira and Morato, 1997). However, Bailey *et al.* (2007) found that a low dose of DCS (50 mg) improved performance on a learning task in a low-anxiety condition (in which participants also received air) but not in a high-anxiety condition (in which participants received CO_2) among healthy volunteers. Given the mixed nature of these results, further investigation on the effect of DCS on stress responses in humans would be of considerable clinical value towards understanding the mechanisms at play in the treatment of clinical depressive and anxiety disorders. Thus, Chapter 6 used psychophysiological and self-reported mood and anxiety measurements to investigate the acute effects of DCS on stress responses in a novel psychosocial stress task in the same cohort of healthy volunteers. Both DCS and placebo produced increases in psychophysiological

measurements (blood pressure, pulse rate, skin conductance response) and subjective anxiety and mood ratings, and DCS did not modify any of the objective or subjective measures in the psychosocial stress paradigm. It is possible that this dose of DCS (250 mg) is insufficient for reducing the stress response in humans. Future studies could test DCS in other stress models at different doses.

7.2 Thesis implications

The neuropsychological model of antidepressant drug action suggests that conventional antidepressants do not primarily affect subjective mood symptoms, but rather, emotional processing. Previous studies on both healthy populations and depressed patients support these positive-biasing effects of conventional antidepressants (SSRIs and SNRIs) and atypical antidepressants, such as mirtazapine and bupropion, on emotional processing (Arnone *et al.*, 2009, Godlewska and Harmer, 2020, Walsh *et al.*, 2018). This thesis presents the first evidence that NMDAR-targeting antidepressants also produce early effects on emotional processes relevant for depression treatment in behavioural and neuroimaging studies. A single administration of ketamine increased activation in the right DLPFC during negative self-referential processing in the absence of subjective mood changes at the time of assessment. Thus, this finding with ketamine further supports the neuropsychological model of antidepressant drug action beyond conventional antidepressants and suggest that novel antidepressant drugs may also act to reduce the negative bias that is known to play an important role in the development and maintenance of depression.

A better understanding of the neuropsychological effects of psychopharmacological therapies may further inform the development of pharmacologic treatments of depression. By implementing these sensitive psychological tasks and fMRI, we can detect early effects of drug

treatment on measures of emotional processing. Effective treatments for depression are known to increase the processing of positive as compared to negative information, an effect that is thought to precede and contribute to changes in mood symptoms. This model can therefore be used to test the efficacy of novel drugs in both patients and healthy volunteers, and can provide an early biomarker of treatment efficacy. Novel candidate drugs are often screened using low predictive validity animal models and without using clinical human models (Berton *et al.*, 2012, Pringle and Harmer, 2015); a lack of efficacy in translation is the main reason for failure in drug development.

As previous studies have suggested, putative antidepressant drugs which lack the ability to positively bias emotional processing in human are more likely to fail as clinically effective antidepressants (Godlewska, 2019). For example, a single dose of memantine did not produce effects on emotional processing in tasks measuring facial emotion recognition or self-referential processing (Pringle *et al.*, 2012). Memantine has also failed to produce antidepressant effects in previous clinical trials (Kishi *et al.*, 2017). In the current study, although patients receiving lanicemine were better at recognising facial expressions across all emotions when compared to the placebo group, it did not produce a positive bias in emotional processing and there was no neural effect either in this respect. In line with this, a key phase 2B clinical trial of lanicemine failed to demonstrate significant antidepressant effects in TRD patients (Sanacora *et al.*, 2017). Thus, as clinical trials involve considerable investment during drug discovery and development, ensuring that new drugs produce positively biased emotional processing in human studies in the early phase of drug development could reduce costs in failed clinical trials. Combining the evidence from this thesis, we also provide a possible direction where the neuropsychological model may be useful for developing new rapid-onset antidepressants in the future.

7.3 Comparison between conventional antidepressants and NMDA receptor-targeting antidepressants in emotional processing

The differences between conventional antidepressants and NMDAR-targeting antidepressants in emotional processing deserves mention. Specifically, NMDAR antagonists and partial agonists do not show the broad range of effects on emotional processing seen with other conventional antidepressants. For example, an acute dose of citalopram and reboxetine led to increased accuracy in the recognition of happy faces and reduced recognition of negative facial expressions, as well as reduced reaction times in response to positive facial expressions and positive self-referential words (Harmer *et al.*, 2003a, Harmer *et al.*, 2003c, Harmer *et al.*, 2009b). Moreover, a single dose of citalopram produced a positive emotional bias in the dot probe task (Browning *et al.*, 2007).

In the current thesis, ketamine was shown to increase the number of misclassifications of ambiguous facial expressions classified as positive, but not negative, and also increased false positive memory intrusions in the emotional recall task. These results provide further evidence that ketamine may eliminate existing negative encoded memories and increase retrieval of more positive encoded memories, which would not be dependent on environmental interactions (e.g., transient forgetting of negative associations), and these factors could contribute to its faster onset of antidepressant effects. However, delayed-onset antidepressants may require inflow of social information to change the way in which this material is encoded, as well as require the interaction between how the patient perceives the environment and social experiences given the new contingencies produced by positive emotional bias (Godlewska, 2019). These results indicate that the neuropsychological model, by using behavioural and neural measurement of emotion processing, may be able to distinguish the differences between

delayed-onset antidepressants and fast-onset antidepressants, consistent with the findings from animal studies (Stuart *et al.*, 2015). However, this hypothesis should be interpreted with caution, as the findings described above involving conventional antidepressants in healthy populations or depressed patients were obtained in the presence of drugs administered either acutely or sub-chronically, whereas ketamine was examined 24 hours post administration.

Similar findings were also found with an acute dose of the NMDA partial agonist DCS. In the self-referential processing task, DCS only significantly improved categorisation and subsequent free recall of positive self-descriptors but there were no effects on reaction time. Further, DCS significantly increased specific autobiographical memory retrieval, and these enhancing effects of DCS persisted for 24 hours. These findings indicate that agents acting to facilitate NMDAR activity may be more likely to affect autobiographical memory as compared to conventional antidepressants in acute dosing. These findings emphasise that DCS, through its interaction with the glycine site of the NMDAR, might underscore its potential role in enhancing cognitive behavioural therapy in depression.

7.4 Lack of anxiogenic effects NMDAR-targeting antidepressants

Importantly, a single dose of an NMDA antagonist (ketamine) and partial agonist (DCS) did not produce anxiogenic effects. This is in contrast to the effects of conventional antidepressants, which may show anxiogenic-like effects in the early stages of treatment. For example, an acute dose of citalopram increased fearful face recognition and produced higher emotional reactivity in a startle response paradigm (Browning *et al.*, 2007, Grillon *et al.*, 2007, Harmer *et al.*, 2003). Preclinical and clinical evidence has suggested that SSRIs can produce anxiogenic effects and exacerbate anxiety symptoms in the early stages of treatment (Burghardt *et al.*, 2004).

A recent study also found that an acute dose of the NMDAR co-agonist, D-alanine, induced a negative bias by significantly increasing the detection speed for fear and anger in the facial emotional recognition task in healthy volunteers, and the authors suggested that D-alanine may produce anxiogenic effects (Capitão *et al.*, 2020). However, neither the NMDAR antagonist ketamine nor NMDAR partial agonist DCS enhanced fearful face recognition in our study. Moreover, neither ketamine nor DCS showed significant effects in the attentional dot probe task, which was used to measure attentional vigilance towards threatening information. The attentional dot probe task has been helpful in determining whether anxiogenic effects can be modulated by a drug (Murphy *et al.*, 2008, Murphy *et al.*, 2009b, Pringle *et al.*, 2011b).

The effects of ketamine on anxiety are inconsistent. Pietersen *et al.* (2006) found that ketamine administration in rats can successfully block the expression of fear-conditioned stress at both behavioural and neural levels. Engin *et al.* (2009) reported that ketamine not only produces antidepressant-like effects, reducing immobility in the forced swim test similar to the conventional antidepressant fluoxetine, but also shows anxiolytic-like properties in the elevated plus-maze test, increasing the number of open-arm entries, a finding comparable to the proven anxiolytic drug diazepam. However, in a study of healthy volunteers (Krystal *et al.*, 1994), a low dose of ketamine (0.1 mg/kg) reduced anxiety symptoms while a higher dose (0.5 mg/kg) increased anxiety. Further studies focusing on the anxiolytic-like effects of ketamine in humans are needed.

In addition, an acute dose of DCS in the current thesis did not elevate the stress response to the novel psychosocial stress task compared to healthy participants who received a placebo. This may provide further evidence that DCS does not produce anxiogenic-like effects. However, the absence of possible anxiolytic-like effects of DCS needs to be confirmed in further studies.

The studies reported in this thesis did not include the emotion-potentiated startle response paradigm, which is a highly sensitive tool to measure stress and the effects of anxiolytics (Davis *et al.*, 1993).

Previous studies have shown that the anxiogenic effects of the startle paradigm can be modulated by an acute dose of drugs such as aprepitant (Pringle *et al.*, 2011b), agomelatine (Harmer *et al.*, 2010), and citalopram (Browning *et al.*, 2007, Grillon *et al.*, 2007). The startle paradigm may also be more sensitive in detecting anxiolytic like effects than the attentional dot-probe, as the results in this task are often variable and the fear vigilance effect is not always seen under placebo conditions in healthy volunteers, as was the case in Chapter 5. Future studies are required to replicate the potential anxiolytic-like effect of NMDAR-targeting antidepressants in human models. Such work could provide important clinical implications towards faster alleviation of comorbid depression and anxiety in the early phase of treatment, as previous studies have suggested that anxious depression involves a higher risk of non-response to treatment and self-harm (Choi *et al.*, 2020, Pfeiffer *et al.*, 2009).

7.5 Thesis limitations

This section discusses some of the methodological limitations of this thesis. The study of emotional processing and autobiographical memory in DCS was conducted on healthy volunteers. Previous studies have demonstrated that healthy volunteers are appropriate for investigating the effects of psychopharmacological treatments (Browning *et al.*, 2007, Capitão *et al.*, 2020, Harmer *et al.*, 2010, Harmer *et al.*, 2008, Murphy *et al.*, 2008, Murphy *et al.*, 2009b, Walsh *et al.*, 2018, Woud *et al.*, 2018), as studying a healthy sample can give insight into drug action without being confounded by mood symptoms, other medication and general cognitive changes that can be present in a sample with clinical depression. However, using

healthy volunteers may mean that the findings are not necessarily applicable to clinical samples. Thus, future studies with a clinically depressed sample should be conducted to examine the ability of the NMDA partial agonist DCS to influence emotional memory enhancement. However, such a study design in a clinical sample was beyond the scope of this thesis due to time and resource constraints.

Another important clinical implication of the neuropsychological model is the prediction of therapeutic response (Godlewska, 2019). Previous studies have shown that early changes in both behavioural measures and neural activity can predict later clinical response. For example, Tranter *et al.* (2009) found a significant positive correlation between recognition of happy faces after two weeks of SSRI (citalopram) and SNRI (reboxetine) treatment and clinical symptom improvement following 6 weeks of treatment. Shiroma *et al.* (2014) reported that improved accuracy in the recognition of happy emotions between baseline and day 7 significantly predicted citalopram antidepressant response on day 56 when considered along with perceived level of social support. Moreover, early changes in the neural response to fearful versus happy faces following 7 days of escitalopram treatment significantly predicted clinical response at week 6 (Godlewska *et al.*, 2016). Delaveau *et al.* (2016) found that DLPFC and DMPFC activation during self-referential processing at baseline can differentiate treatment remitters from non-remitters after 6 months of agomelatine treatment.

Thus, identifying early biomarkers via both behavioural and neural measures of emotional processing has the potential to guide the choice between different classes of psychopharmacological treatment, as the delayed onset of antidepressants may require depressed patients to wait several weeks before mood gradually improves. Moreover, some patients may need to treat multiple medications, which can increase depressed patients' burden.

Although this thesis employed a double-blind design involving MDD and TRD patients receiving placebo or an active drug treatment, we only examined a single drug dose at a single time point rather than multiple doses and time response points in depressed patients, which limited our ability to explore the associations between changes in behavioural and neural measures of emotional processing and treatment response (changes in depressive symptoms).

On the other hand, the effect of NMDAR antagonists was only tested 24 hours after administration here. However, conventional antidepressants have typically been examined in the presence of drug administration (normally 2–3 hours). Thus, it is possible that NMDAR antagonists produce effects on emotional processing following a short period of time after drug administration.

The ETB included several validated measures of emotional processing that have been widely used to investigate antidepressant effects in both healthy volunteers and depressed patients in Western countries. As mentioned in Chapter 4, the ETB, including the facial expression recognition task and emotional categorisation task, has not yet been validated in a Chinese cultural setting. The tasks used in Chapter 4 were developed and modified based on previous studies examining emotional processing in Western countries. The cultural differences were not considered in the task design and future empirical cross-cultural studies should first validate the ETB in the Chinese population before its implementation.

Another limitation to the studies presented in this thesis is the between-groups design. The main disadvantage of using a between-groups design is that the group randomisation may not minimise individual differences across groups. For example, in Chapter 4 we studied a clinical TRD sample, and some patients had higher treatment resistance (with higher depression history,

more failed medication attempts, or different exposures to psychological or ECT treatments) than others, even though there were no significant mean differences between groups in baseline measurements in the several studies discussed here. The advantages of a between-subjects design include shorter sessions and minimising learning and transfer effects across different conditions. The alternative approach is to use a within-subjects design, where each participant undergoes at least two treatment sessions. This can minimise random noise and the impact of individual differences, and requires fewer participants. However, we have observed that the effects of antidepressants are stronger on the first testing session in our laboratory, and the habituation and learning effects in emotional processing tasks are also important factors to consider when delivering repeated presentations of the same emotional materials to the same participant using a within-subject design (Capitão, 2014). However, future studies should consider the use of a within-subject design, which may be more sensitive to detecting the effects of NMDA antagonists.

As previous antidepressant studies using effect sizes suggest that a sample size of 20 participants per group is sufficient to detect significant differences between groups at a power of 0.85, the sample sizes used in two chapters were relatively small. In Chapter 3, we excluded all the imaging data from Manchester University due to a setting issue relating to gantry tilt in the acquisition, which led to 28 participants being excluded from the data analysis. It is possible that there was insufficient power to detect differences in other brain regions in both whole brain and ROI analyses.

In Chapter 4, the study of N_2O was suspended due to the COVID-19 pandemic, leaving 25 patients in the preliminary analysis. In Nagele *et al.* (2015), the changes of depressive symptom ratings (HAMD) for an active treatment group (N_2O) was similar to that in the current study,

and thus, the non-significant difference between placebo and treatment groups in the current study may be due to inadequate power. Thus, the sample sizes in two chapters were less than ideal, which may result in an inability to detect real effects between groups. Future research with a larger sample size should further explore and refine our analysis of these between-group differences.

7.6 Future directions

Further integrative investigations are needed to help us gain a more comprehensive understanding of the mechanisms underlying NMDAR antagonists and partial agonists using a cognitive neuropsychological model approach in the treatment of depression. The specific directions recommended for future research have been mentioned throughout, but this final section will also summarise the key points for future directions of the field.

Future studies may consider testing the effect of NMDAR antagonists shortly after drug administration rather than waiting 24 hours. This research may drive more direct comparisons between NMDAR antagonists and conventional antidepressants. Future studies should also include long-term repeated drug administration with a larger sample size in order to examine whether emotional processing shifts produced by NMDAR antagonists show a predictive therapeutic in depressed patients. More research focused on identifying biomarkers of therapeutic responses is needed, as no actual marker has yet been applied in standard clinical practice.

As mentioned earlier, the between-subjects design used here may not have minimised individual baseline differences, as the profiles of TRD patients are varied (e.g. history of depression, comorbidity of other mental disorders, suicide risk, history of treatment), and future studies may consider adopting a cross-over design to investigate the effects of NMDA

antagonists in emotional processing. Further, some emotional processing tasks may be associated with difficulty focusing or engaging in TRD patients due to their cognitively-impairing depressive symptoms as compared to those with mild-to-moderate symptoms. However, we did not assess how TRD patients actually perceived these tasks. Only a few studies have previously examined emotional processing in a TRD sample (Miskowiak *et al.*, 2017, Reed *et al.*, 2019, Stroud *et al.*, 2018). Understanding the neuropsychological mechanisms of treatments for TRD patients can provide key insights into future drug development, such as rapid antidepressant effects, especially relevant to this population. Thus, it would be worthwhile to conduct comparison studies to explore differences in emotional processing task performance between MDD, TRD, and healthy volunteer groups. Qualitative studies would also aid our understanding of how TRD patients perceive emotional processing tasks such as the ETB. These studies may provide future directions for adjusting task design in order to adequately capture and detect antidepressant effects in emotional processing tasks when applied in TRD populations.

It was found that a single administration of N_2O did not modulate emotional processing as assessed using behavioural tasks in TRD patients, but this thesis did not examine the neural effects of N_2O. It would be interesting to explore the neural effects on brain regions in response to these tasks in future studies, as fMRI BOLD activation may be more sensitive than behavioural measures in studying emotional processing.

As we found the interesting result that a low dose of DCS significantly improved retrieval of specific autobiographic memories and increased positive self-referential processing in healthy volunteers, this should be replicated in clinical samples. If the same effects are also found in depressed patients, a low dose of DCS may provide a positive direction for combination with

psychological interventions, such as cognitive behavioural therapy, in the treatment of depression.

Although Chapter 6 found that a low dose of DCS did not lead to increased arousal or anxiety in response to the acute psychosocial stress paradigm; in fact DCS did not modify any of the measured objective or subjective responses in this study. Future studies should explore the effects of a higher dose of DCS in an acute stress paradigm. On the other hand, future studies should also examine how individual differences (such as low versus high trait anxiety) influence changes in stress responses in a psychosocial stress task. This is because previous human studies showed that a low dose of DCS improved performance on a challenging cognitive task under low levels of anxiety, but not higher levels (Bailey *et al.*, 2007). These studies may be important towards understanding the mechanisms underlying the stress response and may facilitate the development of treatments for depression and other stress-related disorders.

7.7 Conclusion

In conclusion, the several studies presented in this thesis provide evidence that NMDAR targeting antidepressants show effects on emotional processing in both behavioural and neuroimaging studies in the absence of subjective mood symptom improvement, further supporting the cognitive neuropsychological model theory of antidepressant drug action. In the behavioural studies, ketamine did not show the broad range of effects on emotional processing seen with other conventional antidepressants, but significantly increased the error rate for recognising facial expression as positive as opposed to negative, and produced a higher rate of false positive memory intrusions as compared to placebo in the emotional recall task.

These results indicate that the neuropsychological model may be able to distinguish differences between delayed-onset antidepressants and fast-onset antidepressants. In the neuroimaging study, the preliminary analysis revealed that a single dose of ketamine increased brain activation in the right DLPFC when compared to placebo during negative self-referential processing. Moreover, this thesis revealed that an acute dose of DCS can modulate aspects of emotional processing in healthy volunteers, positively biasing emotional memory and, intriguingly, autobiographical memory. However, a potential anxiolytic-like effect was not seen following a single dose of the NMDAR antagonist (ketamine) and partial agonist (DCS). In addition, the results from our exploratory study indicated that a single dose of N_2O did not modulate emotional processing in patients with TRD. Finally, this thesis outlines some methodological limitations and potential next steps for future research in order to further understand the cognitive neuropsychological mechanisms underlying the effects of NMDAR antagonists and agonists in depression and stress-related disorders.

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