Neuropsychological Mechanisms of Ketamine Antidepressant Effect

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Abstract

Ketamine produces rapid (within hours) and sustained (up to 10 days) antidepressant effects that extend beyond its half-life. Although its molecular and cellular mechanisms are well characterised, the neuropsychological processes underlying its clinical effects in depression are less defined.

Animal studies suggest that ketamine reverses negative affective biases in negative memories without significantly impacting positive memories, differently from conventional antidepressants. Further, ketamine targets the lateral habenula, a small, evolutionarily conserved structure in the epithalamus that is key to the brain's reward-punishment circuitry. Finally, emerging evidence suggests that ketamine has lasting effects on stress resilience.

This DPhil project investigates whether these neuropsychological mechanisms translate to humans. Two experimental medicine trials were conducted with participants randomised to receive either ketamine (0.5 mg/kg) or placebo via a 40-minute constant-rate infusion. In the first study, 70 healthy volunteers underwent a battery of assessments including questionnaires, computer-based tasks, and task-based 7 Tesla fMRI using a win-loss-shock Pavlovian paradigm designed to elicit habenula activation. The Emotional Battery Test was performed before and 24 hours post-infusion, and a stress-inducing paradigm was administered one week later to assess stress resilience. In the second study, 60 patients with treatment-resistant depression (TRD) were evaluated for negative biases in autobiographical memories (24 hours and 7-days post-infusion) and self-descriptive words (post-infusion).

Compared to placebo, ketamine significantly reduced negative biases for self-descriptive words in both healthy and depressed subjects, attenuated habenula response to aversive outcomes while engaging reward circuits (including the prefrontal cortex and anterior cingulate cortex) and diminished stress responses (as measured by heart rate variability) at 7 days in healthy volunteers. Interim analysis of the first 43 participants in the second study suggests that ketamine does not alter biases in autobiographical memories in TRD. Further research is needed to translate these effects clinically and further characterise the underlying neurobiological mechanisms of ketamine antidepressant effects.

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This work belongs to all of those people as much as it belongs to me.

Declarations

This dissertation is the result of my own work and includes nothing that is the outcome of work done in collaboration except as specified within the text. No substantial part of my thesis has been previously submitted, or, is currently being submitted, for any degree, diploma, or other qualification at the University of Oxford or any other academic Institution. All submitted publications included herein are directly related to my field of study, and were written whilst holding the status of DPhil student at the University of Oxford. Some parts of this thesis have been published or submitted for publication:

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- Evers A., Murrough J.W., Charney D.S., Costi S. Ketamine as a prophylactic resilience-enhancing agent. Frontiers in Psychiatry 2022

In accordance with the guidelines, this thesis contains approximately 50,000 words, and it contains less than 150 figures.

List of abbreviations

ACC Anterior cingulate cortex

AMPA α-amino-3-hydroxy-5-methyl4-isoxazolepropionic acid

ANCOVA Analysis of Covariance

ANOVA Analyses of variance

ANS Autonomic Nervous System

BBR Boundary-based registration

BDI Beck Depression Inventory

BDNF Brain-derived neurotrophic factor

BMI Body mass index

BOLD Blood oxygenation level dependent

CADSS Clinician Administered Dissociative States Scale

CNS Central nervous system

CORT Chronic Corticosterone

CRF Clinical Research Facility

CS Conditioned stimuli

CSD Chronic Social Defeat

CUS Chronic Unpredictability Stress

DBS Deep brain stimulation

DI Dominant Interaction

DLPFC Dorsal lateral prefrontal cortex

DMPFC Dorsal medial prefrontal cortex

DSM Diagnostic and Statistical Manual of Mental Disorders

ECAT Emotional Categorisation Task

ECG Electrocardiogram

eEF2 Eukaryotic Elongation Factor 2

EMEM Emotional Recognition Memory Task

EPDS Edinburgh Postnatal Depression Scale

EPI Echo-planar imaging

EREC Emotion Recall Task

ETB Emotional Test Battery

FEAT FMRI Expert Analysis Tool

FERT Facial Expression Recognition Task

FOV Field of view

fMRI Functional magnetic resonance imaging

FST Forced Swim Test

GABA Gamma-aminobutyric acid

GAD-7 Generalized Anxiety Disorder-7

GLM General Linear Model

GRE Gradient-recalled echo

HAM-D Hamilton Rating Scale for Depression

Hb Habenula

HF High frequency

HPA Hypothalamic-pituitary-adrenal

HRF Hemodynamic response function

HRV Heart rate variability

IS Inescapable shock

IV intravenous

JSI Juvenile Social Interaction

LF Low frequency

LH Learned Helplessness

MADRS Montgomery-Åsberg Depression Rating Scale

MDD Major depressive disorder

MIST Montreal Imaging Stress Task

MPFC Medial Prefrontal cortex

MNI Montréal Neurological Institute

MRI Magnetic resonance imaging

mTORC1 mammalian target of rapamycin complex 1

NMDA N-methyl-D-aspartate

NMDAR N-methyl-D-aspartate receptor

OAMT Oxford Autobiographical Memory Task

OCST Oxford Cognition Stress Task

PANAS Positive and Negative Affect Schedule

PCIA Patient Controlled Intravenous Analgesia

PDI Peters et al. Delusions Inventory

PFC Prefrontal cortex

PE Parameter estimate

PET Positron Emission Tomography

PPD postpartum depression

PPI Patient and public involvement

POMS-Bi Profile of Mood States - Bipolar

PSS Perceived Stress Scale

PTSD Posttraumatic Stress Disorder

QIDS-SR Quick Inventory of Depressive Symptomatology Self-Report

RMSSD Root Mean Square of Successive Differences

ROI Region of interest

RRS Rumination Response Scale

sAA salivary α-amylase levels

SCID Structured clinical interview for DSM-5 Axis I Disorders

SD Standard deviation

SDNN Standard Deviation of Normal-to-Normal intervals

SERT Serotonin transporter

SNRI Serotonin noradrenaline reuptake inhibitor

SPT Splash Test

SSRI Selective serotonin reuptake inhibitor

STAI-S State-Trait Anxiety Inventory – State subscale

STAI-T State-Trait Anxiety Inventory - Trait subscale

STAR*D Sequenced Treatment Alternatives to Relieve Depression

TCA Tricyclic antidepressant

TFCE threshold-free cluster enhancement

TEPS Temporal Experience of Pleasure Scale

TI Inversion Time

TR Repetition Time

TRD Treatment resistant depression

TrkB Tropomyosin receptor kinase B

TSST Trier Social Stress Test

TST Tail Suspension Test

VAS Visual analogue scales

VTA Ventral Tegmental Area

WB Whole brain

Chapter 1. Introduction

1.1 Major Depressive Disorder: Current Status and Unmet Clinical Needs

Major Depressive Disorder (MDD) is a highly prevalent condition with an estimated lifetime prevalence of 20.6% in the United States alone (Hasin et al., 2018). Similarly, in the United Kingdom, 19.7% of individuals aged 16 and over have reported symptoms of anxiety or depression with higher rates observed in females (22.5%) compared to males (16.8%) (Evans, 2016). Globally, it ranks among the leading causes of disability and disease burden (G. B. D. Mental Disorders Collaborators, 2022; Whiteford et al., 2013). MDD is usually defined by the presence of five or more criteria described in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) (American Psychiatric Association, 2013). These include depressed mood, anhedonia, insomnia/hypersomnia, increased or decreased appetite, psychomotor agitation/retardation, impaired concentration or indecisiveness, worthlessness or inappropriate guilt and thoughts of death. These symptoms must be present for most of the day, nearly every day, and cause a substantial impairment in social, occupational or other important areas of functioning.

A key challenge in MDD treatment lies in its clinical heterogeneity: different combinations of symptoms can lead to the same diagnosis, making personalised and effective treatment difficult. The care pathway is often complex and, even with successful treatment, there is a high risk of relapse and recurrence, and residual symptoms. Conventional antidepressants - most of which target monoaminergic systems - fail to achieve stable remission in 20-30% of patients with depression (Rush et al., 2006), and the lifetime risk of recurrence is

approximately 60% (Monroe & Harkness, 2011). Patients who do not respond to at least two trials of antidepressant treatment of adequate dose and duration are considered to be suffering from treatment resistant depression (TRD) (Ionescu et al., 2015).

1.2 Therapeutic Challenges of Major Depressive Disorder

Despite significant recent advances in the understanding of the biological, psychological, and neuroanatomical underlying depression, current treatment options remain limited. Conventional antidepressants primarily act by increasing levels of specific neurotransmitters - namely serotonin, norepinephrine, or both - in the synapse. While they differ in their precise mechanisms of action, the most common involves inhibiting neurotransmitter reuptake (Harmer et al., 2017; Sheffler et al., 2025). Recent evidence suggests that the initial rise in synaptic neurotransmitter level may trigger downstream effects, including increased expression of neuroprotective proteins such as brain-derived neurotrophic factor (BDNF) and enhanced neuroplasticity (Santarelli et al., 2003), ultimately contributing to the remission of depressive symptoms (Castren & Rantamaki, 2010).

Many individuals on long-term pharmacological therapy do not achieve full symptomatic remission or functional recovery. Adherence remains a critical issue - approximately 50% of patients discontinue treatment prematurely (Garrido & Boockvar, 2014; Gonzalez de Leon et al., 2022; Lee et al., 2010) which increases the likelihood of relapse and recurrence (Gonzalez de Leon et al., 2022; Ho et al., 2016). Furthermore, barriers such as stigma, limited access to mental health care, and financial constraints continue to impede treatment delivery. For example, 36% of individuals in Europe report being unable to access mental

health services due to a shortage of healthcare professionals, and 26% cite financial barriers (Statista, 2021). The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, a large real-world clinical trial aiming at determining the most effective next-steps treatments for subjects with MDD whose symptoms do not remit after an initial treatment (Fava et al., 2003; Rush et al., 2004), highlighted the limitations of current therapeutic approaches. Remission rates declined with each successive treatment step: 36.8% after the first treatment, 30.6% after the second, and only around 13% after the third and fourth steps, with an overall cumulative remission rate of 67% (Rush et al., 2006). Most patients require several treatment steps to achieve remission, and the likelihood of remission significantly decreases after two medication trials, further highlighting the complexities and challenges in the treatment of subjects with TRD (Gaynes et al., 2009). More treatment steps were also associated with higher relapse rates during the follow-up phase (Rush et al., 2006). One of the key reasons treatment options for depression remain limited may lie in the historical path of antidepressant discovery. Many of the medications currently in use were discovered serendipitously over the past few decades, rather than being the result of targeted drug development based on a detailed understanding of the neurobiological and cognitive mechanisms underlying MDD (Insel & Scolnick, 2006).

1.3 The monoamine hypothesis of depression

A prominent theory explaining the pathophysiology of depression is the monoamine hypothesis of depression developed approximately sixty years ago. This hypothesis suggests that depression is associated with a decrease in the functional activity of one or more of the monoamines, including serotonin, norepinephrine and dopamine. First- and second-

generation antidepressants target mostly the monoamine neurotransmitters system and increase the levels of these mediators in the synaptic space. The role of monoamines - especially serotonin- in depression has been studied using “tryptophan depletion” studies. Tryptophan is an essential amino acid required for serotonin synthesis and tryptophan depletion temporarily reduces brain serotonin levels by limiting the availability of its precursor, tryptophan, through an acute dietary intervention. Tryptophan depletion can trigger significant depressive symptoms in unmedicated people who have recovered from depression (Smith et al., 1997). Similarly, medication-free individuals with MDD in remission show significant depressive symptoms when undergoing catecholamine depletion with alpha-methyl-para-tyrosine (Berman et al., 1999). However, tryptophan depletion does not significantly affect mood in healthy individuals with no risk factors for MDD (Abbott et al., 1992; Smith, 1997). These findings suggest that while disrupted monoamine function can lead to clinical depression under certain conditions, it is not neither necessary nor sufficient. Furthermore, the effects of tryptophan depletion are more pronounced in individuals with a personal experience of depression than in those merely at high risk due to factors like family history (Ruhe et al., 2007). This suggests that reduced serotonin activity might affect the mechanisms that sustain recovery from MDD rather than causing low mood in all susceptible individuals (Cowen & Browning, 2015). Overall, these findings suggest that the monoamine system exerts mostly a modulatory role on other neuro-biologic pathways associated with MDD (Charney, 1998; Heninger et al., 1996). Further, the latency of response to antidepressant medications (approximately two weeks) and the significant proportion of MDD patients who do not respond to monoamine-based treatments (approximately one third) (Fava & Davidson, 1996) indicate that the monoaminergic theory of depression is incomplete in describing the neurobiological alterations contributing to the disorder.

1.4 Beyond the monoaminergic hypothesis of depression

Despite the predominant focus on monoamines in recent decades, there has been emerging evidence highlighting alterations in other neurotransmitter systems linked to depression, including the gamma-aminobutyric acid (GABA) system (Croarkin et al., 2011; Sanacora, 2010) and the glutamate system (Sanacora et al., 2008). Several studies have reported a decrease in the density of GABAergic interneurons in the prefrontal and occipital cortices of depressed subjects (Maciag et al., 2010; Rajkowska et al., 2007) and magnetic resonance spectroscopy suggested that the concentration of GABA in the occipital cortex and anterior cingulate cortex (ACC) is diminished in MDD (Sanacora et al., 1999). Additionally, GABA levels in plasma and cerebrospinal fluid appear reduced in depression (Sanacora, 2010).

Finally, the discovery of a rapid and sustained antidepressant effect of a sub-anaesthetic dose of the non-competitive N- methyl-D-aspartate (NMDA) receptor antagonist ketamine (Berman et al., 2000) has brought new focus to the role of glutamate in MDD. Placebo-controlled trials have provided strong evidence for the rapid (within hours) and sustained (up to 7 days) antidepressant effects of a single administration of ketamine in subject with TRD (Newport et al., 2015). Although the exact mechanisms behind ketamine's antidepressant effects are not fully understood, they appear to involve NMDA receptor modulation. Ketamine blocks tonic GABAergic inhibition, leading to an increase in glutamate release in the synaptic cleft. This increase in glutamatergic transmission leads to the synthesis of neural growth factors and contributes to increased synaptogenesis (Aleksandrova et al., 2017; Moore et al., 2022).

Since then, new scientific investigations have examined the role of the glutamate system in the neurobiology of MDD and its potential as a target for novel therapeutics (Duman et al., 2016; Kavalali & Monteggia, 2012).

1.5 A Breakthrough Treatment for Treatment Resistant Depression at the Turn of the Millennium

The first study testing the use of ketamine for depression was conceptualised as a trial to test glutamate synaptic alterations in the context of MDD using the response to ketamine as a probe, and capitalised on previous preclinical work suggesting that NMDA receptor antagonism was associated with antidepressant effects (Krystal et al., 2019; Pilc et al., 2013). In line with previous research on psychosis, ketamine was administered via intravenous (IV) infusion at the dosage of 0.5 mg/kg at a constant rate over 40 min. The study, which had a placebo-controlled crossover design and was conducted on a small sample of medication free MDD subjects (n = 7), reported a marked decrease in depressive symptoms, as measured by the 25-item Hamilton Depression Rating Scale (HAM-D), after 240 min from the administration of the drug. The striking antidepressant response was sustained for 72 hours following the infusion, with symptoms returning to baseline within one to two weeks. Of note, ketamine's rapid and lasting antidepressant effects were temporally distinct from the brief acute dissociative effects of the drug (Berman et al., 2000).

Findings from subsequent clinical trials of a single infusion of ketamine in depressed subjects utilising a crossover design showed similar results (Diamond et al., 2014; Zarate et al., 2006). For example, Zarate and colleagues reported a significant improvement in

depressive symptoms following ketamine infusion compared to placebo at 24 h and at one week in a sample of 17 TRD subjects (Zarate et al., 2006). A larger a two-site trial conducted on a sample of 73 TRD subjects also showed similar rapid improvement in depression severity 24 h after ketamine administration (Murrough et al., 2013).

Overall, a single infusion of ketamine in MDD appears to be well tolerated with response rates (defined as a reduction of $\geq 50\%$ in depression severity scores from baseline) that vary between 50% (Berman et al., 2000) to 71% (Zarate et al., 2006) in clinical trials. The response rate to ketamine appears comparable - or slightly higher - in real-world settings, with approximately one third of TRD subjects achieving remission and approximately 50-75% of patients showing clinical response after a single dose (Wilkinson & Sanacora, 2017).

1.5.1 The Molecular Basis of Ketamine's Antidepressant Effect

Multiple brain regions have been implicated in ketamine's mechanism of action, including the prefrontal cortex (PFC). For example, ketamine has been shown to reverse chronic stress induced reductions in dendritic spine density, through BDNF-driven synaptogenesis (Li et al., 2011; Moda-Sava et al., 2019). The hippocampus, a region critical for mood regulation and memory, also shows rapid synaptic and behavioural changes following ketamine treatment (Autry et al., 2011; Zanos et al., 2016). Recent findings indicate that ketamine reduces bursting activity in the lateral habenula (Hb), which is hyperactive in depression and linked to negative emotional processing (Yang et al., 2018).

Ketamine is a non-competitive NMDA receptor (NMDAR) antagonist that blocks the ion permeation pore located inside the receptor. Preclinical and clinical data suggest that this

property is central to its rapid antidepressant effects, triggering a cascade of intracellular signalling and synaptic changes, particularly in brain regions such as the PFC and hippocampus. These effects are believed to counteract the synaptic deficits, neuronal atrophy, and glial loss observed in MDD, and consequentially restore synaptic plasticity - a fundamental process for information processing, adaptation, and memory formation. Although the mechanisms underlying ketamine's antidepressant action are not fully characterised, two main hypotheses have emerged:

1. Inhibition Hypothesis: This theory suggests that ketamine selectively blocks extra-synaptic NMDARs, which are tonically activated by low levels of glutamate and regulated by astrocytes. Inhibiting these receptors lifts suppression on mTORC1 (mammalian target of rapamycin complex 1), promoting synapse-related proteins and the number of new dendritic spines in the PFC (Li et al., 2010). An infusion of the mTOR inhibitor rapamycin into the PFC inhibited the molecular and behavioural effects of ketamine, which corroborated the hypothesis that mTOR signalling mediates the fast-acting antidepressant effects through increased dendric spine numbers (Li et al., 2010; N. Li et al., 2011). However, recent clinical evidence challenges these findings (Abdallah et al., 2020). In a surprising outcome, a small trial of 20 subjects with TRD, randomised to receive either rapamycin or placebo prior to ketamine treatment, found that rapamycin did not attenuate ketamine's acute antidepressant effects at 24 hours post-infusion. Furthermore, rapamycin pretreatment was associated with higher response and remission rates at two weeks, suggesting it may prolong ketamine's antidepressant effects. However, these findings should be interpreted with caution, as the study was limited by a small sample size and, despite no known interactions, did not assess potential interactions between rapamycin and ketamine metabolites.

Of note, the effects of ketamine on NMDAR are dependent on the basal state of the receptor at the time of drug administration. For active NMDARs, ketamine leads to augmented glutamate release and increased excitatory activity. For inactive NMDARs, ketamine leads to the inhibition of eukaryotic elongation factor (eEF2) kinase, with subsequent up-regulation of BDNF, a key protein that activates signalling that induces synaptic trafficking of AMPARs with synaptic potentiation in the hippocampus. These molecular and synaptic changes in the hippocampus are required for the rapid antidepressant effects of ketamine in animal models (Autry et al., 2011; Kavalali & Monteggia, 2020; Suzuki et al., 2017).

2. Disinhibition Hypothesis: An alternative but not mutually exclusive explanation suggests that ketamine blocks NMDARs on GABAergic inhibitory interneurons, leading to disinhibition of pyramidal neurons (Moghaddam et al., 1997). This results in enhanced glutamate release, activation of postsynaptic α-amino-3-hydroxy-5-methyl4-isoxazolepropionic acid (AMPA) receptors and a downstream cascade involving BDNF release, Tropomyosin receptor kinase B (TrkB) receptor activation, and mTOR signalling. This chain of events ultimately leads to increased dendritic spine density and synaptogenesis, particularly in the PFC (Gerhard et al., 2020).

Ketamine's antidepressant effects persist well beyond its short half-life, and blocking early signalling events critical to its rapid action prevents these sustained benefits (Autry et al., 2011; Ma et al., 2017). It is possible that the active metabolites derived from ketamine metabolization, may contribute to the prolonged pharmacological action. Another possibility is that the intracellular signal transduction (and synaptic changes) associated with ketamine rapid antidepressant action elicits further downstream signalling responsible for the

sustained effects. Additionally, ketamine appears to become trapped within NMDAR channels, resulting in prolonged inhibition even after drug washout. This persistent blockade may contribute to its sustained physiological and behavioural effects. Finally, recent studies suggest that ketamine's effects may extend beyond those mediated by NMDARs. For instance, ketamine interacts with opioid receptors, and pre-treatment with the opioid antagonist naltrexone has been shown to attenuate ketamine's antidepressant effects in both open-label and double-blind studies (Williams et al., 2018). However, small sample sizes and mechanistic uncertainty warrant cautious interpretation.

1.6 A Cognitive Neuroscience Perspective on Ketamine's Effects

Recent advancements in research methodologies have deepened our understanding of how antidepressants influence both neurobiological pathways and psychological functioning. Antidepressants exert effects at the molecular and neurochemical levels that translate into mood improvements and, more broadly, into neurocognitive changes (Godlewska, 2019). While conventional antidepressants are well-characterised pharmacologically, neurobiological models alone cannot fully explain the delayed mood improvements typically observed with treatment (Miller & Hen, 2015). Cognitive neuropsychological models offer a promising integrative framework for understanding the pathophysiology of MDD and the mechanisms through which antidepressants exert their effects (Godlewska, 2019). Experimental medicine trials in both healthy volunteers and clinical populations have been pivotal in demonstrating early shifts in cognitive processing that precede and predict later therapeutic outcomes (Godlewska et al., 2016; Harmer, O'Sullivan, et al., 2009; Shiroma et al., 2014; Tranter et al., 2009), highlighting the potential of cognitive biomarkers

in guiding antidepressant treatment. However, the mechanisms underlying the rapid and sustained antidepressant effects of novel treatments such as ketamine remain less well understood. The following sections summarise emerging evidence from animal models - and, where available, human studies - suggesting that ketamine may exert distinct neurocognitive effects that contribute to its rapid-acting antidepressant action.

1.6.1 Novel Insight into Ketamine Antidepressant Effect: The Role of the Lateral Habenula

A number of studies suggest a novel circuit paradigm through which the lateral Hb is involved in the pathophysiology of MDD. Lateral Hb hosts primarily glutamatergic neurons (Aizawa et al., 2012), receives inputs from the limbic system and projects broadly to the brain's reward centres such as the ventral tegmental area (VTA) (Hu, 2016). Further, it appears involved in multiple cognitive processes that are core components of MDD such as coding of negative emotion, reward processing, stress adaptation, and regulation of monoaminergic neurotransmission (Boulos et al., 2017; Gold & Kadriu, 2019; Matsumoto & Hikosaka, 2007). The Hb appears to be able to modulate the balance between reward and punishment, critically impaired in depressed subjects (Boulos et al., 2017; Gold & Kadriu, 2019): the capacity to revise expectations in response to external feedback represents a fundamental survival mechanism, essential for adapting to a dynamic and changing environment (Kaye & Ross, 2017). Negative reward prediction error (the learning signal generated by a failure to receive an expected reward) appears to be signalled by the lateral Hb (Matsumoto & Hikosaka, 2007). An intriguing case study offered even more direct support for the key role played by this brain region in the experience of depression. An individual with severe depression was treated with a deep brain stimulator (DBS) that

disrupted Hb firing, and the patient experienced a remission of symptoms. Following an accident that led to the device's being shut off, the patient's depression returned - and then remitted again when the device was turned back on (Sartorius et al., 2010). A more recent case report similarly demonstrated that a TRD patient experienced acute antidepressant effects and long-term clinical improvement following lateral Hb deep brain stimulation (Wang et al., 2020)

It is of relevance that, ketamine's ability to block the NMDAR-dependent bursting activity in the lateral Hb appears to mediate the rapid antidepressant actions of ketamine in mouse models of depression (Yang et al., 2018) wherein ketamine, by blocking NMDAR-dependent lateral Hb bursts, releases the inhibition brake onto the reward system. In congenitally learned helpless rats - a validated animal model for depressive behaviour - systemic administration of ketamine (25mg/kg) significantly reduced immobility time in the forced swim test (FST) (B. Li et al., 2011; Shumake et al., 2003), a behavioural measure of negative mood, hopelessness, or despair (Porsolt et al., 1977). Bilateral infusion of ketamine directly into the lateral Hb similarly rescued depression-like behaviours, as measured by reduced immobility in the FST and increased sucrose preference one hour after administration. Administration of ketamine intraperitoneally (10mg/kg) significantly reduced lateral Hb bursting activity and overall firing rate, both increased in animals with depressive-like behaviours. This sustained inhibition is further supported by evidence that ketamine remains trapped in NMDAR channels after washout, continuing to block receptor activity beyond its plasma half-life (Ma et al., 2023; Mealing et al., 1999).

In mice, lateral Hb neurons exhibit distinct firing patterns - silent, tonic firing and burst firing - with burst firing significantly more prevalent in mouse model of depression (Weiss

& Veh, 2011; Yang et al., 2018). Importantly, ketamine reduced lateral Hb bursting activity for up to 24 h following a single ketamine intraperitoneal administration in the chronic stressed mouse, although this effect is no longer observed three days post-administration. Of note, saline did not induce any change in lateral Hb bursting activity (Ma et al., 2023). Interestingly, ketamine acts preferentially on lateral Hb neurons with high basal bursting spike frequency (>2 Hz group) suggesting that ketamine blocks NMDAR channel in the open state (Ma et al., 2023; MacDonald et al., 1987). This suggests that a single systemic dose of ketamine can also induce a sustained suppression of lateral Hb bursting activity in parallel with its behavioural effects. This is further corroborated by evidence that ketamine remains trapped in the NMDAR channel pore after washout, continuing to block receptor activity beyond its half-plasma life (Ma et al., 2023; Mealing et al., 1999). In summary, ketamine appears to rapidly alleviate depressive-like behaviour by blocking NMDAR-dependent burst firing in the lateral habenula in mice, thereby disinhibiting downstream reward pathways and inducing sustained neurophysiological changes beyond its half-life, ultimately providing a mechanistic model for its rapid antidepressant effect.

However, few studies have investigated the effects of ketamine on the Hb in humans. A study using positron emission tomography (PET) to measure regional cerebral glucose metabolism at baseline and following a single sub-anaesthetic ketamine infusion in twenty medication-free participants with TRD reported that Hb glucose metabolism was decreased following ketamine treatment (Carlson et al., 2014). A previous study (Rivas-Grajales et al., 2021) using 3 Tesla resting-state functional magnetic resonance imaging (fMRI) and conducted in 35 subjects with MDD pre- and post- treatment with IV ketamine 0.5mg/kg explored functional connectivity of the Hb and its association with changes in depressive symptom severity, as measured by the Montgomery Åsberg Depression Rating Scale

(MADRS) and the Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR). The authors reported a positive correlation between improvement in MADRS scores and increased functional connectivity between the right Hb and a cluster in the right frontal pole. Similarly, a reduction in QIDS-SR scores was associated with increased functional connectivity between the right Hb and clusters in the right occipital pole, right temporal pole, right parahippocampal gyrus, and left lateral occipital cortex. However, since the authors did not use a high-resolution scan (e.g., 7 Tesla), it was not possible to precisely identify the Hb boarders and exclude a confounding interference from adjacent thalamic areas or ventricles. Further, the lack of a placebo group and non-MDD control group limit the possibility to draw conclusions about the effect of ketamine on the habenula functional connectivity in depression. The role of the Hb in modulating ketamine rapid and sustained antidepressant effect in humans using high resolution fMRI has not been tested to date.

1.7 A cognitive neuropsychological model to explain ketamine antidepressants effects

Antidepressants exert effects at the molecular and neurochemical levels that translate into mood improvements and, more broadly, into neurocognitive changes. Preliminary evidence suggests that ketamine's effects on emotional memories may differ from conventional antidepressants (Hinchcliffe et al., 2024; Stuart et al., 2015).

A well-established cognitive model for identifying and understanding factors that contribute to the development and relapse of MDD is the one formulated by Beck (Disner et al., 2011). According to this model, depression arises from dysfunctional, negatively-biased attitudes or beliefs about the self, the world, and the future. These attitudes drive systematic cognitive

negative bias (*schemas*) that can be triggered by internal or external events and influence how information is processed and translates a specific experience into a distorted negative interpretation. In MDD these are characterised by a focus on negative aspects of experiences, negative interpretations, and blocking of the effects of positive events. This cognitive framework reiterates itself in a vicious cycle wherein negatively-biased cognitive processes consolidate the negatively-biased schemas providing the cognitive infrastructure for perpetuating the disorder (Beck, 1967, 2008).

Experimental medicine models have shown promise in describing early surrogate markers of patient responsiveness to standard antidepressants (Harmer et al., 2011; Harmer, O'Sullivan, et al., 2009) as cognitive biases appear to shift early in antidepressant treatment (Godlewska et al., 2016) and can predict the therapeutic outcome to antidepressant medications (Godlewska et al., 2016; Shiroma et al., 2014; Tranter et al., 2009). Moreover, studies conducted on healthy volunteers tested the effect of conventional antidepressants on emotional processing and provided insight on how the modulation of serotoninergic and/or noradrenergic transmission affects these cognitive processes independently from an effect of medication(s) on mood. Early work showed that a single intravenous administration of the serotonin reuptake inhibitor citalopram was associated with a greater detection of fearful and happy faces and reduced response times compared to placebo in healthy volunteers (Harmer, Bhagwagar, et al., 2003). A subsequent study testing the effect of a single oral dose of citalopram showed increased recognition of facial expression of fear and an attentional bias towards positive words compared to placebo (Browning et al., 2007). Similarly, a single administration of the noradrenergic agent reboxetine induced a greater recognition of happy facial expressions after a single dose in healthy volunteers compared to placebo (Harmer, Hill, et al., 2003), while a seven-day treatment was associated with a

reduction in the identification of the negative facial expressions of anger and fear, an increase in the recall of positive emotional material, and decrease in subjective ratings of hostility (Harmer et al., 2004). In a related study, depressed patients who received placebo showed a reduction in recognition of positive facial expressions and a decrease in recall for positive self-describing information compared to healthy volunteers receiving placebo. Of note, this was reversed in MDD subjects who received a single dose of reboxetine, prior to any changes in mood or anxiety (Harmer, O'Sullivan, et al., 2009). Of clinical relevance, these early change in facial emotion have been shown to predict therapeutic response after a further four weeks of treatment (Tranter et al., 2009). However, whether this early shift in emotional processing that predicts therapeutic outcome to antidepressants applies also to rapid acting antidepressants has not been as extensively investigated yet.

A study conducted on healthy volunteers randomised to either a low dose of the NMDAR partial agonist D-cycloserine or placebo, showed that D-cycloserine increased the categorisation and recall of positive personality words, similarly to conventional antidepressants citalopram and reboxetine, but without exerting any significant effects on facial emotional recognition (R. Chen et al., 2021). Other researchers have examined the effects of sub-anaesthetic doses of ketamine on emotional evaluation tasks in individuals with MDD (n = 31) and healthy controls (n = 24) using magnetoencephalography (Lundin et al., 2021). During the task, participants were required to identify the emotional valence of facial stimuli (happy-neutral or sad-angry). The study revealed diagnosis-specific effects of ketamine: individuals with MDD demonstrated slower reaction times following ketamine administration compared to placebo, whereas healthy controls showed faster reaction times under the same conditions (Lundin et al., 2021). Work from the same group (Reed et al., 2018) also explored the effect of ketamine in MDD (n = 33) and healthy volunteers (n = 26)

using an attentional bias dot probe task with emotional face stimuli during 3T fMRI session. No main effects were observed for emotion, session, or group, nor were any interaction effects found. A subsequent study (Reed et al., 2019), involving an overlapping sample, investigated the effects of ketamine versus placebo in subjects with MDD (n = 33) and healthy controls (n = 24) during an fMRI emotional processing task. In this task, participants were asked to categorize facial emotions as either negative (sad or angry) or positive (happy or neutral). The results indicated no significant differences between MDD participants and healthy controls following ketamine administration. Overall, ketamine appears to exert distinct effects on emotional processing compared to conventional antidepressants. Well-powered studies are needed to assess whether these effects extend to humans and to clarify their implications for ketamine's antidepressant effects.

1.7.1 Echoes of the Past: How Depression Shapes Autobiographical Memories

The last two decades have seen great advances in our understanding of memory processing. Previously, memories were considered 'stable' following a time-dependent process that is commonly known as consolidation. Once consolidated, memories were considered persistent and resistant to any disruptions (McGaugh, 2000). However, more recent insights suggest that memories can again become fragile when reactivated and, similarly to the consolidation phase, memories can undergo a time-dependent stabilisation process known as reconsolidation (Dudai, 2012; Lee, 2009; Nader et al., 2000; Sara, 2000). While memory consolidation describes the process by which a memory is transformed into a stable, long-lasting form, memory reconsolidation is a memory maintenance process whereby reactivated long-term memories are temporarily destabilized in order to incorporate newly available information, and consequentially update their contents (McGaugh, 2000). The

presentation of a memory cue reactivates previously consolidated memories, that become unstable in order to incorporate newly available information (De Oliveira Alvares et al., 2013; Forcato et al., 2014). Subsequentially, the process of reconsolidation allows to store the memory with its updated content (Dudai, 2012; Lee, 2009; Nader et al., 2000).

Interfering with the reconsolidation process offers an opportunity to disrupt memories that may contribute to the development of psychiatric disorders, such as MDD. There is strong evidence suggesting that MDD is associated with memory biases. Specifically, depression tends to be characterised by the preferential recall of negative over positive material (Burt et al., 1995; Mathews & MacLeod, 2005). Furthermore, depressed subjects tend to "overgeneralise" autobiographical retrieval (Dalgleish et al., 2007), as opposed to recollection of specific individual events, and show impaired recollection (Ramponi et al., 2004). These biases contribute to a ruminative thinking that further perpetuates negative thoughts about the self, the world and the future and support a cognitive loop that initiate and sustain an episode of depression (Beck, 1967, 2008). The elucidation of these patterns of biased and categorical processing of the past events in depression has proved of pivotal importance since they have been implicated in the onset, maintenance, and remission from the disorder (Leppanen, 2006; Williams et al., 2007).

1.7.1.1 NMDAR-mediated pharmacological modulation of memory reconsolidation

Pharmacological and cognitive interventions have been tested in animal models and humans to identify drugs that can be potentially relevant for the treatment of conditions characterised by maladaptive memories such as posttraumatic stress disorder (PTSD) and substance dependence (Debiec & Ledoux, 2004; Diergaarde et al., 2008; James et al., 2015; Kindt et

al., 2009; Taubenfeld et al., 2009). Of note, NMDARs appear to be involved with the process of memory reconsolidation through a process of protein synthesis that leads to a reorganisation of the synaptic architecture that ultimately reconsolidate the memories in a new form (McDonald et al., 2005; Milton et al., 2008). A study conducted on young-to-middle aged adults with high drinking levels showed that the administration of ketamine immediately after memory retrieval effectively reduced the long-term drinking levels, compared to ketamine or retrieval alone (Das et al., 2019). This study investigated whether a sub-anaesthetic dose of ketamine could disrupt the reconsolidation of alcohol-related maladaptive memories. These maladaptive reward memories arise from the impact of addictive substances on the brain's reward systems and are formed through associations between drug-related environmental cues and the drug's rewarding effects (Hyman, 2005). Once established, such memories trigger motivated behaviours, including craving, drug-seeking, and substances overconsumption (Litt et al., 2000). In this work, ketamine was administered following the retrieval of maladaptive alcohol memories or non-drinking memories, and compared with placebo. This study found that the administration of ketamine following the retrieval of maladaptive alcohol memories was associated with a reduction in number of drinking days per week, volume of alcohol consumed, and long-term drinking levels, compared to ketamine or retrieval alone (Das et al., 2019). Although the majority of work in this area focused on fear memories and animal models of PTSD (Philippens et al., 2021; Zhang et al., 2015), modulation of the NMDA receptors appears to also affect the decreased autobiographical memory specificity and increased negative bias in memory recall characteristic of MDD. A recent study by Chen and colleagues showed that the NMDA partial agonist D-cycloserine increases specific autobiographical memory retrieval and positive emotional memory (R. Chen et al., 2021). However, to date, no study has been

conducted exploring the potential of ketamine in memory reconsolidation within depressed subjects.

1.7.2 From bed to benches: effect of ketamine on rodent affective biases

The development of a rodent affective bias model allowed the testing of the effects of ketamine, and other rapid acting antidepressant, on positive or negative affective state-induced biases. This test relies on an associative learning where the animals undergo two independent learning experiences (food pellet in a specific digging substrate) wherein they learn to associate a specific digging substrate with a reward (food). While the reinforcer value is consistent, the two learning experiences are acquired during an affective manipulation or control conditions. Affective bias is then defined by the rat's choices using a preference test in which the reinforced substrates previously reinforced are presented together (Stuart et al., 2013). In this paradigm, ketamine has been shown to attenuate biases away from substrates encoded in a negative context (induced either via psychosocial stressor or pharmacologically), but does not affect choice behaviour/affective bias when administered during the substrate-reinforcer association learning phase (Stuart et al., 2015). Overall, these findings suggest that ketamine decreases previously acquired negative biases, but does not induce a bias when administered prior to learning. In contrast, venlafaxine does not reduce previously learnt negative biases but induces a positive bias when administered before learning.

Of note, these actions appear mediated by different brain areas. In order to investigate the neural mechanisms involved in ketamine effects, the authors used pharmacological interventions (the $GABA_A$ agonist muscimol and the local anaesthetic bupivacaine) to

produce local inactivation at the level of the prefrontal cortex together with local administration of ketamine. Venlafaxine effects were tested in animals with or without bilateral excitotoxic lesions of the central nucleus of the amygdala, a key region in emotional processing, and response to antidepressant treatments in MDD (Leppanen, 2006; Victor et al., 2010). Overall, ketamine effects appeared mediated by the medial PFC (mPFC) while venlafaxine induced a positive bias through effects in the amygdala (Hales et al., 2020; Stuart et al., 2015). Recent evidence confirmed that treatment with ketamine selectively attenuated a negative affective bias in the affective bias test and expanded this work to explore the neural mechanisms associated with ketamine sustained antidepressant effects (Hinchcliffe et al., 2024). The authors describe affective bias modification as a two-phase neuropsychological model. In the first phase, ketamine modulates neural circuits in the mPFC, leading to rapid and sustained reduction of negative biases. Consequentially in the second phase, memories are then retrieved and re-encoded with a positive valence. These re-learning effects appear dependent on protein synthesis localised at the level of the mPFC and appear modulated by reward-associated digging substrate cue reactivated one hour post ketamine treatment, suggesting a pivotal role for experience-dependent neural plasticity for ketamine sustained antidepressant effects (Hinchcliffe et al., 2024). However, the translation of these effects to humans has not yet been tested.

1.8 Ketamine: A New Tool to Enhancing Stress Resilience?

Stress resilience is a multidimensional construct with a variety of definitions. It has been defined as the ability to experience stress without developing psychopathology but has also been associated with the concept of adaptation (or the ability to “bounce back”), the ability

to quickly recover after a stressor, or the capacity to maintain functioning following adversity (American Psychological Association, 2024). In recent years, there has been emerging evidence from preclinical data that the administration of ketamine prior to an acute stressor prevents the development of depressive-like or PTSD-like behaviour in animals. In addition, recent human clinical trials suggest that the administration of ketamine may reduce the incidence of postpartum depression (PPD) after Caesarean section (Li et al., 2024).

1.8.1 Ketamine as a prophylactic agent for depressive-like behaviour

Several models reliably induced depression-like behaviours in rodents, including Chronic Social Defeat (CSD), Learned Helplessness (LH), Inescapable Shock (IS), Chronic Unpredictability Stress (CUS), and chronic corticosterone (CORT). In recent years, several authors have demonstrated a single administration of ketamine exerts a protective effect against the depressive-like behaviour induced by these models.

In 2016, Brachman et al. (Brachman et al., 2016) treated male mice with ketamine (10, 30, or 90 mg/kg) or saline one week prior to a two-week CSD and assessed behaviour using the FST one day post- CSD along with the Dominant Interaction (DI) social interaction test two days post- CSD. Ketamine-treated mice (30 mg/kg, but not 10 or 90 mg/kg) showed significantly reduced immobility time during the FST and significantly increased time exploring a social target mouse compared to saline-treated mice during the DI, consistent with a reduction in the pro-depressive effects of CSD and enhancement of stress resilience (Brachman et al., 2016). In 2018, Mastrodonato et al. replicated these results after treating male mice with ketamine (30 mg/kg) 1 week prior to CSD followed by the FST. Mice treated with ketamine exhibited significantly less immobility time compared with saline-treated

mice on day 2 of the FST (Mastrodonato et al., 2018). Furthermore, Amat and colleagues administered ketamine (10 mg/kg) to male rats at varying times (2 h, 1 week, and 2 weeks) before the inescapable shock (IS) procedure, which is known to induce anxiety in rodents (Amat et al., 2016). Ketamine blocked the behavioural impairment of IS at all time intervals compared to saline-treated rats on the Juvenile Social Interaction (JSI), which has been used to test social interest and motivation in rats (Amat et al., 2016).

In another experiment, Brachman et al. (Brachman et al., 2016) treated male mice with ketamine (30 mg/kg) 1 week prior to a two-week LH protocol, during which mice were delivered repeated, inescapable shocks. Ketamine-treated mice had a decreased latency to escape the shock escape protocol during LH testing and significantly shorter sessions, suggesting a blunting of the depressive effect of LH. Brachman et al. also tested the effect of ketamine (90 mg/kg) on male mice prior to a 21-day CORT treatment. CORT administration has been shown to induce depressive-like behaviour in mice as measured with the Tail Suspension Test (TST), Splash Test (SPT), and FST (Brachman et al. 2016, Camargo et al., 2020). Ketamine (90 mg/kg) prevented the chronic CORT-induced depressive-like phenotype and treated mice showed significantly decreased immobility time on the FST, demonstrating the protective effect of ketamine also at a higher dose (Brachman et al., 2016). Similarly, Camargo et al. treated male mice with ketamine (5 mg/kg or 1 mg/kg) 1 week prior to 21-day CORT (20 mg/kg) treatment. Ketamine-treated (5 mg/kg, but not 1 mg/kg) mice showed significantly less immobility time on the TST, grooming latency, and reduced total time grooming on the SPT (Camargo et al., 2020). Grooming during the Splash Test is considered a self-care behaviour and a marker of anhedonia in mice (Camargo et al., 2020). To investigate whether ketamine exerted a similar effect when administered after stress rather than before, ketamine (30 mg/kg) was administered 1 day after a 28-day CORT

treatment. However, post-stress treatment with ketamine did not affect immobility time in the FST (Brachman et al., 2016).

1.8.2 Translating ketamine pro-resilient effect in humans

1.8.2.1 Effect of ketamine in the prevention of postpartum depression

Recent studies have explored the potential of ketamine for the prevention of PPD in humans. In a proof-of-concept demonstration of the translation of the effects seen in rodent models, these studies investigated the effect of ketamine ahead of the development of psychopathology and specifically tested if ketamine exerts a protective effect against the development of mood and anxiety disorders following childbirth. PPD affects an estimated 10-20% of mothers (Hansotte et al., 2017; Meltzer-Brody et al., 2018) and unique challenges face providers in the treatment of PPD, including the potential exposure of the newborn to medications during pregnancy and/or breastfeeding. Currently, only one medication, brexanolone, is available and received Food and Drug Administration approval for the treatment of PPD (Powell et al., 2020).

Ma et al. (Ma et al., 2019) reported the results of 654 women undergoing Caesarean section (c-section) that were randomized to either 0.5 mg/kg of ketamine or saline via epidural bolus 10 min after c-section. Following initial administration of ketamine or saline, participants were provided with a Patient Controlled Intravenous Analgesia (PCIA) device. Patients undergoing c-section were enrolled because the procedure involves the use of anaesthesia regardless of study participation. Further, ketamine has been commonly used as a general anaesthetic in patients undergoing planned c-section since its safety profile in pregnant

patients is well-established (Lema et al., 2017). Participants that were randomized to ketamine received a PCIA device of sufentanil (100 μg), palonosetron hydrochloride (0.25 mg), and ketamine (160 mg). Control subjects were provided the same PCIA device without the addition of ketamine. Participants were assessed with the Edinburgh Postnatal Depression Scale [EPDS; (Cox et al., 1987)] at various time points postpartum. The primary outcome at 6-8 weeks postpartum showed a significantly lower prevalence of PPD in the ketamine group (12.8%) than in the control group (19.6%). The secondary outcome on days 4-6 postpartum showed a significantly lower mean EPDS score and lower prevalence of postpartum blues in the ketamine group (11.9%) than in the control group (18.3%). Notably, a reduction in suicidal ideation 4-6 days postpartum was also significantly greater in the ketamine group compared to the control group. Finally, the effect of ketamine appeared more pronounced in women with a history of moderate stress during pregnancy, antenatal depressive symptoms, and suicidal ideation.

A similar study by Alipoor et al. (Alipoor et al., 2021) was conducted on 134 women undergoing c-section randomized to either ketamine (0.5 mg/kg) plus nesdonal (1-2 mg/kg) or nesdonal (3-5 mg/kg) alone administered intravenously during the induction phase of anesthesia. Participants were assessed with the EPDS at 2 and 4 weeks postpartum. The primary outcome at 4 weeks postpartum showed that PPD prevalence (EPDS score > 9) in the ketamine group was significantly lower compared to the control group and that the mean EPDS score in the ketamine group (10.84) was significantly lower than the control group (12.09). The secondary outcome at 2 weeks postpartum showed a significantly lower prevalence of PPD in the ketamine group and a mean EPDS in the ketamine group (11.82) significantly lower than the control group (14.34). A third randomized trial from Han et al. (Han et al., 2022) explored the effect of a PCIA device with S-ketamine (0.5 mg/kg) given

to patients immediately following c-section delivery. Patients randomized to receive the PCIA device with S-ketamine had significantly less prevalence of PPD at 3 and 14 days postpartum. However, these findings must be interpreted with caution, as the results reported reflect the mean difference and prevalence difference between groups drawn from uncorrected t-tests at the pre-determined time points.

1.8.2.2 A randomized pilot study of the prophylactic effect of ketamine in healthy adults

A small randomised, placebo-controlled, proof-of-concept study testing if the administration of ketamine, compared to the midazolam control condition, can attenuate the behavioural and physiological effects of a laboratory-induced acute stress on healthy volunteers when administered one week prior has been conducted to date (Costi et al., 2023). Twenty-four subjects received a single received IV infusion of either ketamine or midazolam one week prior to stress induction. Midazolam is a benzodiazepine anaesthetic which mimics some of the acute subjective effects of ketamine (hence aiming at preserving the study blind for both participants and researchers) but is expected to have no pro-resilience effect. The Trier Social Stress Test (TSST), a well-validated laboratory stressor, was implemented to induce an acute stress response (Kirschbaum et al., 1993). The TSST is known to provoke moderate stress in most participants and a reliable response in the hypothalamic pituitary adrenal (HPA) axis (Allen et al., 2014; Kirschbaum et al., 1993). Acute elevations of sympatho-adreno-medullary axis activity and self-reports of negative affect using the Profile of Mood States (POMS) and the Positive and Negative Affect Scale (PANAS) have also been reported (Allen et al., 2014). Responses to the TSST appear to be altered in cases of stress-related disorders like MDD or anxiety disorders, and both pharmacological and psychotherapeutic interventions can moderate the TSST response (Allen et al., 2014). A

change on the composed-anxious subscale score of the Profile of Mood States – Bipolar [POMS-Bi; (Lorr et al., 1982)] from pre-TSST to post-TSST in the ketamine group compared to the midazolam group one week after the administration of the study drug represented the primary behavioural outcome. Compared to the midazolam group, the ketamine group did not show a reduction in the POMS-Bi composed-anxious subscale immediately following stress, nor a change in salivary cortisol or salivary alpha amylase (sAA) following stress. However, the change on levels of anxiety was equivalent to a moderate to large effect size (Cohen's d = 0.7) and exploratory analysis conducted on a subgroup showing an expected correlation between plasma and salivary cortisol (n = 23, ketamine n = 11) showed a significant reduction in the level of sAA in the ketamine group compared to midazolam (Cohen's d = 0.7, p = 0.03), and a moderate to large reduction in levels of anxiety immediately following stress, although this was not significant (p = 0.06). However, the small sample size may have may have led to Type II errors that could explain the lack of statistically significant effect of ketamine on psychological and biological readouts following an acute stress. In addition, plasma cortisol data were missing for three subjects, 1 subject in the ketamine group and 2 subjects in the midazolam group. Further, it is unknown if the benzodiazepine anaesthetic midazolam can exert a sustained pro-resilience effect.

1.9 Aims & Hypothesis

Significant progress has been made since the first clinical trial of ketamine's antidepressant effects (Berman et al., 2000). However, many questions remain - particularly regarding its neurocognitive impact. This thesis reports data from two experimental medicine studies

designed to investigate ketamine's effects on key neurocognitive domains, including emotional processing, affective memory, sensitivity to reward and punishment, and stress resilience.

Chapters 2, 3, and 4 present findings from a placebo-controlled experimental medicine trial in which healthy volunteers were randomised to receive either IV ketamine (0.5 mg/kg) or placebo. Chapter 5 reports an interim analysis of data from a separate experimental medicine trial recruiting subjects with TRD, also randomised to receive ketamine (0.5 mg/kg) or placebo. In both studies, assessments were conducted at baseline (pre-infusion), immediately after the infusion, 24 hours post-administration, and one-week post-infusion. Particular focus was given to the 24-hour timepoint, a well-established window during which ketamine's antidepressant effects emerge while its acute (dissociative) effects have typically resolved.

Chapter 2 focuses on clinical questionnaires and emotional processing, assessed using the Emotional Test Battery (ETB), which includes tasks measuring recognition, categorisation, and recall of emotional stimuli, as well as a facial emotion expression recognition task. To parallel findings from animal studies, emotional words were presented prior to infusion and tested for recall 24 hours later. The facial emotion recognition task was administered pre-infusion, immediately post-infusion, and again 24 hours later. These tasks were used to probe the effect of ketamine on affective memory biases for information acquired prior to infusion. We hypothesised that ketamine would reduce negative recall biases for pre-infusion material.

Chapter 3 examines the effects of ketamine on lateral Hb activity in the context of reward and punishment processing. Using ultra-high-field 7-Tesla fMRI during a aversive Pavlovian conditioning paradigm, we investigated ketamine's effects on Hb responses to negative stimuli (peripheral electric shocks), as well as its modulation of reward-related neural responses during win and loss trials 24 hours post-infusion. We hypothesised that ketamine would attenuate Hb activation in response to aversive stimuli compared to placebo.

Chapter 4 explores the effect of ketamine on subjective and physiological responses to stress when administered one week prior to stress exposure. Building on animal research suggesting glutamatergic modulation enhances stress resilience, we employed a computer-based stress-induction paradigm designed to elicit mild-to-moderate stress, while measuring both subjective and physiological outcomes. Subjective outcomes included self-reported mood, anxiety, and stress. Biological endpoints included: (1) salivary cortisol (as a marker of HPA axis activity); (2) sAA; and (3) heart rate variability (HRV), both reflective of Autonomic Nervous System (ANS) activity. These measures were compared between participants who received ketamine or placebo one week before the stress challenge. We hypothesised that ketamine would reduce negative affect and physiological stress markers, supporting its potential pro-resilience effects.

Chapter 5 presents data from an interim analysis of a study investigating the neurocognitive effects of ketamine in patients with TRD. Participants completed clinical assessments, an autobiographical memory task, and emotional processing tasks to assess ketamine's influence on negative emotional memory biases. We hypothesised that ketamine would reduce these biases, thereby contributing to its rapid antidepressant effects. The autobiographical memory task was completed at baseline, 24 hours post-infusion, and one

week later. Emotional processing tasks targeting affective memory were administered immediately post-infusion to explore ketamine's effects on affective memories independently of its antidepressant action.

Overall, this thesis investigates the neuropsychological mechanisms underlying ketamine's rapid antidepressant effects, offering a framework for understanding the neurocognitive basis of fast-acting antidepressants. These findings may ultimately inform the development of novel, rapid-acting treatments for depression.

Chapter 2. The Effect of the NMDA Antagonist Ketamine on Subjective Symptoms and Emotional Processing in Healthy Volunteers: An Experimental Medicine Study

2.1 Introduction

As presented in Chapter 1, recent advances have deepened our understanding of how antidepressants influence neurocognitive pathways and psychological functioning. While the pharmacological properties of conventional antidepressants are well-characterized, neurobiological models alone fail to explain the delayed mood improvements typically observed with treatment (Miller & Hen, 2015). Emerging cognitive neuropsychological models offer a more comprehensive framework, bridging the gap between the pathophysiology of Major Depressive Disorder (MDD) and antidepressant action, thereby addressing critical clinical challenges (Godlewska, 2019).

MDD is characterised by negatively biased attitudes about the self, world, and future, creating a perpetuating a cycle of negative cognitive schemas and biased emotional processing over time (Beck, 1967, 2008; Disner et al., 2011). Emotional processing measures may serve as valuable biomarkers to elucidate therapeutic mechanisms during early drug development (Harmer, Goodwin, et al., 2009). Experimental studies have shown that conventional antidepressants can modulate emotional processing early in treatment, improving the recognition of positive emotional stimuli and reducing negative biases, with these early changes predicting therapeutic outcomes (Godlewska et al., 2016; Harmer, O'Sullivan, et al., 2009; Tranter et al., 2009). However, whether this mechanism is also

relevant to the rapid antidepressant effects of ketamine remains to be tested. Data from animal studies suggest that ketamine's rapid-acting profile may involve distinct neurocognitive mechanisms, some of which may also apply to humans. Understanding these mechanisms is crucial, given ketamine's clinical limitations, including the potential for abuse and concerns regarding long-term safety. Insights into ketamine's neuropsychological effects could guide the development of safer, rapid-acting antidepressants. Specifically, animal studies suggest that ketamine influences emotional memories differently from conventional antidepressants (Hinchcliffe et al., 2024; Stuart et al., 2015). Using a rodent affective bias model, researchers examined the effects of ketamine on biases induced by positive or negative affective states. In this model, animals learn to associate specific cues with a reward (a food pellet) under either an affective manipulation (e.g., restraint stress) or neutral conditions. Affective bias is then assessed by presenting both reinforced cues simultaneously and measuring the animal's preference (Stuart et al., 2013). Despite identical learned reward values, animals typically avoid the stimulus associated with a negative experience, indicating a negative recall bias. Of note, when ketamine is administered before the preference test (after the learning phase), the negative bias is reduced. However, ketamine has no effect when given during the learning phase, suggesting it influences memory recall rather than encoding (Stuart et al., 2015). In contrast, conventional antidepressants like venlafaxine (a serotonin-noradrenaline reuptake inhibitor) have the opposite effect, acting on new information processing but failing to alter previously encoded biases (Stuart et al., 2015). These findings highlight distinct mechanisms underlying antidepressant effects: ketamine appears to modulate negative affective bias in recall, whereas venlafaxine facilitates new learning without modifying existing emotional memories. This difference may help explain the rapid onset of ketamine's clinical effects,

as it reduces negative memory recall without requiring new information processing, unlike conventional antidepressants.

This chapter presents findings from an experimental medicine study in which healthy volunteers were randomised to receive either intravenous ketamine (0.5 mg/kg) or placebo. Participants completed clinical questionnaires and emotional processing tasks to characterise ketamine's effects on emotional processing. The findings offer preliminary insights into the neuropsychological effects of ketamine that could inform translational research targeting clinical populations with TRD. Additionally, these results shaped the study protocol for a related study on affective memory processing in subjects with Treatment Resistant Depression (TRD), which is presented in Chapter 5.

The study examined the recognition, categorisation, and recall of emotional stimuli following ketamine infusion using the Emotional Test Battery (ETB). Key measures included recognition accuracy and reaction times for positive and negative facial expressions (assessed pre-infusion, immediately post-infusion, and 24 hours post-infusion) and recall performance for emotional words, including both correct and incorrect responses (assessed 24 hours post-infusion). To translate findings from animal studies, emotional words were presented pre-infusion and later tested in recall tasks conducted 24 hours post-infusion. Overall, these tasks aimed to evaluate the effects of ketamine on emotional processing and emotional memory. Specifically, the aim of the study was to test whether ketamine attenuates negative biases in the recall of information acquired prior to infusion, as suggested by animal models.

2.2 Methods

2.2.1 Study Participant and design

The study was conducted at the Department of Psychiatry, University of Oxford (UK), where participants were recruited between July 2021 and June 2023. The study was approved by the University of Oxford Central University Research Ethics Committee (Ethics Approval Reference: R73654/RE001), and written informed consent was obtained from all participants prior to any study procedure. The study used a randomised, double-blind, placebo-controlled, experimental medicine study design and was registered on ClinicalTrials.gov (Identifier: NCT04850911). Participants were between the ages of 18 and 45 and did not meet criteria for any lifetime psychiatric diagnosis according to the DSM-5, as assessed by the Structured Clinical Interview for DSM-5 Axis I Disorders (SCID) (First MB, 2015).

The inclusion criteria were as follows: aged 18-45 years; BMI between 18 and 30; willing and able to give informed consent for participation in the study; sufficient knowledge of English language to understand and complete study tasks; willingness to refrain from driving, cycling, or operating heavy machinery, until the following morning or a restful sleep has occurred, whichever is later; willingness to refrain from signing legal documents the day of the infusion visit; willingness to refrain from drinking alcohol for 3 days before the infusion visit and one day before any of the other visits throughout the study. Exclusion criteria included: any current or past significant psychiatric disorder based on the DSM-5; clinically significant abnormalities in blood tests; any unstable medical condition; pregnancy or breastfeeding; recent recreational drug use (within three months), or lifetime

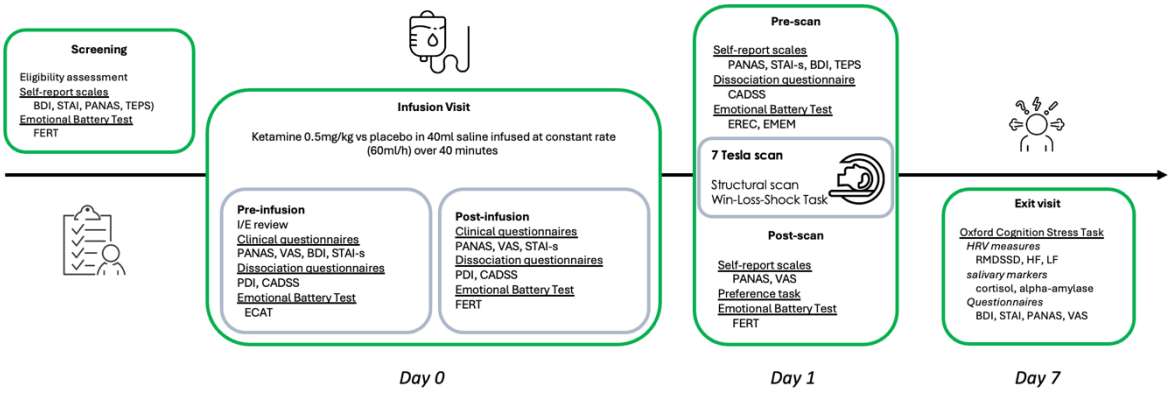
use of ketamine or phencyclidine. A complete list of the inclusion and exclusion criteria is available in the Appendix.

During the screening visit, participants' medical and psychiatric histories were obtained, and comprehensive clinical laboratory tests were performed, including full blood count, liver, thyroid, and kidney function tests, and urine analysis. Toxicology screening and pregnancy testing (for premenopausal women) were also conducted. Eligible participants were randomised to receive either ketamine (0.5 mg/kg) or placebo (0.9% saline) within four weeks of the screening visit.

The randomisation codes were generated by unblinded study team members using the Sealed Envelope randomisation tool (seed: 131846910112388). Participants were randomised in blocks of four and stratified by gender and Facial Emotion Recognition Task (FERT) version to ensure balanced allocation across treatment groups. The randomisation codes were securely stored in the pharmacy of the Clinical Research Facility (CRF) at Warneford Hospital and were accessible only to the unblinded medical team. Blinding was maintained by ensuring that only the medical and nursing staff administering the infusions were unblinded; they had no involvement in participant assessments beyond the infusion visit. To minimise the risk of bias or inadvertent unblinding, team members conducting post-infusion assessments did not participate in any subsequent study visits. The rest of the study team remained fully blinded to treatment allocation and procedures and did not perform any post-infusion tasks on the day of ketamine or placebo administration.

A schematic representation of the study flow is reported in **Figure 2.1**.

Figure 2.1 Study Flow



Abbreviations: BDI, Beck Depression Inventory; CADSS, Clinician-Administered Dissociative States Scale; PANAS, Positive and Negative Affect Schedule; STAI-T, State-Trait Anxiety Inventory - Trait; STAI-S, State-Trait Anxiety Inventory - State; TEPS, Temporal Experience of Pleasure Scale; VAS, Visual Analog Scale. A description of the scan procedures (Day 1) and the exit visit procedures (Day 7) is provided in Chapters 3 and 4, respectively.

2.2.2 Infusion procedures

Before the infusion, participants completed a battery of questionnaires and computerised tasks (see **Figure 2.1**). The infusion of ketamine or placebo took place at one of three locations in the Warneford Hospital: the Clinical Research Facility, the Interventional Psychiatry Service, or the Neurosciences Building. Prior to randomisation, inclusion/exclusion criteria, as well as concomitant medications, were reviewed to ensure the participants continued to meet eligibility requirements. A urine toxicology screen and, when applicable, a pregnancy test was also conducted. During the infusion, blood pressure was monitored at 10-minute intervals, including at the end of the infusion, and every 15 minutes for an hour following its completion. The study participants lay on a bed in a quiet environment and were instructed to avoid listening to music, watching TV, or reading. After the infusion, participants were kept under close observation for approximately 2 hours.

Monitoring included oxygen saturation, vital signs (blood pressure and heart rate), and general observation.

At the end of the visit, a medical doctor discharged the participants, and they were sent home by taxi. Participants were advised not to drive, cycle or operate machinery until they had had a restful sleep or the following morning. Additional precautions included avoiding alcohol, medications, or signing legal documents for up to seven days post-infusion.

2.2.3 Questionnaire measures

Participants completed self-report measures assessing positive and negative affect, anxiety, hedonic capacity, and dissociation. These questionnaires were administered digitally using Qualtrics software. **Table 2.1** provides a summary of the scales used and the specific time points during the trial when they were administered.

Positive and Negative Affect: The Positive and Negative Affect Scale (Watson, 1988) was used to assess affective state throughout the study. It was administered at four time points: baseline, prior to infusion, immediately post-infusion, and 24 hours post-infusion. The PANAS comprises 20 items, with 10 items measuring positive affect and 10 items measuring negative affect. Participants rated each item on a 5-point Likert scale, ranging from 1 (Very Slightly or Not at All) to 5 (Extremely), to indicate the intensity of affect experienced.

Depression: The Beck Depression Inventory (Beck, 1996), a 21-item questionnaire designed to assess cognitive symptoms of depression (e.g., pessimistic thinking, guilt, and difficulties concentrating), was administered at baseline, pre-infusion, and 24 hours post-infusion.

Scores range from 0 to 63, with higher scores indicating greater severity of depressive symptoms.

Anxiety: The State-Trait Anxiety Inventory (Spielberger, 1983) was used to evaluate both trait and state anxiety. Participants rated items on a 4-point Likert scale (1 = Almost Never, 4 = Almost Always), with higher scores reflecting greater anxiety levels. The STAI-Trait (STAI-T) was collected at baseline, and 24-hours post-infusion, while the STAI-State (STAI-S) was administered at baseline, pre-infusion, immediately post-infusion, and 24 hours post-infusion.

Anhedonia: The Temporal Experience of Pleasure Scale (Gard, 2006) was used to assess hedonic capacity by measuring anticipatory and consummatory pleasure. The TEPS provides specific sub-scores for these two dimensions, with higher scores indicating greater pleasure and lower levels of anhedonia severity. The TEPS was administered at baseline and 24 hours post-infusion.

Visual Analogue Scales: The Visual Analogue Scale (VAS) is an instrument used to measure emotions that exist on a continuum and cannot be easily quantified directly. It consists of a 100-mm horizontal line anchored by descriptive labels at each end, such as "Not at all" (0) to "Very much" (10). Participants are asked to indicate their current state by marking a point on the line, and the VAS score is calculated by measuring the distance in millimetres from the left end to the marked point. VAS have been widely used for evaluating the effects of pharmacotherapies on symptom management (Aitken, 1969; Huskisson, 1974). In this study, the words tested were: happy, sad, hostile, alert, stressed, anxious, and calm. The VAS was administered at multiple time points: pre-infusion, post-infusion, 24 hours post-

infusion (both before and after the fMRI scan, as described in Chapter 3), and 7 days post-infusion (as described in Chapter 4).

Dissociation: Dissociative symptoms were assessed using the Clinician Administered Dissociative Symptom Scale (CADSS). This study employed a 6-item simplified version (CADSS-6), which has been validated for its efficacy in measuring ketamine-induced dissociation (Rodrigues et al., 2021). The CADSS-6 evaluates dissociation across three domains: derealisation (items 1 and 2), depersonalisation (items 6 and 7), and amnesia (items 15 and 22) from the full CADSS. Trained raters administered the CADSS-6 at three time points: pre-infusion, immediately post-infusion, and 24 hours post-infusion. At the post-infusion time point, participants were asked to retrospectively evaluate their subjective experience during the infusion, whereas at the 24-hour post-infusion assessment, they were instructed to report on their experiences since leaving the hospital. Additionally, the Peters et al. Delusions Inventory (PDI) was administered as a self-report measure of dissociation (Peters et al., 2004). This 21-item questionnaire evaluates delusional experiences and provides sub-scores for Distress, Preoccupation, and Conviction. Participants completed the PDI at two time points: pre-infusion and post-infusion.

Table 2.1. Schedule of Scale Administration Across Study Visits

Study visit	Scales
Baseline	BDI, PANAS, STAI-T, STAI-S, TEPS, VAS
Infusion visit	
Pre-infusion	BDI, CADSS, PANAS, PDI, STAI-S, VAS
Post-infusion	CADSS, PDI, PANAS, STAI-S, VAS
Post-24 hours	BDI, CADSS, PANAS, STAI-T, STAI-S, TEPS, VAS

Abbreviations: BDI, Beck Depression Inventory; CADSS, Clinician-Administered Dissociative States Scale; PANAS, Positive and Negative Affect Schedule; STAI-T, State-Trait Anxiety Inventory - Trait; STAI-S, State-Trait Anxiety Inventory - State; TEPS, Temporal Experience of Pleasure Scale; VAS, Visual Analog Scale

2.2.4 Affective Bias Tasks

Participants also completed tasks from the Emotional Test Battery (ETB), a set of validated measures designed to assess key aspects of emotional processing (Harmer, O'Sullivan, et al., 2009; Harmer et al., 2004).

2.2.4.1 Emotional Categorisation

The Emotional Categorisation Task (ECAT) required participants to evaluate 40 personality-related adjectives, imagining overhearing someone describing them using these words. Participants indicated whether they would "like" or "dislike" being described by each word by pressing a corresponding button as quickly and accurately as possible. Each word was displayed on the screen for 500 milliseconds in a randomised order unique to each participant. The words were balanced for length, frequency, and meaningfulness. The ECAT was administered at a single time point before the ketamine/placebo infusion. Accuracy of

response and reaction times were analysed separately for positive and negative words. To translate findings from animal studies, emotional words were presented pre-infusion using the ECAT and later tested in recall tasks conducted 24 hours post-infusion using the Emotional Recall Task (EREC) and the Emotional Recognition Memory Task (EMEM), as described below.

2.2.4.2 Emotional memory

Emotional memory was evaluated using two tasks: the EREC and the EMEM. Both tasks were completed 24 hours post-infusion.

The EREC task involved participants freely recalling and writing down as many words as they could remember from the ECAT administered 24-hours prior. Participants were given four minutes to complete the EREC. Words were rated as correct or incorrect, and as positive, negative, or neutral/ambiguous. Each recalled word was categorised as correct or incorrect and further classified as positive, negative, or neutral/ambiguous. Ratings were conducted independently by two researchers, with a third resolving any discrepancies, all blinded to treatment allocation. Recall accuracy was calculated separately for positive and negative words, and the number of positive and negative hits and intrusions was also recorded.

The EMEM task presented participants with a set of personality-related words (40 positive and 40 negative) and asked them to identify whether each word was included in the ECAT (20 positive and 20 negative words were repeated from the ECAT and the others were novel). Words were displayed for 500 milliseconds on a computer screen in a randomized

order, and participants were asked to respond as quickly and accurately as possible. Accuracy and reaction times for correctly identified positive and negative words, which were analysed separately.

2.2.4.3 Facial Recognition

The Facial Expression Recognition Task (FERT), assessed participants' ability to identify facial expressions. Participants were presented with images of faces displaying one of six emotions (fear, anger, sadness, disgust, happiness, surprise) at varying intensity levels (10% - 100% in 10% increments). The facial stimuli were sourced from the Ekman and Friesen Pictures of Affect series (Ekman, 1971).

Participants were shown a total of 250 stimuli, including 40 face stimuli of varying intensity for each of the six emotions, plus 10 neutral face stimuli. Faces were displayed briefly (500 milliseconds) in a pseudorandomised order across three blocks, with emotions and actors balanced. Participants were instructed to identify the emotion displayed on each face as quickly and accurately as possible. The number of correct responses, reaction times for correct identifications, and misclassifications (e.g., incorrectly identifying one emotion as another) were analysed separately for each emotion. The FERT was administered at baseline, immediately following the infusion, and again 24 hours post-infusion.

2.2.5 Statistical analysis

Data from clinical questionnaires were analysed using IBM SPSS V29 and ETB processing and analysis were conducted in R Studio (version 2023.12.1). Continuous baseline clinical

and demographic characteristics were summarized using means or medians and compared by group using t-tests; discrete baseline characteristics were summarised by count and percentage and compared using a Chi-squared. Correlations between measures were computed using Pearson's *r* for parametric distributions and Spearman's rank correlation for non-parametric distributions. When Mauchly's test indicated a violation of the sphericity assumption, the Greenhouse-Geisser correction was applied to adjust the degrees of freedom. A two-sided significance level of $\alpha=0.05$ was set for all statistical comparisons.

The ETB tasks outcomes were analysed by mixed analysis of covariance (ANCOVA), assessing the main effect of treatment group (between-subject factor), emotion (within-subject factor), and the interaction between treatment group and emotion on task performance.

For the ECAT, the primary outcome was percentage of correctly categorised words. Reaction time (calculated in milliseconds) for trials with correct categorisation was also assessed. Trials where participants responded in under 200 milliseconds or over 3000 milliseconds were excluded, and sensitivity analysis excluding participants with low accuracy were completed.

For EREC, the primary outcome was the number of correct positive and words recalled. The number of positive hits (calculated as the proportion of positive words correctly recalled relative to the total number of positive and negative words correctly recalled) and negative hits (calculated as the proportion of negative words correctly recalled relative to the total number of positive and negative words correctly recalled) were analysed. Similarly, positive intrusions (calculated as the proportion of positive words falsely recalled relative to the total

number of positive and negative words falsely recalled) and negative intrusions (calculated as the proportion of negative words falsely recalled relative to the total number of positive and negative words falsely recalled) were also examined. Sensitivity analysis excluding participants with low accuracy in the ECAT were completed.

For the EMEM the primary outcome was the accuracy in identifying positive and negative words. Reaction times for correctly identified words were also analysed. Trials where participants responded in under 200ms or over 4000ms were excluded, and sensitivity analysis excluding participants with a total percentage accuracy below 50% were completed.

For the FERT, the primary outcome was the total percent accuracy. Significant interactions were further analysed using one-way ANCOVAs focusing on contrasts between specific emotions and treatment group. Reaction time (in milliseconds) for trials with correct responses was also assessed. Data were analysed independently for each study visit. All trials were participants responded below 200 milliseconds or over 10 seconds were excluded. Sensitivity analysis excluding participants with a total percentage accuracy below 50% were completed.

2.3. Results

2.3.1 Sample Characteristics

A total of 118 participants were assessed for eligibility, of whom 72 were eligible for randomisation and enrolled in the study. Of these, 70 participants completed the infusion

procedures. **Table 2.2** provides a summary of the clinical and demographic characteristics of the sample.

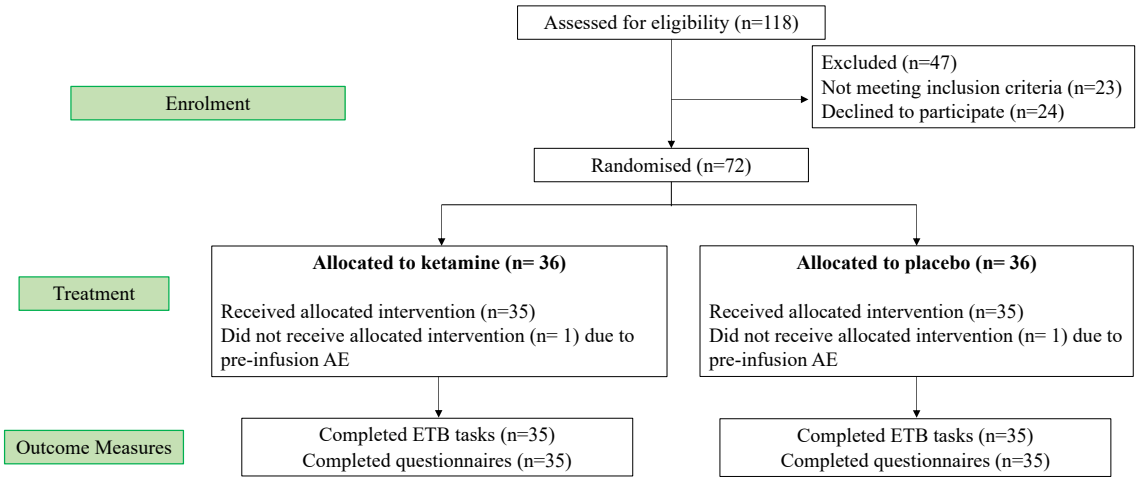
At baseline, no significant differences were found between the ketamine and placebo groups regarding age, gender, educational level, or race. Similarly, self-reported ratings showed no differences between groups at baseline or pre-infusion. All participants completed the ETB tasks, and there were no dropouts during the trial. **Figure 2.2** illustrates the CONSORT diagram, depicting participant flow by study arm (ketamine versus placebo).

Table 2.2. Sociodemographic Characteristic of the Sample

	Ketamine (n=35)	Placebo (n=35)
Gender, M (n)	19	19
Age, years M (SD)	23.5 (5.3)	23.5 (4.5)
Years of education, M (SD)	16.9 (2.6)	17.2 (2.7)
Race		
White/Caucasian (n)	29	26
Asian/Asian British (n)	4	3
STAI-T, baseline M (SD)	29.8 (4.9)	29.3 (8.7)
BDI, baseline M (SD)	1.7 (2)	1.9 (4.4)
TEPS-ant, baseline M (SD)	44.7 (5.2)	45.8 (5.5)
TEPS-con, baseline M (SD)	40.7 (3.8)	41.4 (4.8)

Abbreviations: BDI, Beck Depression Inventory; M, mean; n, number; SD, standard deviation; STAI-T, State-Trait Anxiety Inventory - Trait; STAI-S, TEPS-ant, Temporal Experience of Pleasure Scale - anticipatory score; TEPS-con, Temporal Experience of Pleasure Scale - consummatory score.

Figure 2.2 CONSORT diagram



2.3.2 Subjective Symptom Change

No significant group differences were observed immediately post-infusion, except for dissociative symptoms, or at 24 hours post-infusion. A non-significant numerical difference was noted in the 'Happy' subscale of the VAS immediately post-infusion [$t(67) = -1.954$, $p = 0.055$]. A significant main effect of time [$F(1.27, 85.24) = 74.01$, $p < 0.001$] and a significant interaction between time and treatment group [$F(1.27,85.24) = 74.01$, $p < 0.001$] were observed for CADSS scores, with the ketamine group reporting higher dissociative symptom ratings immediately after the infusion (mean = 6.20, SD = 3.72) compared to the placebo group (mean = 0.56, SD = 1.28). These ratings returned to baseline levels by 24 hours post-infusion (ketamine: mean = 0.63, SD = 1.72; placebo: mean = 0.20, SD = 0.53). No significant main effects of time, treatment, or time-by-treatment interaction were found for any subscale of the PDI.

2.3.3 Emotional Test Battery

2.3.3.1 Emotional Categorisation Task (ECAT)

The ECAT was completed before the infusion and prior to randomisation. Analysis of accuracy revealed no significant differences between treatment groups [$F(1,132) = 0.085$, $p = 0.8$] or valence [$F(1,132) = 0.9$, $p = 0.3$]. Similarly, the interaction between treatment and valence was not significant [$F(1,132) = 2.386$, $p = 0.12$], suggesting comparable performance between the two groups prior to randomisation. A sensitivity analysis excluding the subject with the lowest accuracy (62.5%) confirmed these findings, with no significant main effect of treatment [$F(1,130) = 0.004$, $p = 0.9$], valence [$F(1,130) = 1.543$ $p = 0.2$], nor a significant interaction between treatment x valence interaction [$F(1,130) = 1.43$, $p = 0.2$].

For reaction time, a repeated-measures ANOVA showed no significant main effect of treatment [$F(1,132) = 0.009$, $p = 0.9$] or valence [$F(1,132) = 2.5$, $p = 0.12$], and the treatment x valence interaction was also not significant [$F(1,132) = 0.303$, $p = 0.6$]. These results indicate that the two groups exhibited similar accuracy and reaction times in the ECAT prior to randomisation.

2.3.3.2 Emotional recall task (EREC)

Participants recalled an average of 6.78 ± 3.64 positive words and 4.06 ± 2.88 negative words (correct and incorrect), regardless of treatment allocation. There was a significant effect of word valence on the number of words correctly recalled [$F(1, 134) = 10.348$, $p =$

0.002], but there was neither a significant main effect of treatment [$F(1, 134) = 2.607$, $p = 0.11$] nor a significant treatment × word valence interaction [$F(1, 134) = 0.037$, $p = 0.8$]. Similarly, for incorrectly recalled words, there was no significant main effect of treatment [$F(1,134) = 1.016$, $p = 0.3$], but a significant main effect of valence was identified [$F(1,134) = 13.868$, $p < 0.001$], indicating differences in incorrect recall based on valence. The interaction between treatment and valence was not significant [$F(1,134) = 0.076$, $p = 0.8$]. Subsequent one-way ANOVAs examining the impact of treatment on correctly and incorrectly recalled positive and negative words showed no significant main effect of treatment. A sensitivity analysis excluding the participant with the poorest ECAT performance confirmed no significant deviations from the results of the initial analyses.

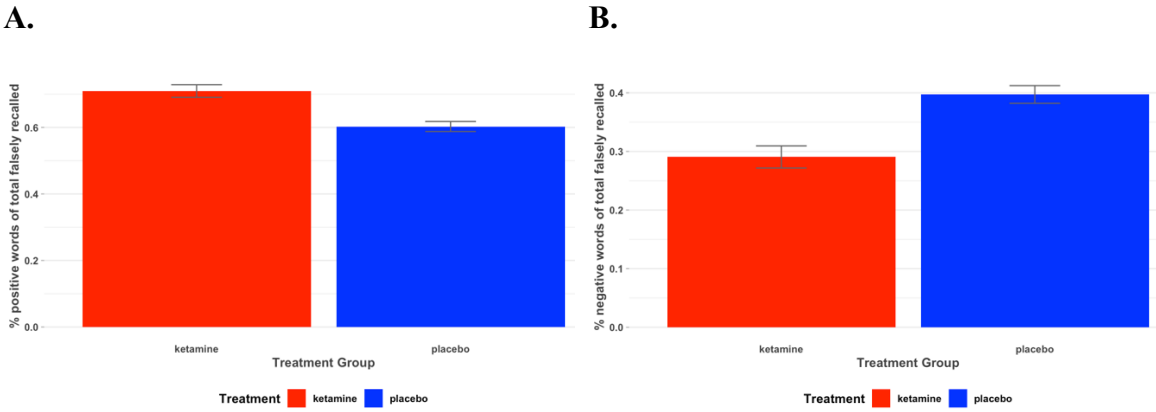
Analysis of hits (percentage of correctly recalled positive or negative words out of total correct recalls) revealed no significant differences between treatment groups. For positive hits, a two-sample t-test indicated no significant effect of treatment, with the ketamine group (mean = 56.94%) and the placebo group (mean = 58.95%) showing comparable rates [$t(67) = -0.236$, $p = 0.8$]. Similarly, for negative hits, no significant difference was found between the ketamine group (mean = 40.12%) and the placebo group (mean = 32.48%), $t(67) = 0.951$, $p = 0.3$.

In contrast, the analysis of intrusions, defined as the percentage of incorrect positive or negative words recalled out of the total incorrect recalls, revealed significant differences between treatment groups (**Figure 2.3**). For positive intrusions, a two-sample t-test showed that the ketamine group exhibited significantly higher intrusion rates (mean = 70.94%) compared to the placebo group (mean = 60.27%), $t(67) = 2.195$, $p = 0.032$. For negative intrusions, the analysis demonstrated significantly lower intrusion rates in the ketamine

group (mean = 29.06%) compared to the placebo group (mean = 39.73%), t(67) = -2.195, p = 0.032.

These findings suggest that ketamine did not significantly affect the recall of correctly remembered positive or negative words compared to placebo in healthy volunteers but significantly increased positive intrusions and decreased negative intrusions, indicating its potential role in modulating emotional memory processes.

Figure 2.3 Effect of Treatment on Positive (Panel A) and Negative (Panel B) Intrusions



Values represent the mean percentage of incorrect positive or negative words recalled out of the total incorrect recalls. Error bars indicate the standard error of the mean (SEM).

2.3.3.3 Emotional recognition memory task (EMEM)

The analysis evaluating the effects of treatment and valence on total correct recall percentages revealed no significant main effect of treatment [$F(1, 136) = 1.37$, $p = 0.2$]. Although there was a significant main effect [$F(1, 134) = 45.30$, $p < 0.001$] for word valence, the interaction between treatment and valence was not significant [$F(1, 134) = 1.07$, $p = 0.3$].

A sensitivity analysis excluding a subject with who showed accuracy of 50%, revealed no significant deviations from the results of the initial analysis performed on the entire sample.

Further analyses were conducted on hits (familiar words considered familiar), misses (familiar words considered novel), correct rejections (novel words considered novel), and false alarms (novel words considered familiar) revealed a consistent effect of word valence across all measures. Specifically, positive valence was associated with higher hits (mean = 14.94, SD = 2.59) and more false alarms (mean = 8.83, SD = 3.40) compared to negative valence (mean = 12.80, SD = 3.07 for hits; mean = 5.61, SD = 2.62 for false alarms). In contrast, negative valence was associated with higher rates of correct rejections (mean = 13.96, SD = 2.65) and more misses (mean = 6.90, SD = 3.01) than positive valence (mean = 10.94, SD = 3.31 for correct rejections; mean = 4.67, SD = 2.41 for misses). These findings suggest that participants were more likely to interpret positive words as familiar, regardless of their actual status, while negative words were more often judged as unfamiliar/novel. However, no significant main effect of treatment or treatment x valence interaction was found for any measure, suggesting that treatment did not influence memory performance or its association with word valence.

Finally, analysis on reaction times revealed no significant effect of treatment [$F(1, 136) = 0.65$, $p = 0.4$].

2.3.3.4 Facial Expression Recognition Task (FERT)

The FERT was used to evaluate participants' ability to identify facial expression at baseline, immediately post-infusion, and 24 hours post-infusion.

Baseline

At baseline, prior to treatment allocation, the two groups did not differ in accuracy [$F(1, 480) = 0.172$, $p = 0.7$], although a significant main effect of emotion was observed [$F(6, 462) = 64.109$, $p < 0.001$]. The interaction between treatment allocation and emotion was not significant [$F(6, 462) = 0.392$, $p = 0.9$]. Similar findings were obtained in a sensitivity analysis excluding participants with an average accuracy below 50%.

There was no significant main effect of treatment allocation on total misclassifications [$F(1, 462) = 0.187$, $p = 0.7$], but a significant main effect of emotion was observed [$F(6, 462) = 215.343$, $p < 0.001$]. The interaction between treatment allocation and emotion was not significant [$F(6, 462) = 0.352$, $p = 0.9$].

For reaction time, a significant main effect of emotional expression was observed [$F(6, 462) = 18.354$, $p < 0.001$], but no significant effect of treatment allocation [$F(1, 480) = 1.341$, $p = 0.2$] or interaction between treatment and emotion [$F(6, 462) = 0.217$, $p = 0.9$]. Overall, these results suggest that the two groups did not differ in performance on facial emotion recognition at baseline prior to randomisation.

Post-infusion

At post-infusion, there was no significant main effect of treatment on accuracy [$F(1, 473) = 0.624$, $p = 0.4$], but a significant main effect of emotion was observed [$F(6, 455) = 37.215$, $p < 0.001$]. The interaction between treatment and emotion was not significant [$F(6, 455) = 0.368$, $p = 0.9$]. Similar findings were obtained in a sensitivity analysis excluding subjects with an average accuracy below 50%.

Misclassification rates revealed no significant treatment effect [F(1, 469) = 0.003, p = 0.9], though a significant main effect of emotion remained [F(6, 469) = 135.809, p < 0.001]. The interaction between treatment and emotion was non-significant [F(6, 469) = 0.225, p = 1.0].

Of note, ketamine was associated with a numeric reduction in reaction time, albeit this difference did not reach statistical significance [F(1, 455) = 3.350, p = 0.07]. A significant main effect of emotion on reaction time was found [F(6, 455) = 22.059, p < 0.001], with no interaction between treatment and emotion [F(6, 455) = 0.668, p = 0.7].

24-hours post infusion

At 24 hours post-infusion, there was no significant main effect of treatment on overall accuracy measured as the total correct percentage [F(1, 481) = 0.21, p = 0.6]. However, a significant main effect of emotion [F(6, 469) = 52.920, p < 0.001] and a significant interaction between treatment and emotion was observed for total accuracy [F(6, 469) = 2.122, p = 0.05; **Figure 2.4**]. In subsequent sensitivity analyses conducted excluding participants with accuracy below 50% (n=8, 4 randomised to ketamine), the main effect of treatment remained non-significant on accuracy, measured as the total correct percentage [F(1, 413) = 0.255, p = 0.6]. However, the main effect of emotion remained highly significant [F(6, 413) = 49.360, p < 0.001] and the treatment-by-emotion interaction became more pronounced [F(6, 413) = 3.234, p = 0.004].

In light of the significant treatment-by-emotion interaction for accuracy observed at 24 hours post-infusion, further exploratory analyses were conducted to investigate the effects of treatment and emotional valence on facial recognition performance. Positive facial expressions (happy, surprise) were compared with negative facial expressions (anger,

disgust, fear, sad). Significant main effects of valence (positive and negative) were observed for total correct percentage [$F(1, 414) = 77.017$, $p < 0.001$], indicating higher accuracy for positive faces (mean = 71.40%, SD = 10.55) compared to negative faces (mean = 51.11%, SD = 16.70). However, there were no significant main effects of treatment on total correct percentage [$F(1, 414) = 0.008$, $p = 0.9$], nor were there significant treatment-by-valence interactions for total correct percentage [$F(1, 414) = 1.143$, $p = 0.3$]. To further explore whether specific emotions contributed to the observed effects, secondary analyses were conducted. While no significant treatment-related differences were found, trend-level effects emerged for disgust, anger, and neutral recognition. Specifically, disgust recognition was higher in the ketamine group (mean = 45.6%, SD = 16.65) compared to placebo (mean = 38.8%, SD = 13.63), with a trend-level effect in the one-way ANOVA [$F(1,68) = 3.533$, $p = 0.06$]. Similarly, neutral recognition was higher in the ketamine group (mean = 91.7%, SD = 10.98) than placebo (mean = 86%, SD = 17.01), showing a trend-level effect in the one-way ANOVA [$F(1,68) = 2.788$, $p = 0.099$]. In contrast, anger recognition was slightly lower in the ketamine group (mean = 58.5%, SD = 12.33) compared to placebo (mean = 64.2%, SD = 14.62), with a trend-level effect in the one-way ANOVA [$F(1,68) = 3.111$, $p = 0.08$]. No significant effects were observed for the remaining emotions (happy, fear, surprise, and sadness). These findings suggest that ketamine may differentially impact recognition accuracy for certain emotions, particularly disgust and neutral expressions, while potentially reducing accuracy for anger recognition, though effects remain at the trend level.

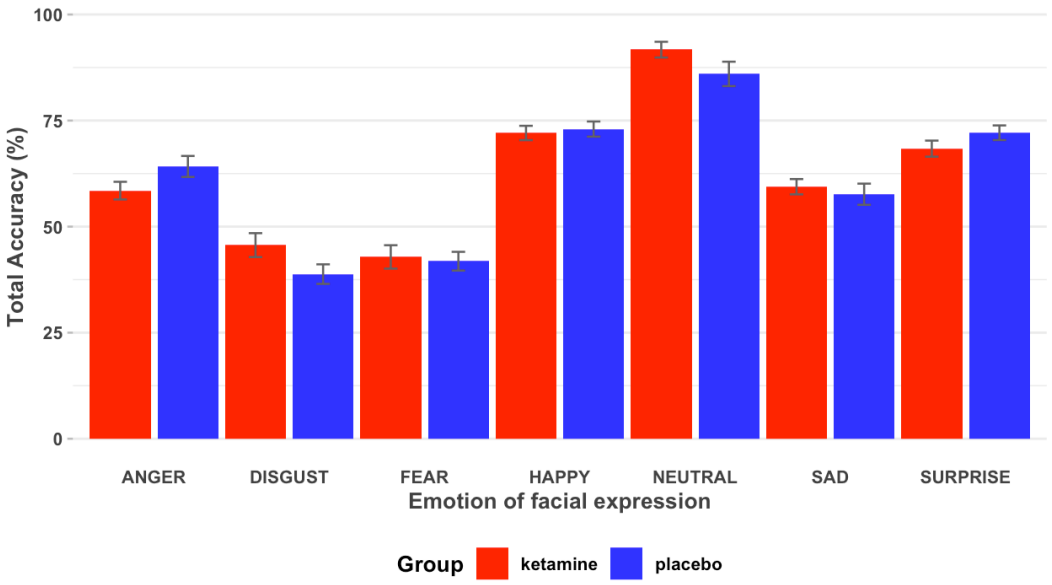
No significant main effect of treatment on total misclassifications was found [$F(1, 469) = 0.003$, $p = 0.9$] though a significant main effect of emotion persisted [$F(6, 469) = 135.809$, $p < 0.001$]. The interaction between treatment and emotion was not significant [$F(6, 469) = 0.225$, $p = 1.0$]. Further analyses of misclassifications revealed a significant main effect of

valence [$F(2, 481) = 358.009$, $p < 0.001$], reflecting differences in misclassification rates based on emotional valence. However, there were no significant main effects of treatment [$F(1, 481) = 0.003$, $p = 0.9$] or treatment-by-valence interactions [$F(2, 481) = 0.153$, $p = 0.9$].

Reaction time analysis revealed a significant main effect of treatment, with the ketamine group displaying faster reaction times (mean = 1398.76 ± 301.14 ms) compared to the placebo group (mean = 1482.51 ± 322.59 ms) [$F(1, 485) = 8.923$, $p = 0.003$]. A significant main effect of emotion on reaction time was also observed [$F(6, 467) = 12.760$, $p < 0.001$], with no interaction between treatment and emotion [$F(6, 467) = 0.312$, $p = 0.9$]. These results suggest that ketamine was associated with faster reaction times 24 hours post-infusion (**Figure 2.5)**.

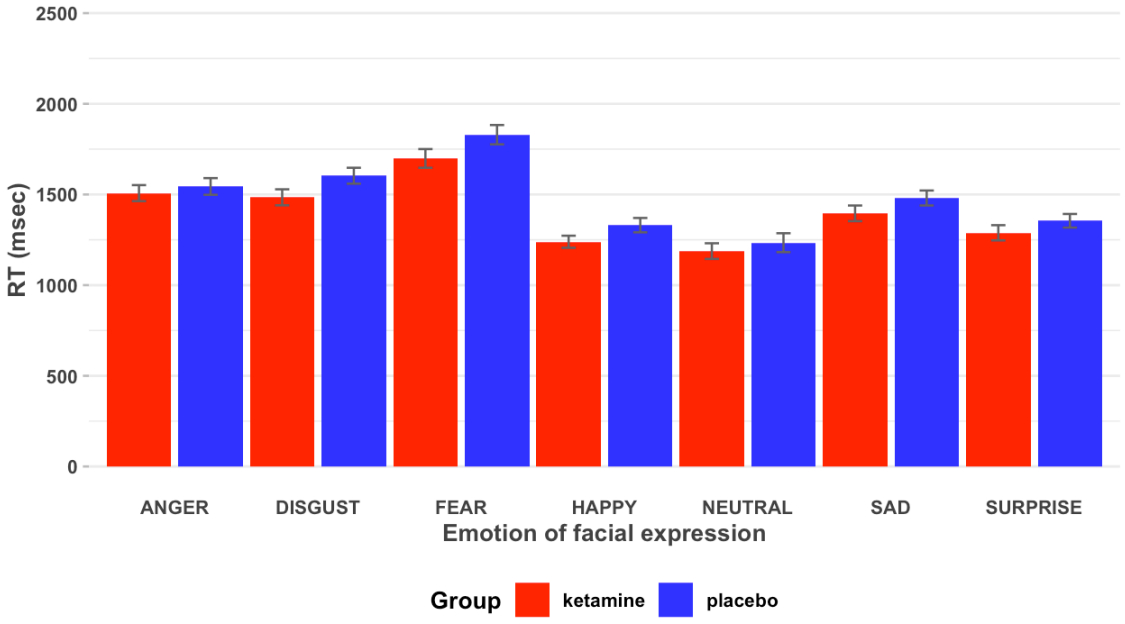
Overall, these results suggest that ketamine influences facial emotional recognition accuracy and may differentially impact recognition of specific emotions, particularly by enhancing accuracy for disgust and neutral expressions while potentially reducing accuracy for anger recognition, though these effects remain at the trend level. However, ketamine did not affect misclassifications across time points. Finally, ketamine was associated with numerically faster reaction times immediately after infusion and significantly lower reaction times at 24 hours post-infusion, suggesting a potential enhancement in processing speed.

Figure 2.4 Effect of Treatment on Accuracy in Facial Emotion Recognition 24-hours Post-Infusion



Values represent accuracy as total correct percent. Error bars indicate the standard error of the mean (SEM)

Figure 2.5 Effect of Treatment on Reaction Time for Facial Emotion Recognition 24-hours Post-Infusion



Values represents mean reaction times (RT, in milliseconds) for each facial emotion. Error bars represent the standard error of the mean (SEM).

2.4. Discussion

This experimental study investigated the neurocognitive effects of a single ketamine infusion, compared to placebo, on emotional processing in healthy volunteers. Ketamine appeared to selectively modulate emotional memory processes for information acquired prior to the infusion, evidenced by an increase in positive intrusions and a decrease in negative intrusions during the recall of self-descriptive words. Notably, while the overall number of correctly and incorrectly recalled words did not differ between groups, the emotional valence of intrusions differed, with the ketamine group showing a higher proportion of positive and a lower proportion of negative intrusions compared to placebo. Although a significant effect of ketamine was observed on facial emotion recognition accuracy 24 hours post-infusion, no specific emotions were identified as primary contributors to this effect. Notably, reaction time for recognizing facial emotions was reduced 24 hours post-infusion, with a trend-level decrease detectable immediately after infusion.

This study did not detect any effects of ketamine on depressive severity, anxiety, or hedonic capacity. Specifically, a previous trial involving healthy controls suggested that ketamine may have a differential mood and emotional effects compared to its impact on individuals with MDD (Nugent et al., 2019). Nugent et al. reported that ketamine increased depressive symptoms in healthy controls, with significant drug-by-time interactions (Nugent et al., 2019) as measured by the Montgomery-Åsberg Depression Rating Scale (MADRS; (Montgomery & Asberg, 1979). In this study, healthy volunteers experienced heightened inner tension, lassitude, inability to feel, and anxiety, along with reductions in hedonic capacity and anticipatory pleasure. These mood-lowering effects, characterized by increased

anxiety, emotional blunting, and anhedonia, resolved within 48 hours post-infusion for most participants. The discrepancy between this previous report and our current findings, which suggest no effect of ketamine on mood or anhedonia, could potentially be attributed to our use of self-report measures rather than clinician-rated assessments. Further, while the current study used the BDI, previous studies relied on the MADRS to assess depression severity. Although the mean BDI score in this trial was 1.8, which is generally comparable to the mean MADRS baseline score of 1.6 reported in previous studies - both indicating minimal to no depressive symptoms at baseline - direct comparisons should be made cautiously due to differences in scale design and scoring interpretation. The BDI is a self-report questionnaire that captures the participant's subjective experience, including emotional, cognitive, and physical symptoms, whereas the MADRS is a clinician-rated scale designed to provide a more objective assessment of depressive severity, making it particularly useful in clinical trials. Notably, while the BDI was developed from a cognitive perspective to measure depression severity subjectively, the MADRS was specifically developed to assess response to antidepressant treatment and is clinician-rated, enhancing its sensitivity to pharmacological treatment effects. Additionally, differences in study design may explain variations in results. Unlike the current study, the previous trial used a crossover design, where the infusion order was randomised, and ketamine and placebo infusions were administered two weeks apart. This design introduces potential carryover effects that may influence observed outcomes. Furthermore, resting-state magnetoencephalography (MEG) recordings were obtained on the day of infusion, which could have interfered with the drug/placebo effects. Finally, differences in sample size may also contribute to the variability in findings. While the current study recruited n = 70 healthy controls, previous studies enrolled only n = 25 healthy controls, potentially limiting the statistical power of their analysis. Larger trials focused on the effects of ketamine in healthy controls would be

valuable in enhancing statistical robustness and further clarifying the impact of sub-anaesthetic doses of ketamine on mood and anxiety symptoms in healthy volunteers.

Animal studies suggest that ketamine can reverse negative affective biases for previously acquired information, distinguishing it from conventional antidepressants like venlafaxine. Stuart et al. demonstrated in a rodent model that ketamine reversed previously encoded negative memories, enabling more positive retrieval, while venlafaxine did not (Stuart et al., 2015). Conversely, venlafaxine, but not ketamine, enhanced positive memory encoding when administered before learning. In the current study, ketamine was associated with increased positive intrusions and decreased negative intrusions compared to placebo for information acquired prior to the infusion. Importantly, these effects occurred without any impact on total performance, as evidenced by accuracy on the EREC or EMEM tasks, suggesting that ketamine did not influence overall memory performance relative to placebo. Although these findings suggest that ketamine's effects on previously acquired emotional material may translate to humans, further studies are needed to fully characterise this phenomenon. It is crucial to determine whether this effect is primarily mediated by ketamine's influence on memory recall or memory encoding of emotional self-describing words. Additionally, future research should explore whether this effect extends to clinical populations of depressed patients, as this would provide valuable insights into ketamine's potential therapeutic neurocognitive mechanisms in mood disorders.

In the current study, ketamine altered facial emotion recognition accuracy and reduced reaction time 24 hours post-infusion compared to placebo. Previous research has explored ketamine's impact on emotional processing, with mixed findings. Reed et al. investigated the effects of ketamine on brain activity during emotional processing in both depressed

patients and healthy controls, reporting differential results between the groups (Reed et al., 2019). While a group-by-session interaction for accuracy approached significance, group differences were observed at baseline and post-placebo, but not post-ketamine. No group-by-session interaction was identified for reaction time. Further, Ebert et al. found that ketamine impaired the recognition of sadness but did not significantly affect overall emotion recognition in 18 healthy male participants using the Ekman 60 Faces Test (Ebert et al., 2012). Similarly, Abel et al. (2003) reported minimal differences in accuracy when identifying fear versus neutral expressions during fMRI, with comparable mean correct scores under ketamine and placebo conditions (Abel et al., 2003). Schmidt et al. (2013) observed that S-ketamine impaired the encoding of both fearful and happy faces in 21 healthy participants, suggesting selective effects on emotional expression processing (Schmidt et al., 2013). However, the use of differing administration protocols and designs focused on emotional blunting in the context of psychotic symptomology limits the generalizability of these findings to mood disorders. Notably, no specific emotions were identified as primary contributors to this effect in the current study. This, combined with the observed reduction in reaction time, suggests the possibility of a general pro-cognitive effect of ketamine, rather than a targeted influence on facial emotion recognition. One potential explanation is that ketamine may enhance cognitive processing speed and efficiency, leading to improvements in task performance independent of emotional content. Alternatively, these findings may reflect ketamine's broader impact on attentional mechanisms and perceptual processing, rather than a selective modulation of emotion recognition. Future studies are warranted to explore whether these effects generalise across other cognitive domains and whether they extend to clinical populations, particularly individuals with mood disorders. Other non-competitive NMDA receptor antagonists, such as memantine and D-cycloserine, have also been investigated for their effects on emotional processing. Memantine, approved

for treating moderate to severe Alzheimer's disease, was studied in a placebo-controlled trial where healthy volunteers received a single 10 mg dose. In this study, memantine reduced false alarms for negatively valenced words in the EREC, a pattern similar to ketamine, and did not have effect on facial emotion recognition (Pringle et al., 2012). Similarly, Chen et al. investigated the effects of a single 250 mg dose of D-cycloserine compared to placebo in a double-blind study involving 40 healthy volunteers (R. Chen et al., 2021). D-cycloserine, an antibiotic commonly used to treat tuberculosis, acts as a partial agonist at the glycine site of the NMDA receptor, functioning as an agonist at low doses and an antagonist at higher doses (Schade & Paulus, 2016). The study found no significant effects of D-cycloserine on facial expression recognition, however, D-cycloserine significantly increased the proportion of positive versus negative words recalled accurately compared to placebo in the EREC.

The implications of these findings for TRD remain uncertain, emphasising the need for further research to determine their clinical relevance. If confirmed, these findings carry significant potential for clinical application. Ketamine's use is constrained by its IV administration route, the transient occurrence of dissociative symptoms, and limited long-term safety data. Employing neurocognitive models could enhance our understanding of ketamine's underlying mechanisms and inform the development of novel, rapid-acting antidepressants with improved safety profiles. Furthermore, these models may refine the integration of psychotherapy with ketamine treatment, offering insights into the optimal timing and conditions for such combinations to maximize therapeutic benefits. This integrated approach is becoming increasingly popular, particularly in clinics across the United States and Canada, where psychotherapy is frequently paired with ketamine treatment to enhance clinical outcomes. Importantly, these changes in memory for emotional material occurred in healthy volunteers without significant alterations in subjective

measures of positive or negative mood, anxiety, or hedonic capacity. This is particularly significant for studying rapid-acting antidepressants like ketamine, as it allows tasks to be conducted without the confounding effects of mood changes, which are challenging to examine within a TRD population, thereby providing unique insights into their neurocognitive mechanisms of action.

2.5. Limitations

The current study has several limitations. The study did not assess other critical aspects of memory, such as working memory and verbal memory, which could have offered a more comprehensive understanding of ketamine's effects on memory functioning. Including a broader range of memory assessments in future studies could help clarify how ketamine influences various domains of memory and its potential therapeutic implications.

Additionally, the findings of this study are limited to healthy volunteers, which may not fully capture the effects of ketamine in clinical populations such as patients with TRD. Extending these findings to TRD populations is essential to evaluate the translational relevance and therapeutic potential of ketamine in alleviating cognitive and emotional impairments associated with depression.

Furthermore, the study did not investigate the neural substrates underlying the observed effects of ketamine on memory. Incorporating functional neuroimaging techniques, such as functional MRI (fMRI), in future research could provide valuable insights into the brain regions and circuits involved in ketamine's effects on memory and emotional processing.

This would not only deepen the understanding of the neurobiological mechanisms but also contribute to the development of biomarkers for predicting treatment response and tailoring interventions for TRD.

2.6. Conclusion

In conclusion, this study showed that ketamine enhances behavioural measures of positive bias in memory recall for information acquired prior to its administration. Such effects could mitigate the negative biases in emotional processing commonly observed in depression, potentially contributing to the therapeutic benefits of this NMDA receptor antagonist in treating depressive disorders. Future research involving clinical populations and incorporating neuroimaging techniques is crucial to understanding ketamine's relevance in clinical settings. Such studies could provide valuable insights into the underlying neural mechanisms and further elucidate the early effects of NMDA antagonists on the brain substrates involved in emotional processing. These insights could inform the development of novel, targeted treatments for depression, with improved safety and efficacy profiles.

Chapter 3. Effects of ketamine in modulating habenula activity within a Pavlovian task

3.1 Introduction

The neurobiological mechanisms underlying major depressive disorder (MDD) remain incompletely understood; however, emerging evidence suggests that the habenula (Hb), a small structure adjacent to the mediodorsal thalamus, and in particular its lateral portion, plays a central role in regulating negatively motivated behaviour (Matsumoto & Hikosaka, 2007). The Hb is extensively connected with the ventral tegmental area (VTA) and the raphe nuclei - the primary sources of the brain's dopamine and serotonin, respectively (Aizawa et al., 2012; Herkenham & Nauta, 1979). Specifically, stimulation of the Hb has been shown to strongly inhibit VTA dopamine neuron firing (Matsumoto & Hikosaka, 2007), thereby suppressing dopaminergic activity (Ji & Shepard, 2007; Matsumoto & Hikosaka, 2007; Stamatakis & Stuber, 2012) and potentially serving as a neural substrate in affective experience and behaviour in MDD, including anhedonia and low motivation (Boulos et al., 2017; Gold & Kadriu, 2019). The Hb responds not only to primary aversive stimuli but also to cues predicting aversive outcomes, as well as to appetitive stimuli that are less rewarding than expected (Matsumoto & Hikosaka, 2007, 2009). Evidence from rodent models of depression implicates the Hb in learned helplessness behaviour (Shumake & Gonzalez-Lima, 2003), supporting the hypothesis that habenula dysfunction may play a critical role in the pathophysiology of MDD (Morris et al., 1999; Sartorius et al., 2010). Often referred to as the brain's "anti-reward" centre (Lecca et al., 2014; Matsumoto & Hikosaka, 2007), the Hb is implicated in processing negative reward signals and mediating aversive responses. In

addition to its role in reward processing, the Hb also influences a range of physiological and behavioural functions, including sexual and feeding behaviour, pain perception, and sleep regulation (Hikosaka et al., 2008; Klemm, 2004).

Recent evidence from animal models of depression suggests that the rapid antidepressant effects of ketamine could be mediated by the blocking of NMDAR-dependent burst firing in the lateral Hb (Yang et al., 2018), which is elevated in depression models (Weiss & Veh, 2011; Yang et al., 2018). This blockade seems to selectively target neurons with high basal bursting activity, reducing overall lateral Hb firing rates (Ma et al., 2023) and leading to a sustained suppression of lateral Hb activity for up to 24 hours in mice (Ma et al., 2023; MacDonald et al., 1987). However, the translation of these effects of ketamine on lateral Hb activity to humans remains to be tested.

This chapter presents findings from an experimental medicine trial in which n = 70 healthy volunteers were randomised to receive either intravenous ketamine (0.5 mg/kg) or placebo. Participants completed a Pavlovian conditioning paradigm task (Lawson et al., 2014) during a 7 Tesla high-field MRI task 24-hours after administration of ketamine/placebo. The study investigated activation of the right Hb in response to anticipation and receipt of monetary wins and losses, and electroshocks. Key measures included brain activation during the presentation of conditioned stimuli (CS) associated with win, loss, and shock, as well as during outcome delivery, comparing the ketamine group to placebo. To isolate this neural activation from the acute dissociative effects of ketamine, the task-based functional MRI (fMRI) scan was conducted 24 hours post-infusion. The primary objective was to assess whether ketamine's rapid inhibitory effects on the Hb, as previously demonstrated in preclinical models, can be translated to humans. Furthermore, this study serves as a proof-

of-concept using an experimental medicine approach to evaluate target engagement, and can potentially inform subsequent clinical studies that will examine whether these neural effects are seen in patients and correlate with clinical outcomes.

3.2 Methods

3.2.1 Study Participant and design

Details of study design and procedures are included in Chapter 2. Briefly, this randomised, double-blind, placebo-controlled experimental medicine study was conducted at the Department of Psychiatry, University of Oxford, between July 2021 and June 2023 (ClinicalTrials.gov ID: NCT04850911). Ethical approval was obtained from the University of Oxford Central University Research Ethics Committee, and all participants provided written informed consent.

Participants were healthy adults aged 18 – 45 with no lifetime psychiatric diagnosis (confirmed via SCID) (First MB, 2015). Inclusion criteria included BMI between 18–30, fluency in English, and willingness to comply with study restrictions (e.g., no alcohol, driving, or signing legal documents during critical study periods). Exclusion criteria included psychiatric history, unstable medical conditions, recent recreational drug use, pregnancy, or prior ketamine/phencyclidine use.

At screening, participants underwent medical and psychiatric evaluation, blood and urine tests, and toxicology screening. Eligible individuals were randomised (block design

stratified by gender) to receive either ketamine (0.5 mg/kg) or placebo (0.9% saline) within four weeks of screening. Randomisation was managed via Sealed Envelope and codes were securely held by unblinded staff at the Warneford Hospital pharmacy.

3.2.2. Infusion procedures

Infusion procedures followed standard procedures, as described in Chapter 2. Prior to ketamine/placebo administration, participants completed a series of questionnaires and computerised tasks. Eligibility was re-confirmed through a review of inclusion/exclusion criteria and concomitant medications. Urine toxicology screening and pregnancy testing (when applicable) were also conducted.

Participants were randomised to receive either ketamine or placebo in a 1:1 fashion. During the infusion, blood pressure was monitored every 10 minutes and subsequently every 15 minutes for an hour post-infusion. Participants rested on a bed in a quiet environment and were instructed to refrain from listening to music, watching television, or reading.

Following the infusion, participants were observed for approximately two hours. Monitoring included oxygen saturation, blood pressure, heart rate, and general clinical observation. Upon completion of the visit, a physician discharged participants, who were transported home by taxi. They were advised to avoid driving, cycling, operating machinery, consuming alcohol, taking medications, or signing legal documents until after a full night's rest, and for up to seven days post-infusion as a precaution. Participants returned to the site the following day to complete computerised tasks, self-report questionnaires, and fMRI scan.

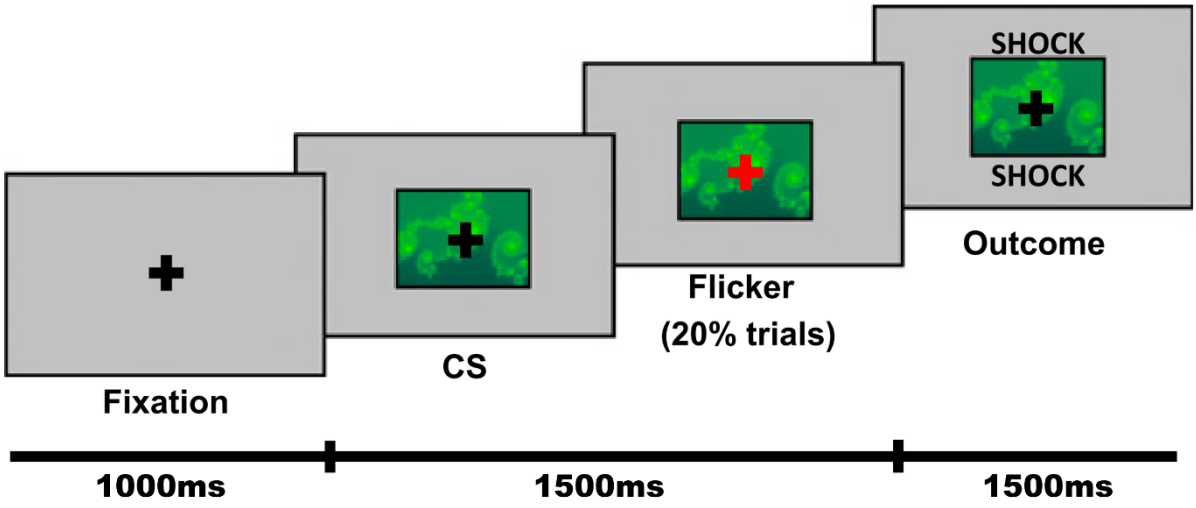
3.2.3 fMRI task

A Pavlovian conditioning task originally developed by Lawson and colleagues was adapted for this study (Lawson et al., 2014) (**Figure 3.1**). Abstract, luminance-matched fractal images served as CSs and were probabilistically paired with win, loss, shock, or neutral outcomes. Each block of the task featured seven CSs with fixed outcome probabilities: 80% chance of £1 win, 20% chance of £1 win, 80% chance of £1 loss, 20% chance of £1 loss, 80% chance of shock, 20% chance of shock, and 100% neutral (no outcome). Stimulus-outcome pairings were randomly assigned across participants. On trials without a reinforcing outcome (i.e., win, loss, or shock), and for all neutral outcomes, the word "nothing" was displayed on-screen. The timeline of each trial is illustrated in Figure 1. A fixation cross was displayed continuously throughout the trial. The CS appeared approximately 1,000 ms after trial onset and remained visible until trial end. Outcomes were presented on average 1,500 ms after CS onset. Trial order and CS-outcome pairings were pseudo-randomised.

Given the observational nature of the task, participants had no control over outcomes. To ensure engagement and reduce the risk of participants falling asleep during scanning, 20% of trials included a brief attention check: the fixation cross flickered from black to red for 300 ms during CS presentation and prior to the outcome, prompting participants to respond with a button press as quickly as possible. Participants were explicitly informed that their responses would not influence the outcomes. In total, the task consisted of 420 trials distributed across three blocks, each lasting approximately 9.3 minutes. Activation of the Hb during the Pavlovian conditioning task in response to the conditioned stimulus associated

with pain stimuli and in response to the receipt of shock represented the primary outcome. To minimise habituation and maintain salience, new fractals were used in each block.

Figure 3.1 Schematic representation of the Pavlovian conditioning task



Participants passively observed abstract fractal images (conditioned stimuli, CS), each followed by one of four possible outcomes: monetary win, monetary loss, electric shock, or a neutral outcome. The task included three blocks, each containing seven distinct fractals (21 in total). For win, loss, and shock conditions, two fractals per outcome type were associated with high (80%) or low (20%) probability outcomes. Neutral fractals were deterministically followed by no outcome (100%). No explicit feedback was given during the task. To maintain attention, 20% of trials featured brief red flickers superimposed on the CS images, requiring a speeded button press. Average durations for each task phase are shown in the timeline. CS: conditioned stimulus. Figure adapted from Lawson et al., 2014.

3.2.4 Pain calibration

Electric stimulation was delivered to the left hand (over the fascia of the adductor pollicis muscle) via a custom magnetically shielded electrode using a single 1,000-Hz electrical pulse. To account for individual differences in skin resistance and pain tolerance, participants underwent a standardised thresholding procedure prior to scanning. During this procedure, shocks of increasing amplitude were administered sequentially, and participants verbally rated each on a scale from 0 ("not painful") to 10 ("extremely painful - comparable to burning one's hand severely or pain that would cause involuntary movement inside the

scanner"). Importantly, participants were not informed of the shock intensities during this process. The stimulation level used during the experiment was individually calibrated to 80% of each participant's maximum tolerable shock. During the experimental task, electrical stimulation was delivered using a Digitimer DS8R constant current stimulator, triggered by custom MATLAB scripts.

3.2.5 fMRI acquisition

MRI data were collected at FMRIB – Wellcome Centre For Integrative Neuroimaging, University of Oxford, John Radcliffe Hospital, using a Magnetom 7T scanner (Siemens Healthineers AG, Germany) equipped with a single-channel transmit and 32-channel receive head coil (Nova Medical Inc., USA). High-resolution structural T1-weighted images were acquired for anatomical registration using a 3D MPRAGE sequence with 0.7 mm isotropic resolution, FOV = 224 × 224 × 179.2 mm^3, TR = 2200 ms, TE = 1.65 ms, inversion time (TI) = 1050 ms, flip angle = 7°, PAT factor = 2, and 256 slices. Functional T2*-weighted images were acquired using a 2D multiband echo-planar imaging (EPI) sequence (Feinberg et al., 2010) with the following parameters: 1.2 mm isotropic resolution, field of view (FOV) = 192 × 192 × 60 mm^3, 50 slices, repetition time (TR) = 1375 ms, echo time (TE) = 19 ms, flip angle = 60°, multiband factor = 2, parallel acquisition (PAT) factor = 3, partial Fourier = 6/8, and bandwidth = 1420 Hz/pixel. The FOV was manually centred on the Hb for each participant to optimise coverage. Each functional block consisted of 418 volumes, with the first five volumes discarded to account for T1 equilibration effects.

To enable distortion correction, a gradient-recalled echo (GRE) field map was acquired with 2 mm isotropic resolution, FOV = 192 × 192 mm^2, 77 slices, TR = 620 ms, Tes = 4.08 ms

and 5.1 ms, and a flip angle of 39°. Additionally, a single EPI volume with increased coverage (120 slices; TR = 3298 ms) but otherwise identical parameters and shim settings to the main functional run was acquired to aid registration.

Physiological data - including peripheral pulse and respiratory effort - were recorded during EPI acquisition using a photoplethysmograph and a pneumatic chest belt connected to a modular data acquisition system (MP150, BIOPAC Systems Inc., USA). These recordings were used to correct for physiological noise (pulse- and respiration-related artefacts) during analysis via physiological noise modelling.

3.2.6 fMRI Preprocessing and First-Level Analysis

Data were analysed using FSL (FMRIB Software Library v6.0; https://fsl.fmrib.ox.ac.uk/fsl). Structural anatomical scans were brain-extracted and B1-biasfield corrected using the fsl_anat pipeline. fMRI data were preprocessed and analysed using FEAT (FMRI Expert Analysis Tool, version 6.0, part of FSL). Preprocessing steps included: 1. brain extraction using BET to remove non-brain tissue; 2. motion correction using MCFLIRT (Jenkinson et al., 2002), along with identification and exclusion of outlier volumes exhibiting excessive motion using fsl_motion_outliers; 3. spatial smoothing using a 3-mm full-width at half maximum (FWHM) Gaussian kernel to enhance signal-to-noise ratio while preserving spatial specificity in small structures such as the Hb; 4. unwarping using field maps to correct for geometric distortions in the echo-planar images; 5. high-pass temporal filtering with a 60-second cut-off to remove low-frequency drifts. Preprocessing of all fMRI data was conducted throughout the trial by study team members who were blinded to treatment allocation. The group-level analyses presented in this chapter were

performed only after the final participant completed the study, incorporating data that had been pre-processed in a blinded fashion during the course of data collection.

Functional images were registered linearly to each participant's structural image via an intermediate high-contrast whole-brain EPI image using boundary-based registration (BBR), optimizing anatomical alignment for regions of interest. First-level analyses were conducted separately for each of the three functional runs using FEAT (FSL v6.0). First-level event-related general linear models (GLM) also included regressors generated by FSL's physiological noise modelling GUI, to improve image quality and signal-to-noise ratio. Regressors were included for both CS and outcome events to model neural responses associated with anticipation and outcome processing. CS-related activity was modelled using four regressors per run (win, loss, shock, and neutral), along with additional contrasts reflecting grouped valence conditions: negative (shock + loss), positive (win) vs. negative (shock + loss), negative vs. positive. The first trial in which participants observed each CS fractal for the first time was excluded from the analysis to control for novelty-related responses. Outcome-related activity was modelled using regressors for each outcome type (win, loss, shock, and neutral) and similar valence-based contrasts (negative, positive vs. negative, negative vs. positive). All regressors were convolved with a canonical double-gamma hemodynamic response function (HRF), and temporal derivatives were included.

3.2.7 Intermediate-Level Statistical Analysis

For each subject, contrast estimates from the three runs were combined using a fixed-effects model to produce an average contrast per condition using a second-level FEAT analysis (level 2).

3.2.8 Group-Level Statistical Analysis

These subject-level averages were then combined across participants with fslmerge. Higher level (group level) analysis was carried out using FSL's tool for nonparametric permutation inference Randomise (5000 permutations) to assess general effects of task-relevant contrasts on both groups, as well as test for group differences. Statistics were assessed using the threshold-free cluster enhancement method with family-wise error correction of 0.05 (or 0.95 threshold within randomise). The GLM included 2 groups: placebo and ketamine. Contrasts were defined as placebo greater than ketamine, ketamine greater than placebo, and the mean across both groups to establish main effect of task.

All reported activation clusters were reported in Montréal Neurological Institute (MNI) standard space (1 mm), and brain regions are reported based on the Harvard-Oxford Cortical and Subcortical Structural Atlas. Main effects of task and treatment group were analysed at the whole-brain (WB) and right Hb levels. A Hb mask was created in standard space as a spherical kernel with a 2 mm radius, approximating the anatomical volume of the habenula (~31 mm^3) (Samanci et al., 2024). MNI coordinates reported by Lawson et al. (2013) were used to identify voxel locations in standard space using FSLeyes. Specifically, the right habenula was identified at MNI coordinates x = 85, y = 102, z = 74, and the left habenula at x = 93, y = 102, z = 74. These coordinates were used to generate the right Hb mask, enabling anatomically informed ROI definition for subsequent analyses.

Significant brain areas were extracted for visualization using the fslmaths and cluster tools, with a threshold of 0.95 (based on the 1-p thresholding from randomise, described above).

To further visualise results, individual parameter estimate (PE) values were extracted from their custom maps, using significant clusters as binary masks using fslmeants.

3.2.9 Statistical Analysis

Statistical analysis of shock calibration values and subjective pain ratings was conducted using an independent samples t-test in SPSS Statistics (version 29.0; IBM Corp).

3.3 Results

3.3.1 Sample Characteristics

A detailed description of the full study sample is provided in Chapter 2. The current chapter reports data from a subset of n = 66 participants who completed the fMRI protocol and had valid imaging data. Of the initial n = 70 individuals randomised and who completed the scan procedure, four participants were excluded from analysis due to technical issues: one was excluded because of severe wraparound artefacts affecting the T1-weighted structural scan; one was lost due to a major power outage in Oxford at the time of scanning; and two were excluded due to scanner failure. The final sample of 66 participants was included in all reported analyses (see **Table 3.1** for demographic and mood-related questionnaire data).

Table 3.1. Sociodemographic characteristic of the study population

	Ketamine (n=35)	Placebo (n=31)
Age, Mean (SD)	23.46 (5.3)	23.52 (4.5)
Gender, Male, n (%)	19 (54.3)	15 (48.4)
Race/Ethnicity, n (%)		
White/Caucasian	29 (82.9)	23 (74.2)
Asian/Asian British	4 (11.4)	3 (9.7)
Years of Education, mean (SD)	16.89 (2.61)	17.39 (2.629)
Questionnaire Measures		
STAI-T, Mean (SD)	29.82 (4.979)	29.32 (9.253)
STAI-S, Mean (SD)	28.2 (6.365)	26.97 (8.758)
BDI, Mean (SD)	1.71 (2.037)	2.06 (4.697)
TEPS Ant, Mean (SD)	44.71 (5.165)	46.29 (4.818)
TEPS Con, Mean (SD)	40.74 (3.830)	41.16 (4.810)

BDI, Beck Depression Inventory; n, number; SD, standard deviation; STAI-S, State-Trait Anxiety Inventory, State subscale; State-Trait Anxiety Inventory, Trait subscale; TEPS-Ant, Temporal Experience of Pleasure Scale, Anticipatory subscale; TEPS-Con, Temporal Experience of Pleasure Scale, Anticipatory subscale.

3.3.2 Shock Calibration and Subjective Pain Ratings

The average shock amplitude across participants was 9.01 ± 1.51 mA (mean ± SD). There were no significant group differences in subjective pain ratings immediately prior to the scan, although a trend toward lower ratings was observed in the ketamine group compared to the placebo group [mean: 5.90 versus 6.81; $t(64) = -1.88$, $p = 0.065$].

3.3.3 Motion Analysis

Given the small size of the habenula ROI, careful consideration was given to motion correction procedures to optimize signal detection while preserving statistical power. Independent samples t-tests were conducted to compare head motion between the ketamine and placebo groups across three runs of the task, using mean absolute and relative motion metrics extracted from FEAT reports. Across all six measures (absolute and relative motion for runs 1, 2, and 3), no statistically significant group differences were found, with motion levels generally comparable across groups. Moreover, visual inspection of MCFLIRT motion correction plots also did not indicate any systematic association between head motion and the timing of electric shock administration.

In run 1, mean absolute motion was 0.8771 (SD = 0.5390) for placebo and 0.7831 (SD = 0.5694) for ketamine, while mean relative motion was virtually identical: 0.1158 (SD = 0.0375) vs. 0.1163 (SD = 0.0488). In run 2, absolute motion increased overall, with means of 1.1319 (SD = 1.0432) in placebo and 0.9394 (SD = 0.8774) in ketamine. Relative motion was similarly close: 0.1310 (SD = 0.0580) for placebo and 0.1283 (SD = 0.0549) for ketamine. For run 3, absolute motion was 1.0135 (SD = 0.8515) in placebo and 0.9534 (SD = 0.8299) in ketamine; relative motion was 0.1274 (SD = 0.0592) and 0.1243 (SD = 0.0504), respectively. All p-values exceeded 0.40 (range: 0.419–0.965), and confidence intervals consistently included zero, indicating no significant differences.

Given the absence of meaningful motion differences between groups, motion-related artifacts are unlikely to have confounded group comparisons in downstream analyses.

3.3.4 Main effect of task

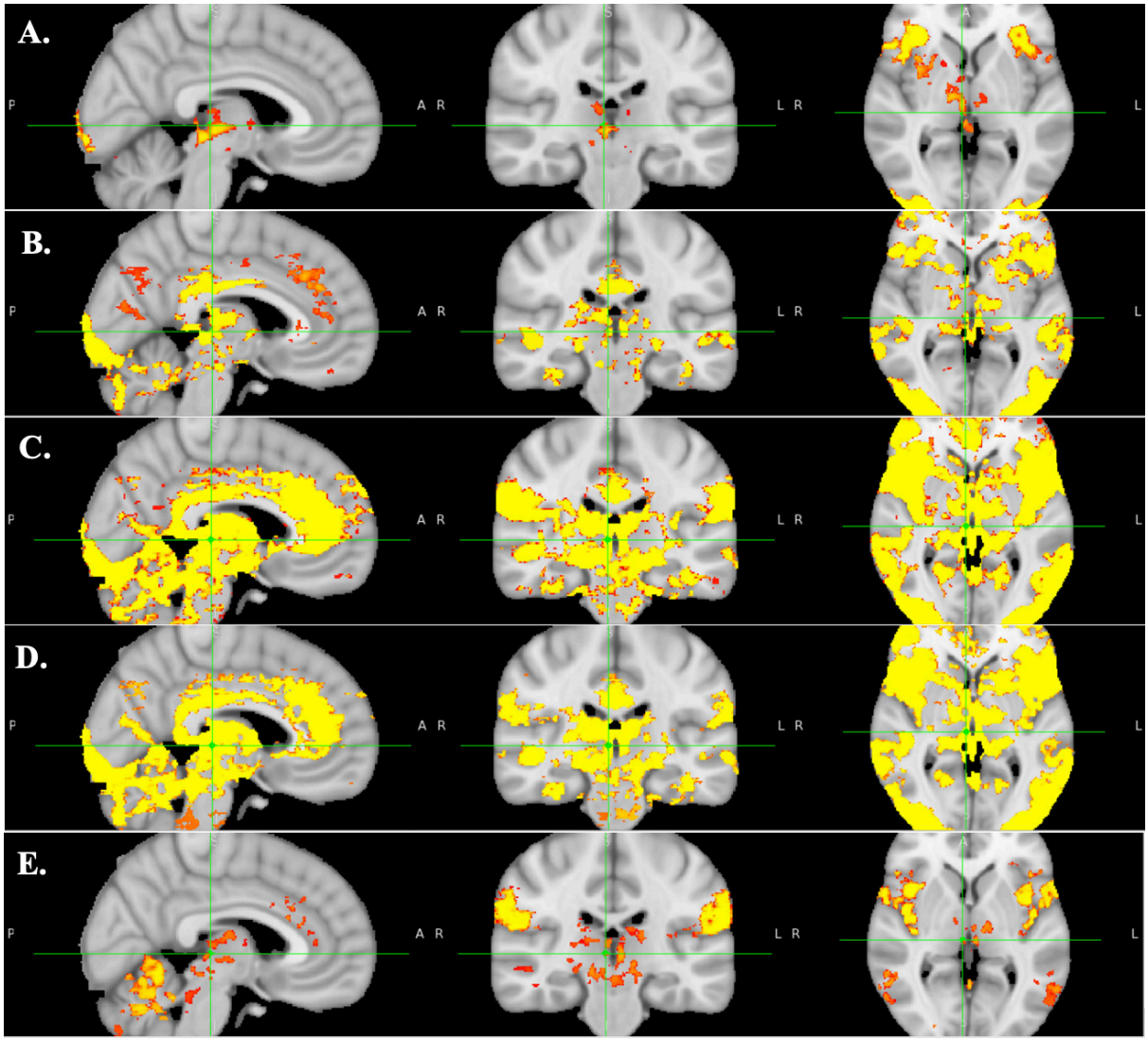
An initial WB analysis was conducted to explore task-related activity across all participants and conditions. To further assess whether the task engaged the right Hb, neural activation associated with the relevant contrasts - independent of treatment allocation - was also examined using a right Hb mask.

3.3.4.1 Whole Brain

Robust WB activation was observed across all individual CS types (see **Table 3.2** for details), including win (85443 voxels, peak voxel location: x = 97, y = 48, z = 41 cerebellum, t-max = 3.756, $p < 0.001$), loss (56735 voxels, peak voxel location: x = 118, y = 44, z = 50 occipital fusiform gyrus and lateral occipital cortex, inferior division, t-max = 3.778, $p < 0.001$), shock (21541 voxels, peak voxel location: x = 52, y = 153, z = 64 frontal orbital cortex, t-max = 4.435, $p < 0.001$), and neutral CS (11995 voxels, peak voxel location: x = 60, y = 35, z = 60 lateral occipital cortex and occipital fusiform gyrus, t-max = 5.991, $p < 0.001$). Notably, negative CS (combined shock and loss; 54574 voxels, peak voxel location: x = 56, y = 86, z = 48 temporal occipital fusiform cortex and temporal fusiform cortex, t-max = 3.764, $p < 0.001$) and the contrast comparing positive versus negative (6589 voxels, peak voxel location: x = 57, y = 75, z = 56 temporal occipital fusiform cortex, t-max = 5.902, $p = 0.007$) also showed strong effects across a network of relevant regions. The reverse contrast (negative vs. positive CS) did not yield significant effects (p = 1-0.414000). During the outcome contrasts (see **Table 3.3** for details), robust activation was observed for win (250986 voxels, peak voxel location: x = 138, y = 54, z = 43 cerebellum, t-max = 3.124, $p < 0.001$), loss (338312 voxels, peak voxel location: x = 59, y = 70, z = 39 cerebellum, t-max

= 3.005 $p < 0.001$), shock (624916 voxels, peak voxel location: x = 71, y = 49, z = 21 cerebellum, t-max = 2.640, $p < 0.001$), and neutral outcomes (87311 voxels, peak voxel location: x = 52, y = 89, z = 44 temporal fusiform cortex and temporal occipital fusiform cortex, t-max = 3.439, $p < 0.001$), as well as for negative outcomes (558199 voxels, peak voxel location: x = 81, y = 52, z = 27 cerebellum, t-max = 2.655, $p < 0.001$) and the negative versus positive contrast (42132 voxels, peak voxel location: x = 50, y = 131, z = 58 Insular cortex, t-max = 3.579, $p < 0.001$). The reverse contrast - positive versus negative - did not reach statistical significance (p = 1-0.494800). Of note, among the significant contrasts, visual inspection suggests that the following conditions elicited activation patterns encompassing the right Hb (see **Figure 3.2**): shock CS, loss, shock, negative and negative versus positive outcomes. These are consistent with previous report using the same task, suggesting that the task was successful in probing Hb activation (Lawson et al., 2017; Lawson et al., 2014). Minimal overlap within the right habenula region was observed for win outcomes (data not shown).

Figure 3.2 Whole-brain (WB) activation patterns for task-related contrasts encompassing the right habenula (Hb)



Main effect of task: Across all participants, the shock CS (A), loss outcome (B), shock outcome (C), negative (shock + loss) outcome (D), and negative vs. positive outcome (E) conditions elicited greater BOLD signal in regions encompassing the right habenula. The area of activation is shown in yellow-red, overlaid with the right Hb mask in green. Images were thresholded using threshold-free cluster enhancement (TFCE), $p < .05$, corrected for multiple comparisons (5000 permutations).

Table 3.2. Whole-Brain Analysis: Cluster Peaks for Activations Related to the Conditioned Stimuli (CSs)

Condition	Cluster Index	Brain areas	Cluster size (voxels)	Voxel max (x, y, z)	MNI max (x,y,z)	TFCE-corrected p (MAX)	p value
Win	1	Cerebellum	4287	97,48,41	-7,-78,-31	1	<.001
	2	Inferior Frontal Gyrus, pars opercularis, Precentral Gyrus, Middle Frontal Gyrus	9217	129,138,98	-39,12,26	0.998	0.002
	3	Precentral Gyrus, Inferior Frontal Gyrus, pars opercularis, Middle Frontal Gyrus	9212	52,132,103	38,6,31	0.998	0.002
	4	Frontal Orbital Cortex, Insular Cortex	2415	119,153,69	-29,27,-3	0.991	0.009
	5	Insular Cortex, Frontal Operculum Cortex, Frontal Orbital Cortex	2349	55,149,75	35,23,3	0.999	0.001
	6	Brain-Stem	9	81,95,69	9,-31,-3	0.953	0.047
	7	Left Thalamus	5	91,116,59	-1,-10,-13	0.954	0.046
Loss	1	Occipital Fusiform Gyrus, Lateral Occipital Cortex, inferior division	56735	118,44,50	-28,-82,-22	1	<.001
	2	Middle Frontal Gyrus, Inferior Frontal Gyrus, pars triangularis	4528	133,154,95	-43,28,23	0.998	0.002
	3	Middle Frontal Gyrus, Inferior Frontal Gyrus, pars opercularis, Inferior Frontal Gyrus, pars triangularis	1896	51,147,98	39,147,98	0.993	0.003
	4	Insular Cortex, Frontal Orbital Cortex	569	60,149,69	30,23,-3	0.986	0.014
	5	Lateral Occipital Cortex, superior division	351	55,57,98	35,-69,26	0.965	0.035
	6	Frontal Orbital Cortex, Insular Cortex	104	120,155,70	-30,29,-2	0.972	0.028

Shock	1	Frontal Orbital Cortex	21541	52,153,64	38,27, -8	1	<.001
	2	Occipital Fusiform Gyrus	21484	114, 42,50	-24,-84,-22	1	<.001
	3	Occipital Fusiform Gyrus	21435	57,53,51	33,-73,-21	1	<.001
	4	Frontal Orbital Cortex, Insular Cortex	4757	122,152,67	-32,26,-5	1	<.001
	5	Precentral Gyrus, Inferior Frontal Gyrus, pars opercularis	4535	133,131,94	-43,5,22	0.999	0.001
	6	Brain Stem	3671	86,98,67	4,-28,-5	0.997	0.003
	7	Right Caudate,	739	80,138,82	10,12,10	0.982	0.018
	8	Left Putamen	285	117,130,68	-27,4,-4	0.965	0.035
	9	Right Putamen, Right Pallidum	90	72,134,72	18,8,0	0.958	0.042
	10	Right Thalamus	89	85,124,74	5,-2,2	0.958	0.042
	11	Right Caudate	53	74,124,94	16,-2,22	0.955	0.045
	12	Subcallosal Cortex	40	100,105,61	-10,-21,-11	0.956	0.044
	13	Parahippocampal Gyrus	34	97,100,62	-7,-26,-10	0.952	0.048
	14	Lingual Gyrus, Occipital Fusiform Gyrus	22	83,49,55	7,-77,-17	0.953	0.047
	15	Left Thalamus	18	98,102,81	-8,-24,9	0.951	0.049
	16	Temporal Occipital Fusiform Cortex	13	52,76,51	38,-50,-21	0.951	0.049
	17	Lateral Occipital Cortex, superior division	2	47,42,98	43,-84,26	0.95	0.05
Neutral	1	Lateral Occipital Cortex, Occipital Fusiform Gyrus	11995	60,35,60	30,-91,12	1	<.001
	2	Lateral Occipital Cortex	9194	112,30,60	-22,-96,-12	1	<.001
Negative	1	Temporal Occipital Fusiform Cortex, Temporal Fusiform Cortex	54574	56,86,48	34,-40,-24	1.0	0.0
	2	Inferior Frontal Gyrus, pars opercularis, Middle Frontal Gyrus	8055	54,145,95	36,19,23	1.0	0.0

	3	Precentral Gyrus, Inferior Frontal Gyrus, pars opercularis	6894	134,130,93	-44,4,21	0.999	0.001
	4	Insular Cortex, Frontal Orbital Cortex	4845	61,149,67	29,23,-5	1.0	0.0
	5	Frontal Orbital Cortex, Insular Cortex	3250	120,154,69	-30,28,-3	0.999	0.001
	6	Right Putamen	430	64,135,84	26,9,12	0.967	0.033
	7	Right Putamen	315	56,127,72	34,1,0	0.968	0.032
	8	Parahippocampal Gyrus, ventromedial Prefrontal Cortex (vmPFC)	295	86,98,67	4,-28,-5	0.973	0.027
	9	Lateral Occipital Cortex	166	119,55,97	-29,-71,25	0.959	0.041
	10	Lateral Occipital Cortex,	71	116,57,104	-26,-69,32	0.955	0.045
	11	Right Putamen	47	65,121,79	25,-5,7	0.953	0.047
	12	Right Caudate	39	78,140,83	12,14,11	0.959	0.041
	13	Subcallosal Cortex, vmPFC, Nucleus Accumbens	21	78,101,63	12,-25,-9	0.956	0.044
	14	Right Cerebral White Matter	1	68,143,82	22,17,10	0.95	0.05
Pos vs Neg	1	Temporal Occipital Fusiform Cortex	6589	57,75,56	33,-51,-16	0.993	0.007
	2	Left Cerebral Cortex	2364	120,47,59	-30,-79,-13	0.983	0.017
	3	Cerebellum	10	64,75,47	26,-51,-25	0.951	0.049
	4	Occipital Fusiform Gyrus, Temporal Occipital Fusiform Cortex, Lateral Occipital Cortex	8	131,62,56	-41,-64,-16	0.951	0.049
	5	Occipital Fusiform Gyrus, Lateral Occipital Cortex, Temporal Occipital Fusiform Cortex	1	132,58,55	-42,-68,-17	0.951	0.049

MNI, Montreal Neurological Institute; Neg, negative; Pos, positive; ventromedial Prefrontal Cortex (vmPFC). Results were thresholded using threshold-free cluster enhancement (TFCE), $p < .05$, corrected for multiple comparisons (5000 permutations).

Table 3.3. Whole brain fMRI activation during the task – outcome

Condition	Cluster Index	Brain areas	Cluster size (voxels)	Voxel max (x, y, z)	MNI max (x,y,z)	TFCE-corrected p (MAX)	p value
Win	1	Cerebellum	250986	138,54,43	-48,-72,-29	1.0	<.001
	2	Cingulate Gyrus, posterior division	2107	86,94,98	4,-32,26	0.986	0.014
	3	Subcallosal Cortex, vmPFC	446	101,108,47	-11,-18,-25	0.957	0.043
	4	Left Cerebral White Matter	73	106,145,89	-16,19,17	0.954	0.046
	5	Left Cerebral White Matter	70	101,172,101	-11,46,29	0.955	0.045
	6	Temporal Pole, Parahippocampal Gyrus	64	67,131,46	23,5,-26	0.957	0.043
	7	Precentral Gyrus, Middle Frontal Gyrus	63	51,124,116	39,-2,44	0.952	0.048
	8	Left Cerebral White Matter	60	108,149,94	-18,23,22	0.956	0.044
	9	Cingulate Gyrus, Paracingulate Gyrus	60	83,165,68	7,39,-4	0.954	0.046
	10	Right Cerebral White Matter	48	74,160,105	16,34,33	0.952	0.048
	11	Right Thalamus	39	83,114,81	7,-12,9	0.952	0.048
	12	Inferior Temporal Gyrus	36	33,86,45	57,-40,-27	0.953	0.047
	13	Superior Temporal Gyrus, Middle Temporal Gyrus	36	139,108,65	-49,-18,-7	0.954	0.046
	14	Lateral Occipital Cortex	29	127,57,107	-37,-69,35	0.951	0.049
	15	Left Cerebral White Matter, Left Putamen	28	121,126,67	-31,0,-5	0.952	0.048
	16	Left Caudate	25	99,132,76	-9,6,4	0.951	0.049
	17	Right Cerebral White Matter	22	63,109,88	27,-17,16	0.952	0.048
	18	Frontal Pole, Superior Frontal Gyrus	20	111,168,116	-21,42,44	0.951	0.049
Loss	1	Cerebellum	338312	59,70,39	31,-56,-33	1	<.001

Shock	1	Cerebellum	624916	71,49,21	19,-77,-51	1	<.001
Neutral	1	Temporal Fusiform Cortex, Temporal Occipital Fusiform Cortex	87311	52,89,44	38,-37,-28	1.0	<.001
	2	Middle Frontal Gyrus, Inferior Frontal Gyrus, pars triangularis	13968	45,159,94	45,33,22	0.996	0.004
	3	Middle Frontal Gyrus, Inferior Frontal Gyrus, pars triangularis	10030	127,155,93	-37,29,21	0.997	0.003
	4	Frontal Operculum Cortex, Insular Cortex	2801	119,147,83	-29,21,11	0.993	0.007
	5	Cingulate Gyrus, posterior division	388	89,88,94	1,-38,22	0.975	0.025
	6	Left Cerebral White Matter, Left Putamen	39	112,140,82	-22,14,10	0.961	0.039
	7	Left Caudate	35	99,135,85	-9,9,13	0.953	0.047
	8	Left Frontal Cortex	32	118,179,62	-28,53,-10	0.958	0.042
Negative	1	Cerebellum	558199	81,52,27	9,-74,-45	1	<.001
Neg vs Pos	1	Insular Cortex	42132	50,131,58	40,5,-14	1.0	0.0
	2	Cerebellum	31871	74,75,51	16,-51,-21	0.999	0.001
	3	Insular Cortex, Frontal and Central Operculum Cortex	24486	126,137,76	-36,11,4	1.0	0.0
	4	Middle Temporal Gyrus, Angular Gyrus, Supramarginal Gyrus	3166	142,74,82	-52,-52,10	0.985	0.015
	5	Cerebellum	1991	118,75,40	-28,-51,-32	0.977	0.023
	6	Cingulate Gyrus	1452	90,148,93	0,22,21	0.985	0.015
	7	Cingulate Gyrus, anterior division, Parahippocampal Gyrus	226	94,91,52	-4,-35,-20	0.975	0.025
	8	Subcallosal Cortex	174	85,89,50	5,-37,-22	0.965	0.035

9	Middle Temporal Gyrus, Superior Temporal Gyrus	156	40,102,65	50,-24,-7	0.961	0.039
10	Right Cerebral White Matter	76	42,105,56	48,-21,-16	0.962	0.038
11	Cerebellum	64	116,44,28	-26,-82,-44	0.955	0.045
12	Temporal Pole, Frontal Orbital Cortex	46	135,151,50	-45,25,-22	0.96	0.04
13	Temporal Pole, Frontal Orbital Cortex	44	121,135,49	-31,9,-23	0.951	0.049
14	Cingulate Gyrus, anterior division	34	86,163,81	4,37,9	0.965	0.035
15	Cingulate Gyrus, anterior division, Paracingulate Gyrus	31	98,142,107	-8,16,35	0.951	0.049
16	Cerebellum	23	124,44,27	-34,-82,-45	0.952	0.048

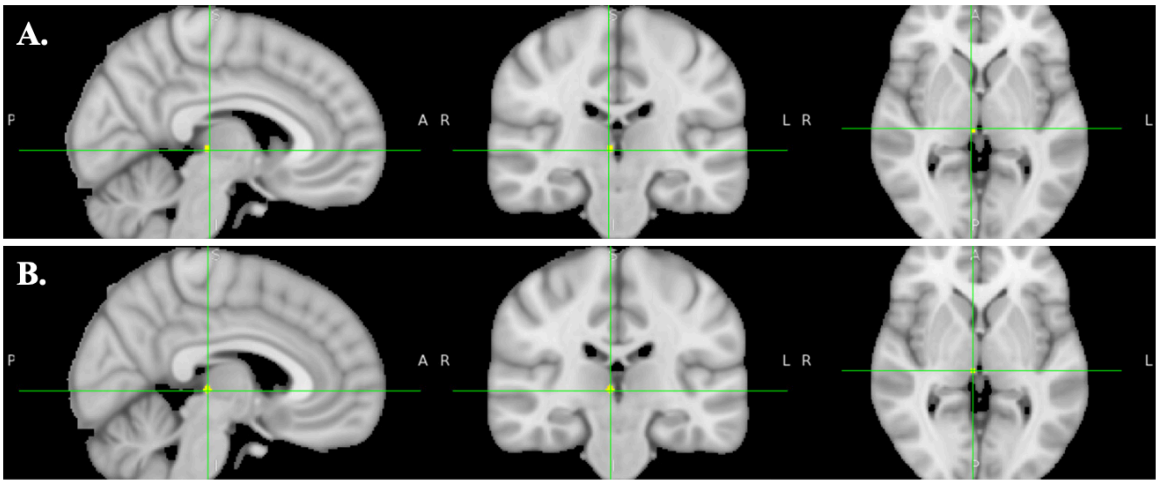
MNI, Montreal Neurological Institute; Neg, negative; Pos, positive. Results were thresholded using threshold-free cluster enhancement (TFCE), $p < .05$, corrected for multiple comparisons (5000 permutations). Note: For win, neutral, and negative vs positive outcomes only clusters with a minimum size of 20 voxels have been included in the table.

3.3.4.2 Right Habenula

Using the right Hb mask, significant task-related activation was observed in the right Hb across several conditions, particularly those involving aversive or negatively valenced stimuli (**Figure 3.3** and **Table 3.4**). During the cue (CS) phase, robust activation was detected for shock CS (29 voxels, peak voxel location: x = 84, y = 103, z = 73, t-max = 4.103, $p < 0.001$), negative CS (27 voxels, peak voxel location: x = 85, y = 103, z = 73, t-max = 3.384, $p < 0.001$), and win CS (10 voxels, peak voxel location: x = 86, y = 101, z = 73, t-max = 2.879, p = 0.021), although the latter was limited to a smaller cluster of 10 voxels. Loss CS and neutral CS elicited weaker effects (p = 1-0.890800 and p = 1-0.907800, respectively). During the outcome phase, the right Hb again showed significant engagement in response to shock (33 voxels, peak voxel location: x = 85, y = 102, z = 72, t-max = 4.349, $p < 0.001$), loss (25 voxels, peak voxel location: x = 86, y = 101, z = 73, t-max = 3.485, p = 0.004), negative (33 voxels, peak voxel location: x = 86, y = 101, z = 73, t-max = 5.154, $p < 0.001$), and negative versus positive outcomes (9 voxels, peak voxel location: x = 86, y = 101, z = 73, t-max = 3.298, p = 0.014), the latter localized to a small cluster of 9 voxels. By contrast, win and neutral outcomes did not elicit significant activation, and positive versus negative outcome contrasts showed no detectable signal (p = 1-0.000000).

Overall, these findings support the engagement of the right Hb in response to aversive and negatively valenced stimuli during both the anticipatory and outcome-related phases of the task.

Figure 3.3 Right habenula (Hb) activation patterns for task-related contrasts



Sagittal, coronal and axial images depicting neural activation during the task using the right Hb ROI mask. Main effect of task: across all groups, the task blocks led to greater BOLD signal response during the shock CS (panel A) and shock outcome (panel B). Notably, right Hb activation was also evident for win CS, negative CS, loss outcome, negative outcome, and negative vs. positive outcome conditions. For full details, see Table 3.4. Images were thresholded using threshold-free cluster enhancement (TFCE), $p < .05$, corrected for multiple comparisons (5000 permutations).

Table 3.4. Right habenula fMRI task activations

Condition	Cluster Index	Cluster size (voxels)	Voxel max (x, y, z)	MNI max (x,y,z)	TFCE-corrected p (MAX)	p value
CS						
Shock	1	29	84, 103, 73	6, -23, 1	1.00	<.001
Neg	1	27	85, 103, 73	5, -23, 1	1.000	<.001
Win	1	10	86, 101, 73	4, -25, 1	0.979	0.021
Outcomes						
Shock	1	33	85, 102, 72	5, -24, 0	1.00	<.001
Loss	1	25	86, 101, 73	4, -25, 1	0.996	0.004
Neg	1	33	86, 101, 73	4, -25, 1	1.00	<.001
Neg vs Pos	1	9	86, 101, 73	4, -25, 1	0.986	0.014

CS, condition stimulus; Neg, negative; Pos, positive. results thresholded using threshold-free cluster enhancement (TFCE), $p < .05$, corrected for multiple comparisons (5000 permutations). Error bars represent the standard error of the mean.

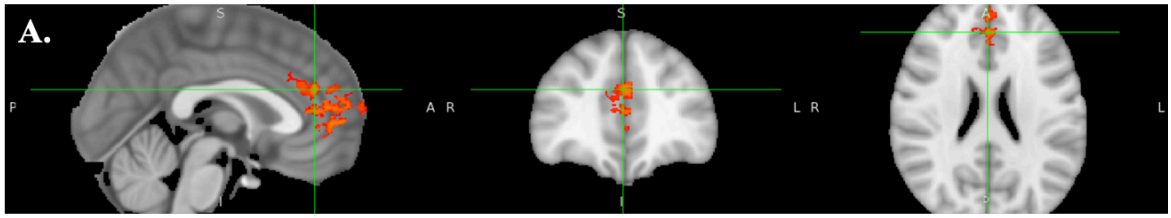
3.3.5 Group differences

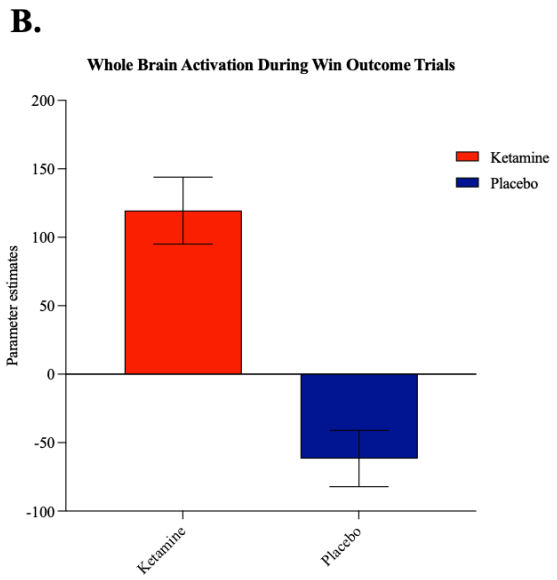
3.3.5.1 Whole Brain

At WB level, a significant cluster of activation was identified for the contrast ketamine > placebo during the win outcome phase (4287 voxels, peak voxel location: x = 89, y = 166, z = 95, t-max = 0.998), with a maximum corrected p-value of p = 1-0.9764 = 0.0236 (**Figure 3.4**; **Table 3.5**). The largest cluster (4287 voxels) showed peak activation at voxel coordinates x = 89, y = 166, z = 95, corresponding approximately to MNI coordinates 1, 40, 23. These results indicate greater BOLD signal in the ketamine group relative to placebo in response to win outcomes. The cluster encompassed the paracingulate gyrus and the anterior division of the cingulate gyrus, which include key medial prefrontal areas such as the dorsal anterior cingulate cortex (dACC), as well as parts of the rostral and pregenual anterior cingulate cortex. In contrast, no significant activation was detected for the reverse contrast (placebo > ketamine), as the maximum corrected p-value did not reach statistical threshold (p = 1-0.0106=0.9894). There were no significant differences between the ketamine and placebo groups during the CS phase, as indicated by non-significant TFCE-corrected p-values across all contrasts (win CS: placebo > ketamine p = 1-0.5794 = 0.42 and ketamine > placebo p = 1-0.6558 = 0.344; loss CS: placebo > ketamine p = 1-0.1808 = 0.819 and ketamine > placebo p = 1-0.6726 = 0.327; shock CS: placebo > ketamine p = 1-0.2052 = 0.795 and ketamine > placebo p = 1-0.8410 = 0.159; neutral CS: placebo > ketamine p = 1-0.6774 = 0.323 and ketamine > placebo p = 1-0.3042 = 0.696; negative CS: placebo > ketamine p = 1-0.0892 = 0.91 and ketamine > placebo p = 1-0.7686 = 0.231; positive versus negative CS: placebo > ketamine p = 1-0.7020 = 0.298 and ketamine > placebo p = 1-0.2078 = 0.792; negative versus positive CS: placebo > ketamine p = 1-0.1926 = 0.807 and ketamine

> placebo p = 1-0.6934 = 0.307). Similarly, in the outcome phase, aside from the win outcome condition reported above, no significant differences were observed between the ketamine and placebo groups across the remaining conditions (loss outcome: placebo > ketamine p = 1-0.0478 = 0.952 and ketamine > placebo p = 1-0.7524 = 0.248; shock outcome: placebo > ketamine p = 1-0.3472 = 0.653 and ketamine > placebo p = 1-0.7974 = 0.203; neutral outcome: placebo > ketamine p = 1-0.0514 = 0.949 and ketamine > placebo p = 1-0.843 = 0.157; negative outcome: placebo > ketamine p = 1-0.1242 = 0.876 and ketamine > placebo p = 1-0.8276 = 0.172; positive versus negative outcome: placebo > ketamine p = 1-0.126 = 0.874 and ketamine > placebo p = 1-0.8098 = 0.19; negative versus positive outcome: placebo > ketamine p = 1-0.8230 = 0.177 and ketamine > placebo p = 1-0.1376 = 0.862).

Figure 3.4 Effects of ketamine compared to placebo on whole brain activation during win outcome





A main effect of treatment revealed that ketamine was associated with greater activation in the paracingulate gyrus and the anterior division of the cingulate gyrus compared to placebo (panel A). Additionally, a smaller cluster (n = 5 voxels) showing a similar effect was identified in the left thalamus (data not shown). The top image was thresholded using threshold-free cluster enhancement (TFCE), $p < 0.05$, corrected for multiple comparisons (5000 permutations). Parameter estimates extracted from the most significant cluster for win outcome trials versus baseline (panel B). Error bars represent the standard error of the mean.

Table 3.5. Effect of ketamine compared to placebo on whole brain fMRI activation during the win outcome

Cluster Index	Brain areas	Cluster size (voxels)	Voxel max (x, y, z)	MNI max (x,y,z)	TFCE-corrected p (MAX)	p value
1	Paracingulate Gyrus, Cingulate Gyrus, anterior division	4287	89,166,95	1,40,23	0.976	0.0236
2	Left Thalamus	5	99,111,86	-9,-15,14	0.951	0.049

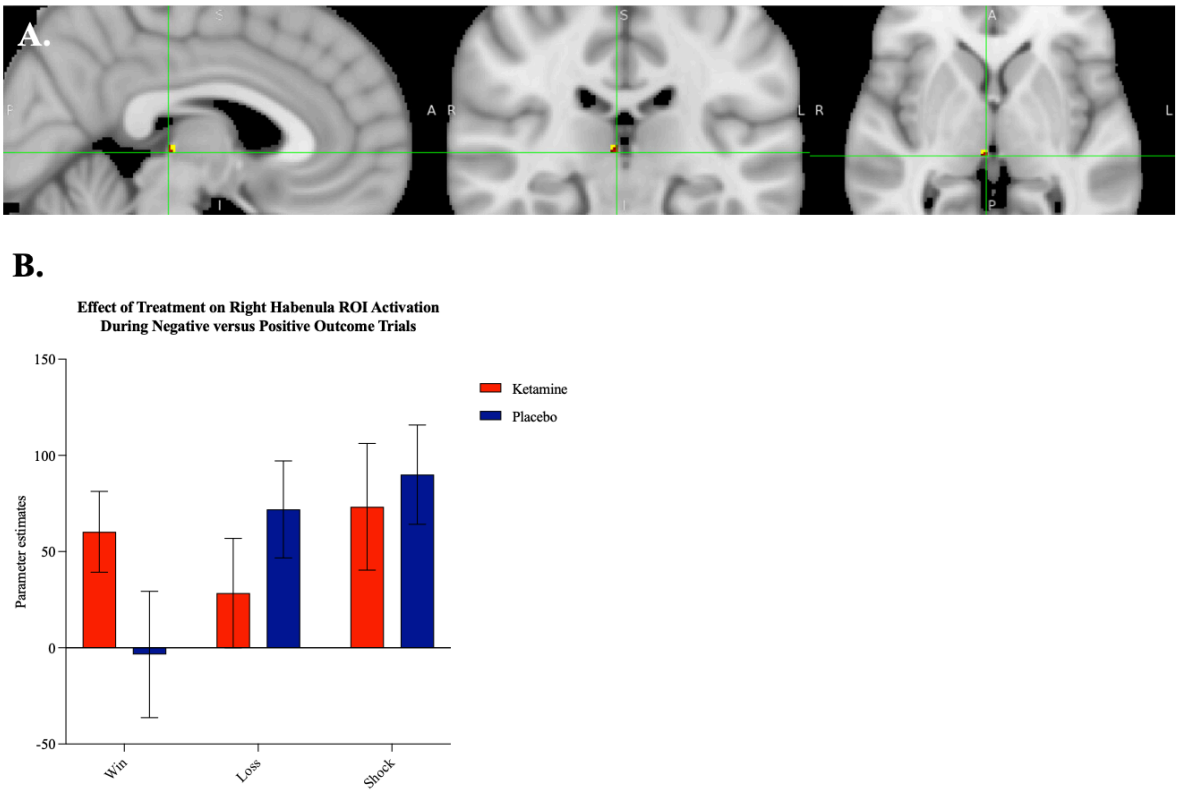
Results were thresholded using threshold-free cluster enhancement (TFCE), $p < 0.05$, corrected for multiple comparisons (5000 permutations).

3.3.5.2 Right Habenula

When analysing the right Hb ROI, a statistically significant cluster was observed for the negative versus positive outcome contrast in the placebo group compared to ketamine (23 voxels, peak voxel location: x = 85, y = 104, z = 74, t-max = 2.957; **Figure 3.5**). The peak corrected p-value was p = 1-0.991 = 0.009 and the significant cluster comprised 23 voxels, with peak activation at voxel coordinates x = 85, y = 104, z = 74. Additionally, the contrast win outcome vs baseline showed numerically higher activation in the ketamine group compared to placebo; however, this difference did not reach statistical significance (p = 1-0.9490 = 0.051). Finally, a small cluster of activation (1 voxel) was identified during the shock CS in the placebo group compared to ketamine *(*1 voxel, peak voxel location: x = 85, y = 101, z = 75, t-max = 2.637). The peak corrected p-value was p = 1-0.954 = 0.046, with the voxel located at coordinates x = 85, y = 101, z = 75. While the area was minimal in size, this result suggested a trend toward reduced right Hb engagement in response to aversive

cues in the ketamine group compared to placebo. Of note, the reverse contrast (ketamine > placebo) was not significant p = 1-0.405 = 0.595).

Figure 3.5 Effects of treatment on right habenula ROI activation during negative versus positive outcomes



A main effect of treatment revealed that placebo was associated with greater activation in the right habenula (Hb) region of interest (ROI) compared to ketamine for the contrast negative versus positive outcomes. The area of activation is shown in red, overlaid with the right Hb mask in yellow. The top image (panel A) is thresholded using threshold-free cluster enhancement (TFCE), $p < 0.05$, corrected for multiple comparisons (5000 permutations). Parameter estimates extracted from significant cluster for each condition versus baseline (panel B). Error bars represent the standard error of the mean.

3.4 Discussion

This chapter aimed to test the effects of a single infusion of ketamine on lateral Hb activity in healthy volunteers, 24 hours post-infusion. This is the first experimental test of the

translation of the rapid reduction in lateral Hb activation reported following ketamine administration in animal models of depression. Within the right Hb ROI, greater activation was observed in the placebo group compared to ketamine for the negative versus positive outcome contrast, suggesting attenuated right Hb engagement following ketamine. Furthermore, greater activation in the right Hb in response to shock CS was observed in the placebo group compared to the ketamine group, although this effect was limited to a small area. Additionally, there was a trend toward increased right habenula activation in the ketamine group during win outcomes relative to placebo, albeit not statistically significant. At the whole-brain level, ketamine was associated with greater activation than placebo in medial prefrontal regions, including the paracingulate and anterior divisions of the cingulate gyrus, during win outcomes. These findings provide preliminary support for ketamine's modulation of the Hb during aversive processing and offer further evidence of its effects on prefrontal circuitry during reward processing in humans.

Relatively few studies have examined the role of the Hb in the context of MDD, partially due to the challenges associated with imaging this small structure and distinguishing it from adjacent regions such as the thalamus - a limitation that can be partially addressed through the use of high-field MRI. Furthermore, most research to date has focused on resting-state functional connectivity (Cameron et al., 2024), with fewer studies investigating Hb activity during task-based fMRI paradigms (Furman & Gotlib, 2016; Lawson et al., 2017; Lawson et al., 2014; Willinger et al., 2022). However, Hb responses to aversive stimuli have previously been shown to be abnormal during a Pavlovian conditioning task involving monetary loss and electric shocks in MDD (Lawson et al., 2017). In this study n = 25 MDD subjects and n = 25 healthy controls underwent task-based 3 Tesla fMRI to investigate the role of Hb in depression. While healthy controls showed increased Hb activation as

conditioned stimuli became more strongly associated with electric shock, MDD participants exhibited a significant decrease in Hb activation, potentially reflecting a reduced capacity to avoid negative stimuli in depression.

In our study, the right Hb showed greater activation in the placebo group compared to ketamine for the negative versus positive outcome contrast, suggesting attenuated right Hb engagement under ketamine. Only a few studies have directly assessed the impact of antidepressant treatment on the Hb. One PET imaging study found that pre-treatment serotonin transporter (SERT) availability in the Hb predicted treatment response to SSRIs in MDD (Lanzenberger et al., 2012). Another study using structural MRI assessed habenula volume changes following 3 months of venlafaxine treatment in 50 antidepressant-free MDD patients, revealing a significant increase in total Hb volume (particularly in the left Hb) though no direct association was found between volumetric changes and clinical response (Etienne et al., 2024). Of particular relevance, a recent study by Salas et al. (2021) examined the effects of ketamine on Hb connectivity in 35 patients with MDD. Improvements in depressive symptoms were associated with increased functional connectivity between the right Hb and several cortical regions, including the frontal pole, occipital pole, temporal pole, parahippocampal gyrus, and lateral occipital cortex. While these findings suggest a possible role for the Hb in mediating ketamine's antidepressant effects, it remains unclear whether these changes are specific to ketamine or could be reflective of a general antidepressant response. Moreover, the absence of a control group (placebo or healthy volunteers) and the limited spatial resolution of standard 3T MRI in accurately delineating the Hb constrain the strength of these conclusions.

At the WB level, greater activation was observed in the medial prefrontal cortex (PFC), dACC and pgACC in the ketamine group compared to placebo during win outcomes. This pattern aligns with a growing body of literature showing ketamine's capacity to normalize depression-related dysconnectivity in prefrontal and striatal networks. The ACC - particularly its dorsal and subgenual subregions - has emerged as a key hub in ketamine's mechanism of action, showing both rapid and sustained changes in activity following administration (Alexander et al., 2021). These modulations have also been linked to ketamine's ability to ameliorate reward-processing deficits. For example, increased dACC activity within hours of ketamine infusion has been associated with improvements in anhedonia (Lally et al., 2014; Lally et al., 2015), while reductions in sgACC overactivation during reward anticipation have similarly been related to symptom improvement (Morris et al., 2020). Of note, a recent study investigated the effects of ketamine compared to placebo in individuals with treatment-resistant depression (TRD; n = 29) and healthy controls (n = 21), with a specific focus on ACC subregions. These findings suggest that forty-eight hours post-infusion, ketamine induced differential changes in resting-state functional connectivity between ACC subregions and regions such as the right insula and the ventromedial PFC, especially in TRD. Importantly, these changes in functional connectivity were significantly associated with improvements in anhedonia, highlighting a potential neural mechanism underlying ketamine's therapeutic effects (Alexander et al., 2023).

Notably, the ACC - particularly the sgACC and pgACC subregions - projects directly to the lateral Hb (Yang et al., 2021). A growing body of preclinical research suggests a complex functional interplay between the ACC and lateral Hb, particularly in mediating behavioural adaptation following negative outcomes. For instance, during reversal learning tasks in macaques, phasic lateral Hb activity is sensitive to immediate 'no reward' outcomes,

whereas tonic activity in the pg/dACC reflects integrated outcome information over several trials (Kawai et al., 2015). These two structures appear to have distinct roles while the lateral Hb is more reactive, the ACC encodes learning and behavioural shifts. In sum, both the Hb and ACC could play complementary roles in monitoring and adjusting behaviour in response to negative feedback - a mechanism that may underlie their shared relevance in depression and its treatment with ketamine (Alexander et al., 2021).

Finally, there was a trend toward lower subjective pain ratings immediately prior to the scan in the ketamine group compared to the placebo group. Notably, the ACC is rich in opioid receptors. This observation aligns with growing interest in the role of opioid signalling in mediating ketamine's antidepressant effects. Although ketamine's direct affinity for opioid receptors is limited and likely insufficient to fully explain its antidepressant properties (Sanacora, 2019), opioid pathways have nonetheless emerged as potential contributors. Two small double-blind randomized controlled trials reported that pre-treatment with naltrexone (an opioid receptor antagonist) blocked ketamine's antidepressant effects in TRD subjects (Jelen, 2024; Williams et al., 2018), supporting the possibility of opioid system involvement. However, given the limited sample sizes and unresolved questions regarding whether ketamine acts directly or indirectly on opioid receptors, these findings warrant cautious interpretation. Further, the ACC's involvement remains speculative; while it is a central node in pain processing, it also plays key roles in reward, emotion regulation, and decision-making (Hiser & Koenigs, 2018), all of which may mediate ketamine's therapeutic effects. Future research is needed to delineate the contribution of opioid signalling - and specifically in the context of the ACC - to ketamine's mechanisms of action in alleviating depressive symptoms.

3.5 Limitations

The current study has several limitations that should be acknowledged. First, these analyses were completed in the time constrains of a DPhil submission and represent only an initial analysis of this dataset. Future analysis including sensitivity analysis for motion correction and Hb manual segmentation should be completed. Specifically, the use of a right Hb mask derived from previously published publications was used for analysis rather than manual segmentation of the Hb individually from each participant's anatomical scan. Although this approach ensures consistency, it may reduce spatial specificity given the habenula's small size and anatomical variability. Similarly, no participants were excluded based on motion, given the exploratory and hypothesis-generating nature of the analysis. While this approach maximized sample retention, it may have introduced variability due to motion-related artefacts. Furthermore, this dataset may benefit from a computational modelling approach. By generating trial-by-trial estimates of latent cognitive processes, including prediction error and learning rate, it may enable a more nuanced characterisation of how individuals learn from and adapt to feedback over time. This approach could be especially valuable when investigating the effects of pharmacological interventions, as it can reveal subtle changes in reward learning and belief updating that may underlie a drug's rapid antidepressant properties.

Finally, the use of a healthy participant sample limits the ability to draw conclusions about ketamine impact on Hb function as a mediator of its antidepressant effect. As such, it was not possible to assess whether ketamine-induced changes in right Hb activation are directly related to its antidepressant or anti-anhedonic effects. Furthermore, while preclinical models often describe the Hb as hyperactive in depression, human data suggest that Hb activity may

in fact be reduced in individuals with MDD (Lawson et al., 2017). This discrepancy underscores the importance of clarifying whether ketamine restores normative Hb functioning in depression or exerts its effects through modulation of broader neural circuits involving the Hb - such as those connecting with the ACC.

3.6 Conclusion

In conclusion, this study provides preliminary evidence that ketamine, when administered 24 hours prior, attenuates right Hb activation in response to negative versus positive outcomes compared to placebo. Additionally, and in line with existing literature, ketamine was associated with increased activation in medial prefrontal regions, including the dACC and pgACC, relative to placebo. These findings represent the first attempt to translate preclinical data into humans, supporting the hypothesis that ketamine's rapid antidepressant effects may be mediated, at least in part, by inhibition of habenula activity. Future research in clinical populations is warranted to confirm and extend these findings, and to further understand the neural mechanisms underlying ketamine's rapid therapeutic action.

Chapter 4. An Experimental Medicine Study on the Prophylactic Effect of Ketamine on Laboratory-Induced Stress in Healthy Volunteers

4.1 Introduction

The concept of stress resilience has evolved from a 'passive' notion - defined as the absence of psychopathology following stress - to an 'active' and multifaceted framework (Southwick et al., 2023). It is now broadly characterised as the capacity to adapt effectively, maintain functioning after adversity, and withstand stress without developing clinically significant symptoms (American Psychological Association, 2018). However, the cognitive neuroscience underlying resilience, as well as current strategies to enhance it in the context of psychiatric disorders, remains limited, with existing interventions predominantly focusing on psychotherapy and exercise (McEwen, 2016; McKercher et al., 2014; Reynolds, 2019).

As outlined in Chapter 1, recent preclinical studies provide compelling evidence that ketamine, administered one week before a stressor, can block the development of depression- and anxiety-like behaviours in rodent models (Brachman et al., 2016). These findings marked the first demonstration that a pharmacological agent can provide long-term prophylactic protection against stress-induced depression or anxiety. In this study, animals pre-treated with ketamine or saline underwent chronic social defeat (CSD) procedures, and behaviour was assessed two weeks later using the Forced Swim Test (FST) and the Dominant Interaction (DI) social interaction test. Ketamine-treated mice exhibited significantly reduced immobility during the FST and increased social exploration compared to saline-treated mice, indicating a blockade of the pro-depressive effects of CSD and

enhanced stress resilience. While additional preclinical studies further support these findings (Amat et al., 2016; McGowan et al., 2017), evidence remains limited regarding whether ketamine's pro-resilient effects observed in animal models can be effectively translated to humans. A recent small, randomized, placebo-controlled proof-of-concept study aimed to translate these findings into humans by testing whether ketamine could mitigate behavioural and physiological responses to an acute stressor when administered one week prior (Costi et al., 2023). In this study, 24 healthy participants received either ketamine or midazolam before undergoing the Trier Social Stress Test (TSST) (Allen et al., 2014; Kirschbaum et al., 1993). While no statistically significant differences in stress-related behavioural or biological outcomes were observed, ketamine demonstrated a moderate-to-large effect in reducing anxiety (Cohen's d = 0.7). Additionally, in a subset of subjects who exhibited the expected correlation between plasma and salivary cortisol, ketamine significantly reduced salivary alpha-amylase levels. These findings suggest potential resilience-enhancing effects through modulation of the autonomic nervous system that warrant further investigation in larger studies. Further, ketamine's potential as a prophylactic agent has also been investigated in the context of postpartum depression (PPD). Recent studies (Alipoor et al., 2021; Han et al., 2022; Ma et al., 2019; Wang et al., 2024; Yao et al., 2020) explored the effects of ketamine administered during scheduled Caesarean sections to prevent mood and anxiety disorders following childbirth. These studies indicate that ketamine may help prevent the onset of PPD symptoms when administered pre-emptively, but further research is needed to confirm its efficacy and safety in this population.

This chapter presents findings from an experimental medicine study investigating whether ketamine can attenuate subjective and physiological stress responses in healthy volunteers when administered one week prior to stress exposure. Informed by preclinical rodent studies

and emerging human research, this translational study explores glutamate modulation as a potential strategy for managing stress-related disorders and enhancing resilience. The study employed the Oxford Cognition Stress Task (OCST), a computer-based paradigm designed to induce mild-to-moderate stress, while collecting subjective and physiological responses. Changes in self-reported mood, anxiety, and stress served as subjective outcomes. Biological endpoints included: (1) salivary cortisol concentrations as a marker of hypothalamic-pituitary-adrenal (HPA) axis activity; (2) salivary α-amylase levels (sAA); and (3) heart rate variability (HRV), both reflecting autonomic nervous system (ANS) activity. These measures were compared between participants who received ketamine and those given placebo one week prior undergoing the OCST. We hypothesized that ketamine would attenuate self-reported stress and negative affect while reducing cortisol, sAA, and HRV levels compared to placebo, reflecting its potential impact on stress reactivity and autonomic regulation when administered one week prior to an acute stress (OCST).

4.2 Methods

4.2.1 Study Participant and design

A detailed description of screening and infusion procedures is provided in Chapter 2. This randomised, double-blind, placebo-controlled study was conducted at the University of Oxford's Department of Psychiatry between July 2021 and June 2023 and registered on ClinicalTrials.gov (NCT04850911). Ethical approval was granted by the University of Oxford Central University Research Ethics Committee (R73654/RE001), and all participants provided written informed consent.

The study included adults aged 18 to 45 years without a lifetime DSM-5 psychiatric diagnosis. Eligibility required a BMI of 18–30, English proficiency, and adherence to safety protocols (e.g., alcohol abstinence, avoiding machinery use post-infusion). Exclusion criteria included psychiatric or medical conditions, pregnancy, recent recreational drug use, and prior ketamine or phencyclidine exposure.

Screening involved a medical and psychiatric history review, laboratory tests, toxicology screening, and pregnancy testing. Eligible participants were randomized within four weeks to receive ketamine (0.5 mg/kg) or placebo (0.9% saline).

On infusion day, eligibility was re-verified, medications reviewed, and urine tests conducted (toxicology and pregnancy, if applicable). Participants underwent a closely monitored infusion, followed by two hours of post-infusion observation before discharge under medical supervision.

Seven days after the infusion visit, participants returned to the Department of Psychiatry at the Warneford Hospital to complete the stress/exit visit.

4.2.2 Psychological Stress Task: The Oxford Cognition Stress Task

The OCST is a computer-based laboratory paradigm delivered through a website. It is designed to induce psychosocial stress by requiring participants to complete difficult mathematical, verbal, and visuospatial cognitive tasks under time pressure. Inspired by established stress-induction protocols such as the TSST, the OCST offers several advantages: it is simpler to administer, more cost-effective, and provides enhanced control

over contextual variables. Additionally, its computerized format increases flexibility and eliminates the need for confederates, streamlining the experimental process.

The task consists of a series of challenges designed to test mental arithmetic, verbal anagram solving, and visuospatial search abilities. In the mental arithmetic component, participants are asked to solve mathematical exercises that are displayed on the screen. For the verbal anagram task, a series of 11 anagrams is presented and participants are asked to rearrange jumbled letters to form meaningful English words. In the visuospatial search task, participants are required to locate a target (blue "T") within an array of distractors (blue "L" and green "T"). Each task increases in difficulty as participants progress. Participants first complete 10 practise mental arithmetic trials with an unlimited duration to respond. Following the practise trials, participants complete the following tasks in the following order: 3 minutes of mental arithmetic tasks, 11 anagrams (maximum of 30 seconds to complete each one), 3 minutes of mental arithmetic tasks, 5 minutes of visual search tasks, 3 minutes of mental arithmetic tasks.

To induce a state of uncontrollability, known to increase stress response, a high failure rate (less than 50% accuracy across the task) is enforced. The mental arithmetic questions can be presented at five difficulty levels. In Levels 1 and 2, two or three single-digit integers are used respectively, with addition and subtraction operators. Level 3 and level 4 questions involve multiplication of two single-digit integers and addition or subtraction of a two-digit integer or two single-digit integers. Level 5 questions comprise the multiplication, addition, or subtraction of two single-digit integers, from which the product of two further single-digit integers and two two-digit integers are added/subtracted. The visual search challenges can

be presented at four difficulty levels corresponding to distractor arrays of increasing size: 5*5, 6*6, 7*7, and 8*7.

Further, the OCST algorithm varies task timing and difficulty to be just beyond participant's abilities. Performance on the practice trials is used to set the initial task timing; on the first set of mental arithmetic tasks, participants are given 2% less time than their average practice trial response time and task difficulty is set to Level 1. The percentage of test questions answered correctly is monitored over the preceding 5 trials. Time allowed is varied in 2% increments according to performance (decreased if achieving >40% correct, increased if <20% correct) up to a maximum of 10 increments, at which point the difficulty level is increased/decreased. In the visual search component, each level has an initial time allowed which is incrementally altered by 5% depending on performance, up to a minimum/maximum at which point the difficulty level is altered. In order to ensure a high failure rate on the anagram task, 6 of the 11 anagrams are impossible to solve. Across all tasks, time constraints are made salient by the presentation of a time bar on each trial, accompanied by a ticking clock sound.

Finally, the OCST includes elements of social evaluative threat. An introductory instructional video informs the participant that their results will be used to produce a cognition profile with their strengths and weaknesses compared to the general population. This video is designed to produce the expectation of high performance: participants are led to believe task difficulty is tailored to their ability based on their answers to a set of pre-test questions, and are informed that most individuals achieve a 70–90% accuracy rate throughout the task. Participants are given on-screen and audio feedback which indicates they are performing badly, comprising an angry face and an aversive sound on incorrect

trials, reinforcing the perception of poor performance. At the end of each phase, participants are shown their results, which included their score and a comparison to the high accuracy rates typical of a (fictitious) general population.

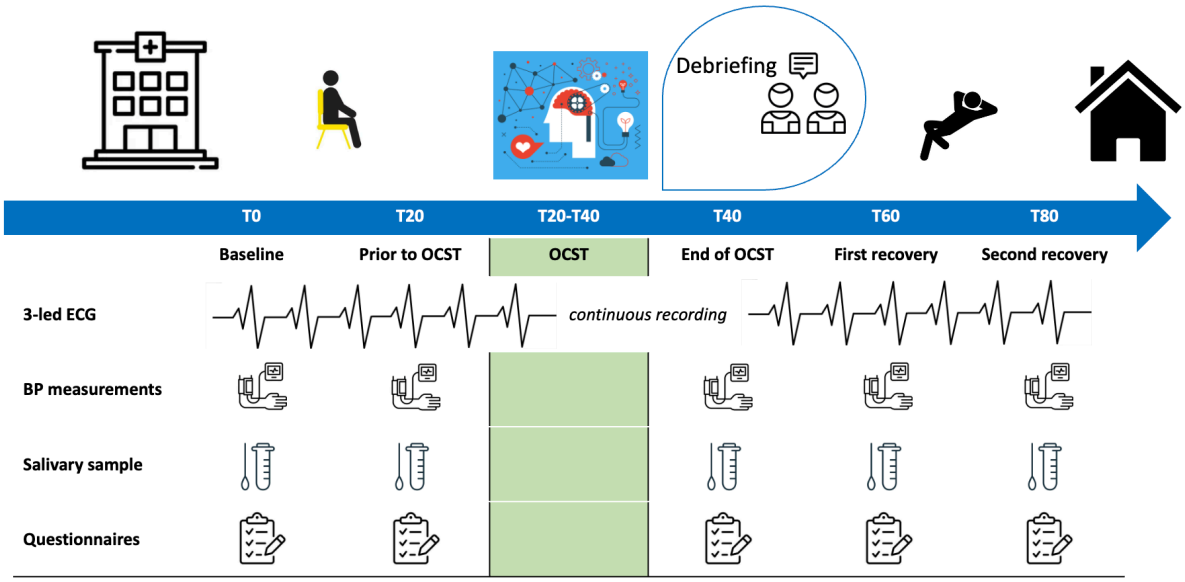
Participants were initially informed that the study aimed to explore the relationship between problem-solving abilities and physiological and hormonal responses. They were briefed on their upcoming activities, which included tasks designed to assess mathematical ability, verbal intelligence through anagram solving, and visual search skills by locating a target among distractors.

Physiological measures, including saliva samples (for cortisol and amylase analysis), were taken at baseline, during the task, and after task completion, while a three-lead electrocardiogram (ECG) continuously monitored cardiac activity throughout the visit. To ensure data accuracy, participants were instructed to minimise movement during the task, as the measurements were highly sensitive to motion. Due to the collection of saliva samples, participants were also advised that drinking would not be permitted during the task. A schematic representation of the study visit, detailing the timing and type of measures collected, is presented in **Figure 4.1**.

Before starting, participants provided information about factors that could influence physiological responses, including caffeine consumption, recent sleep duration, timing of their last meal, recent cigarette use (if applicable), and their highest mathematics qualifications.

At the end of the test session, participants were debriefed and informed of the true aim of the task. They were informed that the tasks were intentionally designed to be beyond their abilities, and that comparisons to the general population's performance were fabricated. They were also reassured that no video recordings had been made. This debriefing ensured ethical transparency and provided participants with a clear understanding of the experimental design.

Figure 4.1 Schedule of event during the stress/exit visit



Overview of the procedures during the stress/exit visit with stress parameter sampling times. T0 = starting of the baseline rest; T20 = immediately prior to the stress procedure; T40 = immediately after the end of the stress procedure; T60 = 20 minutes after the end of the stress procedure; T80 = 40 minutes after the end of the stress procedure and discharge. BP = Blood Pressure; OCST = Oxford Cognition Stress Task; Salivary samples collected for analysis of salivary cortisol and alpha amylase; Questionnaires included the Positive and Negative Affect Schedule (PANAS), the State-Trait Anxiety Inventory-State (STAI-S), and visual analogue scales (VAS). Note that the timeline is not to scale.

4.2.2.1. Subjective psychological measures of stress

Participants completed self-report measures assessing positive and negative affect, and anxiety levels. These questionnaires were administered digitally using Qualtrics software.

For this analysis, the following instruments were utilised: the Positive and Negative Affect Schedule (PANAS) (Watson, 1988), the State-Trait Anxiety Inventory-State version (STAI-S) (Spielberger, 1983), and visual analogue scales (VAS) (Aitken, 1969; Huskisson, 1974). A description of these questionnaires is included in Chapter 3. The VAS included the following descriptors: happy, sad, hostile, alert, stressed, calm, and anxious. Questionnaires were administered at five time points during the visit: upon arrival (t0), before the stress task (t20), immediately after the stress procedure (t40), and during the recovery period at 20- (t60) and 40-minutes post-task (t80). **Figure 4.1** summarises the procedures and measurements obtained at the specific time points during the stress/exit visit. On screening and stress/exit visit, participants completed also the Beck Depression Inventory (BDI) (Beck et al., 1961) and Perceived Stress Scale (PSS) (Cohen et al., 1983).

4.2.2.2. Physiological measures

Various stress-related parameters were assessed throughout the visit (see **Figure 4.1** for an overview). Salivary was collected using Sarstedt Salivettes (Hellhammer et al., 2009), and later analysed for cortisol, a biomarker of HPA axis activity. Alpha-amylase concentration, a marker of ANS activation, was also measured from the same saliva samples (Nater & Rohleder, 2009). Additionally, HRV, another key marker of ANS activity, was derived from a 3-electrode ECG recording using the Biopac BSL MP46 2-Channel System. HRV is widely utilised as a physiological measure of stress response, as it serves as a non-invasive marker of beat-to-beat variation in heart rate, which provides information about the autonomic control of the heart rate (Berntson et al., 1997). Short-term recordings (e.g., 5-minute intervals) primarily reflect parasympathetic nervous system activity, making HRV a reliable indicator of autonomic regulation under stress.

Salivary hormonal collection

The stress/exit visit was scheduled for late morning to early afternoon to align with the cortisol circadian rhythm (Kirschbaum et al., 1996; Pace et al., 2006). To minimise potential confounding effects of food, smoking, and alcohol on hormonal measurements, participants were instructed to refrain from eating for 30 minutes before the visit, avoid smoking for at least 2 hours beforehand, and abstain from alcohol for 24 hours prior to the session (Allen et al., 2014).

Salivary samples were collected using the Sarstedt Cortisol Salivette system, which employs synthetic swabs. Collection time points included baseline (t0), prior to the practice questions (t20), at the end of the OCST (t40), and at the conclusion of the two resting periods (20- and 40- minutes post-task). At the end of the visit, samples were centrifuged at 1000 x g for 2 minutes at ambient temperature and then stored at −20 °C until analysis at until the end of the study. Salivary cortisol and alpha-amylase levels were measured using commercially available immunoassay kits. Cortisol levels were assessed using the Salimetrics assay kit (Cat. No. 1-3002, Lot 2309515, Exp. 2025-06-01). Each sample was analysed in duplicate, with final values calculated as the average of the two measurements. Results originally reported in µg/dL were converted to nmol/L and subsequently log-transformed for data analysis. A sustainable stress response (responder) was defined as a cortisol increase of at least 1.5 nmol/l from baseline to the end of the OCST (t40), according to Miller et al. (2013). sAA activity was measured using the Salimetrics kinetic enzyme assay kit (Cat. No. 1-1902, Lot 2310516, exp. 2025-11-30). Results were run in duplicate and initially reported in U/ml and converted to SI units (nKat/L) by multiplying by 0.01667. According to the manufacturer, sAA activity ranges from 3.1 to 423.1 U/ml. Samples with values below 2 U/ml were considered unreliable and excluded from subsequent sensitivity analyses.

Heart rate and heart rate variability measurements

Heart rate measurements were collected using the Biopac BSL MP46 2-Channel System (Biopac Systems, 2021). Three electrodes were attached to the right and left clavicle and on the right leg, above the ankle. To minimise movement artefacts, the electrodes were secured with a micropore tape, and participant were instructed to keep their feet flat on the floor (not crossed) and to avoid moving their shoulders. ECG data were recorded continuously from the start of the baseline period until 40 minutes after the task concluded. The ECG data was acquired using the Biopac Student Lab software.

Heart-related physiological data cleaning and analysis were performed using Kubios HRV Scientific software version 4.1.0 (2023). Raw ECG data underwent visual inspection for artifacts, and an automatic artifact correction algorithm within Kubios HRV was applied (Tarvainen et al., 2014). The final datasets included in the analysis had less than 5% artifacts. HRV analysis adhered to recommendations by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Electrophysiology, 1996). The primary time-domain measure analysed was the root mean square of successive differences (RMSSD), which estimates vagally mediated changes in HRV (Malik, 1996). Additional analysed variables included frequency-domain measures such as the high-frequency (HF) band (0.15–0.4 Hz), indicative of parasympathetic activity and related to respiratory cycles, and the low-frequency (LF) band (0.04–0.15 Hz), reflecting both parasympathetic and sympathetic contributions, as well as baroreflex activity. The LF/HF ratio was also calculated. For clarity and consistency, these values were then averaged into 5-minute segments for each period of the visit (rest, stress exposure, and recovery).

4.2.3 Statistical analysis

Repeated measures ANOVAs were performed to analyse stress responses across various parameters, including cortisol, sAA, HR, RMSSD, and self-report scales, with treatment group (placebo, ketamine) included as a between-subject factor. When the assumption of sphericity was violated, Greenhouse-Geisser corrections were applied to adjust for the deviation. Differences at specific time points were analysed using independent *t*-tests.

The statistical analyses were performed using IBM SPSS Statistics version 28.0 (IBM, 2021). The significance level was set at $\alpha = 0.05$. Given the exploratory nature of this study, no formal adjustments for multiple testing were applied, as secondary analyses were considered hypothesis-generating. The analysis reported in this chapter include only the subjects that completed the stress/exit visit.

4.3 Results

4.3.1 Sample Characteristics

A detailed description of the recruited sample is provided in Chapter 2. Of the enrolled subjects, a total of 66 participants completed the OCST, with 33 receiving ketamine and 33 receiving placebo. The sample comprised 35 males and 31 females, with a similar gender distribution across the ketamine and placebo groups. Approximately 80% of the sample identified as White/Caucasian, and 10% as Asian. The two groups were also comparable in age (mean = 23.58, SD = 4.9) and years of education (mean = 17.09, SD = 2.7). Reasons for

not completing the stress/exit visit included two participants who declined to participate, one who was lost to follow-up, and one who tested positive for COVID-19 between the infusion visit and the stress induction visit (one week post-infusion).

4.3.2 Subjective Symptom Change

No significant differences were observed between the ketamine and placebo groups across various baseline and pre-infusion measures, as assessed through self-reports, in the cohort of participants who completed the stress/exit visit. At 24 hours post-infusion, no statistically significant differences were observed between the ketamine and placebo groups across most psychological measures. Notably, for the calm item on the VAS, the ketamine group reported higher levels of calmness (mean = 70.36, SD = 21.97) compared to the placebo group (mean = 61.61, SD = 17.56) following the scan procedures 24 hours after the infusion (see Chapter 2 for more details). However, although the difference approached statistical significance [$t(64) = -1.785$, $p = 0.079$], it did not reach the threshold for significance. Finally, no significant differences were observed between the groups across these measures at the beginning of the stress/exit visit.

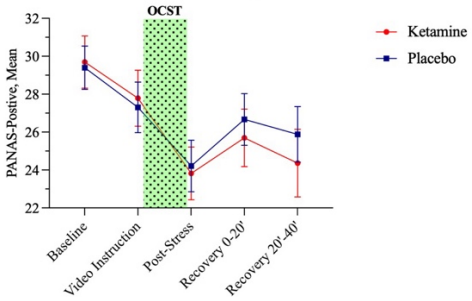
On the stress/exit visit day, repeated-measures ANOVA revealed significant main effects of time on all measures, including STAI-S [$F(2.554, 163.432) = 106.582$, $p < 0.001$], PANAS-Positive [$F(2.749, 175.912) = 22.368$, $p < 0.001$], and PANAS-Negative [$F(1.799, 115.162) = 46.175$, $p < 0.001$]. Specifically, negative affect and anxiety scores increased immediately after the task and returned to baseline during recovery, while positive affect scores decreased, reaching their lowest level immediately after the stress test. However, no significant time x treatment interactions were found for any of the measures [STAI-S:

F(2.554, 163.432) = 1.315, p = 0.272; PANAS-Positive: F(2.749, 175.912) = 0.858, p = 0.456; PANAS-Negative: F(1.799, 115.162) = 0.964, p = 0.377], indicating that changes in these variables over time were not differentially influenced by treatment. A graphical representation of these changes is included in **Figure 4.2**.

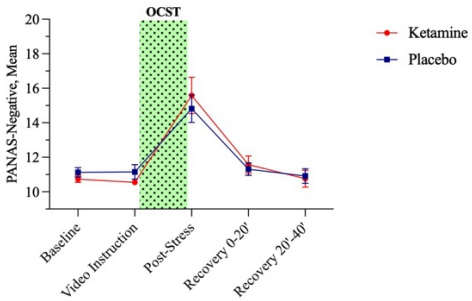
Similarly, significant changes over time were observed for VAS measures of happiness [F(1.857, 117.000) = 67.681, p < .001], sadness [F(1.5, 94.528) = 16.644, p < 0.001], hostility [F(1.548, 97.538) = 19.069, p < 0.001), alertness [F(2.365, 149.005) = 5.331, p < 0.001], stress [F(1.740, 109.645) = 57.519, p < 0.001], calmness [F(2.877, 181.263) = 61.436, p < 0.001], and anxiety [F(1.744, 109.886) = 11.469, p < 0.001]. Despite these changes, no significant interaction effects between time and treatment were found for any measure, suggesting that the patterns of change were similar for both ketamine and placebo groups. Overall, across the treatment groups, an increase in negative affect rating was observed after the OCST, suggesting that participants experienced a heighten subjective perception of stress following the task.

Figure 4.2 Effect of treatment on positive (Panel A) and negative affect symptoms (Panel B)

A. Effect of Ketamine Compared to Placebo on Positive Affects



B. Effect of Ketamine Compared to Placebo on Negative Affects



Change in PANAS Positive (Panel A) and Negative (Panel B) scores during the Stress/Exit Visit in healthy volunteers randomised to ketamine (0.5 mg/kg) or placebo (saline 0.9%) one week prior. PANAS, Positive and Negative Affect Schedule; OCST, Oxford Cognition Stress Task. Note that the timeline is not to scale.

Finally, analyses for the BDI and PSS across the screening and final visit timepoints revealed no significant main effects of time or time x treatment interactions. Specifically, there were no significant temporal changes in BDI scores [$F(1, 64) = 0.283$, $p = 0.597$] or PSS scores [$F(1, 64) = 0.521$, $p = 0.473$), nor any significant time x treatment interactions for BDI [$F(1, 64) = 0.121$, $p = 0.923$) or PSS [$F(1, 64) = 0.081$, $p = 0.776$). These findings further confirm that changes in depressive symptoms and perceived stress over time were comparable between the ketamine and placebo groups.

4.3.3 Biological endpoints

4.3.3.1. Salivary cortisol

Of the 66 subjects who completed the task and collected salivary samples, n=65 had samples for all the 5 datapoints (n = 33 ketamine) of the stress/exit visit. Repeated-measures ANOVA revealed a significant main effect of time [$F(1.981, 124.803) = 4.334$, $p = 0.015$], indicating temporal variations in cortisol levels. However, there was no significant time x treatment interaction [$F(1.981, 124.803) = 0.363$, $p = 0.695$], suggesting that changes in cortisol levels over time were comparable between the treatment groups (see **Figure 4.3**). Subsequent analyses controlling for pre-stress cortisol values (including baseline, OCST video instruction cortisol values, and the combination of both) led to similar results, with the time x treatment interaction remaining non-significant across all models [baseline: $F(2.436, 151.007) = 0.151$, $p = 0.896$; video instruction: $F(2.040, 126.458) = 0.241$, $p = 0.790$; combined: $F(2.115, 128.990) = 0.149$, $p = 0.872$). Finally, given the well-established effect of gender on cortisol levels, a subsequent analysis was conducted, incorporating gender as a covariate within the repeated-measures framework. The results remained consistent, with

no significant interaction between time and treatment [F(1.773, 109.947) = 0.126, p = 0.859)].

Of note, only a subset of participants (n = 11; n = 5 in the ketamine group) showed a sustained stress response, defined as an increase in cortisol levels of at least 1.5 nmol/L from baseline to peak [end of the OCST (t40)], as defined by Miller et al. (2013). This finding suggests that the OCST did not significantly impact the stress response via the HPA axis.

4.3.3.2. Salivary alpha amylase

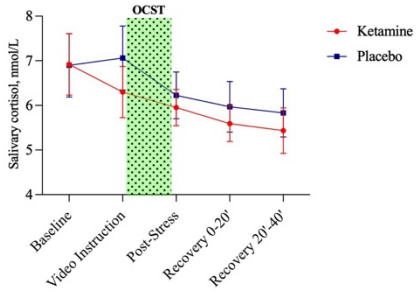
Of the 66 subjects who completed the task and collected salivary samples, n=60 had samples for all the 5 OCST datapoints (n = 31 ketamine). The analysis of sAA across timepoints revealed significant main effects of time [F(3.145, 182.389) = 2.668, p = 0.046], indicating temporal changes in salivary alpha-amylase activity. However, no significant time x treatment interactions were observed [F(3.145, 182.389) = 0.573, p = 0.641], suggesting that the changes over time were comparable between the groups (see **Figure 4.3**). Subsequent analyses controlling for pre-stress sAA values (including baseline, OCST video instruction, and the combination of both) yielded consistent results, with the time x treatment interaction remaining non-significant across all models [baseline: F(3.066, 174.755) = 0.430, p = 0.736; video instruction: F(3.143, 179.161) = 0.569, p = 0.644; combined: F(2.462, 137.869) = 0.506, p = 0.642], suggesting that adjusting for pre-stress values did not influence the overall pattern of results.

Similarly, an analysis excluding participants whose sAA values fell outside the assay kit's reliable range (n = 3 randomised to ketamine, n = 3 randomised to placebo) led to similar

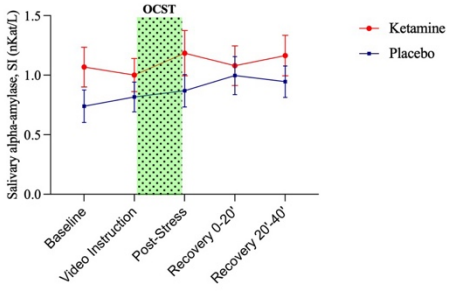
results, with no significant time x treatment interaction observed [F(3.195, 175.702) = 0.976, p = 0.409). Additionally, controlling for pre-stress baseline values produced comparable findings, with no significant time x treatment interaction when adjusting for baseline sAA values [F(3.113, 168.095) = 0.822, p = 0.487), OCST video instruction values [F(3.168, 171.097) = 1.313, p = 0.271), or both combined [F(2.489, 131.925) = 0.945, p = 0.408).

Figure 4.3. Effect of Ketamine Compared to Placebo Administered One Week Prior on Salivary Cortisol (Panel A) and Salivary Alpha Amylase (Panel B) Levels in Healthy Volunteers during the Oxford Cognition Stress Task (OCST)

A. Effect of Ketamine Compared to Placebo on Salivary Cortisol



B. Effect of Ketamine Compared to Placebo on Salivary Alpha Amylase



Change in salivary cortisol (Panel A) and salivary alpha amylase (Panel B) during the Stress/Exit Visit in healthy volunteers randomised to ketamine (0.5 mg/kg) or placebo (saline 0.9%) one week prior. OCST, Oxford Cognition Stress Task. Note that the timeline is not to scale.

4.3.3.3. Heart rate and heart rate variability

Among the 66 participants who completed the stress/exit visit, HRV data were available for analysis from 60 subjects (n = 29 ketamine, n = 31 placebo). A repeated-measures ANOVA for RMSSD, a measure of HRV, identified a significant main effect of time [F(2.237, 129.773) = 9.862, p < 0.001] and a significant time × group interaction [F(2.237, 129.773) = 3.843, p = 0.020], suggesting that HRV changes differed between the groups over time (**Figure 4.4**). A subsequent analysis controlling for baseline RMSSD values corroborated

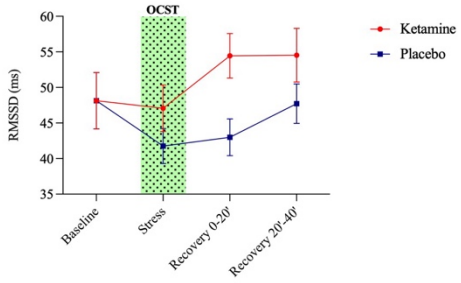
these findings, with a significant time x group interaction remaining evident [F(2.371, 135.138) = 3.404, p = 0.029].

Repeated-measures ANOVAs for other HRV parameters, revealed no significant main effects of time or time × group interactions. For LF values, no significant changes over time were observed (F[1.494, 86.679] = 1.686, p = 0.197), and the time x treatment interaction was also not significant (F[1.494, 86.679] = 0.229, p = 0.730). For HF values, the main effect of time and the time x treatment interaction approached statistical significance [F(1.671, 96.933) = 1.872, p = 0.164; F(1.671, 96.933) = 2.979, p = 0.062, respectively). Finally, for the LF/HF ratio, neither the main effect of time [F(1.937, 118.176) = 1.135, p = 0.324) nor the time x treatment interaction [F(1.937, 118.176) = 1.019, p = 0.385] was significant.

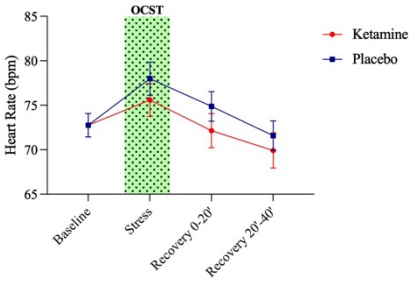
Finally, a repeated-measures ANOVA for heart rate (HR) revealed a significant main effect of time [F(2.318, 134.441) = 33.899, p < 0.001], but was no significant time x treatment interaction [F(2.318, 134.441) = 1.994, p = 0.129], suggesting that the changes in HR over time were not significantly different between the groups.

Figure 4.4. Effect of Ketamine Compared to Placebo Administered One Week Prior On Heart Rate Variability (Panel A) and Heart Rate (Panel B) In Healthy Volunteers During the Oxford Cognition Stress Task (OCST)

A. Effect of Ketamine Compared to Placebo on Heart Rate Variability



B. Effect of Ketamine Compared to Placebo on Heart Rate



Change in heart rate variability (HRV; Panel A) and heart rate (HR; Panel B) during the Stress/Exit Visit in healthy volunteers randomised to ketamine (0.5 mg/kg) or placebo (saline 0.9%) one week prior. OCST, Oxford Cognition Stress Task; RMSSD, Root mean square of successive differences. Note that the timeline is not to scale, and baseline values have been adjusted for graphical representation

4.4. Discussion

The present study investigated the effects of ketamine, administered one week prior, compared to placebo in modulating the stress response during a moderate acute stressor in a laboratory setting. The ultimate goal was to determine whether the pro-resilient effects of ketamine observed in animal models also apply to humans. The psychosocial stress test induced an increase in biological and psychological readout of stress across the whole sample, as documented by self-reports and HRV, respectively. Specifically, there was a significant effect of time and treatment on HRV, as evidenced by reduced RMSSD values in the ketamine group compared to the placebo group, alongside a numeric trend towards higher HF values in the ketamine group in response to a stressor, albeit not reaching statistical significance. This suggests a greater vagal tone and parasympathetic activity during the stress response, indicating that participants randomised to ketamine may have

exhibited better autonomic balance and a reduced physiological stress response. Overall, these findings indicate that participants randomised to ketamine were potentially better able to regulate their stress responses. No significant effects were observed for LF or LF/HF ratio. Other measures of stress, including self-reported assessments and salivary levels of stress hormones such as cortisol and alpha-amylase, did not reveal any significant effects.

Ketamine was first shown to have prophylactic effects against stress-induced behaviours using a CSD stress model (Brachman et al., 2016). In this study, male mice received a single intraperitoneal injection of ketamine (30 mg/kg) or saline one week prior to undergoing a CSD paradigm. Ketamine-treated mice exhibited reduced immobility in the FST, an effect specific to stressed mice, while no behavioural changes were observed in non-stressed mice. Subsequently, although similar protective effects were observed in other models of chronic stress (Camargo et al., 2021; Krzystyniak et al., 2019), the prophylactic benefits appeared to be unique to ketamine (Chen & Denny, 2023). Other treatments, such as fluoxetine and NMDAR antagonists (e.g., memantine), did not produce comparable protective effects when administered prior to the stressor (Brachman et al., 2016; B. K. Chen et al., 2021). Ketamine's protective effects towards stress were also observed using acute stress paradigms. A single dose of ketamine (30 mg/kg) administered one week prior to learned helplessness (LH) or inescapable shock (IS) stress prevented the development of helplessness and stress-induced deficits in social behaviour in mice (Brachman et al., 2016; Zhang et al., 2016). Of note for translational purposes, preclinical studies have shown that the timing of ketamine administration is critical for its optimal prophylactic effects. Maximum efficacy appears to be achieved when ketamine is administered one week prior to the stressor, while administration at earlier (one month) or later time points (one day or

one hour before stress exposure) does not significantly affect behavioural outcomes (McGowan et al., 2017).

These findings also expand previous data from a small randomised, placebo-controlled proof-of-concept study exploring whether ketamine could mitigate the behavioural and physiological effects of stress when administered one week before stress induction in healthy volunteers (Costi et al., 2023). In that study, 24 participants were randomised to receive either ketamine or midazolam before undergoing the TSST. Although no statistically significant differences in overall behavioural or biological stress markers were observed, ketamine demonstrated a moderate-to-large effect in reducing anxiety (Cohen's d = 0.7) and significantly lowered sAA levels in the ketamine group.

In the current study, however, no effects of ketamine on subjective self-rated measures of stress were observed. Additionally, the task did not elicit significant increases in salivary cortisol or sAA, limiting the ability to assess ketamine's effects on these specific stress markers. It is important to note that the overall lack of elevation in these biomarkers may have contributed to the absence of significant effects. Interestingly, studies using the Montreal Imaging Stress Task [MIST; (Dedovic et al., 2005)] - an fMRI-based stress-inducing paradigm similar to the OCST - have reported stronger cardiovascular responses compared to other stress-inducing tasks (Brugnera et al., 2018). The MIST has been associated with a distinct cardiovascular profile, including a significant reduction in SDNN (a measure of overall heart rate variability) during the task compared to baseline and recovery periods. During recovery, all HRV indices typically return to baseline, highlighting the sensitivity of this paradigm to cardiovascular stress responses (Brugnera et al., 2018). These differences in task-induced physiological activation may explain variations in

outcomes across studies and provide insight into the optimal experimental conditions for assessing ketamine's prophylactic effects on stress. Additionally, from a translational perspective, a recent meta-analysis indicates that ketamine (and esketamine) can effectively prevent PPD within the first one to four weeks following Caesarean delivery (Li et al., 2024). Specifically, higher dosages, administration through patient-controlled intravenous analgesia (PCIA), and the use of esketamine are associated with significant reductions in the incidence and severity of depression of PPD during these periods (Li et al., 2024). However, ketamine was also associated with adverse effects, including dizziness, diplopia, hallucinations, and headaches (Li et al., 2024). Although promising, future studies are required to confirm the potential of ketamine as a preventative strategy for PDD. The implementation of standardised protocols is also advisable in order to limit the heterogeneity in administration methods, dosages, and depressive severity. Further, incorporating neurocognitive measures into future studies is advisable to better understand the potential cognitive effects of ketamine in the prevention of PPD. This approach could also inform our understanding of how ketamine impacts key cognitive domains, such as memory, attention, and executive function, which are often impaired in PPD. Evaluating these measures alongside clinical outcomes could provide valuable insights into the neurocognitive mechanisms underlying PPD and ultimately clarify how to foster resilience in at-risk subjects. Finally, replication in different countries is also important to assess the generalisability of these findings, since most studies to date were conducted in a single country (China).

Finally, these findings in healthy volunteers do not support the notion that prophylactic ketamine treatment should be broadly considered as a preventive strategy against the development of stress-related disorders, such as MDD or PTSD, in the general population.

However, if these results are replicated in larger, well-controlled studies, ketamine could emerge as a potential tool to reduce the risk of developing stress-related disorders in high-risk populations. Nevertheless, the current lack of data on the long-term safety and potential side effects of repeated or sustained ketamine use warrants caution (Sanacora et al., 2017). However, aside from the potential clinical applications, this data offers valuable insights into the mechanisms of stress resilience and potentially on the biological processes that differentiate adaptive from maladaptive responses to stress. By further investigating how ketamine modulates key pathways - such as ANS and glutamatergic transmission - this data can contribute to a deeper understanding of the pathophysiology of stress-related disorders. Moreover, it highlights the importance of identifying biomarkers and temporal windows during which interventions like ketamine may be most effective, paving the way for precision-based preventive approaches in psychiatry.

Future studies should also aim to clarify the durability of ketamine's protective effects, optimal dosing schedules, and individual differences in treatment response. Additionally, research into combining ketamine with other resilience-enhancing interventions, such as pharmacotherapies, cognitive-behavioural therapy or lifestyle modifications, may further enhance its prophylactic potential while minimizing side effects and risks in the short and long-term. In conclusion, these findings may support the field of preventive psychiatry, particularly by informing targeted interventions for at-risk populations while enhancing the broader understanding of stress-related psychopathology.

4.5. Limitations

Some limitations of this study should be considered. First, the study enrolled a relatively young and highly educated population, which may limit the generalizability of the findings to broader or more diverse groups.

Second, the task failed to elicit significant increases in key stress biomarkers, such as cortisol and sAA. Furthermore, these biomarkers were measured indirectly through saliva concentrations rather than directly in plasma. Although salivary assessments have been validated as reliable (Hellhammer et al., 2009; Nater & Rohleder, 2009), a potential loss of sensitivity cannot be excluded. Furthermore, sAA was analysed from samples initially collected for salivary cortisol measurement, a procedure that may not provide the optimal conditions for precise and reliable sAA quantification (Bosch et al., 2011).
Additionally, the study design evaluated only a single dose and single time point for ketamine administration, based on findings from animal studies. Future research is needed to determine optimal dosing and timing strategies for ketamine administration relative to stressor exposure in humans.

Finally, the present study did not include a control condition (e.g., a placebo OCST) without a stress-inducing component. While the primary goal of this study was to assess whether ketamine, compared to placebo, could reduce the response to a lab-induced stressor when administered one week prior, including a non-stress control condition in future research would be valuable. This could help clarify individual differences in stress reactivity and better identify the factors contributing to ketamine's pro-resilient effect.

4.6. Conclusions

In conclusion, this randomised, placebo-controlled study evaluated the potential resilience-enhancing effects of ketamine in healthy adults and found that a single dose of ketamine administered one week prior to an acute stressor was associated with a reduced physiological stress response compared to placebo. However, no significant effects were observed on other key stress measures, including self-reported stress assessments and salivary concentrations of stress hormones such as cortisol and alpha-amylase. These mixed findings suggest that while ketamine may influence certain aspects of the physiological stress response, its broader impact on subjective and biochemical stress markers requires further investigation.

To build on these results, larger and more comprehensive randomised controlled trials are recommended to confirm ketamine's pro-resilience effects and clarify the role of glutamatergic modulation in stress prevention. Future studies should also include mechanistic investigations to explore the underlying neurobiological pathways through which ketamine exerts its stress-prophylactic effects, including its modulation of the glutamatergic system, HPA axis and ANS. Additionally, the inclusion of diverse populations, optimal dosing schedules, and varying time points of administration relative to stress exposure could provide a more complete understanding of ketamine potential pro-resilient effects across different contexts and individuals. By addressing these gaps, future research could help understanding the neurocognitive mechanisms of resilience with the ultimate goal of developing targeted interventions aiming at preventing the development of stress-related disorders.

Chapter 5. The Effect of the NMDA Antagonist Ketamine on Negative Biases in Emotional Memories in Treatment Resistant Depression

5.1 Introduction

Depression is characterised by significant disruptions in autobiographical memory, including impaired recollection, enhanced memory for negative events, reduced memory for positive events, and a tendency to recall general, negatively valenced autobiographical memories rather than specific individual events (Burt et al., 1995; Dalgleish & Werner-Seidler, 2014; Williams et al., 2007). These memory biases are clinically important as they contribute to the onset, maintenance, and remission of depression, yet their neurocognitive and neural underpinnings remain poorly understood (Dalgleish & Werner-Seidler, 2014; Williams et al., 2007). Stress is a major factor contributing to memory disruptions in MDD, as suggested by animal models of depression (Finsterwald & Alberini, 2014) and by the neural processes that mediate memory encoding and retrieval (Dillon & Pizzagalli, 2018). In particular, overgeneral autobiographical memory retrieval, a characteristic of MDD, has been linked to these neurocognitive and structural deficits. Further, enhancing autobiographical memory specificity has been shown to alleviate depressive symptoms, underscoring the importance of targeting these cognitive biases in therapeutic interventions (Dalgleish & Werner-Seidler, 2014).

Memory processing relies greatly on NMDAR activity to reorganize and reconsolidate memory traces in an updated form. While traditionally consolidated memories were thought to be persistent and resistant to disruption (Dudai & Eisenberg, 2004), recent advances in memory research highlight the pivotal role of reconsolidation in long-term memory. Reconsolidation is

a dynamic process where reactivated stable memories temporarily destabilize, allowing for the incorporation of new information and subsequent updating of memory content (Nader et al., 2000). This process relies on NMDAR-mediated mechanisms and protein synthesis within the medial prefrontal cortex (Dudai, 2012; Lee, 2009; Milton et al., 2008; Sara, 2000). Intervening during this period of memory instability can weaken negative-biased memories (Schwabe & Wolf, 2014) and potentially rewrite maladaptive ones (Milton & Everitt, 2012).

Early evidence suggests that ketamine, an NMDAR antagonist, may disrupt maladaptive memory reconsolidation, which could play a key role in its antidepressant effects. For example, Chen and colleagues (R. Chen et al., 2021) demonstrated that the NMDA partial agonist D-cycloserine enhances specific autobiographical memory retrieval and increases positive emotional memory. Further, to explore ketamine's potential to disrupt maladaptive memories - implicated in the development and persistence of substance and behavioural addictions - Das and colleagues (2019) showed that administering ketamine immediately after the retrieval of alcohol-related memories reduced drinking behaviour in individuals with high alcohol consumption. This change in behaviour included fewer drinking days, decreased alcohol consumption, and lower long-term drinking levels, suggesting ketamine can disrupt maladaptive appetitive memories in humans. Despite these findings, the effects of ketamine on affective memory recall in patients with TRD remain unexplored, representing a significant gap in understanding its antidepressant mechanisms. Furthermore, as discussed in Chapter 2, animal studies suggest that ketamine has a distinct ability to reverse negative affective biases associated with information acquired prior to its administration, differentiating it from conventional antidepressants (Stuart et al., 2015). This data is supported by findings from the RELAKS healthy volunteer study presented in Chapter 2, suggesting that ketamine enhances behavioural measures of positive bias in affective memory recall for information acquired prior

to its administration without affecting overall memory performance. The current study extends these findings by investigating whether similar effects occur in a TRD population immediately after a ketamine infusion, a timepoint when ketamine's clinical effects are not yet evident.

This chapter presents preliminary findings from an interim analysis of an experimental medicine trial in subjects with TRD, who were randomised to receive either ketamine (0.5 mg/kg) or placebo. Participants completed clinical questionnaires, the Oxford Autobiographical Memory Task (OAMT), and emotional processing tasks to characterise ketamine's effects on negative biases associated with emotional memories. This research aims to elucidate the neurocognitive mechanisms underlying ketamine's rapid antidepressant effects and assess its potential to disrupt negative biases associated with emotional memories in TRD.

5.2 Methods

5.2.1 Study Participant and design

The study was conducted at the Department of Psychiatry, University of Oxford, in Oxford (UK), and includes data from an interim analysis of participants recruited between May 2022 and August 2024. The study called for the enrolment of a total of 60 TRD participants. Previous research has shown that ketamine can induce rapid changes in depressive symptoms with a large effect size observed at 24 hours post-infusion (Hedge's $g \approx 1.2$) (Coyle & Laws, 2015). However, the neurocognitive outcome measures used in the current study were novel, and thus the expected effect size was uncertain. To account for this, we adopted a conservative approach,

calculating the required sample size based on a moderate effect size of g = 0.7. This indicated that 30 participants per group would provide 80% power to detect between-group differences.

The study used a randomised, double-blind, placebo-controlled, experimental medicine design. It was registered on ClinicalTrials.gov (Identifier: NCT05809609) and approved by the NHS Research Ethics Committee (NHS REC; IRAS Project ID: 302265). Written informed consent was obtained from all participants prior to any study procedure. Participants were required to be between 20 and 60 years of age and demonstrate the ability to provide informed consent. They needed sufficient proficiency in English to understand and complete study tasks. Participants were required to meet several clinical inclusion criteria, including a diagnosis of MDD according to the SCID-5 criteria (First MB, 2015), with a current major depressive episode lasting no longer than two years. Additionally, they must have shown an inadequate response to at least one and no more than three antidepressant treatments during the current episode, as determined through a clinical interview. All participants had to be registered with a general practitioner (GP) and consent to their GP being informed of their participation in the study. Other requirements included agreeing to refrain from driving, cycling, or operating heavy machinery until the following morning or after a restful sleep, whichever came later; abstaining from signing legal documents on the day of the infusion visit; and avoiding alcohol consumption for three days prior to the infusion visit and one day before any other study visits. For pre-menopausal women and male participants, the use of a highly effective method of contraception was required from the screening visit until 30 days after receiving the study medication.

Exclusion criteria for the study included a history or current diagnosis of bipolar disorder, schizophrenia, emotionally unstable personality disorder, or any other significant psychiatric

disorder as defined by the DSM-5. Participants were also excluded if they presented a clinically significant risk of suicide, abnormal laboratory findings, or any unstable medical condition. Additional exclusions included current pregnancy or breastfeeding, recent recreational drug use within the past three months, or lifetime use of ketamine or phencyclidine. A complete list of the inclusion and exclusion criteria is available in the Appendix.

During the screening visit, participants' medical and psychiatric histories were obtained, and comprehensive clinical laboratory tests were performed, including full blood count, liver, thyroid, and kidney function tests, and urine analysis. Toxicology screening and pregnancy testing (for premenopausal women) were also conducted. Eligible participants were invited to return for the baseline visit within four weeks of the screening visit.

Participants were randomised using an online randomisation tool (www.sealedenvelope.com; seed: 250661842916354) to either placebo or ketamine. The randomisation code was drawn up by a researcher not involved in the study. Randomisation was stratified for gender and order of conditions on the version of the Facial Emotion Recognition Task (FERT). The list was maintained by the NIHR Oxford Cognitive Health Clinical Research Facility (CRF) at the Warneford Hospital. Blinding was maintained by restricting knowledge of treatment allocation to only the medical and nursing staff responsible for administering the infusions; these individuals were not involved in any participant assessments beyond the infusion visit. To further reduce the risk of bias or accidental unblinding, staff conducting post-infusion assessments did not take part in any subsequent study visits. All other members of the study team remained fully blinded to treatment allocation and procedures and did not carry out any post-infusion tasks on the day of ketamine or placebo administration.

5.2.2 Baseline visit

Participants who met all inclusion criteria and none of the exclusion criteria were invited to attend baseline assessments at the Neurosciences Building, University of Oxford. During this visit, participants completed the Autobiographical Memory Retrieval and Word Sorting tasks, components of the OAMT. Additionally, a trained staff member administered the Hamilton Depression Rating Scale (HAM-D), while participants completed self-report questionnaires evaluating depression (QIDS-SR), anxiety (GAD-7), and ruminative thinking (RRS). At the end of the visit, participants were scheduled to return for the infusion visit within one week.

5.2.2.1 The Oxford Autobiographical Memory Task

The Oxford Autobiographical Memory Task (OAMT) used in this trial was adapted from the fMRI autobiographic memory task described by Levine et al. (2002) and Parlar et al. (2018) and the Life Structure Card-Sorting Task described by Dalgleish et al. (2011). This task is designed to assess autobiographical memory processing and was conducted at baseline, at 24-hours post-infusion, and at exit (one week post-infusion). The OAMT was designed to investigate changes in autobiographical memory and emotional processing, providing insights into the potential effects of interventions like ketamine on memory and mood-related mechanisms. The OAMT comprises two main components: Part A: Autobiographical Memory Retrieval and Part B: Word Sorting Task. In addition, participants were asked to indicate how frequently they had thought about the recalled memory during the week preceding each assessment.

Part A: Autobiographical Memory Retrieval

At baseline, participants identified two positive, two negative, and two relatively non-emotional autobiographical events from the past five years, excluding those occurring within the week prior to testing. An additional non-emotional event was recalled for training purposes during the assessment visit. Participants were instructed to select events that were specific in time and place and to ensure they had a personal recollection of being actively involved. For each event, participants created a title and segmented the event into five distinct temporal periods. These segmented titles were used as retrieval cues for the subsequent word-sorting task (described below).

Part B: Word Sorting

Following the recollection of each memory, participants reviewed a list of 10 words, each representing an adjective or noun with either positive or negative valence. Participants used a sliding bar (0 = "not at all," 10 = "extremely") to rate the relevance of these words to how they felt during the event. They repeat this procedure to assess the relevance of these words to their current feelings about the event. The dual rating captures both past and present emotional appraisals related to the recalled events. Additionally, participants were asked how frequently they thought about each memory in the previous week. The word sorting component of the OAMT was administered digitally using Qualtrics software. A list of the adjectives and nouns used in the study as part of the Word Sorting Task is provided in the Appendix.

To incorporate patient and public perspectives into the design of this new task, a patient and public involvement (PPI) process was conducted via five structured Zoom interviews during March - April 2021. Participants included individuals with personal lived experience of depression, particularly TRD, and/or a first-degree relative with depression. The discussions

provided valuable feedback on the study design and the OAMT. Suggestions included adjusting the timeframe for memory recollection, emphasizing clear task instructions with examples, and confirming participants' willingness to recall personal memories, given the potential distress involved. Additionally, participants contributed to improving the word sorting section by recommending the inclusion of more descriptive adjectives and a blank space for customized input. These suggestions were integrated into the updated task instructions and materials, enhancing the study's relevance and participant engagement.

5.2.3 Infusion visit

Within one week of the baseline assessment, participants returned to the NIHR Oxford Cognitive Health CRF at Warneford Hospital for the infusion. Upon arrival, participants underwent a brief re-assessment to confirm continued eligibility. This included a review of inclusion/exclusion criteria, assessment of concomitant medications, a urine pregnancy test for female participants, drug screening, vital sign measurements, and completion of baseline side-effect checklists. Participants also completed computer-based tasks and questionnaires as part of the pre-infusion assessment.

Immediately prior to the infusion, a partial memory reactivation procedure was conducted using the negative autobiographical memory rated as most negative during the baseline word-sorting task. Subsets of retrieval cues - such as event titles - were presented by a researcher to prompt recall. Once the participant began to recall the memory, they were interrupted and redirected to an ongoing minor medical procedure (e.g., blood pressure monitoring). This approach aimed to induce a negative prediction error, hypothesised to destabilise the memory trace.

Following the memory reactivation procedure, participants received an infusion of ketamine 0.5 mg/kg or placebo (saline NaCl 0.9%) over the course of 40 minutes. Blood pressure was monitored every 10 minutes during the infusion, including at the end of the infusion, and then every 15 minutes for an hour post-infusion. Participants remained under close observation for approximately 2 hours, with monitoring of oxygen saturation, vital signs, and general well-being.

Thirty minutes after the infusion ended, participants completed questionnaires to assess dissociative symptoms - recalling how they felt during the infusion - and emotional processing tasks. Before discharge, a medical professional assessed participants to confirm readiness for safe departure. Participants returned home by taxi and were advised to refrain from driving, cycling, operating machinery, consuming alcohol, taking other medications, or engaging in activities requiring high alertness until the following morning or after a restful sleep. Participants were also provided with 24-hour contact details for medically qualified personnel in case of questions or concerns.

5.2.4 Clinical Questionnaire measures

Throughout the study, participants completed a combination of rater-administered and self-report measures to evaluate depressive severity, anxiety levels, rumination patterns, and dissociation. Most clinical questionnaires were administered digitally using Qualtrics software at baseline and 24 hours post-infusion. An exception was the CADSS-6, which was specifically collected during the infusion visit, both before and after the administration of ketamine or placebo, to assess dissociative symptoms. Depressive severity scales were administered at baseline, 24 hours post-infusion, and at study exit (one week post-infusion).

5.2.4.1 Depressive and Anxiety-Related Symptoms

The Hamilton Rating Scale for Depression (HAM-D) is a clinician-administered tool designed to assess the severity of depressive symptoms before, during, and after treatment (Hamilton, 1960). Administered by trained raters blinded to treatment allocation, the HAM-D consists of 21 items, though scoring is based on the first 17 items. These items are rated on a 5-point scale. A total HAM-D score below 16 suggests mild depression, scores between 17–23 indicate moderate depression, and scores above 25 reflect severe depression.

The Quick Inventory of Depressive Symptomatology-Self-Report (QIDS-SR) is a self-reported measure evaluating the severity of depressive symptoms in adults aged 18 and older (Rush et al., 2003). It comprises 16 items derived from the Inventory of Depressive Symptomology (IDS, 2000) and corresponds to the DSM-IV diagnostic criteria. Respondents use a 4-point Likert scale to rate their mood and behaviours over the past week. Scores below 10 suggest mild depression, scores between 11-15 indicate moderate depression, and scores above 16 represent severe depression.

The Generalized Anxiety Disorder-7 (GAD-7) is a widely validated and efficient screening tool for generalized anxiety disorder and its severity in both clinical and research settings (Spitzer et al., 2006). The scale includes 7 items rated on a 4-point scale ranging from “not at all” to “nearly every day.” The total GAD-7 scores range from 0 to 21, with scores categorised as follows: 0-4 (minimal anxiety), 5-9 (mild anxiety), 10-14 (moderate anxiety), and 15-21 (severe anxiety).

5.2.4.2 Ruminative thinking

Ruminative thinking was assessed using the Rumination Response Scale (RRS), a 22-item self-report questionnaire designed to measure the tendency to ruminate in response to distress, particularly depressive symptoms (Treynor et al., 2003). Participants rate items on a 4-point Likert scale ranging from "Almost never" to "Almost always". The scale allows the assessment of two main dimensions: Brooding, a maladaptive form of repetitive, passive focus on distress, and Reflection, a more adaptive form of purposeful introspection aimed at problem-solving. Higher scores indicate greater ruminative tendencies, with subscale scores distinguishing between adaptive and maladaptive rumination.

5.2.4.3 Dissociation

Dissociative symptoms were assessed using the CADSS-6. A description of this instrument is provided in Chapter 2.

5.2.5 Affective Bias Tasks

Building on the findings from the RELAKS study and focusing on emotional memories, this study employed psychological tasks aimed at assessing different aspects of emotional processing. These included the Emotional Categorisation Task (ECAT), Emotional Recall Task (EREC), and Emotional Recognition Memory Task (EMEM) from the Emotional Test Battery (ETB; (Harmer, O'Sullivan, et al., 2009; Harmer et al., 2004). The ECAT was conducted prior to the infusion, while the EREC and EMEM were administered approximately 30 minutes post-

infusion of ketamine or placebo. A description of these tasks, including their methodologies, is provided in Chapter 3.

5.2.6 Statistical analysis

The statistical analysis of the OAMT word-sorting task involved averaging positive and negative adjectives across positive, negative, and non-emotional memories, resulting in two composite scores: positive and negative. These scores were analysed separately for both feelings at the time of the event and current feelings related to that event. The positive and negative scores were used for group comparisons and analyses of covariance, conducted using IBM SPSS (version 29). The primary outcome of the trial was assessed through a repeated measures ANOVA, with time (baseline, post-treatment, exit) as the within-subject factor, to evaluate changes in negative and positive valence adjectives relative to negative autobiographical memories. Similar analyses were subsequently conducted for positive and non-emotional memories. The frequency of memory recall was averaged and analysed separately for positive, negative, and non-emotional memories at baseline, 24 hours post-infusion, and at exit.

The statistical analysis of clinical questionnaire and ETB task data used in this study followed a methodology consistent with that described for RELAKS (Chapter 2). Clinical questionnaire data were analysed using IBM SPSS (version 29). Baseline clinical and demographic characteristics were described as means or medians for continuous variables and as counts or percentages for categorical variables. Correlations between measures were assessed using Pearson's r for parametric data and Spearman's rank correlation for non-parametric data. For repeated measures analyses, the Greenhouse-Geisser correction was applied to account for

violations of sphericity. Statistical significance was determined at a two-sided $\alpha = 0.05$ for all analyses. The ETB task outcomes were analysed using R Studio (version 2023.12.1) with mixed ANCOVA models to assess the main effects of treatment group (between-subject factor), emotion (within-subject factor), and their interaction on task performance. For the ECAT, analysis focused on the percentage of correctly categorized words and reaction times, excluding trials with response times below 200 milliseconds or above 3000 milliseconds. Sensitivity analyses were conducted excluding participants with low accuracy. For the EREC, analyses examined the number of correctly recalled positive and negative words, positive and negative hits (correct recalls as a proportion of total recalls), and positive and negative intrusions (false recalls as a proportion of total false recalls), with additional sensitivity analyses for low ECAT accuracy. The EMEM outcomes included accuracy in identifying positive and negative words and reaction times for correct identifications, excluding trials with response times under 200 milliseconds or over 4000 milliseconds. Sensitivity analyses were conducted for participants with overall accuracy below 50%.

5.3 Results

5.3.1 Sample Characteristics

These analyses were an interim analysis of the data set, conducted on participants who were randomised and completed infusion procedures by 31st August 2024. At this point, 43 participants were eligible for randomisation with 19 assigned to the ketamine group and 24 to the placebo group. Of these, 42 participants completed the 24-hour post-infusion visit. One participant from the ketamine group was unable to attend the visit due to a positive COVID-19

test, resulting in a final count of 18 participants in the ketamine group and 24 in the placebo group for this timepoint. At baseline, no significant differences were found between the ketamine and placebo groups regarding age, gender, educational level, or race. **Table 5.1** provides a summary of the clinical and demographic characteristics of the sample.

Table 5.1. Sociodemographic and Clinical Characteristics of the Sample

	Ketamine (n=19)	Placebo (n=24)
Gender, M (n)	7	7
Age, years M (SD)	29.5 (11.6)	33.38 (10.7)
Years of Education, M (SD)	16.6 (2)	17.6 (3)
Race		
White/Caucasian (n)	16	16
Asian/Asian British (n)	1	4
QIDS-SR, baseline M (SD)	15.4 (3.5)	14.9 (3.9)
HAM-D, baseline M (SD)	15.8 (3.8)	16.9 (2.6)
GAD-7, baseline M (SD)	17.4 (5.3)	17.5 (4.3)

Abbreviations: GAD-7, Generalized Anxiety Disorder-7; HAM-D, Hamilton Rating Scale for Depression; M, mean; QIDS-SR, Quick Inventory of Depressive Symptomatology-Self-Report; SD, standard deviation.

5.3.2 Symptom Change

The two groups did not differ significantly in depressive severity at baseline (prior to randomisation), as measured by QIDS-SR [$t(41) = -0.197$, $p = 0.845$] and HAM-D [$t(40) = 1.155$, $p = 0.2$]. Repeated-measures ANOVA for QIDS-SR scores revealed a significant main

effect of time [F(2, 78) = 87.681, p < 0.001], indicating an overall reduction in depressive symptoms in both groups. A significant time x treatment interaction [F(2, 78) = 4.576, p = 0.013] suggested greater improvement in the ketamine group compared to placebo over time. Similarly, the repeated measures ANOVA for HAM-D scores showed a significant main effect of time [F(2, 72) = 100.395, p < 0.001] and a trend level time x treatment interaction [F(2, 72) = 2.954, p = 0.06], though it did not reach statistical significance. Specifically, by 24 hours post-infusion, significant between-group differences emerged, with the ketamine group exhibiting lower depressive symptom scores compared to the placebo group. This was evident in HAM-D scores [t(36.928) = 2.375, p = 0.02, mean difference = 2.734, 95% CI [0.402, 5.067]] and QIDS-SR ratings [t(36.24) = 2.386, p = 0.022, mean difference = 2.403, 95% CI [0.361, 4.444]]. These findings support the efficacy of ketamine in reducing depressive symptoms, with the most pronounced effects observed within the first 24 hours post-infusion. Notably, these improvements were sustained at the exit visit, with significant between-group differences observed for both the QIDS-SR [t(39.06) = 2.402, p = 0.021, mean difference = 2.903, 95% CI [0.458, 5.347]] and the HAM-D [t(38.84) = 3.064, p = 0.004, mean difference = 3.99, 95% CI [1.36, 6.64]]. These results indicate a persistent reduction in depressive symptoms in the ketamine group relative to placebo. **Figure 5.1** provides a visual representation of the changes in depressive severity scores.

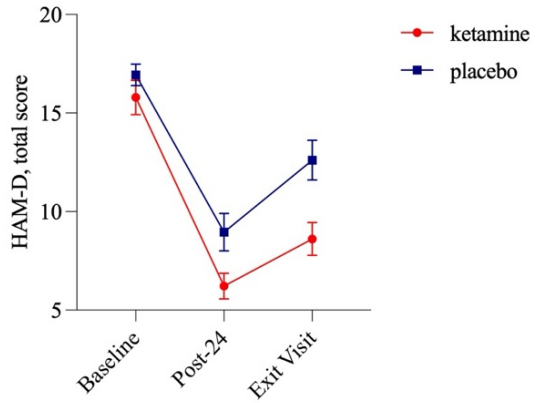
Repeated-measures ANOVA revealed no significant time x treatment interactions for GAD-7 [F(1,41) = 0.006, p = 0.9] or RRS [F(1,41) = 0.999, p = 0.3], indicating no differential changes over time between the groups. Consistent with this, t-tests at both baseline and 24 hours post-infusion did not reveal any significant group differences. At baseline, there were no significant differences in anxiety symptoms (GAD-7) [t(41) = 0.025, p = 1.0, mean difference = 0.037, 95% CI [-2.933, 3.008]] or ruminative symptoms (RRS) [t(41) = 0.678, p = 0.5, mean

difference = 1.904, 95% CI [-3.763, 7.570]]. At 24 hours post-infusion, there were no significant group difference in GAD-7 scores [t(41) = 0.161, p = 0.9, mean difference = 0.154, 95% CI [-1.768, 2.075]], or in ruminative symptom levels [t(41) = 1.588, p = 0.1, mean difference = 6.404, 95% CI [-1.742, 14.549]].

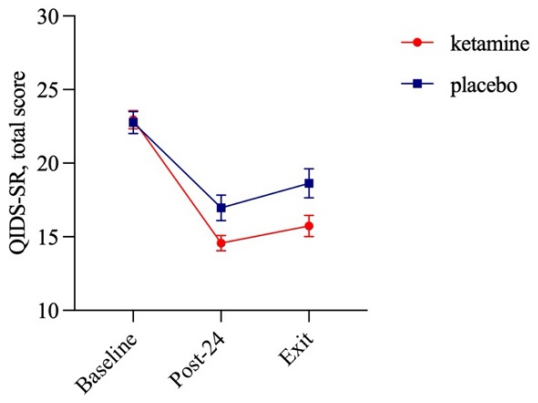
Dissociative symptoms, as measured by the CADSS-6, were significantly different between groups, with a repeated-measures ANOVA revealing a significant main effect of time [F(1, 41) = 105.2, p < .001], treatment [F(1, 41) = 51.7, p < 0.001], and a significant time x treatment interaction [F(1, 41) = 60.5, p < 0.001]. There were no significant differences between the ketamine and placebo groups prior to infusion [t(41) = 0.162, p = 0.87, mean difference = 0.02, 95% CI [-0.23, 0.27]], but a significant group difference emerged post-infusion, with the ketamine group exhibiting significantly higher CADSS-6 scores [t(41) = -7.524, p < 0.001, mean difference = –7.56, 95% CI [-9.59, -5.53]]. A subsequent Spearman's correlation between changes in CADSS-6 scores (post-infusion minus pre-infusion) and changes in HAM-D scores (baseline minus 24 hours post-infusion) revealed a weak-to-moderate positive association [$r_s(42) = 0.307$, p = 0.054], which approached but did not reach statistical significance. This trend may suggest a possible link between the intensity of acute dissociative effects and short-term antidepressant response. Similarly, a correlation between CADSS-6 and QIDS-SR score changes showed a weaker, non-significant positive association [$r_s(42) = 0.238$, p = 0.13].

Figure 5.1 Effect of Ketamine Compared to Placebo on Depression Severity in Subjects with Treatment Resistant Depression (TRD)

A. Change in Hamilton Depression Rating Scale (HAM-D) Scores



B. Change in Quick Inventory of Depressive Symptomatology - Self-Report (QIDS-SR) Scores



Values reflect means with associated standard error of the mean (SEM). HAM-D, Hamilton Rating Scale for Depression; QIDS-SR, Quick Inventory of Depressive Symptomatology - Self-Report

5.3.3 Oxford Autobiographical Memory Task (OAMT)

The OAMT was used to evaluate the negative biases associated with autobiographical memories at baseline, 24 hours post-infusion, and at exit (one week post-infusion). As part of the word-sorting questionnaires, participants reviewed a list of 10 words, each representing an adjective or noun with either positive or negative valence. They rated the relevance of these words to their feelings during the event. This process was repeated to evaluate the relevance of these words to their current feelings about the event. At baseline, prior to randomisation, there were no significant differences between the treatment groups in their use of positive or negative adjectives when rating feelings both at the time of the event and currently for negative, positive, or non-emotional memories**. Table 5.2** presents descriptive statistics for emotional ratings of negative, positive, and non-emotional memories, both for current feelings and for feelings at the time the event occurred.

5.3.3.1 Negative memories

A repeated-measures ANOVA on average negative ratings revealed a significant main effect of time for both current feelings [$F(2, 74) = 19.745$, $p < 0.001$] and feelings at the time of the event [$F(2, 74) = 12.539$, $p < 0.001$], indicating a significant change in ratings over time. The mean values indicate a decline in negative ratings over time. Average negative ratings for feelings at the time of the event decreased from 6.47 at baseline to 5.78 at 24-hours post-infusion, remaining relatively stable at 5.79 at the exit visit. Similarly, average negative ratings for current feelings declined more substantially, from 4.38 at baseline to 3.07 24-hours post-infusion, and further to 2.91 at the exit visit. This pattern suggests a gradual reduction in negative emotional responses over time, particularly for current feelings. There was no significant effect of treatment on either current feelings [$F(1, 37) = 0.152$, $p = 0.70$] or feelings at the time of the event [$F(1, 37) = 0.735$, $p = 0.40$]. Similarly, the time x treatment interaction was not significant for current feelings [$F(2, 74) = 0.125$, $p = 0.88$] or for recalled feelings at the time of the event [$F(2, 74) = 0.439$, $p = 0.64$]. **Figure 5.2** illustrates the changes in average negative emotion ratings - both current feelings and feelings at the time of the event - for negative memories across the study timepoints.

A separate repeated-measures ANOVA found no significant main effect of time and no time x treatment interaction for positive ratings of current feelings in the context of negative memories. However, there was a significant main effect of time for feelings at the time of the event [$F(1.2, 20.84) = 17.31$, $p < 0.001$], indicating a change in recalled emotional intensity for positive adjectives over time. However, there was no significant main effect of group [$F(1, 37) = 2.29$, $p = 0.14$] and no significant time x treatment interaction [$F(1.4, 50.4) = 1.22$, $p = 0.29$]. Mean values indicate that positive ratings remained relatively stable between baseline (0.872)

and 24 hours post-infusion (0.875), followed by an increase at the exit visit (1.08), suggesting a delayed shift toward more positive emotional appraisal over time.

5.3.3.2 Positive memories

A repeated-measures ANOVA revealed a significant main effect of time on average negative ratings for current feelings [$F(1.2, 43.2) = 7.145$, $p = 0.008$], reflecting a reduction in negative ratings across time in both groups. However, there was no significant effect of treatment [$F(1, 37) = 0.13$, $p = 0.72$] and no significant time x treatment interaction [$F(1.2, 43.2) = 0.075$, $p = 0.82$] for average negative ratings for current feelings. Additionally, there was no significant effects of time, treatment, or time x treatment interaction on average negative ratings for feelings at the time of the event.

There was no significant effect of time, treatment, or time x treatment interaction for average positive ratings of current feelings or feelings at the time of the event.

5.3.3.3 Non emotional memories

There was no significant effect of time or time x treatment interaction for average negative or positive ratings of current feelings or feelings at the time of the event.

Table 5.2. Average Emotional Ratings for Negative, Positive, and Non-Emotional Memories Throughout the Study

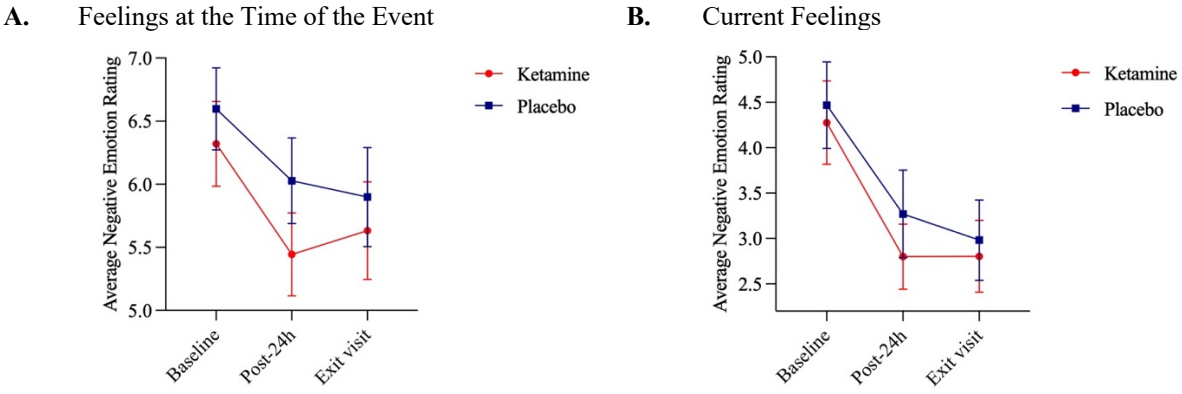
	Baseline		24-hours post-infusion		Exit	
Negative memories	Ketamine	Placebo	Ketamine	Placebo	Ketamine	Placebo
Negative ratings for feelings at time of event	6.3 (1.5)	6.6 (1.6)	5.44 (1.4)	6 (1.7)	5.6 (1.6)	5.9 (1.9)
Negative ratings for current feelings	4.3 (2)	4.5 (2.3)	2.8 (1.5)	3.3 (2.4)	2.8 (1.6)	3 (2.1)
Positive ratings for feelings at time of event	0.8 (0.8)	0.9 (0.9)	0.8 (1.1)	0.9 (1.0)	0.8 (0.8)	1.3 (1.4)
Positive ratings for current feelings	0.9 (1.)	1.0 (1.3)	1.1 (1.8)	1.5 (1.8)	1.2 (1.5)	1.6 (2.1)
Positive memories						
Negative ratings for feelings at time of event	0.7 (0.9)	0.6 (0.8)	0.7 (0.6)	0.4 (0.5)	0.7 (0.7)	0.6 (0.6)
Negative ratings for current feelings	1.1 (1.6)	1.0 (2.1)	0.6 (1.2)	0.5 (1.4)	0.3 (0.6)	0.6 (1.6)
Positive ratings for feelings at time of event	6.1 (0.9)	6.3 (1.3)	5.9 (1.9)	6.7 (1.8)	5.8 (1.8)	6.7 (1.8)
Positive ratings for current feelings	5.1 (2.2)	5.3 (2.4)	4.6 (2.2)	5.7 (2.4)	5.1 (2.2)	5.3 (2.6)
Non-emotional memories						
Negative ratings for feelings at time of event	0.8 (0.9)	0.9 (1)	0.8 (1.0)	0.7 (1.0)	0.7 (0.8)	0.7 (0.9)
Negative ratings for current feelings	0.7 (1.4)	0.9 (1.8)	0.5 (0.8)	0.4 (1.3)	0.4 (0.6)	0.6 (1.6)
Positive ratings for feelings at time of event	1.6 (1.4)	2 (1.2)	1.8 (1.8)	2.3 (1.5)	1.6 (1.7)	2.2 (1.4)
Positive ratings for current feelings	1.1 (1.1)	1.4 (1.4)	1.6 (1.8)	2.3 (2.0)	1.3 (1.9)	2 (2.2)

Ratings reflect the average of self-reported adjective scores from the word sorting component of the Oxford Autobiographical Memory Task (OAMT) for each timepoint during the study. Scores have been averaged across memories of the same type. Values indicate group means, with standard deviations (SD) reported in brackets

Finally, no significant differences between the ketamine and placebo groups were observed in the frequency of recalling positive, negative, or neutral memories, either at baseline or at the 24-hour post-infusion visit. Additionally, no significant time x treatment interaction effects were detected for positive, negative, or neutral memory recall frequencies, indicating that the treatment did not influence changes in frequency of memory recall.

In conclusion, there was no significant effect of treatment on ratings of positive or negative adjectives across negative, positive, and neutral autobiographical memories. While significant changes over time were observed for some measures, these changes were consistent across both groups, with no significant time x treatment interactions detected.

Figure 5.2 Effect of Ketamine Compared to Placebo on Negative Emotion Ratings for Negative Memories



Panel A: Average negative emotion ratings related to participants' feelings at the time of the event. Panel B: Average negative emotion ratings related to current feelings about the event. Average scores were calculated by averaging the ratings for negative adjectives selected by participants during the word-sorting component of the Oxford Autobiographical Memory Task (OAMT). Ratings were averaged across two autobiographical negative memories collected at baseline. Values reflect means with associated standard error of the mean (SEM)

5.3.4 Emotional Test Battery

5.3.4.1 Emotional Categorisation Task (ECAT)

The ECAT was completed prior to ketamine/placebo infusion, with data available for n = 43 subjects. Analysis of accuracy revealed no significant main effects of treatment allocation [$F(1,80) = 0.939$, $p = 0.3$] or valence [$F(1,80) = 1.392$, $p = 0.2$], and no significant interaction between treatment allocation and valence [$F(1,80) = 0.086$, $p = 0.8$]. A sensitivity analysis excluding three subjects in the placebo group with under 85% accuracy demonstrated findings consistent with the main analysis. No significant main effects of treatment allocation [$F(1,74) = 1.766$, $p = 0.2$] or valence [$F(1,74) = 0.375$, $p = 0.5$] were observed, and the interaction between treatment allocation and valence remained non-significant [$F(1,74) = 0.537$, $p = 0.5$].

For reaction time, repeated-measures ANOVA showed no significant main effect of treatment allocation [$F(1,80) = 0.521$, $p = 0.5$] or valence [$F(1,80) = 1.025$, $p = 0.3$], and the treatment allocation x valence interaction was also not significant [$F(1,80) = 0.000$, $p = 1.0$]. Overall, these results indicate that the two treatment groups did not differ in accuracy and reaction time on the ECAT prior to infusion.

5.3.4.2 Emotional recall task (EREC)

Participants completed the EREC approximately 30 minutes after the end of the infusion, with data available for n = 42 subjects. Participants recalled an average of 6.13 ± 2.63 positive words and 3.6 ± 2.4 negative words (correct and incorrect), regardless of treatment

allocation. There was a significant effect of word valence on the number of words correctly recalled [$F(1, 148) = 14.121$, $p = 0.002$], but there was neither a significant main effect of treatment [$F(1, 148) = 0.224$, $p = 0.6$] nor a significant treatment x valence interaction [$F(1, 148) = 0.351$, $p = 0.5$]. A two-way ANOVA on the number of incorrectly recalled words post-infusion revealed a significant main effect of valence [$F(1,72) = 12.96$, $p < 0.001$]. However, no significant main effect of treatment [$F(1,72) = 0.063$, $p = 0.8$] or treatment-by-valence interaction [$F(1,72) = 0.063$, $p = 0.8$] was found. Subsequent one-way ANOVAs examining the impact of treatment on correctly and incorrectly recalled positive and negative words showed no significant main effect of treatment. A sensitivity analysis excluding the three participants with ECAT performance below 85% (all of whom were randomized to the placebo group) confirmed no significant deviations from the results of the initial analyses.

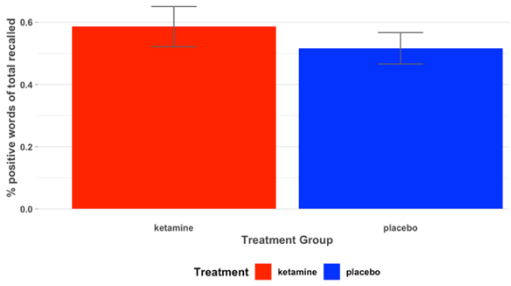
Analysis of hits (percentage of correctly recalled positive or negative words out of total correct recalls) revealed no significant differences between treatment groups for positive hits [$F(1,36) = 0.738$, $p = 0.4$]. However, a significant group difference was observed for the proportion of negative words correctly recalled, with the ketamine group demonstrating fewer negative hits compared to the placebo group [$F(1,36) = 7.264$, $p = 0.01$] (**Figure 5.3**). In contrast, the analysis of intrusions, defined as the percentage of incorrect positive or negative words recalled out of the total incorrect recalls, revealed no significant differences between treatment groups. Specifically, there were no significant group effects for positive intrusions [$F(1,36) = 0.629$, $p = 0.4$] or negative intrusions [$F(1,36) = 0.045$, $p = 0.8$].

Overall, these findings indicate that ketamine did not affect overall recall of positive or negative words compared to placebo in subjects with TRD. However, it significantly

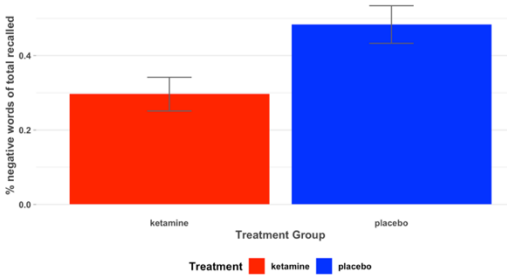
reduced the proportion of recalled negatively valenced words, suggesting its potential to selectively attenuate negative emotional biases shortly after infusion.

Figure 5.3 Effect of Treatment on Positive Hits (Panel A) and Negative Hits (Panel B)

A. Effect of Treatment on Positive Hits at Post-infusion



B. Effect of Treatment on Negative Hits at Post-infusion



Values reflect means with associated standard error of the mean (SEM)

5.3.4.3 Emotional recognition memory task (EMEM)

The analysis of accuracy defined as total correct recall percentages revealed no significant main effects of treatment [$F(1,82) = 0.011$, $p = 0.9$] or valence [$F(1,80) = 2.31$, $p = 0.9$], and no significant interaction between treatment and valence [$F(1,80) = 0.821$, $p = 0.4$]. These findings, consistent with the analysis using total correct recall as an indicator of accuracy, indicate that ketamine did not significantly influence accuracy, regardless of emotional valence, compared to placebo. A sensitivity analysis for accuracy was conducted excluding subjects with accuracy below 50% who appeared to respond at random (1 ketamine and 2 placebo). There were no significant main effects of treatment or valence, and no significant treatment x valence interaction. These findings confirm that excluding participants with random response patterns did not alter the results, indicating consistent null effects across measures.

Further analyses were conducted on hits (familiar words considered familiar), misses (familiar words considered novel), correct rejections (novel words considered novel), and false alarms (novel words considered familiar), which revealed a consistent effect of word valence across all measures. Specifically, participants showed higher hit rates for positive words (mean = 15.31, SD = 2.60) than for negative words (mean = 11.79, SD = 4.70), while negative words were associated with more misses (mean = 7.86, SD = 4.79) compared to positive words (mean = 4.36, SD = 2.55). False alarms were more frequent for positive novel words (mean = 6.98, SD = 4.75) than for negative ones (mean = 3.67, SD = 2.90), whereas correct rejections were higher for negative novel words (mean = 16.14, SD = 2.87) relative to positive ones (mean = 12.71, SD = 4.86). These findings suggest a general tendency toward greater familiarity responses for positive words and more accurate rejection of negative ones. However, there were no significant main effect of treatment or treatment x valence interaction for any measure, suggesting that treatment did not influence memory performance or its association with word valence. Across sensitivity analyses, treatment did not significantly impact hits, misses, correct rejections, or false alarms.

Finally, analysis of reaction times revealed no significant main effect of treatment [$F(1, 82) = 0.0$, $p = 0.99$], nor a significant main effect of valence [$F(1, 82) = 0.634$, $p = 0.428$]. Additionally, the interaction between treatment and valence was not significant [$F(1, 80) = 0.219$, $p = 0.641$], indicating that reaction times did not differ as a function of treatment, valence, or their interaction.

5.4 Discussion

This chapter presented the results from an interim analysis of an experimental study investigating the neurocognitive effects of a single ketamine infusion, compared to placebo, on negative biases associated with emotional memories in TRD. Ketamine appeared to selectively modulate emotional memory processes for information acquired prior to the infusion, as evidenced by a reduced proportion of correctly recalled self-descriptive words of negative valence (negative hits) immediately after the infusion. No significant effects of ketamine on emotional ratings associated with autobiographical memories were observed.

Treatment with ketamine was associated with a greater reduction in depressive symptoms compared to placebo, with significant between-group differences observed in both HAM-D and QIDS-SR scores. Of note, alternative outcome measures may offer even greater sensitivity in detecting the rapid onset of ketamine's antidepressant effects, as traditional depression rating scales may be limited in addressing the rapid onset of antidepressant effects seen with ketamine. Originally designed to assess symptom changes over extended periods (one to two weeks), these scales may lack the temporal precision needed to capture the swift therapeutic responses elicited by rapid-acting treatments like ketamine (McIntyre et al., 2021). To address this gap, novel assessment tools such as the McIntyre and Rosenblat Rapid Response Scale (MARRRS) have been developed specifically to measure early symptom improvements associated with rapid-acting antidepressants (McIntyre et al., 2021). Additionally, while efforts have been made to adapt existing scales by modifying the timeframes for symptom assessment and incorporating patient-reported outcomes to reflect rapid changes in mood and cognition, the Assessment Methods and Endpoints for Rapid-Acting Antidepressants (RAADs) Working Group of the International Society for CNS

Clinical Trials and Methodology (ISCTM) has emphasized the inherent limitations of traditional depression rating scales (Yavorsky et al., 2023). These limitations arise from their fixed structure, reliance on retrospective symptom review, and conceptual biases. Moreover, such scales fail to capture the nuanced subjective experiences of patients, which could provide crucial additional insights into treatment effects (Sedway et al., 2024). Incorporating measures that prioritize subjective experience and rapid symptom changes could greatly enhance the understanding and evaluation of rapid-acting antidepressants' clinical impact. These advancements are crucial for evaluating and optimizing treatments with rapid-onset mechanisms, ensuring accurate assessment of their therapeutic benefits (Sanacora et al., 2017).

There were no significant treatment effects on anxiety or ruminative symptoms at 24 hours post-infusion. Of note, at baseline, participants exhibited severe anxiety levels, while depressive symptoms were rated as mild according to the rater-administered HAM-D and moderate according to the self-reported QIDS-SR. This discrepancy may reflect the differences in assessment modality, as the GAD-7 (anxiety) and QIDS-SR (depression) are both self-report measures and may be more closely aligned in capturing subjective symptom burden. However, it is important to note that these findings are based on an interim analysis, and conclusions should be interpreted with caution until the full sample has been analysed. Clinical data on the effects of ketamine on anxiety remain limited compared to the extensive literature on depression. Beyond case reports and small trials, there is limited evidence supporting the use of IV ketamine for anxiety (Banov et al., 2020; Tully et al., 2022). In a randomised, double-blind, psychoactive-controlled study of patients with suicidal ideation (n = 24), including some with comorbid anxiety disorders (n=12), Murrough et al. showed that a single intravenous infusion of ketamine (0.5 mg/kg) was associated with reductions in

irritability and panic at 24 hours, though no changes in GAD were observed (Murrough et al., 2015). Similarly, a randomised, double-blind, placebo-controlled crossover study by Taylor et al. in patients with social anxiety disorder (n=18) found that single-dose ketamine led to reduced anxiety symptoms for up to 14 days post-infusion (Taylor et al., 2018). Overall, the evidence supporting ketamine's potential to reduce anxiety symptoms in TRD is limited, highlighting the need for further large-scale randomized controlled trials.

In this study, a reduction in negative bias was observed, as ketamine decreased negative hits compared to placebo immediately after the infusion. As discussed in Chapter 2, animal studies suggest that ketamine has the unique ability to reverse negative affective biases associated with previously acquired information, setting it apart from conventional antidepressants like venlafaxine. Stuart et al. (Stuart et al., 2015) demonstrated in a rodent model that ketamine, but not venlafaxine, reversed previously encoded negative memories, facilitating more positive retrieval. In contrast, venlafaxine enhanced positive memory encoding when administered prior to learning. More recent animal studies further suggest that low-dose ketamine may promote re-learning with a positive affective valence 24 hours after administration - shifting an initially negative bias toward a positive one - potentially via circuit-level changes in neural plasticity (Hinchcliffe et al., 2024). Overall, the findings reported in this chapter alongside the data from the RELAKS trial, presented in Chapter 2, provide translational support for ketamine's effect in modulating negative biases toward information acquired prior to infusion. Specifically, the current results demonstrate a reduction in negative hits immediately after infusion compared to placebo, reflecting ketamine's rapid-acting capacity to reverse previously encoded negative memories. In parallel, Chapter 2 reports a reduction in negative intrusions 24 hours post-infusion,

suggesting that ketamine may also promote a sustained positive memory bias without impairing overall memory performance.

Despite the effect of ketamine on negative biases associated with self-referred words, there was no effect of ketamine on negative biases associated with autobiographical memories collected prior to randomisation. The effects of ketamine on memory (re)consolidation have been explored in the literature, particularly in relation to post-traumatic stress disorder (PTSD), a condition often comorbid with MDD. However, rodent studies investigating ketamine's effects on fear memory provide mixed and inconclusive evidence (Choi et al., 2020). Outcomes vary depending on the dose, timing, and duration of administration. Post-conditioning ketamine shows dose-dependent effects: low doses (10 mg/kg) administered 24 hours after fear conditioning reduce freezing behaviours during extinction, while higher doses (30 mg/kg) decrease fear generalization for up to two weeks. However, delayed low-dose administration has also been linked to impaired extinction and heightened fear renewal, indicating a narrow therapeutic window. When ketamine was given prior to extinction testing, higher doses (25-30 mg/kg) have minimal impact on freezing but enhance fear recall days later. In contrast, lower doses (10 mg/kg) administered an hour before extinction improve early extinction learning observed 10 days post-treatment. These findings underline ketamine’s complex and context-dependent role in fear modulation, suggesting that precise dosing and timing have a critical impact on its memory effects.

A recent study in non-human primates explored the effects of ketamine on fear memory reconsolidation using a passive avoidance task (Philippens et al., 2021). Marmoset monkeys, conditioned to associate a dark compartment with an aversive experience, received either ketamine (0.5 mg/kg) or saline during memory reactivation one week later. Ketamine-

treated monkeys displayed reduced fear memory, as they willingly entered the previously aversive compartment, unlike their saline-treated counterparts. Similarly, a study in individuals with PTSD examined the potential of ketamine to enhance post-retrieval extinction of traumatic memories. Participants received a single infusion of ketamine (0.5 mg/kg) or an active placebo (midazolam, 0.045 mg/kg) following traumatic memory retrieval, coupled with four days of trauma-focused psychotherapy (Duek et al., 2023). While PTSD symptoms improved similarly across groups, the studies collectively highlight ketamine's potential role in modulating fear memories and enhancing therapeutic interventions. Finally, Das et al. explored the potential of ketamine to disrupt maladaptive alcohol-related memories in heavy drinkers (Das et al., 2019). By targeting the memory reconsolidation process, ketamine was administered during the reconsolidation window following memory retrieval. Results showed that ketamine, only when paired with alcohol memory retrieval, significantly reduced drinking levels, with lasting effects. These findings suggest that ketamine can effectively rewrite reward structures associated with alcohol when combined with memory retrieval. However, the focus on targeting appetitive memories in a non-clinical population, rather than negative biases associated with autobiographical memories in TRD, may account for the differing outcomes observed in this study. Additionally, the infusion protocol diverged from the standard approach used in MDD, as it maintained a steady plasma concentration rather than adhering to the typical clinical practice of administering a 0.5 mg/kg dose at a constant rate over 40 minutes.

Finally, these findings contribute to the broader body of literature exploring the impact of psychopharmacotherapy on autobiographical memories. Research exploring the effect of psychopharmacotherapy on autobiographical memories, has often utilised the Autobiographical Memory Test. For example, Carvalho et al., examined whether the

antidepressants bupropion and sertraline could enhance emotional memory retrieval in healthy volunteers (Carvalho et al., 2006). Their findings showed that neither antidepressant improved memory performance or demonstrated specific memory-enhancing effects from acute monoaminergic stimulation. In contrast, another study using the NMDAR partial agonist D-cycloserine in healthy controls reported that the drug enhanced the retrieval of more specific autobiographical memories, with this effect persisting 24 hours later (R. Chen et al., 2021). These findings suggest that modulation of the NMDAR and glutamate levels, rather than monoaminergic pathways, plays a more critical role in memory processing. Although the current findings do not support the hypothesis that ketamine modulates negative biases associated with autobiographical memories, future studies - including analyses of the complete dataset from this study - exploring its effects on memory specificity could provide deeper insights into ketamine's impact on memory processing in TRD.

5.5 Limitations

The current study has several limitations that should be addressed in future research. First, these findings are based on an interim analysis of a subset of participants, and the final sample will include 60 individuals. Conducting analyses on the complete dataset will be essential to confirm the observed results and provide a more robust understanding of the effects. A larger sample size will also allow for greater generalisability of the findings and more nuanced subgroup analyses.

Second, the study would benefit from incorporating advanced neuroimaging techniques to elucidate the underlying neural mechanisms associated with memory and emotional

processing. Imaging modalities such as functional MRI could provide detailed insights into key circuits, including the prefrontal cortex, hippocampus, and amygdala, which are central to these cognitive and emotional functions.

Lastly, the current study primarily focuses on a limited aspect of memory processing. To achieve a more comprehensive evaluation, future studies should include a broader range of cognitive assessments, particularly those targeting working memory and verbal memory. These additions would offer a more holistic view of memory function and its interplay with emotional regulation.

5.6. Conclusion

In conclusion, these findings complement results from the RELAKS trial conducted in healthy controls, supporting the hypothesis that ketamine reduces behavioural markers of negative bias in memory recall immediately following infusion for information acquired prior to its administration. However, in individuals with TRD, ketamine did not significantly alter negative biases associated with autobiographical memories, highlighting its selective impact on emotional memory processes.

Future research with a larger sample size is needed to validate these findings, and the incorporation of neuroimaging techniques will be crucial for elucidating ketamine's effects on autobiographical memories and emotional processing in the context of TRD.

Chapter 6. General Discussion

Ketamine elicits rapid (within hours) and sustained (up to 7-10 days) antidepressant effects, which extend well beyond its short half-life. While its molecular and cellular mechanisms have been well characterised, the neuropsychological processes underlying its clinical efficacy remain only partially understood. Preclinical studies suggest that ketamine's unique antidepressant profile arises, in part, from its modulation of the lateral habenula - an evolutionarily conserved structure located in the epithalamus, central to the brain's reward-punishment circuitry - which regulates dopamine and serotonin release from the VTA and the raphe nuclei, respectively. Additionally, animal models have shown that ketamine reverses negative affective biases in established negative memories without significantly affecting positive ones, distinguishing it from traditional antidepressants such as venlafaxine. Further, emerging evidence suggests that ketamine (and glutamatergic transmission more broadly) has enduring effects on stress resilience. However, the extent to which these mechanisms translate to humans has not yet been fully investigated.

This DPhil project aimed to assess whether these neuropsychological mechanisms observed in animal models also apply to humans. It draws on data from two experimental medicine trials: the Reward, Emotion Learning and Ketamine Study (RELAKS) and the Glutamate Emotion Memory Study (GEMS). Both trials employed similar designs, with participants randomised to receive either ketamine (0.5 mg/kg) or placebo via a standard 40-minute infusion (60 ml/hour) and completing four (RELAKS) or five (GEMS) visits.

In RELAKS, n = 70 healthy volunteers aged 18-45 completed self-report questionnaires and computerised tasks, including the Emotional Battery Test (ETB), administered before and 24 hours after infusion to assess changes in emotional processing. At 24 hours, participants underwent 7 Tesla task-based fMRI using a win-loss-shock Pavlovian paradigm designed to elicit Hb activation. Seven days post-infusion, participants completed a stress-inducing computer-based task to explore ketamine's effects on stress resilience. During this session, both subjective (self-report questionnaires) and physiological responses to stress were collected, including salivary cortisol (as a proxy for HPA axis activation), salivary alpha-amylase, and heart rate variability (as a marker of autonomic nervous system activation).

In GEMS, n = 60 participants with treatment-resistant depression (TRD) completed the Oxford Autobiographical Memory Task (OAMT) at baseline, 24 hours, and 7 days post-infusion. The OAMT is designed to evaluate emotional biases in autobiographical memories and consists of two components: (A) Autobiographical Memory Retrieval and (B) Word Sorting. At baseline, participants were asked to identify two positive, two negative, and two non-emotional events from the past five years (excluding the prior week). For each memory, participants rated 10 positive or negative adjective/words in terms of how they felt at the time of the event and how they feel about it now, using a sliding scale (0 = "not at all," 10 = "extremely"), aiming at capturing both past and present emotional appraisals.

Results from RELAKS suggest that, compared to placebo, ketamine reduces negative biases for self-descriptive words at 24 hours post-infusion, attenuates the habenula response to negative versus positive outcomes, and enhances activation in reward-related brain regions, including the prefrontal cortex (PFC) and anterior cingulate cortex (ACC). It also reduces the physiological stress response, measured via heart rate variability (HRV), one-week post-

infusion. Interim findings from GEMS (n = 43) suggest that ketamine attenuates negative biases for self-descriptive words shortly after infusion but does not significantly affect emotional biases associated with autobiographical memories compared to placebo.

Taken together, these findings suggest that ketamine modulates negative self-referential biases in both healthy and clinical populations and alters neural responses to aversive stimuli as well as engaging brain reward circuitry. Its effect on stress resilience further reinforces its potential effect on modulation of stress resilience and expand on previous animal and small proof of concept trials. Further research is needed to explore the relationship between ketamine's neuropsychological effects, clinical outcomes and modulation of affective biases at the neuronal level.

6.1 Summary of findings

6.1.1 Clinical outcomes

In RELAKS, no significant effect of treatment was observed for self-report questionnaires of mood, anxiety or anhedonia throughout the study. Interestingly, previous work has reported a transient increase in depressive symptoms in healthy individuals following ketamine infusion. Notably, those effects were identified using a clinician-rated scale (MADRS), while in the present study, data from healthy controls were limited to self-reported depressive symptoms (BDI). This raises the possibility that clinician and self-report measures may capture different facets of the mood response to ketamine, particularly in non-

clinical populations. Further, in RELAKS, the average BDI score (1.9 ± 4.4) was higher than that reported by Nugent et al. (2019) (0.2 ± 0.6), suggesting a greater baseline variability in mood symptoms within our cohort. Additionally, while Nugent et al. (2019) collected data at multiple time points immediately post-infusion (40, 80, and 120 minutes, as well as 1- and 2-days post-infusion), our study assessed depressive symptoms only at pre-infusion and 24 hours post-infusion. This may have limited our ability to capture transient changes in mood occurring in the hours immediately following administration. Similarly, anticipatory pleasure, as measured by the TEPS-Anticipatory subscale, did not differ between the ketamine and placebo groups in our sample, indicating no detectable short-term effect of ketamine on reward anticipation in this context.

In GEMS, depressive symptoms decreased over time, with greater improvement observed in the ketamine group, particularly within the first 24 hours following infusion. Specifically, depressive symptoms decreased over time across both treatment groups. In contrast, no differences between groups were found in measures of anxiety or rumination at baseline or 24 hours post-infusion. Symptoms in both domains remained relatively stable over time, with no evidence of differential change between the ketamine and placebo groups. It is important to note that these findings are based on interim data from a subset of participants (n = 43) rather than the full intended sample of 60, and therefore should be interpreted with caution and re-evaluated once full data are available.

In both trials, as anticipated, ketamine elicited a greater dissociation compared to placebo as measure by the CADSS. While separate study personnel completed the infusion and post-infusion procedures in order to maintain the blind it is possible that this different experience modulated expectation and hence subsequent response of study participants.

6.1.2 Emotional processing

Emotional processing was assessed in both studies using the ETB, although the timing of task administration differed. In RELAKS, assessments occurred pre-infusion and at 24 hours post-infusion, whereas in GEMS, tasks were administered immediately (20 - 30 minutes after the end of the infusion) following drug/placebo administration. This discrepancy reflects a deliberate methodological choice to distinguish ketamine's cognitive effects from its well-established mood-enhancing properties. Specifically, RELAKS recruited healthy volunteers and focused on the 24-hour post-infusion window, when mood changes are expected to peak. In contrast, GEMS, which enrolled participants with TRD, scheduled tasks shortly after infusion to capture early shifts in emotional processing prior to observable mood improvement.

Since the two studies were not launched simultaneously, insights gained from RELAKS informed the design of GEMS. For instance, due to the limited impact of ketamine (versus placebo) on facial expression recognition in RELAKS, this ETB component was not administered immediately after infusion in GEMS. Further, the primary aim of this interim analysis was to examine ketamine's effects on emotional biases associated with previously acquired information, focusing on tasks such as the Emotional Recognition and Emotional Memory (EREC and EMEM) components, alongside the OAMT. Future studies should extend these findings by examining ketamine's influence on the encoding of newly acquired emotional information after infusion, helping to clarify whether its cognitive effects differ based on the timing of information acquisition.

Moreover, ongoing analysis of the GEMS dataset will inform whether the initial emotional bias shift observed immediately post-infusion persists at the 7-day follow-up and how this correlates with depressive symptoms. Such data could help determine whether early neurocognitive changes not only predict antidepressant response - as has been shown for conventional treatments - but may also serve as early indicators of relapse, which often occurs 7-10 days post-ketamine administration.

6.1.3 Reward-punishment neuronal response to ketamine

In RELAKS, ketamine was associated with a greater activation than placebo in medial prefrontal regions, including the paracingulate and anterior divisions of the cingulate gyrus, during win outcomes at the WB level. At the Hb ROI level, ketamine was associated with a higher activation in the positive vs negative contrast compared to placebo. These findings confirm previously observed data on the effect of ketamine at the level of the prefrontal cortex in response to reward stimulus and also apport novel information on the neural effect of ketamine at the level of the Hb. It is noteworthy that we did not observe a significant effect of ketamine on any CS, aside from a very small activation for shock presentation, or for any of the outcomes compared to baseline. It is possible that our focus on the right Hb may have lacked capacity to detect an effect when bilateral habenula is considered, for example in response to loss.

This study is novel in demonstrating that ketamine administration leads to altered activity in the Hb 24 hours post-infusion in healthy individuals, relative to placebo. This finding provides important evidence that ketamine can perturb Hb function even in the absence of clinical depression. However, the activity of the Hb response deviates from expected

patterns in depression compared to healthy controls and raises critical questions about how well these findings would translate to clinical populations. Importantly, there is a paucity of research examining how conventional antidepressants affect Hb function, and it remains unknown whether their mechanisms involve similar modulation of this structure. Understanding how the Hb responds to both rapid-acting and traditional antidepressants could be key to identifying its role in treatment response and relapse. Furthermore, it is noteworthy that it remains unclear whether altered Hb responses are state-dependent - emerging only during acute depressive episodes - or represent a more stable, trait-like vulnerability marker. Clarifying this distinction will be critical for understanding the role of the Hb in the pathophysiology of depression and for determining whether it may serve as a biomarker for risk, illness progression, or treatment response.

Moreover, the present study employed a task-based fMRI approach but did not include resting-state (rs) imaging data, limiting our ability to assess ketamine-induced changes in functional connectivity between the Hb and key regions such as the VTA. Future work incorporating rs-fMRI would provide valuable insight into the network-level dynamics involving the Hb. Additionally, the Pavlovian design of the current task, which required passive observation of conditioned stimuli and outcomes, may not have been optimal for probing learning-related processes. Given the Hb's established role in encoding prediction errors and guiding trial-by-trial behavioural updating, the use of a probabilistic instrumental learning paradigm in future studies may offer a more sensitive approach to capture the computational mechanisms by which ketamine modulates Hb function.

6.1.4 Stress resilience

Chapter 4 presents data on the effects of ketamine compared to placebo on stress resilience when administered one week prior to testing. Participants who received ketamine showed reduced HRV, as measured by RMSSD, during the stress-inducing task, suggestive of enhanced vagal tone and increased parasympathetic activity. This physiological pattern indicates a more adaptive autonomic response to stress, suggesting that ketamine may support greater autonomic balance and reduced physiological reactivity to laboratory-induced stressors. Notably, no significant effects of treatment or task were observed for salivary cortisol or alpha-amylase, commonly used markers of HPA axis and sympathetic nervous system activation, respectively.

Given the one-week interval between drug administration and stress testing, these findings likely reflect a sustained effect of ketamine that is not attributable to its acute pharmacological action. Interestingly, these effects are tested and detected at a time point when the antidepressant response to ketamine typically begins to wane in patients with TRD. This raises the possibility that ketamine's pro-resilience effects may be mediated by distinct mechanisms from its antidepressant properties. Preclinical studies (Mastrodonato et al., 2018) seem to support to this hypothesis: in animal models, prophylactic ketamine administration increases ΔFosB expression in the ventral dentate gyrus and ventral CA3 (vCA3) of socially defeated mice, but not in controls. Inhibition of ΔFosB activity in vCA3 abolishes ketamine's prophylactic efficacy, while overexpression mimics and occludes its stress protective effects. In mice, ketamine also alters memory traces related to contextual fear conditioning specifically within vCA3. These findings suggest that ketamine may induce stress resilience by modulating neural ensembles encoding aversive experiences.

Whether similar hippocampal mechanisms underlie ketamine's pro-resilience effects in humans remains unknown. Future studies should investigate this question, as it may help clarify the role of ketamine (and glutamatergic transmission more generally) in modulating stress-related disorders and stress-associated memory representations.

6.1.5 Autobiographical memories

Interim analyses from the GEMS study using the OAMT revealed a significant main effect of time on negative emotional ratings for negative autobiographical memories, suggesting a reduction in negative affect over the course of the study. This effect was observed for both current emotional responses and retrospective ratings of how participants felt at the time of the event. While both ketamine and placebo groups showed reductions in negative ratings between baseline, 24 hours post-infusion, and the exit visit, the decline was more pronounced for current feelings, with a slightly greater (albeit non-significant) reduction observed in the ketamine group. Ratings of positive emotion remained stable over time, with no significant group differences. These findings indicate that, although both groups experienced reduced negative emotional responses to negative memories, ketamine did not significantly impact memory-related negative biases in this preliminary subsample. For positive and non-emotional memories, there were no significant effects of time or treatment on either positive or negative emotional ratings, with the exception of a modest reduction in negative ratings for current feelings in response to positive memories over time, observed in both groups. No significant treatment-related differences emerged for either memory type.

One of the aims of this study was to translate findings from preclinical models, such as those reported by Stuart et al. (2015), where ketamine, but not venlafaxine, reversed previously

encoded negative memories in rodents. However, the effects of conventional antidepressants, or their interaction with ketamine, on the OAMT remain unknown. Notably, across the trial, a general reduction in negative emotions associated with negative memories was observed, raising the possibility that factors such as expectancy or repeated memory reactivation during participation may have contributed to these changes. This process may reflect mechanisms more aligned with memory reconsolidation rather than a direct pharmacological effect. Of note, this effect may be relevant in the context of psychotherapeutic engagement, where repeated reactivation and emotional reprocessing of memories could contribute to reduced negative affect. However, this hypothesis remains speculative and warrants rigorous investigation in future research.

Finally, as described earlier, we observed an effect of ketamine on emotional biases associated with self-descriptive words in both healthy individuals and TRD participants. However, no treatment-related effects emerged for emotional biases linked to autobiographical memories. If confirmed in the full dataset, these findings may suggest that ketamine's modulation of negative biases in previously acquired information could primarily influence self-referential processing, rather than emotional appraisals tied to external life events. This distinction could have important implications for understanding the specific cognitive-affective domains targeted by ketamine’s antidepressant action.

6.2 Study Limitations

6.2.1 Samples

In addition to the project-specific limitations discussed in the respective chapters, these studies present additional methodological constraints. In GEMS, findings are based on an interim analysis and should be interpreted with caution until confirmed in the full dataset. The role of concomitant antidepressant medications remains uncertain and may have confounded treatment and neuropsychological effects. Furthermore, to fully contextualize the behavioral findings, it will be important to integrate neuroimaging data across the entire sample. In RELAKS, the sample primarily consisted of young, highly educated individuals, which may limit the generalizability of the findings to the wider population. The smaller subgroup of participants who completed 7T imaging and stress resilience assessments also reduced the statistical power for those analyses.

6.2.2 Study duration

Across both studies, the investigation focused exclusively on the rapid effects of a single ketamine infusion, with outcome assessments limited to the first 24 hours and up to one-week post-administration. While this design offers valuable insight into the short-term neuropsychological and physiological effects of ketamine, it does not capture the trajectory of symptom change or the durability of therapeutic benefit over a longer period. Given that ketamine's antidepressant effects are known to wane in approximately 7-10 days post-administration, future research should evaluate the impact of repeated dosing protocols, such

as multiple infusions over a period of days or weeks, and explore how these influence relapse timing, symptom recurrence, and sustained clinical improvement. Longitudinal designs incorporating follow-up beyond the first week would also be important to assess the potential for ketamine to modify long-term mood symptoms and cognitive-affective processing, and to identify patient subgroups who may benefit most from maintenance strategies or combination therapies.

6.2.3 Study design

Another limitation of the studies is the use of a between-groups design. While this approach offers practical advantages (reducing participant burden and learning effects), it also introduces greater variability due to potential differences between groups, which may not be fully accounted for by randomisation. A within-subjects crossover design could, in theory, mitigate such variability by allowing each participant to serve as their own control, thereby enhancing statistical power. However, in the context of ketamine's prolonged and variable effects on neuropsychological mechanisms, a crossover design presents substantial challenges. The persistence of pharmacological and psychological effects beyond the initial treatment session complicates washout requirements and increases the risk of carry-over effects, which could bias results and hinder interpretation. For these reasons, despite its limitations, a between-groups design was considered the most appropriate methodological choice for the present studies.

6.2.4 Blinding

Finally, both studies faced challenges in maintaining effective blinding, which may have influenced participants' expectations and introduced potential bias. Although procedural blinding was implemented - whereby there were study team members who administered the infusion and conducted immediate post-infusion assessments, while a separate, blinded team managed all subsequent visits - participants themselves may have (correctly) inferred their treatment allocation. This is particularly relevant given the well-documented dissociative effects of ketamine, which contrast sharply with the subjective experience of receiving a placebo. In support of this, differences in CADSS scores post-infusion suggested that participants in the ketamine group were more likely to experience noticeable psychoactive effects, potentially compromising blinding. This contextual factor, particularly in the GEMS study enrolling TRD participants, may have interacted with expectancy effects ultimately confounding treatment outcomes. Future studies might consider the use of an active placebo, such as midazolam, to better mask treatment allocation and reduce expectancy-related biases.

6.3 Ketamine in the landscape of the cognitive neuropsychological model of antidepressant action

It is relevant to contextualize the impact of ketamine within the broader framework of emotional processing models, particularly in the context of other antidepressants.

In this thesis, ketamine showed similar emotion-processing shifts to conventional antidepressants wherein the acute administration of SSRIs was shown to enhance the recognition of positive emotional stimuli (Harmer et al., 2008; Harmer, Hill, et al., 2003; Harmer et al., 2004). In RELAKS, ketamine reduced the number of negative intrusions during emotional recall, while in the GEMS study, it reduced the number of negative hits - both findings suggesting a shift towards a positive emotional bias compared to placebo. Such shifts in emotional memory processing may contribute to ketamine's rapid antidepressant effects, aligning with findings from preclinical studies (Stuart et al., 2015). These observations are also consistent with the neuropsychological model of antidepressant action, which proposes that behavioural and neural indices of emotional processing can distinguish between the mechanisms of delayed-onset (standard antidepressants such as venlafaxine) and rapid-acting antidepressants (ketamine). As suggested in animal models, fast-acting agents like ketamine may exert their effects by rapidly modifying emotional biases and memory retrieval patterns - processes that typically change more gradually with conventional treatments (Stuart et al., 2015).

Future studies should aim to elucidate the pharmacological mechanisms underlying these neuropsychological effects, particularly as research into the therapeutic potential of psychedelics continues to expand. While ketamine has some influence on monoaminergic systems (Gigliucci et al., 2013; Li et al., 2015), its primary mechanism of action lies in modulating glutamatergic transmission (Zanos & Gould, 2018). In contrast, classical psychedelics, such as psilocybin, exert their effects primarily through serotonergic (specifically 5-HT_{2A} receptor agonist) pathways, yet they too demonstrate rapid-acting antidepressant properties (Carhart-Harris et al., 2021; Gonzalez-Maeso et al., 2007; Goodwin et al., 2022). It will be of particular interest to determine whether the

neuropsychological distinctions observed in preclinical models - such as shifts in emotional bias and memory retrieval - extend across agents with differing pharmacological profiles. Clarifying whether these effects are drug-specific or represent a shared downstream mechanism of rapid-acting antidepressants could have important implications for treatment development and mechanistic understanding.

6.4 Experimental design approach

Studying healthy volunteers offers a valuable platform for investigating the mechanisms underlying antidepressant action, as it eliminates confounding factors such as current mood symptoms, psychiatric comorbidities, or concomitant medications. This approach allows for a more controlled examination of how pharmacological agents influence emotional and cognitive processes. Previous research has demonstrated the utility of healthy participants in modelling the early cognitive and affective changes induced by antidepressants (Browning et al., 2007; Harmer et al., 2008; Murphy et al., 2008; Walsh et al., 2018). However, it is important to acknowledge that findings in healthy individuals do not always generalise to clinical populations. For example, right Hb shows different pattern of activation in healthy controls and in individuals with depression (Lawson et al., 2017), underscoring the need for caution when extrapolating results from non-clinical to clinical contexts.

The use of healthy volunteer studies offers clear advantages, particularly in disentangling the neuropsychological effects of a treatment from its impact on mood. This distinction is especially important in the context of rapid-acting antidepressants, such as ketamine, where

mood improvements can emerge within hours and potentially confound behavioural outcomes. For instance, findings from RELAKS suggest that ketamine may induce a positive bias in self-referential word recall 24 hours post-infusion - a time point typically associated with peak antidepressant effects. However, this effect could not be directly replicated in GEMS. Repeating the tasks at the same 24-hour timepoint in TRD participants may coincide with the emergence of the clinical antidepressant response, potentially confounding or interacting with the underlying neuropsychological mechanisms of interest. This highlights the importance of including assessments immediately post-infusion, when mood improvements are unlikely to have taken full effect. Such early assessments allow for a more precise characterization of ketamine's cognitive and affective effects, independent of its clinical efficacy.

6.5 Clinical implication

While immediate clinical implications from this work may not be readily apparent, the findings offer promising avenues for therapeutic innovation. The observed ketamine-induced reduction in negative biases toward self-describing words highlights a potential window of opportunity to enhance psychotherapeutic engagement. If this finding is confirmed, delivering psychotherapy in conjunction with ketamine treatment could capitalize on the ketamine-induced cognitive-affective shift and potentially enhance and sustain the antidepressant response over time. This integrative approach may offer a more robust and enduring therapeutic effect than pharmacological treatment alone. However, whether such transient changes in emotional processing yield lasting antidepressant effects remains an open question. Additionally, completing the analysis of emotional biases

associated with autobiographical memories is essential. These insights could deepen our understanding of how ketamine modulates self-referential affective processing and help tailor personalized, mechanism-based interventions.

6.6 Future direction

Future studies may benefit from assessing the effects of ketamine shortly after drug administration, especially in the context of clinical population. Early timepoint assessments would allow for more direct comparisons with conventional antidepressants and help determine whether shifts in emotional processing precede clinical improvement. In addition, future trials should examine whether the effects observed following a single ketamine infusion are sustained or altered with repeated administration. Parallel long-term follow-up studies are also needed to monitor depressive relapse and elucidate the neuropsychological mechanisms underlying relapse following rapid-acting antidepressant treatment. This line of inquiry could contribute to the development of early biomarkers of relapse risk and inform strategies for maintaining treatment response.

More specifically, further research should aim to replicate and extend the current findings to clinical populations. For instance, the ketamine-related modulation of right Hb activity observed in healthy volunteers should be investigated in individuals with TRD, to explore whether ketamine's antidepressant effects are mediated by changes in Hb function. Including resting-state data in future studies would also be particularly valuable to better capture Hb connectivity with other brain regions implicated in ketamine's mechanism of action, such as the prefrontal cortex and ACC. This approach could offer important insights

into the network-level dynamics underlying ketamine's antidepressant effects. Additionally, future studies should go beyond measures of emotional bias to include objective assessments of memory performance. Although total memory performance appeared unaffected in the tasks used in these studies (as indicated by the overall number of words recalled or misclassified), these findings suggest that ketamine's effects on emotional bias may not reflect a general impact on memory processes. Nonetheless, future studies should incorporate dedicated memory assessments to further clarify this relationship.

Finally, findings related to ketamine's effects on stress resilience warrant deeper investigation into the neural circuitry underlying these responses. Mapping these mechanisms could provide valuable insight into how ketamine modulates adaptive responses to stress and inform future therapeutic targets for stress-related psychopathology

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Appendix

RELAKS. List of inclusion/exclusion criteria

Inclusion criteria

- Age between 18 to 45 years
- BMI between 18 and 30
- Participant is willing and able to give informed consent for participation in the study
- Sufficient knowledge of English language to understand and complete study tasks
- Willingness to refrain from driving, cycling, or operating heavy machinery, until the following morning or a restful sleep has occurred, whichever is later.
- Willingness to refrain from signing legal documents the day of the infusion visit.
- Willingness to refrain from drinking alcohol for 3 days before the infusion visit and one day before any of the other visits throughout the study

Exclusion criteria

- The participant may not enter the study if ANY of the following apply:
- Any current or past DSM-V significant psychiatric disorder including any psychotic, mood and anxiety and borderline personality disorders
- History of, or current medical conditions which, in the opinion of the investigator, may interfere with the safety of the participant or the scientific integrity of the study, including epilepsy/seizures, brain injury, hepatic or renal disease, diabetes, severe gastro-intestinal problems, Central Nervous System (CNS) tumours, neurological conditions

- First-degree relative with a diagnosis of schizophrenia-spectrum or other psychotic disorder, or bipolar disorder
- History of unexplained hallucinations or impulse control problems (e.g. pathological gambling)
- Current or past history of heart rhythm disorders
- Clinically significant hypertension
- Increased intraocular pressure/glaucoma
- Current pregnancy (as determined by urine pregnancy test taken during Screening and Infusion Visits) or breastfeeding
- Clinically significant abnormal values for clinical chemistry (e.g. liver function tests), urine drug screen, blood pressure measurement and ECG. A participant with a clinical abnormality or parameters outside the reference range for the population being studied may be included only if the Investigator considers that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures
- Current or previous intake (last three months) of any medication that has a significant potential to affect mental functioning (e.g. benzodiazepines, antidepressants, neuroleptics etc.)
- Any intake of recreational drugs in the last 3 months (e.g. marijuana, ecstasy etc.)
- Lifetime recreational use of ketamine or phencyclidine
- Regular alcohol consumption of more than 14 units a week or excessive alcohol consumption up to three days before any of the in-person study visits · Inability to abstain from alcohol for more than 1 week
- Regular smoker (> 5 cigarettes per day)
- Excessive caffeine user (> 6 caffeinated drinks per day)

- History of recurrent rashes or history of allergic reactions to relevant substances (ketamine treatment, placebo treatment)
- Previous participation in a study using the same or similar tasks, as assessed by an interview to the participants by a trained member of the study team wherein a brief description of the tasks will be provided (to the best of participant's recollection).
- Current participation in another study or participation in similar study within the last 6 months
- Participant is unlikely to comply with the clinical study protocol or is unsuitable for any other reason, in the opinion of the Investigator
- Claustrophobia
- Any implants (including dental implants) or pacemaker
- Tattoos above the chest
- Any other MRI contraindications outlined in FMRIB 7 Tesla scanning safety form

GEMS. List of inclusion/exclusion criteria

<u>Inclusion criteria</u>

- Male or female;
- Aged 20-60 years;
- Willing and able to give informed consent for participation in the study;
- Sufficiently fluent English to understand and complete the tasks;
- Registered with a GP and consents to GP being informed of participation in the study;

- Participants need to meet a number of concurrent clinical criteria:

- Current criteria for Major Depressive Disorder, in a current major depressive episode with a duration of no longer than two years, as determined by the SCID-5;
- Inadequate response to at least one and no more than three antidepressant treatments within the current episode as documented during the clinical interview
- Currently taking a licensed antidepressant at a therapeutic dose for at least four weeks

- Pre-menopausal women and male participants engaging in sex with a risk of pregnancy must agree to use a highly effective method of contraception from Screening Visit until 30 days after receiving the study medication treatment. Acceptable methods of contraception include:
  - Condoms
  - Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal or transdermal;
  - Progestogen-only hormonal contraception associated with inhibition of ovulation: oral, injectable or implantable;
  - Intrauterine device (IUD);
  - Intrauterine hormone-releasing system (IUS);
  - Bilateral tubal occlusion;
  - Vasectomy (or vasectomised partner);
  - Sexual abstinence. [Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), and spermicides only are not acceptable methods of contraception.]

- Male participants must not donate sperm until 30 days after receiving the study medication.
- Participants taking non-prescription/prescription medication may still be entered into the study, if, in the opinion of the Investigator, the medication received will not interfere with the study procedures or compromise safety
- Willingness to refrain from driving, cycling, or operating heavy machinery, until the following morning or a restful sleep has occurred, whichever is later.
- Willingness to refrain from drinking alcohol for 3 days before the infusion visit and one day before any of the other visits throughout the study

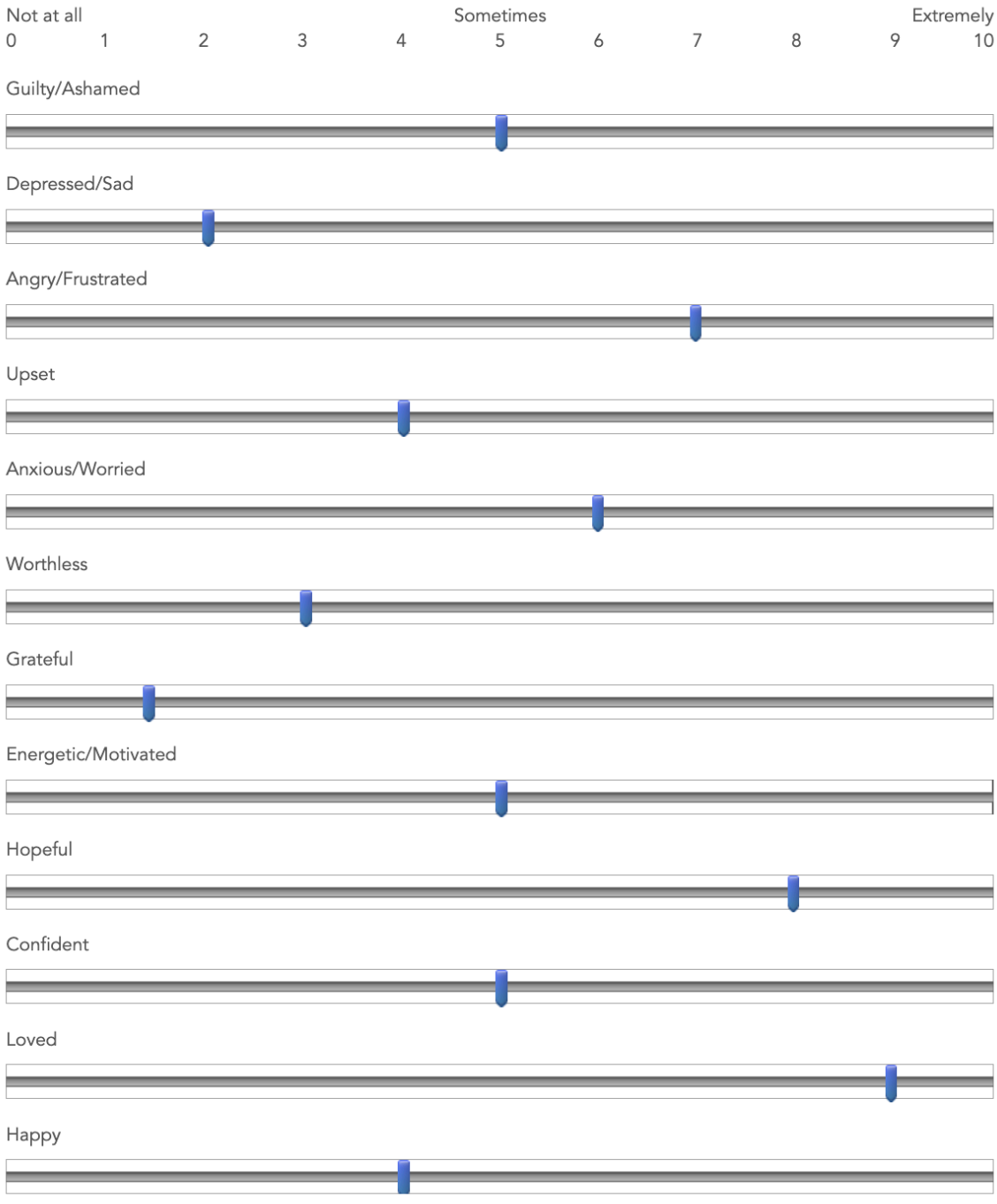
Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- History of /or current DSM-5 bipolar disorder, schizophrenia or emotionally unstable personality disorder [co-morbid anxiety disorders (including agoraphobia, generalized anxiety disorder, social anxiety disorder and panic disorder) and Posttraumatic Stress Disorder (PTSD) are allowed];
- Participants who fulfil current criteria for other comorbid disorders may still be entered into the study, if, in the opinion of the Investigator, the psychiatric diagnosis will not compromise safety or affect data quality;
- Diagnosis of a major cognitive disorder or evidence of cognitive impairment;
- Clinically significant risk of suicide;
- Participants undergoing or who have undergone electroconvulsive therapy for the treatment of the current episode of depression;
- Substance or alcohol use disorder over the past 6 months;

- Regular alcohol consumption of more than 21 units a week or excessive alcohol consumption up to three days before any of the in-person study visits or inability to abstain from alcohol for more than 3 days
- Moderate cigarette use (> 10 cigarettes per day)
- History of, or current general medical conditions that in the opinion of the Investigator may interfere with the safety of the participant or the scientific integrity of the study;
- Current pregnancy (as determined by urine pregnancy test), breastfeeding, planning a pregnancy, or unwillingness to practice birth control during the course of the study;
- Clinically significant abnormalities of laboratory tests, physical examination, or ECG. A participant with a clinical abnormality or parameters outside the reference range for the population being studied may be included only if the Investigator considers that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures;
- Current or past history of heart rhythm disorders
- Clinically significant untreated hypertension
- Any contraindication to MRI including claustrophobia, any trauma or surgery which may have left magnetic material in the body, magnetic implants or pacemakers, and inability to lie still for 1 hour or more;
- Previous participation in a study using the same, or similar, emotional processing tasks in the last three months;
- Previous lifetime use of ketamine or phencyclidine;
- Participant with planned medical treatment within the study period that might interfere with the study procedures;
- Participant who is unlikely to comply with the clinical study protocol or is unsuitable for any other reason, in the opinion of the Investigator.

Word Sorting Interface of the Oxford Autobiographic Memory Task (OAMT)



Participants were presented with the adjectives/nouns listed below and asked to rate the extent to which each word reflected their feelings currently and at the time of the event related to a specific autobiographical memory. Ratings were provided on a visual analogue scale ranging from 0 ("Not at all") to 10 ("Extremely"). This interface constituted the Word Sorting Component of the OAMT and was used to quantify the subjective emotional tone associated with personal memories.