Using pharmacological reconsolidation-interference strategies to attenuate maladaptive appetitive memories

by

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

Abstract

Under certain conditions memories can re-enter a transient, labile state in which they are susceptible to modification. 'Reconsolidation' thus describes the hypothetical process by which a reactivated memory is returned to a stable state. The current thesis will explore the potential of pharmacological reconsolidation-interference strategies in attenuating the maladaptive appetitive memories underlying alcohol dependence and binge eating disorder (BED). **Chapter 1** presents an overview of the reconsolidation literature and its potential to treat disorders of maladaptive appetitive memory. In **Chapter 2**, a review and meta-analysis of the efficacy of treatments utilising behavioral and pharmacological reconsolidation strategies in clinical or sub-clinical populations is presented. In **Chapter 3**, the requirement for the inclusion of a prediction error (PE) at retrieval in a population of hazardous drinkers is assessed in a randomised, between subjects design (N=60). Although no effect of post-retrieval N_2O (a predicted blocker of reconsolidation) was observed initially, exploratory analysis showed a memory-weakening effect only when administration occurred after cue-alcohol retrieval *and* PE. **Chapter 4** presents a single blind, randomised, between subjects (N=90) study of the efficacy of the NMDA receptor antagonist ketamine. Relative to placebo and a no-reactivation group, ketamine produced significant reductions in drinking and putative measures of cue-alcohol memory strength. **Chapter 5** explores the efficacy of rapamycin, a proven blocker of reconsolidation in pre-clinical models, to attenuate non-drug reward memory in a population with a tendency of overeat or binge on chocolate (N=75). No effect of rapamycin was observed, although this may represent the limited scope to see improvement in measures of disordered eating within this sample. Finally, **Chapter 6** summaries and integrates the current findings into the existing literature. A discussion of the implications, limitations, and suggestions for future research on reconsolidation is given.

Impact statement

Disorders of appetitive memory, including substance use disorders and binge eating disorder are associated with enormous social, economic, and health impacts. Current treatments for these disorders suffer from high relapse rates, even after prolonged abstinence and long after acute withdrawal symptoms have passed. This is likely due to their failure to treat the maladaptive reward memories that underlie the formation and maintenance of these disorders. Memory reconsolidation, the process by which memories can re-enter a labile state (resembling the pre-consolidated state), therefore offers a promising mechanism through which these memories might be attenuated or modified. Despite this, in 2015 (at the outset of this PhD) just six studies of human appetitive reward memory reconsolidation had been published. The three studies of human appetitive reward memory reconsolidation-interference presented here therefore represent a significant contribution to the reconsolidation literature.

Given that maladaptive reward memories implicated in appetitive disorders are targeted in the studies outlined in this thesis, it can be argued that the results presented here are of significant clinical relevance. Perhaps most strikingly, when ketamine was administered after alcohol memory reactivation, total consumption of alcohol was more than halved over a period of nine months. Despite being tested in a sub-clinical (heavy drinkers) sample, it is likely that similar effects would be observed in patients with alcohol dependence. The ketamine study provides a strong rationale for a formal clinical trial to be conducted.

This thesis also presents the first study to attempt to attenuate the appetitive memories associated with overeating in a binge-eating phenotype using reconsolidation-interference. Further, this is only the second to use the mTOR inhibitor rapamycin with a human reconsolidation-interference paradigm.

All the work presented here has, or is in the process, of publication within academic journals (details of published studies given below). Where possible, the outlined studies used open science practices. A pre-print of the meta-analysis presented in Chapter 2, for instance, was published on bioRxiv (http://tiny.cc/ex92az) prior to submission for publication. Further, all data collection and analytic procedures for the study in Chapter 5 were pre-specified and pre-registered on the Open Science

Framework database (*http://osf.io/tqxdb*). Beyond academia, the work presented in Chapter 4 has been disseminated to the public, both within a newspaper article (The Guardian *http://tiny.cc/4aa3az*), and in a television documentary (VICE *http://tiny.cc/oha3az*).

The work presented in the current thesis gave rise to the following publications:

Walsh, K., Das, R. K., & Kamboj, S. K. (2017). The Subjective Response to Nitrous Oxide is a Potential Pharmaco-Endophenotype for Alcohol Use Disorder: A Preliminary Study with Heavy Drinkers. *International Journal of Neuropsychopharmacology, 20*(4), 346-350. doi: 10.1093/ijnp/pyw063

Walsh, K. H., Das, R. K., Saladin, M. E., & Kamboj, S. K. (2018). Modulation of naturalistic maladaptive memories using behavioural and pharmacological reconsolidation-interfering strategies: a systematic review and meta-analysis of clinical and 'sub-clinical' studies. *Psychopharmacology 235*(9), 2507-2527. doi: 10.1007/s00213-018-4983-8

Das, R. K., **Walsh, K.,** Hannaford, J., Lazzarino, A. I., & Kamboj, S. K. (2018). Nitrous oxide may interfere with the reconsolidation of drinking memories in hazardous drinkers in a prediction-error-dependent manner. *Eur Neuropsychopharmacol, 28*(7), 828-840. doi: 10.1016/j.euroneuro.2018.05.001

Walsh, K., Iskandar, G., Kamboj, S. K., & Das, R. K. (2019). An assessment of rapamycin for weakening binge-eating memories via reconsolidation: A pre-registered, double-blind randomised placebo-controlled experimental study. *Psychol Med*, 1-10. doi: 10.1017/S003329171900312X

Das, R. K., Gale G., **Walsh, K.**, Hennessy, V., Iskandar, G., Mordecai, L., Brandner, B., Kindt, M., Otten, L., Curran, H. V. &, Kamboj, S. (in press). Ketamine can reduce harmful drinking by pharmacologically rewriting drinking memories. *Nat Commun*.

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List of abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
A**PKC**	protein kinase
AUD	alcohol use disorder
BED	binge eating disorder
Ca^{+}	calcium
CaMK	Ca^{2+}/calmodulin-dependent kinase
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CR	conditioned response
CREB	cAMP response element binding protein
CS	conditioned stimulus
DCS	D-cycloserine
ERK	extracellular signal-regulated kinase
ES	effect size
GABA	γ-aminobutyric acid
HPF	high palatable foods
LPF	low palatable foods
LTD	long term depression
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
Mg^{2+}	magnesium
MRM	maladaptive reward memories
mTOR	mammalian target of rapamycin
Na^{+}	sodium
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMDA	*N*-methyl-d-aspartate
NMDAR	*N*-methyl-d-aspartate receptor
PE	prediction error
PIT	Pavlovian-to-instrumental transfer
PKA	protein kinase
SUD	substance use disorder
UCS	unconditioned stimulus
UR	unconditioned response
Zif268	zinc finger protein 225

Glossary

Reconsolidation	The hypothetical process by which a memory renters an active, labile state, from which restabilisation is required
Retrieval	The process by which memories are returned to a state in which they can be expressed (i.e. recalled) and used for behaviour. May also refer to experimental procedures that are intended to reactivate/destabilise memories, but which may or may not be successful in this regard.
Reactivation	The first stage of the reconsolidation process, as well as a memory state that is highly accessible and malleable.
Destabilisation	The molecular process by which the memory is returned to an active state. Induced by memory reactivation.
Restabilisation	The molecular process in which the memory is once again returned to an inactive, stable state.
Naturalistic memories	Memories that are naturally- (rather than experimentally-) acquired. The learning history of naturally-acquired maladaptive memories is usually unknown (except perhaps, in the case of recent, single-incident traumas underlying posttraumatic stress disorder; PTSD), although it is assumed that in general, these memories are formed through multiple, intermittent reinforcements (Pavlovian and instrumental) in a variety of contexts and over a prolonged period
Natural	Used here to refer to non-drug rewards (e.g. food)

Chapter 1.

General Introduction

1.1 Reinforcement and reward learning

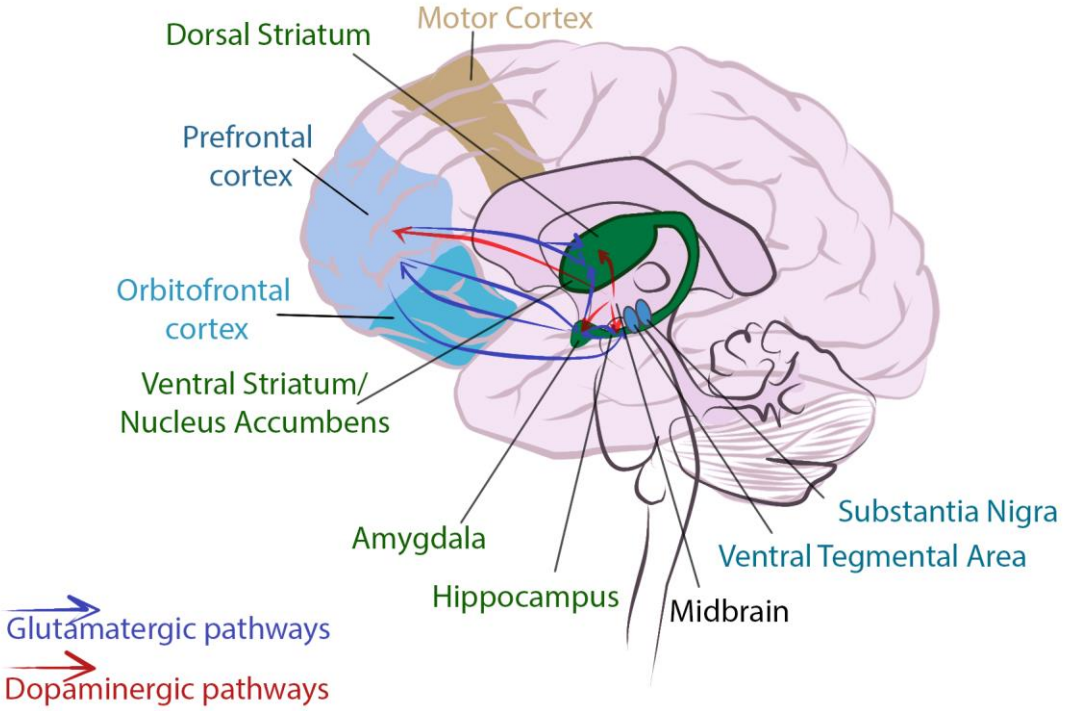
Under typical circumstances, reward learning plays a central role in adaptive behaviour. Primary rewards such as food and water are intrinsically motivating due to their survival value, and thus drive behavioural approach. Associative learning describes the processes by which discrete stimuli, or a behavioural response and stimuli, become associated with one another. During Pavlovian conditioning, previously neutral cues and environmental stimuli (conditioned stimuli; CS) are associated with a reinforcer (unconditioned stimuli; US) that elicits a behavioural response (Pavlov, 1927). Through paired-presentation with the US, the presentation of the CS alone eventually produces the response that was previously elicited by the US. This is referred to as a conditioned response (CR). Importantly, in Pavlovian conditioning, while associations between CSs and outcomes are learned, the learning agent is largely not in control of the contingency between CS and outcome. Conversely, in **instrumental,** or **operant conditioning**, a behavioural response itself is rewarded or punished, and thus the association between this response and its consequence is learnt (Skinner, 1938). Adaptive responses important for survival are therefore more likely to be repeated, while those that predict punishment are not. Existing Pavlovian conditioning can influence subsequent instrumental conditioning via Pavlovian-to-instrumental transfer (PIT), whereby an instrumental response can be invigorated in the presence of a Pavlovian cue associated with a reward (conditioned motivation; Cartoni, Puglisi-Allegra, & Baldassarre, 2013). Environmental stimuli paired with rewards may also acquire reinforcing properties, and conditioned approach occurs when the CS elicits approach behaviour (Fitzpatrick & Morrow, 2016). Finally, conditioned reinforcement describes the level to which an individual will work to acquire the CS (Everitt, Dickinson, & Robbins, 2001; Taylor & Robbins, 1984). Together, these processes of adaptive learning allow organisms to predict the presentation of rewards central to survival and elicit goal-directed behaviours to obtain them.

1.2 Neuroanatomy of reinforcement and reward

The basic mechanisms through which reward learning occurs depends primarily on dopaminergic and glutamatergic (but includes noradrenergic, endocannabinoid, opioid, glucocorticoid and GABAergic) signalling and the cellular and molecular

cascades downstream of their associated receptor sites. These pathways and associated brain structures are depicted in *Figure 1.1.*

***Figure 1.1**. Brain structures and circuitry associated with reward learning and memory*



Schematic representing the brain structures and pathways implicated in reinforcement and reward learning. The mesocorticolimbic dopamine pathway (in red) consists of projections from the ventral tegmental area and substantia nigra in the midbrain to the dorsal striatum and ventral striatum, which is inclusive of the nucleus accumbens (Ikemoto, 2010). Glutamatergic pathways (in blue) project between the pre-frontal cortex, amygdala, hippocampus, ventral striatum (including the nucleus accumbens), and ventral tegmental area (Kelley & Berridge, 2002).

Dopaminergic projections from the ventral tegmentum and substantia nigra in the midbrain, to the dorsal striatum and ventral striatum comprise the **mesocorticolimbic** dopamine pathway, known also as the primary "reward pathway". Other brain areas including the amygdala, prefrontal cortex, and hippocampus are also associated with the processing of primary rewards. For instance, activation of the ventral striatum and nucleus accumbens is associated with stimulus-outcome learning (Everitt & Robbins, 2005), while pre-clinical studies implicate the amygdala in some types of reward-representation and stimulus-reward learning (Baxter & Murray, 2002). Lesions to the central nuclei of the amygdala, for example, abolish conditioned orientating to food rewards (Holland, 1977) and PIT (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001).

Exposure to both primary and secondary rewards is associated with the activation of the prefrontal cortex (e.g. Rolls et al., 2003). This brain structure exhibits top-down cognitive control over reward, and may be involved in the encoding of reward value and expectations (Duverne & Koechlin, 2017). Via its interaction with the amygdala, the prefrontal cortex is implicated in executive control over conditioned reinforcement and PIT (Everitt & Robbins, 2005). The hippocampus, together with the prefrontal cortex, likely encodes contextual information about the reward, while interactions between the prefrontal cortex and the nucleus accumbens and dorsolateral striatum are associated with goal-directed actions (for a review, see Everitt & Robbins, 2005).

1.2.1 Dopamine and reward

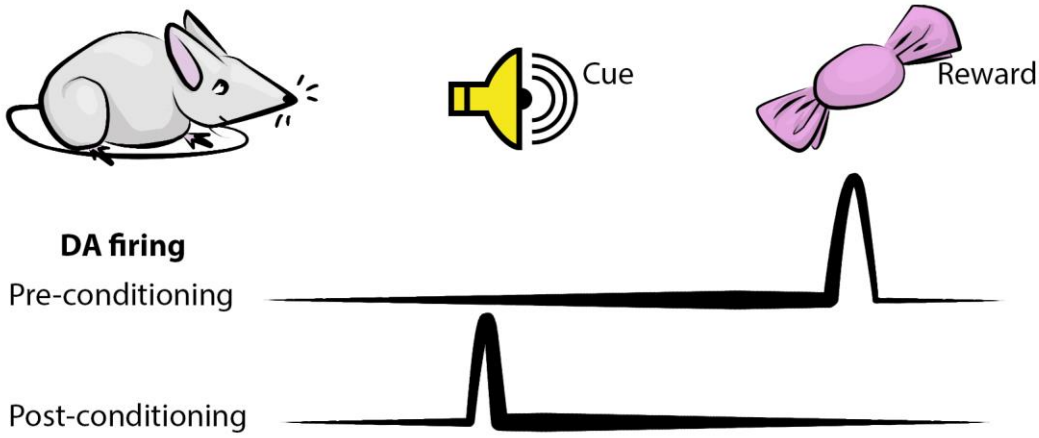
Primary rewards are sought as they confer pleasurable, rewarding effects. These pleasurable properties of rewards were initially thought to be mediated by the neurotransmitter dopamine. The first studies to elucidate the role of dopamine demonstrated that rats will repeatedly self-stimulate specific dopaminergic areas of the brain (e.g. Crow, 1972) and many drugs of abuse are associated with the production of dopamine in the mesolimbic system (Boileau et al., 2003; Di Chiara & Imperato, 1988; Urban et al., 2010). Studies correlating dopamine with the euphoric effects of drugs (e.g. Drevets et al., 2001) led to the assumption that dopamine mediated reward and pleasure. Disappointingly, treatments for substance use disorders (SUD) which target the dopamine system have not proved efficacious (e.g. Kreek, LaForge, & Butelman, 2002; Rothman, 1994) and blockade of the dopamine receptors does not prevent the pleasurable effects from stimulant drugs (e.g. Brauer & De Wit, 1997).

In 1989, Berridge, Venier, and Robinson (1989) demonstrated how rats depleted of dopamine will stop consuming sucrose, despite continuing to elicit behaviours indicative of enjoying the taste. Rats depleted of dopamine can also starve to death even when food is readily available (e.g. Szczypka et al., 1999), suggesting a role for dopamine in *motivation*, rather than pleasure. Indeed, several human neuroimaging studies have since demonstrated correlations between mesolimbic dopaminergic brain activity and wanting of food and drug rewards, but not for liking of them (Evans et al., 2006; Leyton, 2002; Volkow, Fowler, Wang, Baler, & Telang, 2009). On this basis, Berridge et al. (1989) proposed the **incentive-sensitisation** model of addiction, suggesting that the 'wanting' (or 'incentive salience') and 'liking' of drugs are dissociable processes, with the former mediated by the dopaminergic system. This differentiation and the

incentive sensitisation model have been hugely influential on contemporary models of reward learning and reward-related disorders.

Dopamine may therefore assign motivational salience to cues. When rewards are first experienced, phasic dopamine release from the ventral tegmental area and substantia nigra is thought to code the predictability of these events. A reward which is better than predicted, for instance, elicits increased phasic dopamine release, while a reward that is exactly as predicted does not (Day, Roitman, Wightman, & Carelli, 2007). This discrepancy between predicted and actual reward (i.e. a 'prediction error'; PE) acts as a signal to the reward circuitry to initiate adaptive behavioural responses (e.g. Jay, 2003). Over time, the reward and the environmental stimuli which consistently predict its occurrence become associated, and dopamine firing occurs in response to the environmental stimulus itself (see *Figure 1.2*; Day et al., 2007; Schultz, Dayan, & Montague, 1997; Volkow et al., 2008). The dopaminergic system is therefore thought to orientate behaviour towards rewards and cues that are important for survival.

Figure 1.2. *Dopaminergic firing to environmental stimuli and associated reward pre- and post-conditioning*



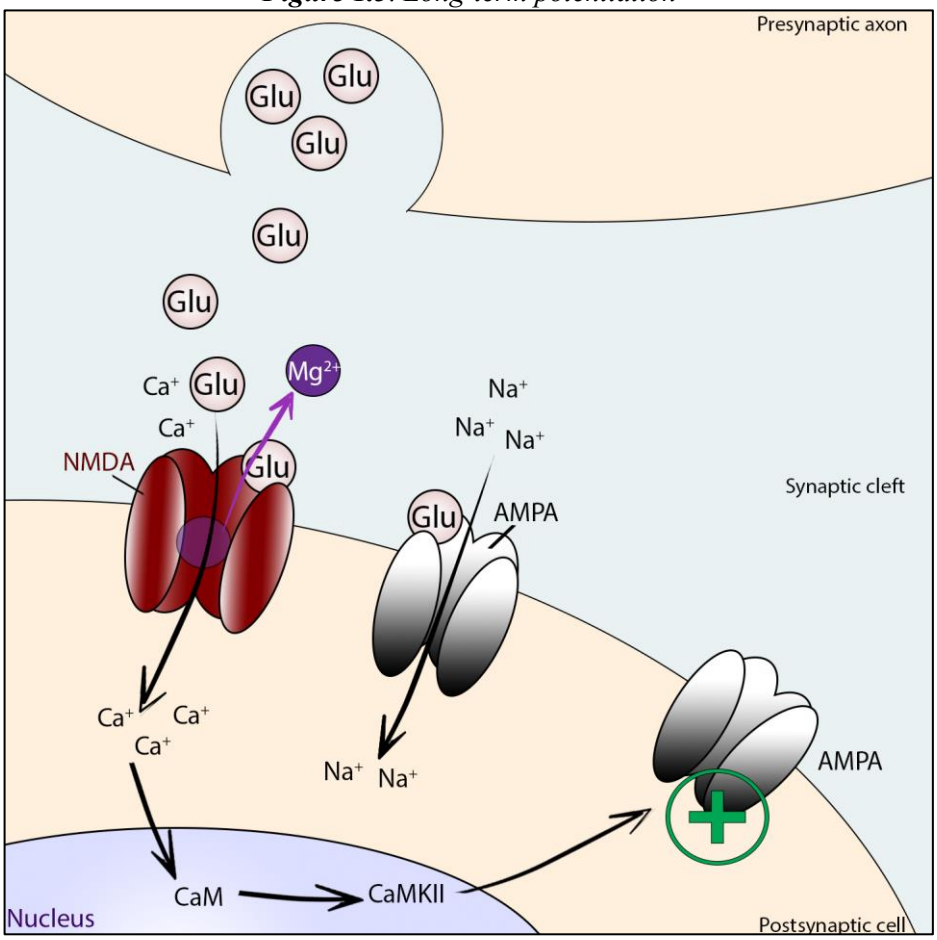
Initially, dopaminergic firing occurs in response to the receipt of a reward. Over time, environmental stimuli ('cues') which consistently predict the occurrence of the reward become associated, and dopaminergic firing occurs in response to the presentation of the associated stimuli. Subsequently (after responses to the cue are well established), omission of the reward results in a reduction in ventral tegmental area DA neuron firing, representing a negative prediction error (Schultz, 2016).

1.2.2 Glutamate and reward

Glutamatergic projections from the pre-frontal cortex (PFC) to the ventral striatum are implicated in learning about rewards (Everitt & Robbins, 2005), and the excitatory glutamatergic N-Methyl-D-Aspartate (NMDA) and α-amino, 3–methyl 5-hyroxy 4-isoxazolepropionic acid (AMPA) receptors are associated in long-term plasticity. Crucially, activation of NMDA receptors (NMDAR) is required for the induction of **long-term potentiation (LTP)**, the process by which the connections between neurons are strengthened with frequent activation (see *Figure 1.3*), and **long-term depression (LTD)**, in which these connections are weakened. Although LTP and LTD were initially discovered in the hippocampus (and thus were assumed to underlie only learning and memory; Cajal, 1894), these processes have since been demonstrated across the central nervous system (Malenka & Bear, 2004).

Interactions between the glutamate and dopamine systems mediate plasticity throughout the reward network. For instance, LTP at the hippocampal-prefrontal cortex synapses is dependent on the coactivation of dopaminergic D1 and NMDA receptors, while in the prefrontal cortex and striatum, NMDAR activation is potentiated by activation of the D1 dopamine receptors (Seamans, Durstewitz, Christie, Stevens, & Sejnowski, 2001; Wang & O'Donnell, 2001).

***Figure 1.3**. Long-term potentiation*



Adapted from Malenka and Nicoll (1999)

Long-Term Potentiation (LTP) describes the process through which the connections between neurons are strengthened following repeated activation. LTP occurs when glutamate is released from the presynaptic axon and binds to NMDA and AMPA receptors on the post-synaptic cell. Na^+ enters the postsynaptic cell through the AMPA channel and depolarises the cell. Depolarisation removes the voltage-dependent Mg^{2+} block from the NMDA receptor, producing an influx of Ca^{2+} through the NMDA receptor. This results in the activation intracellular signalling cascades inclusive of CaMKII (Malenka & Nicoll, 1999), and the phosphorylation and insertion of additional AMPA receptors. These structural changes to the post-synaptic cell increases the connectivity between the two neurons, the maintenance of which is dependent on protein synthesis (Lynch, 2004).

1.3 Maladaptive reward learning

Drugs such as alcohol, cocaine and amphetamines are associated with the dysregulated, prolonged release of dopamine in the mesolimbic structures (Boileau et al., 2003; Wolf, 2002). This has led to the suggestion that drugs, and potentially other 'hyper-normal' rewarding substances like highly palatable foods (HPF, foods high in fat and/or sugar; Volkow, Wang, Tomasi, & Baler, 2013) can 'hijack' the reward system, producing hyper-motivated responding to cues predictive of drug or HPF availability and continued over-consumption of drugs or HPFs. Indeed, the acute release of dopamine caused by drug use is thought to underlie conditioned Pavlovian and instrumental cue-associations between environmental stimuli, actions and the drug itself (Wyvell & Berridge, 2000). Repeated drug use continually strengthens these memories via this dopaminergic mechanism, producing powerful behavioural responses to drug associated stimuli that lead to maladaptive over-use (Vanderschuren & Everitt, 2005). Over time, the formation of these maladaptive reward memories (MRM) means subsequent exposure to reward cues can trigger 'wanting' or craving of the reward, and drive behaviours required to obtain it (Robbins, Ersche, & Everitt, 2008). This cue-elicited behaviour may underlie appetitive disorders, in which initially voluntary reward seeking behaviour becomes habitual, and then compulsive (Everitt & Robbins, 2005).

Repeated, intermittent, and prolonged drug use can also produce sustained sensitisation of the mesolimbic dopamine and glutamate system in the brain (e.g. Kalivas & Stewart, 1991; Post, 1980; Robinson & Becker, 1986; Wolf, 2010; Wolf, Mangiavacchi, & Sun, 2003), reducing dopaminergic tone and phasic responses to non-drug reward and increasing dopaminergic responses to drug rewards. These physical changes to the mesolimbic neurons are assumed to leave chronic drug users hypersensitive to the use of drugs and hyper-reactive to environmental cues that predict their use (Leyton & Vezina, 2013; Robinson, Browman, Crombag, & Badiani, 1998; Robinson & Kolb, 2004). Indeed, human brain imaging studies demonstrate greater activity within the mesocorticolimbic circuitry following the presentation of alcohol and alcohol-related cues, relative to neutral beverages and cues in those with alcohol use disorder (AUD; Claus, Ewing, Filbey, Sabbineni, & Hutchison, 2011; Filbey et al., 2008; George et al., 2001; Hanlon et al., 2018; Kareken et al., 2004; Wrase et al., 2007). Furthermore, these responses correlate with severity of alcohol use problems,

suggesting that greater activity in the prefrontal cortex, striatum, ventral tegmentum and substantia nigra in response to alcohol cues are observed in those with more disordered alcohol consumption (Claus et al., 2011; Filbey et al., 2008; George et al., 2001). This same reward-specific pattern of activity is observed in overweight or obese samples, whereby exposure to HPF cues is associated with mesolimbic activity, with greater activity seen in those who reported more binge-eating symptoms (Filbey, Myers, & Dewitt, 2012).

Beyond the dopaminergic system, long-term alcohol consumption is associated with the attenuation of LTD in glutamatergic synapses in the dorsal striatum (Munoz, Fritz, Yin, & Atwood, 2018), as well as dysregulated synaptic activity in the central nuclei of the amygdala (McBride, 2002). Glutamatergic projections from the basolateral amygdala and nucleus accumbens core are also implicated in cocaine seeking in rodents (Di Ciano & Everitt, 2004). Ultimately, these neural changes lead to shifts in the neurocircuitry underlying reward learning, hypothetically leading to over-learned, habitual stimulus-response associations that persist even following aversive outcomes and become largely resistant to modification (Robbins et al., 2008).

1.3.1 Individual differences in reward processing

Despite the effects of drugs of abuse on reward, the majority of individuals who try drugs of abuse, or eat HPFs, will not become dependent or develop disordered behaviours. The underlying causes of susceptibility to appetitive disorders is hugely complex and multifaceted. Both substance use disorders (SUD) and obesity are highly heritable (e.g. Ducci & Goldman, 2012; Haworth et al., 2008), and a number of genes have been linked to the development and persistence of addiction (Agrawal, Edenberg, & Gelernter, 2016; Li, Mao, & Wei, 2008). For example, lower levels of AUD are seen in those with a gene that disrupts the normal metabolism of alcohol (e.g. Hurley & Edenberg, 2012; Li, Zhao, & Gelernter, 2012). Environmental factors including stress exposure (e.g. Goeders, 2003; Koob, 1999; Sinha, 2001), a negative emotional state (e.g. Koob, 2015), and social economic status (e.g. Galea & Vlahov, 2002), can also impact an individual's risk of drug abuse and relapse, and likely interact with genetic susceptibility (e.g. Caspi & Moffitt, 2006; Sinha, 2009).

These factors may also influence reward processing, and changes in the dopaminergic reward circuitry likely interact with existing genetic vulnerabilities for appetitive disorders. Lower striatal dopamine D2 receptor availability is observed in

those who are dependent on cocaine (Martinez et al., 2011; Volkow et al., 1993) and alcohol (Volkow et al., 2007), as well as in those who are obese (Wang et al., 2001). Although the direction of these effects is not clear, detoxification from alcohol is not associated with a recovery of striatal D2 receptors (Volkow et al., 2002). As such, it might be assumed that reduced D2 expression reflects a pre-existing vulnerability factor for appetitive disorders (although see Benton & Young, 2016). Pre-clinical models similarly suggest that knock-down of striatal D2 receptors leads to compulsive overeating (Johnson & Kenny, 2010), while in humans, a genetic vulnerability for decreased striatal D2 receptor expression is predictive of substance use disorders (SUD; Noble, 2000) and obesity (Stice, Spoor, Bohon, & Small, 2008). D2-mediated fast spiking of DA neurons is thought to underlie reward prediction error signalling and D2 dysfunction may therefore interfere with adaptive PE detection, resulting in the attribution of excessive incentive-salience towards reward-associated cues (Heinz et al., 2004). Via its association with the prefrontal cortex, reductions in striatal D2 may also lead to a reduction in overall cognitive-control, increasing impulsive behaviour and drug seeking (e.g. Simon et al., 2013). A genetic predisposition for dysregulated striatum D2 expression may therefore form a feedback loop with the effects of chronic drug use itself, whereby increased impulsivity leads to drug use, which itself leads to dysregulation of the dopaminergic system (Porter et al., 2011) and further down-regulation of D2 receptors (Heinz et al., 2004).

Exposure to both acute and chronic stress can also trigger drug craving and seeking, and is implicated in relapse in dependent individuals (for a review see Sinha, 2001). Early life stress, for instance, is associated with increased drug use in humans (Enoch, 2011; Widom, Weiler, & Cottler, 1999). Through its effects on the mesolimbic dopaminergic circuitry (e.g. Piazza & Le Moal, 1996; Sinha, 2001) stress can induce an internal state that amplifies the rewarding effects of drugs. Indeed, acute stress is associated with altered activity in the dorsal striatum and orbitofrontal cortex region of the prefrontal cortex (e.g. Kumar et al., 2014; Porcelli, Lewis, & Delgado, 2012). The internal states induced by drugs or the disorders themselves can also influence existing cue-reward associations. Dopaminergic and opioid stimulation to limbic structures, including the central nucleus of the amygdala and nucleus accumbens, is associated with greater incentive salience towards reward associated cues (e.g. DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009). Similarly, the repeated, intermittent style of

food consumption that typifies binge eating disorder (BED) can perpetuate the formation and persistence of MRMs (Corwin & Grigson, 2009).

Individual differences in conditioned responding may also explain vulnerabilities to appetitive disorders. During Pavlovian conditioning, a subsection of animals will attribute motivation salience towards the cue itself. Termed 'sign-trackers', these animals will approach the cue, which itself can act as a secondary reinforcer and drive behavioural approach even in the absence of the original reward. Comparatively, 'goal-trackers' assign *predictive* value to the cue and will approach the location of US delivery upon presentation of the cue (Robinson, Yager, Cogan, & Saunders, 2014). As sign-trackers attribute greater reward value to associative cues, this paradigm is used to explain why some individuals are more susceptible to developing appetitive disorders. For instance, an animal's tendency to sign-track predicts cocaine seeking (Tunstall & Kearns, 2015), as well vulnerability to relapse following cue exposure (Robinson et al., 2014). A tendency to over-attribute incentive salience to associative cues may also be observed in humans (Garofalo & di Pellegrino, 2015; Versace, Kypriotakis, Basen-Engquist, & Schembre, 2016). For instance, 'sign-tracking' towards food-related cues, as assessed by late positive potentials during EEG, is associated with a greater risk of obesity relative to 'goal-tracking' (Versace et al., 2016). Obese sign-trackers also reported higher levels of emotional eating, food craving, and loss of control over eating. Whether this reflects the same process observed in animal models is less clear, as Versace et al. (2016) measured reactivity to predictive cues, rather than approach behaviour (as in animal studies). Indeed, if humans 'sign-tracked' to cookbooks rather than food, you might expect them to *lose* weight. These nuances reflect the complexity of addiction in humans, and the potential limitations of animal models of addiction (for a review see Fuchs, Higginbotham, & Hansen, 2019).

1.4 Appetitive Disorders

The reinforcing and learning-promoting properties of appetitive rewards are therefore thought to contribute towards the development and persistence of disorders of 'overconsumption'; including substance use disorder (SUD), obesity, and binge eating disorder (BED). These disorders are associated with significant personal and economic burdens. A reported 1.4% of individuals in the UK and Northern Ireland are dependent on alcohol, while harmful alcohol use is associated with more than 5% of the global disease burden (WHO, 2018) and costs UK taxpayers a reported £21 billion

a year (The Centre for Social Justice, 2013). Obesity is similarly implicated with significant economic costs (Tremmel, Gerdtham, Nilsson, & Saha, 2017) and is associated with multiple key health risks, including diabetes, cardiovascular disease, and cancer (Kopelman, 2007; Van Gaal, Mertens, & De Block, 2006). Worldwide, obesity rates have nearly tripled since 1975, suggesting a pressing need for the development of disease-modifying treatments.

Within the current thesis, *Chapters 3* and *4* will specifically address the role and targeting of MRMs underlying alcohol use disorders (AUD), while in *Chapter 5*, the appetitive MRM associated with overeating and BED will be targeted. These disorders will be briefly introduced below.

1.4.1 Alcohol Use Disorder (AUD)

Alcohol Use Disorder (AUD), a type of Substance Use Disorder (SUD), is a chronically relapsing disorder characterised by compulsive drug use despite negative consequences. SUD is typically diagnosed using the Diagnostic and Statistical Manual of Mental Disorders, currently in its fifth edition (DSM-V). While substance abuse and substance dependence were considered separate diagnoses in the former incarnation of the DSM (the DSM-IV), the distinction between abuse and dependence was later removed in the DSM-V. In its place, a criteria count (from two to 11) was added to indicate overall severity (mild, moderate, severe; Hasin et al., 2013). These diagnostic criteria are given in *Table 1.1.*

Table 1.1. *DSM-V diagnostic criteria for substance use disorder (SUD)*

1. The substance is often taken in larger amounts or over a longer period than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control use of the substance.
3. A great deal of time is spent in activities necessary to obtain the substance, use the substance, or recover from its effects.
4. Craving, or a strong desire or urge to use the substance.
5. Recurrent use of the substance resulting in a failure to fulfil major role obligations at work, school or home.
6. Continued use of the substance despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of its use.
7. Important social, occupational or recreational activities are given up or reduced because of use of the substance.
8. Recurrent use of the substance in situations in which it is physically hazardous.
9. Use of the substance is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance.
10. Tolerance, as defined by either of the following: a) A need for markedly increased amounts of the substance to achieve the desired effect b) A markedly diminished effect with continued use of the same amount of the substance.
11. Withdrawal, as manifested by either of the following: a) The characteristic withdrawal syndrome for the substance b) The substance is taken to relieve or avoid withdrawal symptoms.

SUD is characterised by cyclic relapse, encompassing intoxication, binging, withdrawal, and craving (Koob, 2015). Chronic use of some drugs, including alcohol and opiates, is associated with acute withdrawal symptoms. Five percent of individuals with alcohol use disorder, for example, experience severe withdrawal symptoms including seizures, anxiety and delusions (Yeramaneni, 2010). Removal of physically addictive substances therefore engenders a state of allostasis that underlies these unpleasant and maladaptive symptoms (e.g. Becker & Mulholland, 2014; Koob & Le Moal, 2001), driving continued use and relapse in the short term. Even after detoxification and the allostatic processes underlying relapse have passed, exposure to the cues and environmental stimuli that previously predicted the reward may trigger craving and subsequent relapse in the long-term (Berridge & Robinson, 2016).

1.4.2 Binge Eating Disorder (BED)

While the existence of food- or eating-addiction is under some debate (e.g. Avena, Rada, & Hoebel, 2008; Benton, 2010; Bruinsma & Taren, 1999; Davis & Carter, 2009; Hebebrand et al., 2014; Schulte, Avena, & Gearhardt, 2015; Westwater, Fletcher, & Ziauddeen, 2016; Wilson, 1991, 2010; Ziauddeen & Fletcher, 2013), there is some overlap in the symptoms of BED and SUD. For instance, BED is characterised by cyclic relapse, loss of control, and craving. The diagnostic criteria for BED are given below (see *Table 1.2*), with a criteria count grading the severity of BED as follows: mild: 1 to 3 episodes

per week, moderate: 4 to 7 episodes per week, severe: 8 to 13 episodes per week, extreme: 14 or more episodes per week.

Table 1.2. *DSM-V diagnostic criteria for binge eating disorder (BED)*

Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following: Eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat in a similar period of time under similar circumstances The sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating)
2. Binge-eating episodes are associated with three (or more) of the following: Eating much more rapidly than normal Eating until feeling uncomfortably full Eating large amounts of food when not feeling physically hungry Eating alone because of being embarrassed by how much one is eating Feeling disgusted with oneself, depressed, or very guilty after overeating
3. Marked distress regarding binge eating is present.
4. The binge eating occurs, on average at least 1 day a week for 3 months (DSM-V frequency and duration criteria.
5. The binge eating is not associated with the regular use of inappropriate compensatory behaviour (e.g., purging, fasting, excessive exercise) and does not occur exclusively during the course of anorexia nervosa or bulimia nervosa.

Importantly, for the current thesis, SUDs and BED are thought to share common underlying neurobiological substrates with regard to discussed maladaptive reward learning and dysregulation.

1.4.3 Existing treatments for appetitive disorders

Temporary alleviation of acute withdrawal symptoms is a primary target of SUD treatment. Nicotine replacement therapy can improve the likelihood of an individual stopping smoking by 50-60% (Hartmann-Boyce, Chepkin, Ye, Bullen, & Lancaster, 2018), while buprenorphine and methadone can be used effectively for the maintenance treatment of opioid dependence (Mattick, Kimber, Breen, & Davoli, 2004). Analogous treatments for alcohol are, however, limited (Amato, Minozzi, & Davoli, 2011; Chick & Nutt, 2012) and despite the apparent short-term effectiveness of the aforementioned treatments, the long-term prognosis of SUD is poor. Within a year, 85% of people who attempt to stop smoking will relapse (Hughes, Keely, & Naud, 2004) and it appears there is little that can improve this risk (Hajek et al., 2013). To illustrate this point, The Centre for Social Justice (2013) reported that in one year, 800 individuals were *readmitted* to a single hospital in Manchester for alcohol-related issues. As little as 11.5% of individuals with SUD (across substance types) leave

residential treatment non-dependent, with an upper figure of 60% in the best treatment centres (The Centre for Social Justice, 2013). Thus, while treatment of the acute withdrawal symptoms associated with SUDs is necessary, it is insufficient as it fails to prevent relapse in the long term.

Treatment figures suggest that BED is similarly vulnerable to relapse. For example, 36% of individuals in residential treatment retained a diagnosis of BED after 12 years (Fichter, Quadflieg, & Hedlund, 2008), with 34% of patients continuing to binge eat more than twice a week after six years (Fichter, Quadflieg, & Gnutzmann, 1998). NICE guidelines suggest that BED should be treated primarily through self-help programmes, with cognitive behavioural therapy (CBT) or interpersonal psychotherapy (IPT) offered secondarily (NICE, 2017). However, these treatments may also suffer from relapse, and individual CBT for BED is associated with a relapse rate of 26% at one year follow up (Agras, Telch, Arnow, Eldredge, & Marnell, 1997). More broadly across BED and bulimia nervosa (in which binges are followed by compensatory behaviours such as purging), 34.5% of patients who completed CBT treatment still met criteria, dropping to 31% at 60 weeks follow-up. Comparatively, 66.7% of patients who received IPT still met criteria post-treatment, dropping to 51% at follow-up (Fairburn et al., 2015).

It might be argued that these treatments for BED and SUDs fail as they do not address the MRM formed between rewards and the environmental stimuli that predict their availability (Berridge & Robinson, 2016). Thus, treatments that directly target these MRM are required for the long-lasting, efficacious treatment of disorders of appetitive reward memory. Cue-exposure therapy (CET) for appetitive disorders aims to suppress MRMs via extinction, wherein the drug-associated cues, context, or behavioural responses are repeatedly presented in the absence of drug-reinforcement (Torregrossa & Taylor, 2013). Extinction consequently produces a new memory trace, which competes with the original and interferes with its expression. However, extinction-based treatments are susceptible to relapse via *renewal, reinstatement and spontaneous recovery* (Bouton, 2002; Bouton, Winterbauer, & Todd, 2012). During renewal, a change in context can lead to the return of the extinguished behaviour (e.g. Bouton & King, 1983), while during reinstatement, the extinguished behaviour returns by itself when the US is presented before the CS is presented again (e.g. Rescorla & Heth, 1975). Finally, spontaneous recovery refers simply to the return of the extinguished behaviour following the passage of time (Bouton, 1993). Together, these issues associated with the return of the extinguished behaviour suggest that extinction

leaves the original memory intact. Permanent modifications to the original memory trace may therefore be required to produce long-lasting, relapse-resistant treatments for appetitive disorders of MRM.

1.5 Memory Reconsolidation

It was originally assumed that once consolidated, memories were stable and resistant to modification. Synaptic consolidation was first described more than a century ago, when Müller and Pilzecker (1900) demonstrated the susceptibility of new learning to interference. When new information was learnt shortly after original learning took place, memory for the original learning was disrupted. This time-dependent process of memory consolidation has also been demonstrated via the administration of protein synthesis inhibitors (Flexner, Flexner, De La Haba, & Roberts, 1965), and electroconvulsive shock (Duncan, 1949), shortly following learning. In these cases, interference disrupts memory when administered shortly after learning, but not at longer intervals of an hour and above. As such, the consolidation hypothesis posits that newly learnt information is labile (i.e. 'active') and requires consolidation to persist in the long-term (i.e. become 'inactive'; see *Figure 1.4*).

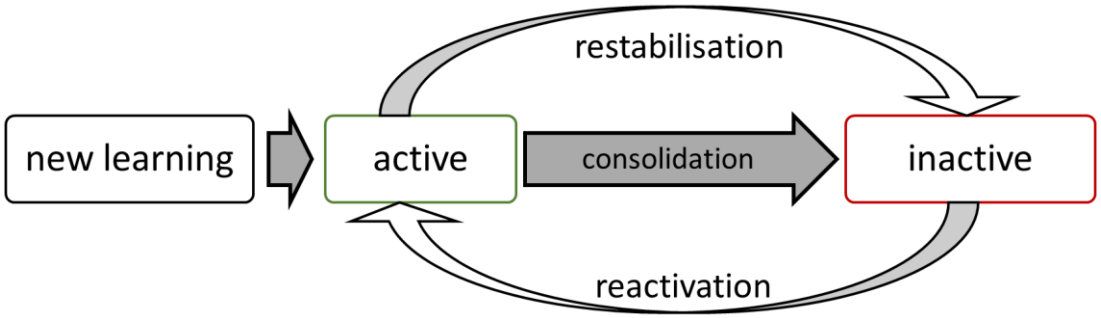
Figure 1.4. *Model of memory consolidation*

Memory consolidation describes the time-dependent process by which new learning is taken from an active, unstable state, and stabilised into an inactive state (Lewis, 1979).

The idea that memories could re-enter this active, labile state (i.e. *'reconsolidate'*) was first demonstrated in the late 1960's (c.f. Spear, 1973). In rodents, fear memories conditioned 24 hours prior (and thus consolidated) were weakened when electroconvulsive shock was administered after brief presentation of the CS (Misanin, Miller, & Lewis, 1968; Schneider & Sherman, 1968). Brief visual exposure to the start box, coupled with the sound of the door opening, also reactivated the trained memory of a complex maze task, such that rats who received electroconvulsive shock following cue exposure experienced memory erasure (Lewis, Bregman, & Mahan, 1972). It was

therefore suggested that consolidation was not a one-time occurrence; rather, reminders of the memory can return it to a labile, modifiable state (see *Figure 1.5*).

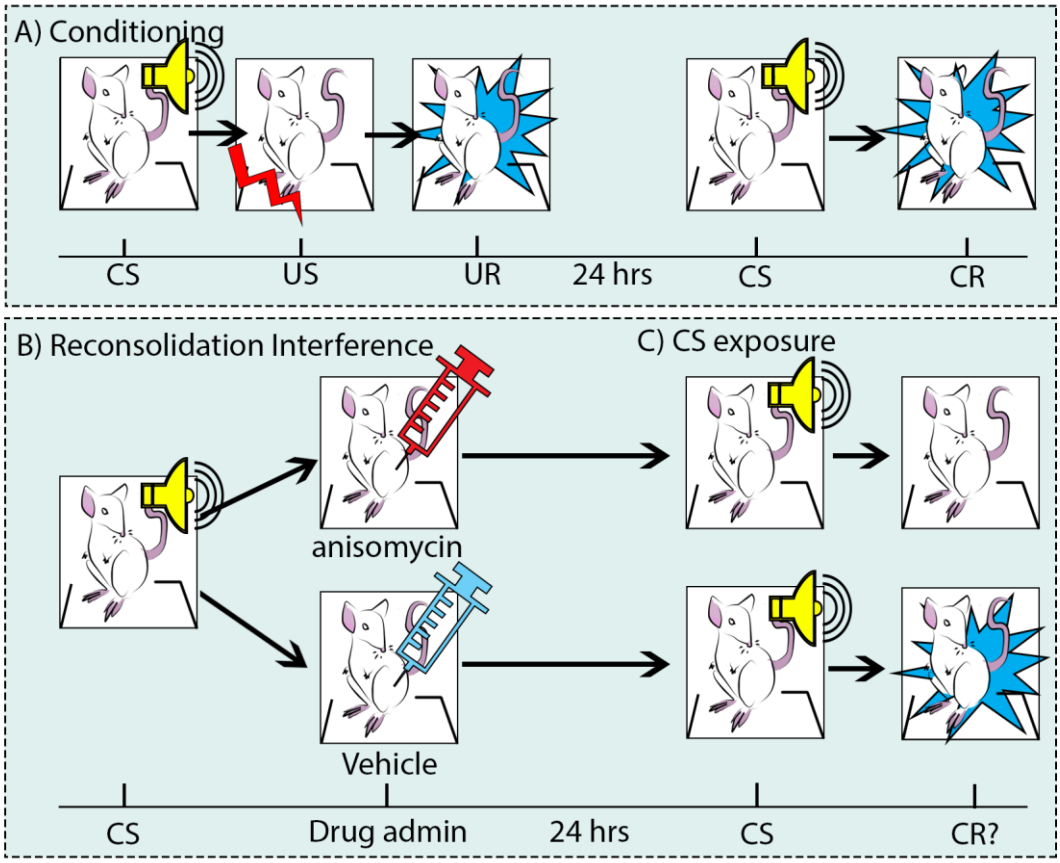
Figure 1.5. *Model of memory reconsolidation*



Memory reconsolidation describes the process by which a previously consolidated memory is reactivated, returning to an active, unstable state. The memory subsequently requires reconsolidation to return it to an inactive state, in which it is resistant to modification.

Despite the potential clinical relevance of a method through which established memories can be modified, relatively few papers were published until 2000 (see Schiller & Phelps, 2011, for a comprehensive review), when Nader, Schafe, and Le Doux (2000) published their seminal paper in the journal *Nature*. Here, rats were first conditioned to associate the sound of a tone (CS) with a foot-shock (US). Twenty-four hours later (when the memory was assumed to have consolidated), the associative memory was retrieved by playing the tone (CS), after which the rats received anisomycin, a protein synthesis inhibitor, or vehicle. Twenty-four hours later, freezing in response to the tone (used as an index of fear) reduced only in the rats that received anisomycin, and only when the reminder CS was played before anisomycin administration (see *Figure 1.6* for a graphical depiction of this experiment).

Figure 1.6. *Schematic of a typical reconsolidation experiment*



Memory reconsolidation as demonstrated via the disruption of conditioned Pavlovian auditory fear memory. A) During conditioning, the sound of a tone (conditioned stimuli; CS) is paired with a footshock (unconditioned stimuli; US), such that the sound of the tone elicits freezing (conditioned response; CR) in the absence of the footshock. B) Twenty-four hours later (when the conditioned memory is assumed to have been consolidated), the rats are returned to the chamber and briefly exposed to the tone (CS) after which protein synthesis inhibitor or vehicle is administered. C) Twenty-four hours later the rats are returned to the chamber and exposed to the tone (CS) again. Rats who received anisomycin demonstrate significantly lower levels of freezing (CR), relative to rats that received vehicle.

Following the publication of Nader et al'.s (2000) paper, evidence for the occurrence of reconsolidation has been demonstrated across species including mice, rats, chicks, pond snails, crabs, and, critically, humans (see Nader & Hardt, 2009; Schiller & Phelps, 2011 for relevent reviews). Reconsolidation has similarly been demonstrated for spatial, object recognition, Pavlovian threat conditioning, Pavlovian reward conditioning, and instrumental reward conditioning (for a review see Dudai & Eisenberg, 2004; Lee, Nader, & Schiller, 2017), suggesting reconsolidation is a fundamental memory plasticity process.

1.6 'Boundary conditions' of reconsolidation

The ubiquity of reconsolidation implies it serves an adaptive function in behaviour, acting as a mechanism through which existing memories can be updated or strengthened (Lee, 2009). Given the dynamic nature of memory, it makes sense that there exist 'boundary conditions' which prevent adaptive memories from constantly destabilising. Indeed, relative to their newer, more weakly conditioned counterparts, older (e.g. Baratti, Boccia, Blake, & Acosta, 2008; Eisenberg & Dudai, 2004; Frankland et al., 2006; Inda, Muravieva, & Alberini, 2011; Milekic & Alberini, 2002; Robinson & Franklin, 2010; Suzuki et al., 2004) and more strongly conditioned (e.g. Eisenberg, Kobilo, Berman, & Dudai, 2003; Inda et al., 2011; Morris et al., 2006; Robinson & Franklin, 2010; Taylor, Olausson, Quinn, & Torregrossa, 2009; Wang, de Oliveira Alvares, & Nader, 2009) memories are less likely to undergo reconsolidation following simple retrieval procedures (although see Debiec, LeDoux, & Nader, 2002).

Given that appetitive MRM appear to be susceptible to modification via reconsolidation, it has been suggested that this process should be harnessed to treat appetitive disorders. However, given the aforementioned boundary conditions of memory age and strength, it is likely that these 'naturalistic' reward memories will be relatively resistant to destabilisation upon retrieval, particularly when compared than those conditioned experimentally over a single or few days. To illustrate, during development of AUD it is likely that an individual will have consumed alcohol thousands of times, amounting to a significant number of conditioning trials. Similarly, those who develop AUD will likely have started drinking many years prior (DeWit, Adlaf, Offord, & Ogborne, 2000; although see Maimaris & McCambridge, 2014). Overcoming these boundary conditions is therefore a key consideration for the clinical utility of reconsolidation-based treatments.

Fortunately, these boundary conditions do not appear absolute. Labialisation of stronger fear memories can be achieved using longer reactivation sessions (Suzuki et al., 2004). Although, paradoxically, retrieval trials that are *too* long may engender extinction rather than reconsolidation (Pedreira & Maldonado, 2003; Suzuki et al., 2004 but see Duvarci, Ben Mamou, & Nader, 2006). Administration of protein synthesis inhibitors is associated with the blockade of memory reconsolidation when retrieval procedures are brief (3 minutes), yet administration during longer retrieval procedures (30 minutes) is associated with the blockade of new memory formation (i.e.

the consolidation of extinction; Eisenberg et al., 2003; Pedreira & Maldonado, 2003; Suzuki et al., 2004). Memory reconsolidation and extinction therefore appear dissociable, whereby blockade of extinction did not attenuate the original memory trace and suggesting retrieval induces either extinction *or* reconsolidation. This dissociation is further supported by Mamiya et al. (2009), who demonstrated an increase in CREB activity in the hippocampus and amygdala, but not in the medial prefrontal cortex, following a brief, 3-minute, retrieval procedure. Activity in the medial prefrontal cortex and amygdala, but not the hippocampus, was alternatively observed when the retrieval procedure was longer (30 minutes). CREB-mediated transcription is associated with both extinction and reconsolidation of contextual fear memories, suggesting that the duration of the retrieval procedure acts as a switch, determining the initiation of either reconsolidation *or* extinction (e.g. Bustos, Maldonado, & Molina, 2009; Cassini, Flavell, Amaral, & Lee, 2017; Suzuki et al., 2004). Beyond the pre-clinical literature, Hu et al. (2018) systematically varied the reminder duration at retrieval. When reminder duration at retrieval was short (1 and 4 seconds), no recovery of memory was observed following retrieval extinction procedures. Conversely, when reminder duration was long (30 seconds) or when no reminder cue was presented, reinstatement of the threat-related memory was observed. In all, these results suggest retrieval procedures (and duration of cue presentation) should be time limited to prevent extinction, rather than reactivation, from occurring.

Alternatively, retrieval procedures of intermediate durations may induce neither reconsolidation nor extinction. Rather, Cassini et al. (2017) observed reconsolidation blockade only when retrieval procedures were shorter than five minutes, and attenuation of extinction when retrieval procedures were 30 minutes in duration. Interestingly, no effect of amnesic treatment was observed when the duration of the retrieval was between five and 20 minutes long, suggesting that memories may enter a 'limbo state', in which they are insensitive to amnesic treatment. This limbo state may also be induced when exposure to retrieval cues exceeds a single presentation (i.e. as is the case for the induction of reconsolidation) but does not meet the number of CS presentations required to induce extinction. Merlo, Milton, Goozee, Theobald, and Everitt (2014), for instance, demonstrated the molecular independence of reconsolidation and extinction, wherein the limbo state might represent an intermediate stage where the original memory trace is no longer labile, yet the number

of CS exposures is not sufficient to produce new extinction learning (Merlo, Milton, & Everitt, 2018; Merlo et al., 2014).

An additional boundary condition constraining reconsolidation is the content and context of the retrieval procedure. Reminder cues that are too discrepant from the original learning can also produce a limbo state (Sevenster, Beckers, & Kindt, 2014), or new learning (i.e. extinction; Bos, Beckers, & Kindt, 2012; Eisenberg et al., 2003). For instance, reconsolidation was not observed when retrieval occurred in a different environment to the original learning (Forcato, Argibay, Pedreira, & Maldonado, 2009; Misanin et al., 1968).

On the other hand, if the new event is *too* similar to the original it may also fail to induce destabilisation. Given the hypothesised role of reconsolidation in maintaining memory relevance, it might be expected that destabilisation will occur only when there is something new to be learnt. Indeed, increasing evidence suggests that the retrieval procedure itself must include surprising information relevant to the original learning to engender destabilisation of more stable memories. The inclusion of PE (the mismatch between expected and actual events) may therefore be important for the destabilisation of stronger memories (e.g. Alfei, Ferrer Monti, Molina, Bueno, & Urcelay, 2015; Diaz-Mataix, Ruiz Martinez, Schafe, LeDoux, & Doyere, 2013; Exton-McGuinness, Lee, & Reichelt, 2015; Forcato et al., 2009; Kindt & van Emmerik, 2016; Morris et al., 2006; Pedreira, Perez-Cuesta, & Maldonado, 2004; Sevenster, Beckers, & Kindt, 2013; Sevenster et al., 2014; Sinclair & Barense, 2018). To illustrate, Sevenster et al. (2013) administered the noradrenergic β-blocker propranolol following reactivation inclusive of a positive, negative, or no PE procedure. Attenuation of the memory was observed only when PE (both positive and negative) was included at retrieval, with discrepancy between the original learning and retrieval procedure positively related to destabilisation. The requirement for surprising information at retrieval fits with the role of reconsolidation as a mechanism for memory updating, with PE acting as a signal that there is something new to be learnt about the current situation. Together, these numerous boundary conditions highlight the challenge of inducing destabilisation within a given retrieval procedure, representing a need to better understand the key governing parameters for clinical translation.

1.7 The cellular and molecular basis of memory reconsolidation

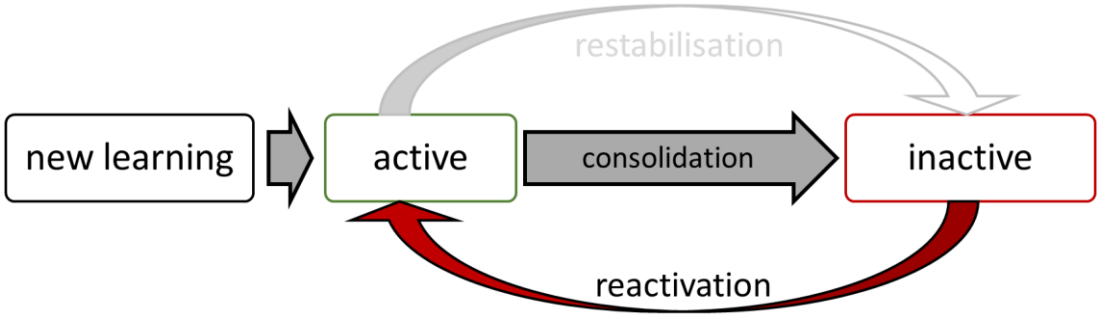
The process of memory reconsolidation is comprised of several stages (see *Figure 1.5*), whereby *reactivation* of a memory returns it to an active state, and *restabilisation* is required to return it to an inactive, stable state. Confusingly, the terms retrieval, reactivation, and destabilisation are used interchangeably throughout the reconsolidation literature. The current thesis will refer to ***retrieval*** as a process by which memories are returned to a state in which they can be expressed (i.e. recalled), while memory ***reactivation*** refers to the process of destabilisation. These stages are molecularly and behaviourally distinct. Thus, understanding the biological and molecular components of each stage is important when developing reconsolidation-based treatments. For the sake of clarity, the molecular underpinnings of each of these processes will be briefly discussed below.

1.7.1 Memory retrieval

Retrieval is required for the expression or experience of a previously consolidated memory. As discussed, several boundary conditions can prevent retrieved memories from re-entering a labile state (i.e. be 'reactivated'). Memory retrieval can therefore occur *without* reactivation (and associated destabilisation) of the memory trace (Cammarota, Bevilaqua, Medina, & Izquierdo, 2004). Consistent with this, memory expression (retrieval) is doubly dissociable from memory reactivation (Ben Mamou, Gamache, & Nader, 2006). Pre-retrieval administration of glutamatergic AMPA receptor inhibitors, for instance, is associated with attenuation of the expression of a taste aversion and auditory fear memory, without influencing the effect of post-retrieval anisomycin on reconsolidation (Ben Mamou et al., 2006; Rodriguez-Ortiz, Balderas, Garcia-DeLaTorre, & Bermudez-Rattoni, 2012). Antagonism of hippocampal AMPA receptors is similarly associated with attenuated expression of inhibitory avoidance (Bianchin et al., 1993; Szapiro et al., 2000), object recognition (Winters & Bussey, 2005), and spatial (Bast, da Silva, & Morris, 2005; Liang, Hon, Tyan, & Liao, 1994; Riedel et al., 1999) memory. Requirement of AMPA appears site-specific, as retrieval of fear memories in rats is dependent on hippocampal AMPA receptors, whereas a requirement for NMDARs has been demonstrated in the neocortical structures (e.g. Barros et al., 2000).

Blockade of the molecular cascades downstream of glutamatergic receptors is similarly implicated in the expression of a memory trace. Intrahippocampal blockade of mitogen-activated protein kinase (MAPK) or protein kinase A (PKA) is associated with the attenuated expression of a single trial inhibitory avoidance memory, while an agonist of PKA transiently enhances retrieval (Szapiro et al., 2000). Antagonism of the mammalian target of rapamycin (mTOR) pathway downstream of these intracellular activators is similarly associated with the reduced expression of an inhibitory avoidance and object location memory (e.g. Pereyra, Katche, de Landeta, & Medina, 2018), while ongoing protein synthesis induced by the mTOR1 pathway in the amygdala is implicated in the retrieval of an auditory fear memory (Lopez, Gamache, Schneider, & Nader, 2015).

1.7.2 Memory reactivation

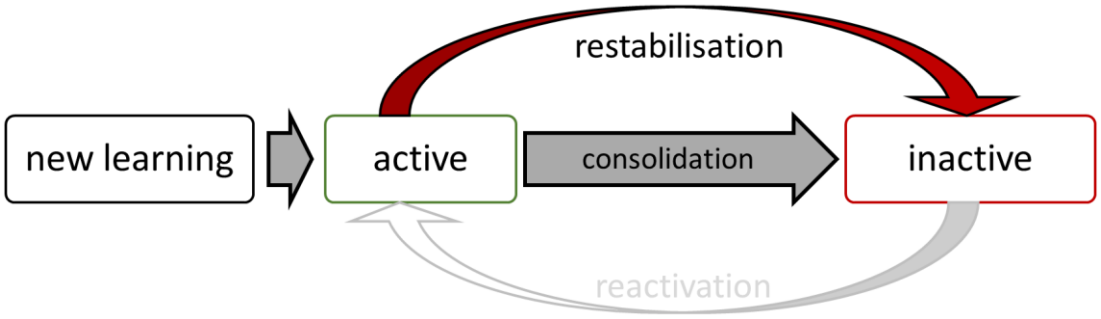


Memory reactivation describes the process by which the inactive, consolidated memory is returned to an active state (c.f. Spear, 1973). Studies attempting to elucidate the mechanisms of memory reactivation typically administer an inhibitor of the predicted molecular component prior to memory retrieval, followed by a known blocker of reconsolidation. If memory reactivation is indeed dependent on the predicted component, the memory will be unaffected by the pharmacological blocker of reconsolidation. Using this procedure, a requirement for glutamatergic signalling at amygdala NMDARs has been demonstrated for the reactivation of conditioned fear memory (e.g. Ben Mamou et al., 2006). Consistent with this, agonism of the NMDAR is associated with the potentiation of fear memory destabilisation. Intraperitoneal administration of the partial NMDAR agonist D-cycloserine (DCS) prior to retrieval is associated with the destabilisation of reactivation-resistant fear memories (Bustos, Giachero, Maldonado, & Molina, 2010).

The cannabinoid receptor CB_1 and L-type voltage-gated calcium channels are also implicated in the destabilisation of fear memories. Administration of CB_1 and L-type voltage-gated calcium channel blockers prevents memory weakening from protein synthesis inhibitors (Suzuki, Mukawa, Tsukagoshi, Frankland, & Kida, 2008). Further, L-type voltage-gated calcium channels exert upstream control over the ubiquitin-proteasome pathway and as with consolidation, memory destabilisation is dependent on proteasome-dependent protein degradation (Artinian et al., 2008). For instance, increased expression of polyubiquitinated protein was observed in the hippocampi of mice 15 minutes and one hour after fear memory retrieval, but not in mice who did not undergo retrieval (Lee et al., 2008). The post-retrieval memory impairment produced by the protein synthesis inhibitor anisomycin is also blocked when a proteasome inhibitor is co-administered into the hippocampus, suggesting a role for protein degradation in the destabilisation of previously consolidated memories (Lee et al., 2008). Protein degradation has also been observed for fear memory reactivation in the amygdala (Jarome, Werner, Kwapis, & Helmstetter, 2011) and in the nucleus accumbens core for cocaine reward memory (Ren et al., 2013). Given that glutamatergic transmission activates the ubiquitin and proteasome system in cultured neurons (Bingol & Schuman, 2006; Guo & Wang, 2007), it is speculated that downstream signalling of NMDAR activation may increase protein degradation, subsequently producing memory destabilisation (Lee et al., 2008).

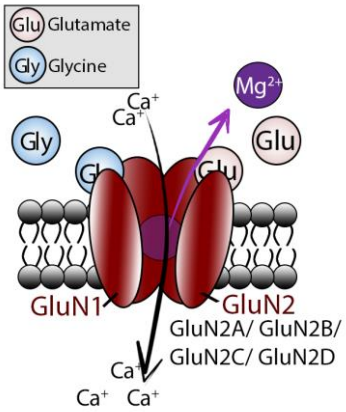
Consistent with the requirement of PE at retrieval for the destabilisation of older and more strongly conditioned memories, the dopaminergic system is also implicated in memory destabilisation. The presentation of a non-reinforced CS (i.e. a PE), is associated with the release of dopamine in the basolateral amygdala during appetitive memory reactivation (Merlo et al., 2015), and the ventral tegmental area during both appetitive (Schultz et al., 1997) and fear memory reactivation (Brischoux, Chakraborty, Brierley, & Ungless, 2009; Cahill, Wood, Everitt, & Milton, 2019).

1.7.3 Restabilisation



Following reactivation, memories must undergo memory restabilisation to return to a stable, inactive state. Restabilisation likely occurs as a result of the molecular cascades downstream of the NMDA and β-adrenergic (βAR) receptor sites. This seemingly paradoxical involvement of the NMDAR in both the destabilisation and restabilisation of memory can be explained by its structure. NMDARs are tetramers, comprised of two obligate GluN1 and two GluN2 subunits (Dingledine, Borges, Bowie, & Traynelis, 1999; Furukawa, Singh, Mancusso, & Gouaux, 2005; see *Figure 1.7*). There are four different types of GluN2 subunits (GluN2A-D) which are differentially responsive to glutamate. The GluN2A- and GluN2B subunits couple with different proteins, and thus are associated with separate intracellular signalling pathways (Ivanov et al., 2006; Kim, Dunah, Wang, & Sheng, 2005). A double dissociation between the GluN2B- and GluN2A- containing NMDARs in reconsolidation was demonstrated by Milton et al. (2013), where selective blockade of GluN2B-NMDARs with ifenprodil was associated with the prevention of fear memory destabilisation, such that memory attenuation by anisomycin was prevented by pre-retrieval ifenprodil. Conversely, selective inhibition of GluN2A-NMDARs via pre-retrieval NVP was associated with fear memory attenuation, suggesting NVP prevented the restabilisation of fear memories without blocking destabilisation. This double dissociation between GluN2A and GluN2B-containing NMDARs is consistent with their associated intracellular signalling pathways. GluN2B-containing NMDARs are associated with cell death with greater efficiency than GluN2A-containing NMDAR (Martel et al., 2012), and are associated with the blockade of CREB and the activation the ubiquitin-proteasome pathway.

Figure 1.7. *The NMDA receptor*



Adapted from Balu (2016)

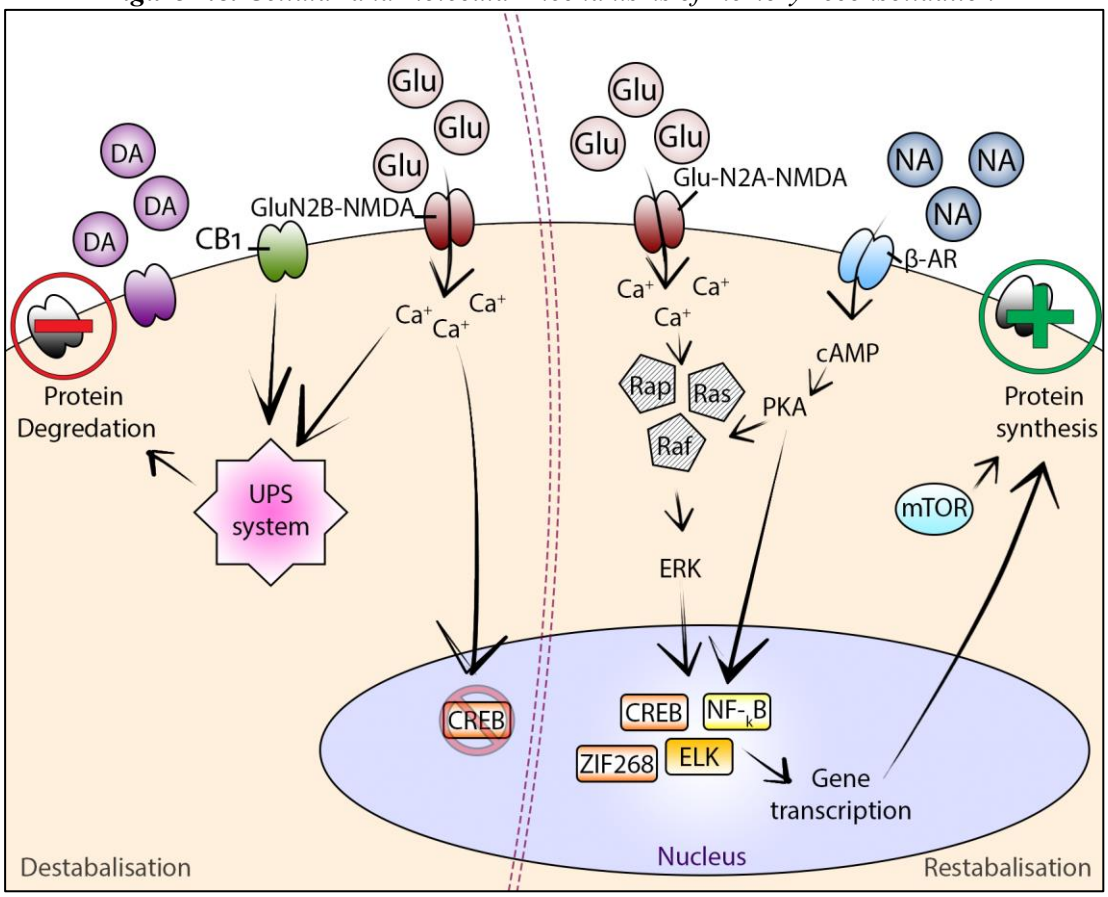
The excitatory glutamatergic N-Methyl-D-Aspartate receptor (NMDAR) is comprised of two obligatory GluN1 subunits with either two GluN2 subunits or a combination of GluN2 and GluN3 subunits. There are four GLuN2 subunits (GluN2A-D) which are differentially responsive to glutamate. In addition to glutamate binding at the GluN2 subunit, activation of the NMDAR is dependent on glycine or D-serine binding at the GluN1 subunit, and on post-synaptic depolarization relieving the magnesium (Mg^{2}) block in the channel. Once activated, the receptor becomes calcium (Ca^{+}) permeable.

Post-reactivation administration of βAR antagonists is similarly associated with attenuation of fear (Debiec & Ledoux, 2004; Huang, Zhu, Zhou, Liu, & Ma, 2017; Kindt & Soeter, 2018; Thomas, Saumier, Pitman, Tremblay, & Brunet, 2017; Zaichenko, Markevich, & Grigor'yan, 2017) and appetitive reward memory (Bernardi, Lattal, & Berger, 2006; Milton, Lee, & Everitt, 2008b; Otis, Dashew, & Mueller, 2013; Zhu et al., 2018). Downstream of the NMDA and βAR receptors, restabilisation is likely dependent on signalling molecules including protein kinase A (PKA; Duvarci & Nader, 2004; Kelly, Laroche, & Davis, 2003; Tronson, Wiseman, Olausson, & Taylor, 2006) and extracellular signal regulated kinase (ERK; Cestari, Costanzi, Castellano, & Rossi-Arnaud, 2006; Duvarci, Nader, & LeDoux, 2005; Kelly et al., 2003; Krawczyk et al., 2015; Krawczyk, Millan, Blake, Feld, & Boccia, 2019). In turn, these activate transcription factors including cyclic AMP response element-binding protein (CREB; Kida et al., 2002), nuclear factor-κB (NFkB; Boccia et al., 2007; Freudenthal et al., 2005; Merlo, Freudenthal, Maldonado, & Romano, 2005), and zinc finger 268 (Zif268; Besnard, Laroche, & Caboche, 2014), which initiates gene transcription and synaptic protein synthesis.

Finally, restabilisation of memories may depend on replacing the proteins degraded during reactivation. A requirement for *de novo* protein synthesis following reactivation has been demonstrated across memory types, including fear (Biedenkapp & Rudy, 2004; Duvarci et al., 2006; Duvarci & Nader, 2004; Eisenberg et al., 2003; Nader et al., 2000; Parsons, Gafford, Baruch, Riedner, & Helmstetter, 2006a; Pedreira, Perez-Cuesta, & Maldonado, 2002; Schafe & LeDoux, 2000), appetitive (Gotthard, Kenney, & Zucker, 2018; Hernandez & Kelley, 2004; Hernandez, Sadeghian, & Kelley, 2002; Torregrossa et al., 2019; Zhu et al., 2018), and object recognition memory (Rossato et al., 2007; Sharp, Scott, Mehta, & Wise, 2006). A subset of protein synthesis is modulated by the mammalian target of rapamycin (mTOR), which is implicated in the translation of new proteins for memory restabilisation (e.g. Barak et al., 2013; Blundell, Kouser, & Powell, 2008; Gafford, Parsons, & Helmstetter, 2011; Jobim et al., 2012a).

How these numerous molecular mechanisms interact to produce reconsolidation has not been entirely elucidated. An attempt to reconcile the cellular and molecular processes discussed above is presented in *Figure 1.8*.

Figure 1.8. *Cellular and molecular mechanisms of memory reconsolidation*



Adapted from Tronson and Taylor (2007)

Memory reconsolidation occurs as a result of the molecular cascades downstream of the N-methyl-d-aspartate (NMDA) and β-adrenergic (βAR) receptor sites. Memory destabilisation occurs following the activation of GluN2B-containing NMDA, cannabinoid, and dopamine receptors. With L-type voltage-gated calcium channels, this activates the ubiquitin and proteasome system that in turn, produces protein degradation. Restabilisation is initiated when extracellular neurotransmitters glutamate and noradrenaline bind respectively to GLuN2A-containing NMDA and βAR receptor sites. Following NMDAR activation, calcium (Ca^{+}) flows into the cell, activating in turn small GTPases such as Ras, Raf, and Rap. These small proteins transmit signals to the extracellular signal-regulated kinase pathway (ERK). The βAR receptor is coupled to the G protein and associated with L-type calcium channels. When noradrenaline blinds to the receptor site, the regulatory enzyme adenylyl cyclase is activated, which then catalyses the formation of cyclic adenosine monophosphate (cAMP). CAMP-dependent protein kinase (known also as protein kinase A; PKA) acts either directly, or indirectly through small GTPases and ERK, to activate the transcription factors cAMP response element-binding protein (CREB), zinc finger (ZIF268) and ELK1. Through the mTOR pathway, expression of these transcription factors ultimately initiates gene transcription and protein synthesis.

1.8 Translation of the pre-clinical reconsolidation literature to humans

Discussion of the molecular components of memory reconsolidation reveals many potential targets in which reconsolidation could be modulated. However, despite the clinical utility of a treatment through which maladaptive memories could be modified, the ratio of human to animal studies remains relatively small. This is likely due to several difficulties that arise when attempting to extend the existing pre-clinical literature into human populations. A full meta-analysis and review of the human reconsolidation in naturalistic (i.e. non-experimentally conditioned) memories is presented in *Chapter 2*, thus, only a brief description of the human literature will be given below.

Many of the pharmacological probes described, including protein synthesis inhibitors, are highly toxic in humans. Consequently, the number of drugs available for use in humans is severely limited, at least relative to those available for use in non-human animals. Those that are safe tend to target reconsolidation upstream, via the βAR and NMDA receptor sites. The most frequently used pharmacological blocker of reconsolidation is propranolol, a βAR antagonist traditionally used for the treatment of hypertension. In rats, direct administration of propranolol to the amygdala is associated with the attenuation of auditory fear memory (e.g. Debiec & Ledoux, 2004), with complimentary results in humans. Merel Kindt's lab, for instance, has consistently demonstrated attenuation of fear memory in healthy and clinical populations using pre- and post-retrieval propranolol (e.g. Kindt & Soeter, 2018; Kindt, Soeter, & Vervliet, 2009; Sevenster, Beckers, & Kindt, 2012; Sevenster et al., 2013, 2014; Soeter & Kindt, 2010, 2011, 2012a, 2012b, 2015a, 2015b, although see Schroyens, Beckers, & Kindt, 2017). Across the wider literature, meta-analysis of propranolol's efficacy in healthy participants has generated a medium effect size (Hedge's g=0.56; Lonergan, Olivera-Figueroa, Pitman, & Brunet, 2013), suggestive of a potential clinical utility for post-retrieval blockade of fear memories via propranolol. Interestingly, reconsolidation-interference appears to attenuate only the emotional component of the fear memory (i.e. the amygdala-dependent startle reflex), while declarative memory (threat expectancy) remained intact (Kindt et al., 2009; Sevenster et al., 2013; Soeter & Kindt, 2010, 2011, 2012a). This selectivity may be particularly relevant given the potential clinical application of reconsolidation.

Despite pre-clinical literature suggesting a requirement for the βAR receptor in the reconsolidation of appetitive drug reward memories (e.g. Bernardi et al., 2006; Bernardi, Ryabinin, Berger, & Lattal, 2009; Diergaarde, Schoffelmeer, & De Vries, 2006; Milton & Everitt, 2010; Milton et al., 2008b; Zhu et al., 2018), the efficacy of propranolol in treating disorders of appetitive memory in humans is limited to just four studies. Saladin et al. (2013) administered propranolol following the reactivation of a cocaine associative memory in a population of cocaine-dependent individuals. Relative to placebo, reductions in craving and cue-reactivity were observed in those that received post-retrieval propranolol. However, it is notable that at 1-week follow-up this difference was no longer significant, potentially reflecting a lack of power for follow-up analyses. Zhao et al. (2011) similarly administered pre-retrieval propranolol in a sample of abstinent heroin addicts, observing reductions in memory for heroin-related words. A double-blind pilot study in just 17 participants (nine in the propranolol group) also demonstrated a reduction in craving across a drug dependent sample (Lonergan et al., 2016) while Xue et al. (2017) noted reductions in cue-induced nicotine craving following reconsolidation-interference via pre-retrieval propranolol. Together, these studies provide mild evidence for the use of propranolol in attenuating reward memory, and it is notable that none of the above studies demonstrated reductions in drug use following treatment.

Meta-analysis of the basic reconsolidation literature suggests superior reward memory attenuation following post-retrieval NMDA, relative to βAR receptor antagonism (Das, Freeman, & Kamboj, 2013). Despite this, just one study had administered an NMDAR antagonist as a potential blocker of human reconsolidation at the outset of this PhD. In Das et al. (2015a), pre-retrieval memantine (an NMDAR antagonist) failed to attenuate reward memories in a group of hazardous drinkers. This may reflect memantine's unique pharmacological profile, or the timing of administration. As discussed previously, NMDARs are implicated in both the destabilisation and restabilisation of memory (Milton et al., 2013). Thus, the administration of memantine prior to retrieval may have prevented initial destabilisation of the memory trace. When conditioned fear memories were retrieved in humans following the administration of ketamine (a potent NMDAR antagonist), an *increase* in fear was observed (Corlett et al., 2013). This effect on fear was thought to reflect greater PE via ketamine, generating greater destabilisation of the memory trace. The use of NMDAR antagonists for the blockade of human reconsolidation may

therefore be limited to those that can be rapidly administered *after* memory reactivation.

Beyond NMDA and βAR receptor antagonism, there may be some pharmacological probes of reconsolidation that can be safely administered to humans. For instance, Shi et al. (2009) administered the mTOR inhibitor rapamycin prior to retrieval of a fear memory. No effect of pre-retrieval rapamycin was observed; however, it is unclear whether the dose was appropriate, or if pre-retrieval administration interfered with reactivation of the memory trace. Again, further replication is required to elucidate whether targeting the mTOR system if a viable method of reconsolidation-interference in humans.

Despite the apparent failings of extinction to produce lasting treatments for disorders of maladaptive memory, administering extinction within the reconsolidation window may provide a promising alternative to pharmacological reconsolidation-interference. The most commonly used behavioural-interference technique is retrieval-extinction, in which extinction procedures are administered after memory retrieval (Schiller et al., 2009). While extinction is typically assumed to produce new learning that competes with and (temporarily) supresses the original, delivering extinction within the reconsolidation window (while the reactivated memory is labile) may instead modify the original memory. Meta-analysis of retrieval-extinction procedures suggest a large, significant effect for animal appetitive memories, and a significant, small-to-moderate, effect for human fear (Kredlow, Unger, & Otto, 2016). In human reward memory, retrieval-extinction is associated with a significant reduction in cue-induced heroin (Xue et al., 2012), and smoking (Germeroth et al., 2017) craving. Promisingly, effects on heroin craving persisted for 6 months (Xue et al., 2012). Whether this reflects a *reconsolidation*-dependent effect is, however, less clear. The control group used by Xue et al. (2012) received extinction six hours after retrieval, when the reconsolidation window was assumed to have closed. Further, despite the impressive findings on craving, no actual drug-use outcomes were reported as the participants were maintained drug-free on an inpatient unit. Similarly, while post-treatment daily cigarette use differed between the retrieval + propranolol and no retrieval + propranolol control group in Germeroth et al. (2017), no differences in relapse or days abstinent were observed at 1-month follow-up. Rodent studies in which retrieval was administered immediately *after* extinction (and presumably outside of the reconsolidation window), did not report a difference in alcohol seeking relative to

rodents that underwent retrieval prior to extinction (Millan, Milligan-Saville, & McNally, 2013). Retrieval-extinction may therefore reflect enhanced consolidation of extinction. Indeed, as retrieval-extinction appears to occur independently of PE (and thus presumably without memory destabilisation; Cahill et al., 2019), it is possible this does not reflect a reconsolidation-dependent effect.

Although less commonly studied, alternative behavioural reconsolidation strategies for weakening or modifying MRMs have been described. Counter-conditioning (Das, Lawn, & Kamboj, 2015b; Goltseker, Bolotin, & Barak, 2017), in which inhibitory avoidance training is delivered within the reconsolidation window, is associated with the attenuation of alcohol MRM in hazardous drinkers (Das et al., 2015b). Similarly, reappraisal of maladaptive alcohol-related cognitions within the reconsolidation window has been shown to impair verbal fluency for alcohol-related words (Hon, Das, & Kamboj, 2016).

Null effects are frequently observed in human reconsolidation studies, likely due to a failure to overcome the boundary conditions that constrain destabilisation. Bos, Beckers, and Kindt (2014), for instance, failed to demonstrate a reduction in conditioned fear responding 24 hours after administrating 40 mg of propranolol. The authors note that they did not measure PE, which, given that PE is a putative pre-requisite for the destabilisation of strong memories, may explain the failure to observe an effect. Negative finding have been demonstrated elsewhere (e.g. Spring et al., 2015; Wood et al., 2015), however, as retrieval procedures are discrepant in length, and do not include a PE, these results should not be taken as evidence that reconsolidation does not occur in humans. Rather, it highlights the need to conduct studies in which these boundary conditions are carefully considered and measured.

1.9 Alternative hypotheses for reconsolidation

The traditional view of reconsolidation-interference outlined above has been challenged. Gisquet-Verrier and Riccio (2018) posited that post-retrieval drug effects reflect integration of the internal drug state into the memory trace, rather than interference with reconsolidation. Thus, when the individual is no longer in the drug state, the targeted memories are no longer accessible. This criticism of the reconsolidation-interference model is drawn from studies demonstrating recovery of memory following protein-synthesis reconsolidation-interference (Gisquet-Verrier et

al., 2015; Gisquet-Verrier & Riccio, 2018). For instance, when the protein-synthesis inhibitor cycloheximide was administered after retrieval, rats demonstrated amnesia for an inhibitory avoidance memory. However, when re-tested after re-administration of cycloheximide, rats demonstrated recovery of the inhibitory avoidance memory. This same pattern of results was also observed with lithium chloride, a drug that induces a drug state but does not affect protein synthesis. Thus, under this interpretation the amnesia observed after reconsolidation-interference occurs as memories are no longer accessible. Under a similar interpretation, Lattal and Abel (2004) demonstrated memory-rebound following reconsolidation-interference procedures in a pre-clinical model. As such, it might be argued that reconsolidation-interference reflects a transient failure to retrieve the memory, rather than memory weakening.

However, these alternative hypotheses for reconsolidation do not preclude the potential clinical utility of reconsolidation as a treatment for disorders of appetitive MRM. Rather, these reveal a requirement for further research determining the mechanisms underlying memory reconsolidation.

1.10 Aims of the current thesis

The limited number of human reconsolidation studies, particularly those that attempt to attenuate appetitive MRMs, presents a pressing need for further study. The current thesis presents an investigation of the utility of pharmacological reconsolidation-interference strategies to attenuate maladaptive appetitive memories.

1.10.1 Research questions

This thesis will aim to answer the following questions:

1) Given putative boundary conditions of memory strength and age, is reconsolidation interference a viable treatment strategy in clinically relevant populations where memories are more strongly conditioned (i.e. naturalistically formed)?

2) Prediction error (mismatch between expected and actual events) is considered necessary for the destabilisation of older, stronger memories. In a clinically relevant population, is prediction error necessary and sufficient for the destabilisation of strongly conditioned naturalistic cue-alcohol memories?

3) Is ketamine (an NMDA receptor antagonist) a viable blocker of the reconsolidation of naturalistic cue-alcohol memories in a sample of hazardous drinkers?

4) Is rapamycin (an mTOR inhibitor) an effective blocker of natural (non-drug) appetitive reward memory reconsolidation in those with a tendency to overeat chocolate?

1.10.2 Methodological approach

Question 1 will be addressed using a systematic review and meta-analysis of the existing literature. *Chapter 2* will therefore present a meta-analysis the existing literature, looking primarily at the utility of reconsolidation in treating disorders of maladaptive memory in clinical and sub-clinical populations. Existing meta-analyses have either focused on a single drug or encapsulated all current studies regardless of the age or strength of the memory trace. Given the direct relevance of these boundary conditions for treating naturalistic memories, analysis restricted to this population is warranted. Building on the findings of *Chapter 2*, *Chapter 3* will directly assess the requirement for PE in the destabilisation of MRMs procedure within a population of hazardous drinkers. Measures of memory age and strength will be obtained, to assess whether PE is sufficient to destabilise memories regardless of strength, or if some remain resistant to destabilisation even after PE inclusion. *Chapter 4* will continue this route of enquiry, examining the effects of the NMDA receptor antagonist ketamine as a potential blocker of reconsolidation in a sample of hazardous alcohol drinkers, examining clinically relevant markers of intervention efficacy. *Chapter 5* will assess the transdiagnostic utility of reconsolidation interference by utilising a different population to preceding chapters, instead focusing on cue-associative memories associated with the over-consumption and craving of chocolate. Whereas previous chapters focused on NMDA receptor antagonists as blockers of reconsolidation, in this experimental study the utility of the mTOR inhibitor rapamycin will be tested. Finally, *Chapter 6* will present a synthesis of the previous chapters and evaluate how these findings can be integrated into the existing literature.

Chapter 2.

Modulation of naturalistic maladaptive memories using behavioural and pharmacological reconsolidation interfering strategies: A systematic review and meta-analysis of clinical and 'subclinical' studies

Walsh, K. H., Das, R. K., Saladin, M. E., & Kamboj, S. K. (2018). Modulation of naturalistic maladaptive memories using behavioural and pharmacological reconsolidation-interfering strategies: a systematic review and meta-analysis of clinical and 'sub-clinical' studies. Psychopharmacology (Berl), 235(9), 2507-2527.

2.1 Introduction

2.1.1 Phobia, Traumatic Stress and Substance Use Disorders as Disorders of Memory

As discussed in *Chapter 1*, threat-related (phobia and traumatic stress-), and substance use disorders (SUDs) can be conceptualised as disorders of maladaptive associative memory (Fanselow & Sterlace, 2014; Hyman, 2005; McCarthy, Baker, Minami, & Yeh, 2011). The processes underlying the formation and maintenance of maladaptive memories are thus highly relevant to the treatment of these disorders. The failure of existing therapies to attenuate the emotional/motivational influence of maladaptive memories is one reason why treated individuals are vulnerable to relapse, even after prolonged remission/abstinence. Reconsolidation, the process by which previously consolidated memories are returned to a labile state, may therefore be manipulated to ameliorate anxiety/trauma and SUD symptoms by targeting the naturalistic maladaptive memories that underlie them.

The term 'naturalistic memories' refers here to memories that are naturally- (rather than experimentally-) acquired. The learning history of naturally-acquired maladaptive memories is usually unknown (except perhaps, in the case of recent, single-incident traumas underlying posttraumatic stress disorder; PTSD), although it is assumed that in general, these memories are formed through multiple, intermittent reinforcements (Pavlovian and instrumental) in a variety of contexts and over a prolonged period. This is generally radically different from the situation for experimentally acquired memories, upon which the vast majority of animal and human reconsolidation research has been conducted.

2.1.2 Memory Reconsolidation

Historically, consolidated memories were assumed stable and resistant to modification (cf., McGaugh, 2000). However, over the past two decades numerous studies have convincingly demonstrated that under certain retrieval conditions, even apparently long-established, putatively cortically-distributed memories can enter a transient labile state during which they are susceptible to modification (e.g. Graff et al., 2014; Robinson & Franklin, 2010; Suzuki et al., 2004). This process of *reconsolidation* consists of two temporally and pharmacologically dissociable stages: (a) retrieval-induced *reactivation* or *destabilisation,* and (b) *restabilisation* in which the memory is updated strengthened form (Lee, 2009). Under typical circumstances, reactivation

engenders a period of memory instability that is required for normative memory strengthening and updating. However, this period of instability might also represent a state in which memories are susceptible to disruption via pharmacological agents and behavioural procedures.

2.1.3 Weakening maladaptive memories: Disruption of restabilisation with pharmacological agents

The restabilisation phase of the reconsolidation cycle is protein synthesis-dependent and drugs that (in)directly interfere with protein synthesis can disrupt this phase. The most potent of these drugs (e.g. anisomycin or cyclohexamide) interfere directly with cellular translational machinery and macromolecule biosynthesis. However, these drugs are toxic and not safe for human use. As such, an alternative approach has involved indirect inhibition of protein synthesis through, for example, upstream neurotransmitter blockade. While a number of studies have examined such indirect modulation via diverse drugs (e.g. glucocorticoid, glutamatergic and GABAergic compounds), there are relatively few human studies using these drug classes (c.f. Das et al., 2015a; Das, Walsh, Hannaford, Lazzarino, & Kamboj, 2018b; Drexler, Merz, Hamacher-Dang, Tegenthoff, & Wolf, 2015; Meir Drexler, Merz, Hamacher-Dang, & Wolf, 2016; Rodriguez et al., 2013; Wood et al., 2015). By contrast, the β-blocker, propranolol, has proven to be a particularly popular tool for probing reconsolidation in humans, especially in laboratory studies of fear conditioning (e.g. Bos et al., 2014; Kindt et al., 2009; Schroyens et al., 2017; Sevenster et al., 2012, 2013, 2014; Soeter & Kindt, 2010, 2011, 2012a, 2015b). Other studies have extended these experimental findings with propranolol to clinical populations, showing enduring retrieval-dependent reductions in trauma symptoms in people with PTSD (Brunet et al., 2018) and fear in spider phobics (Soeter & Kindt, 2015a), as well as drug craving among addicted individuals (e.g. Xue et al., 2017).

2.1.4 Rewriting maladaptive memories using behavioural techniques

An alternative approach involves disrupting memory expression via purely behavioural reconsolidation-interference strategies (e.g. Monfils, Cowansage, Klann, & LeDoux, 2009). By targeting memory networks that are causally implicated in symptom expression, this approach aims to overcome the limitations of traditional inhibitory training (extinction) strategies. As described in *Chapter 1*, initially successful extinction is often followed by the 'return of fear' - or in the case of substance use disorders, the recurrence of craving and drug seeking - following re-exposure to

unconditioned stimuli (US; reinstatement), the simple passage of time (spontaneous recovery), or change in context (renewal). This strongly suggests that maladaptive associative memories persist following typical extinction-based therapies and might contribute to relapse (Bouton, 2002; Conklin & Tiffany, 2002). Reconsolidation-based behavioural (and pharmacological) treatments can potentially overcome these issues through *direct updating* of reactivated memory networks.

Consistent with this, retrieval-extinction (i.e. post-retrieval extinction learning) eliminates, and prevents the return of fear in rats (e.g. Monfils et al., 2009) and humans (Johnson & Casey, 2015; Schiller et al., 2009). Relative to extinction without prior retrieval, retrieval-extinction also leads to enduring reductions in reactivity to drug cues in rodent models of addiction (e.g. Cofresi et al., 2017; Xue et al., 2012) and in human substance users (Germeroth et al., 2017; see Kredlow et al., 2016 for a review of post-retrieval extinction effects; Xue et al., 2012), suggesting that this procedure may be a general-purpose strategy for lasting modification of maladaptive memories. Other therapeutically applicable post-retrieval learning strategies might also be suited to updating appetitive and threat memories in humans, although these have received less attention (c.f. Das et al., 2015b; Hon et al., 2016).

2.1.5 Putative boundary conditions on memory destabilisation

Despite the therapeutic implications of reconsolidation interference hinted at above, there appear to be some inbuilt limits on the regular destabilisation-restabilisation of naturally acquired memories. In particular, ongoing and indiscriminate memory interference following retrieval is constrained by a number of 'boundary conditions' that limit destabilisation. Of particular relevance to naturalistic maladaptive memories, older and more strongly-encoded associations appear to be relatively resistant to destabilisation following simple retrieval procedures (e.g. Alfei et al., 2015; Milekic & Alberini, 2002; Robinson & Franklin, 2010; Suzuki et al., 2004). In contrast, experimental studies showing robust reconsolidation effects, particularly in humans, often involve experimentally generated memories (especially conditioned fear), which are often reactivated mere days after training. These simulated maladaptive memories reflect profoundly different learning intensities compared to their naturalistic counterparts in phobia/trauma and SUDs. Associative learning in these disorders involves highly salient USs at encoding (supporting single trial learning) or reinforcement over many years in multiple contexts. For example, the typical 'pack-a-day' smoker will experience close to 10^6 reinforcements (puffs on a

cigarette) over 12 years of regular smoking. These distinct properties of naturalistic memories (asymptotic learning and temporal remoteness) relative to experimentally learned associations (sub-maximal learning and recency) potentially severely limit the application of findings from experimental conditioning studies to the treatment of psychological disorders with reconsolidation-interference strategies.

In addition, variation in stimulus predictability at retrieval may moderate the ability of retrieval procedures to labilise naturalistic maladaptive memories. In particular, accumulating experimental evidence suggests that a relevant prediction error (PE) at retrieval may be important for enabling full destabilisation of memory networks (e.g. Alfei et al., 2015; Exton-McGuinness et al., 2015; Kindt & van Emmerik, 2016; Pedreira et al., 2004; Sevenster et al., 2013, 2014). As an illustration, Das et al. (2015b) showed that while simple retrieval cues (followed by counter-conditioning) produced intermediate levels of memory updating, incorporation of a PE at retrieval appeared to result in more pronounced rewriting of alcohol memories. More recently, Das et al. (2018b; see *Chapter 3*) found no evidence of a reconsolidation-blocking effect of post-retrieval nitrous oxide gas (a putative NMDAR antagonist) in heavy drinkers. However, re-analysis that took account of the level of experienced PE (subjective surprise ratings) at retrieval revealed a significant reduction in craving and drinking behaviour in the retrieval + nitrous oxide group among participants experiencing high PE following reward omission during retrieval. As such, extant studies in humans that demonstrate weakening/updating of naturalistic maladaptive memories without the use of explicit PE-generating procedures during retrieval (most published studies) may reflect a lower bound of efficacy of such interventions, due to sub-optimal reactivation of maladaptive memory networks. However, while evidence of the PE-dependence of destabilisation has been demonstrated in studies of experimentally acquired memories in humans (e.g. Fernandez, Boccia, & Pedreira, 2016; Sevenster et al., 2013), this has yet to be tested through systematic variations in degree of PE during reactivation of memories with fixed and unknown learning histories (i.e. naturalistic memories). More generally, optimal retrieval parameters (e.g. the duration or number of conditioned stimulus (CS) presentations or the use of USs rather than CSs at retrieval; Exton-McGuinness et al. 2015; Merlo et al. 2014) have not been thoroughly studied in humans, leaving some uncertainty about the suitability of the retrieval procedures used in extant studies of naturally acquired memories.

2.1.6 The current review

To date, reviews and meta-analyses on reward and fear memory reconsolidation have either largely focused on non-human animals (e.g. Das et al., 2013) or, in the case of human studies, primarily on experimentally-generated memories focusing on a single reconsolidation interference strategy (e.g. Kredlow & Otto, 2015; Lonergan et al., 2013) or memory system (Scully, Napper, & Hupbach, 2017). Such analyses are critical for furthering our understanding of the modulators of this fundamental memory process. However, a determination of the utility of reconsolidation modulation as a therapeutic strategy requires a synthesis of studies in which clinically important symptoms are targeted in appropriate populations. To date, no comprehensive synthesis has been conducted on the effects of reconsolidation modulation strategies specifically directed at clinically relevant reward and threat-related memories in humans. As alluded to above, the distinct properties of strongly encoded and remote naturalistic maladaptive memories versus those formed during experimental procedures may be extremely important in determining the translational utility of laboratory findings. Moreover, it might be that differences in the neural substrates of learning, and the distinctive learning histories associated with appetitive memories versus threat-related memories, render addictive and phobia/traumatic-stress disorders differentially susceptible to reconsolidation treatments due to differences in 'reactivation-potential' of their underlying maladaptive memories. However, this has yet to be formally tested. Finally, a systematic comparison of behavioural versus pharmacological strategies has not be conducted. The current meta-analysis therefore addresses the lack of a systematic synthesis of behavioural *versus* pharmacological reconsolidation-interference strategies applied to human substance using and anxious/trauma-exposed (clinical and sub-clinical) samples.

2.2 Methods

2.2.1 Search Strategy

Psychinfo, PubMed, Web of Science, and Scopus databases were searched on 20/03/2018 using search terms based on a scoping search on experimental and therapeutic modulation of reconsolidation. The search terms were: (memory) AND (((((((reactivat*) OR destabilis*) OR destablis*) OR memory reconsolidation) OR reconsolidation) OR reconsolidation-extinction) OR extinction) OR retrieval) AND ((((((((((((((((pharmacologic*) OR NMDA) OR N-methyl-D-aspartate) OR adrenoceptor) OR adrenergic) OR noradrenergic) OR beta adreno) OR adrenoreceptor) OR sympathetic) OR sympathetic nervous system) OR dopamine) OR dopaminergic) OR glucocorticoid*) OR cortisol) OR benzodiazepine) OR calcium channel) OR extinction) OR exposure) AND ((((((((((avers*) OR appetit*) OR fear) OR anxiety) OR PTSD) OR addiction) OR substance use disorder) OR substance use) OR drug use) OR drug) OR reward). The search was re-run on the 28/06/2019 to identify any additional studies published over the course of the current PhD.

The search was limited to human studies and excluded reviews. The international clinical trials registry platform and *clinicaltrials.gov* were searched using the term "reconsolidation", after which a search of the identified authors' current publications was conducted. The reference lists of the following reviews were also checked for relevant studies: (Centonze, Siracusano, Calabresi, and Bernardi (2005); de Kleine, Rothbaum, and van Minnen (2013); de Quervain, Roozendaal, Nitsch, McGaugh, and Hock (2000); Dennis and Perrotti (2015); Elsey, Van Ast, and Kindt (2018); Farach et al. (2012); Gisquet-Verrier and Riccio (2012); Kredlow et al. (2016); Lee et al. (2017); Makkar, Zhang, and Cranney (2012); Milton (2013); Milton and Everitt (2010); Pitman (2011); Schwabe, Nader, and Pruessner (2014)). Authors of all included studies were contacted regarding unpublished data.

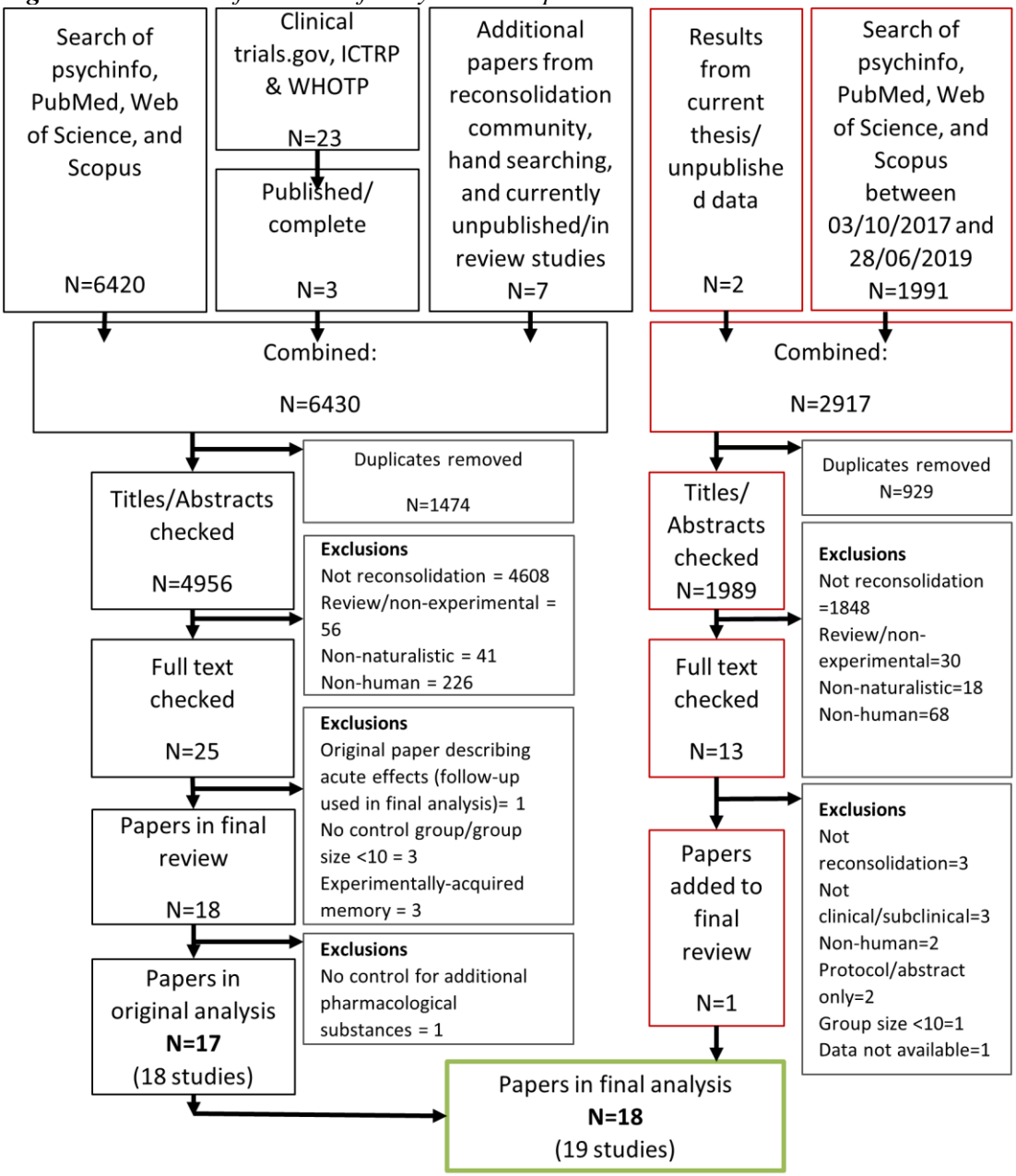
2.2.2 Study inclusion criteria

Figure 2.1 outlines the search, screening and section process, in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Note that details of the initial search (conducted on data sourced in February 2018 and available in (Walsh et al. 2018) are denoted in black, with details of the additional search (conducted in June 2019) and data collected as a part of the current thesis denoted in red. Analyses of studies were restricted to those that examined a

reconsolidation-modulating (retrieval-dependent) pharmacological or behavioural strategy targeting naturally, rather than experimentally, acquired memories. In addition, studies were required to assess symptoms relevant to substance use or anxiety/trauma disorders reflecting effects on long-term (≥24 hr) memory. Participants were required to be recruited on the basis of elevated anxiety, experience of trauma or problematic alcohol/substance use. There was no requirement for a formal diagnosis or for participants to be seeking treatment. Studies were required to randomise adult participants to a 'Retrieval + (reconsolidation interfering) Treatment' or control group (see below) and contain n≥15 per condition *at randomisation* (although the final ES calculation was based on the eventual sample, after exclusions/dropouts). The decision to include only studies with n≥15/group was based on pragmatic considerations relating to the limited number of studies with substantial sample sizes. Only studies reported in English were included. Abstracts were reviewed for eligibility by the author. Nineteen studies that examined pharmacological or behavioural strategies for modifying naturalistic appetitive or threat-related memories via reconsolidation in clinical or subclinical human samples were included.

Note, one study (Jobes et al., 2015) that initially met inclusion criteria was excluded following discussion due to the complex nature of the design, which involved participants receiving methadone at various times during the intervention (either pre- or post-retrieval). This was in addition to the reconsolidation-interfering study-medication (propranolol), making it impossible to disentangle opioid from β-adrenergic treatment effects. In addition, a study on the effects of propranolol on smoking memories (Xue et al., 2017) did not meet criteria because the effects primarily related to experimentally acquired, rather than naturalistic smoking memories. The study presented in *Chapter 5* of the current thesis was also excluded as it assessed natural (i.e. non-drug) reward memories, and thus did not represent problematic substance use. Finally, one study (Kaag, Goudriaan, De Vries, Pattij, & Wiers, 2018) was identified as meeting the criteria in the more recent search but was not integrated into the final analysis due to a lack of published data and time constraints associated with sourcing data from authors.

Figure 2.1: *PRISMA flowchart of study inclusion process.*



Note: original search (conducted on 20/03/2018) in black. Updated search (conducted 28/06/2019) in red.

2.2.3 Methodological evaluation of studies

Identifying information (authors, institutions, journal details and mention of significance of results) was obscured from included papers before assessment of methods sections by two (nominally blind) investigators. The tool for methodological appraisal was a modified version of an instrument used in Das et al.'s (2013) previous meta-analysis of reconsolidation studies (*Appendix item 1*). The level of inter-coder agreement was 83% and any discrepancies in ratings were resolved through discussion.

2.2.4 Data Extraction

Details regarding the study protocol, including memory retrieval procedure, outcome measures, treatment timing (relative to retrieval), type (behavioural or pharmacological) and dose, as well as 'disorder' type were extracted from the selected articles.

2.2.5 Outcome measures

A preliminary review of the selected studies identified specific outcomes for use in effect size (ES) calculation. These were selected based on the regularity with which these measures were reported across studies. We chose this approach in preference to determining ESs for published significant effects in order to minimise bias, since some of the included studies were not identified as clinical trials, and therefore had no pre-determined (registered) outcomes. As such, subjective craving, an important clinical target in SUD treatment, reflecting conditioned responding to drug cues (i.e. the subjective expression of retrieved drug-related memories) was the primary outcome in the current analysis of substance use studies, as it was reported in all but one of the relevant publications. For the study in which craving was not measured (*Chapter 4*), ES was calculated based on total alcohol consumption (total number of units per week) as a measure of drug-seeking. Studies of phobias consistently used the behavioural approach (/avoidance) test (BAT), although the nature of outcomes from this test varied from study to study (e.g. distance between participant and feared object, Shiban, Brutting, Pauli, & Muhlberger, 2015; subjective fear ratings during proximal approach, Telch, York, Lancaster, & Monfils, 2017). Finally, of the five trauma-related studies most commonly reported PTSD symptom severity (four studies; *Table 2.1*) and one study reported memory performance (number of recalled trauma event details; Kredlow & Otto, 2015).

Table 2.1. *Study details*

Study	N Cont:Tx	% Male	Mean Age	Retrieval-dependent treatment	Control condition	Reconsolidation procedure	Outcome (ES calculation)	Effects reported in publication (bold=result used for ES calculation)
Phobia/trauma studies								
Bjorkstrand et al. (2016) & Bjorkstrand et al. (2017) Specific (spider) phobia	20:20	26.7%	26.20	Retrieval-extinction	RETRIEVAL+ 6hr + Tx*	Retrieval (12s) → 10-min → Exposure	Approach behaviour (BAT)	↓ in BOLD response to familiar and novel cue (1 day) ↑ in approach behaviour (1 day) ↑ **Proportion of spider versus neutral pictures views (6 months)** ↓ in BOLD response to cue in right amygdala (6 months) No Δ BOLD response to cue in left amygdala (6 months)
Brunet et al. (2018) Patients with PTSD	30:30	41.7%	39.4	Propranolol	RETRIEVAL + NTx	0.67 mg/kg propranolol (SA) → 120 min; session 1) →1.0 mg/kg propranolol (LA; concurrently administered after session 1) → 90 minutes → Retrieval (10-20 min)	Symptom severity (CAPS)	↓ **CAPS**, PCL-S (6 weeks; **6 months**)
Kredlow and Otto (2015) Indirect witnesses of a terrorist act	22:23	20.2%	19.30	Negative story - interference	RETRIEVAL + NTx	Retrieval (4-min) → Negative story	details for traumatic event	↓ **number of details for event (1 week)**
Maples-Keller et al. (2017) Specific (flying) phobia	30:27	21.1%	42.11	Retrieval-extinction (VRET)	NRETRIVAL + Tx	Retrieval (15s) → 10-min → VRET	Fear (FFI)	No Δ FFI, QAF (posttreatment; 3 months; 6 months) **No Δ FFI,** QAF **(12 months)** ↑HR (3 month) ↓SCR (3 month)
Shiban et al. (2015) Specific (spider) phobia	15:13	10.0%	31.14	Retrieval-extinction (VRET)	NRETRIEVAL +Tx	Retrieval (5s) → 10-min → In vivo and VRET	Approach behaviour (BAT)	**No Δ in approach behaviour during BAT (1 day)** No Δ fear ratings during spontaneous recovery (1 day) No Δ in SCR (1 day) No Δ in vivo fear ratings (1 week) No Δ self-reported avoidance or FSQ (6 months)

Study	N Cont:Tx	% Male	Mean Age	Retrieval-dependent treatment	Control condition	Reconsolidation procedure	Outcome (ES calculation)	Effects reported in publication (bold=result used for ES calculation)
Phobia/trauma studies (continued)								
Soeter and Kindt (2015a) Specific (spider) phobia	15:15	9.0%	21.60	Propranolol	NRETRIEVAL +Tx	Retrieval (2-min) → 40mg propranolol	Approach behaviour (BAT)	No Δ in numerical fear scale (4 days) ↑ in approach behaviour during BAT (11 days) No Δ in SPQ (11 days) ↑ in approach behaviour during BAT (3 months) No Δ in self-reported fear (3 months) **↑ in approach behaviour during BAT (1 year)** ↓ in numerical fear scale (1 year)
Suris, Smith, Powell, and North (2013) Patients (Vietnam and post-Vietnam era veterans) with PTSD	24:27	100%	43.02	Rapamycin	RETRIEVAL+NTx	15 mg rapamycin ('sirolimus') → retrieval(30-75 min; average:45-min)	Symptom severity (CAPS)	↓ CAPS in post-Vietnam subgroup (1 month) **No Δ CAPS,** PCL, QIDS-SR (1 month) No Δ CAPS, PCL, QIDS-SR (3 months)
Telch et al. (2017) Specific (spider/snake) phobia	17:15	12.5%	21.31	Retrieval-extinction	Tx+RETIEVAL**	Retrieval (10s) → 30 minutes → In vivo exposure (3-min x6)	Peak fear during behavioural approach (BAT)	No Δ peak fear during BAT (1 day) No Δ expected fear during BAT (1 day) **↓ in peak fear during BAT renewal test (1 month)** No Δ expected fear during BAT (1 month) No Δ FSQ (1 month)
Wood et al. (2015) Study 2 Patients (civilians and veterans) with PTSD	15:13	71.43%	45.75	Mifepristone	NRETRIEVAL+Tx	1800mg mifepristone → 90-min → retrieval (duration not specified)	Symptom severity (IES)	**No Δ IES-R**, Physiological 'PTSD probability score', HR, SCR, F-EMG, C-EMG **(1 week)**
Wood et al. (2015); Study 3 Patients (civilians) with PTSD	15:16	45.2%	38.50	D-Cycloserine & Mifepristone	RETRIEVAL+NTx	100 mg D-Cycloserine → 240-min → 1800mg mifepristone → 90-min → retrieval (duration not specified)	Symptom severity (IES)	**No Δ IES-R**, Physiological 'PTSD probability score', HR, SCR, F-EMG, C-EMG **(1 week)**

Study	N Cont:Tx	% Male	Mean Age	Retrieval-dependent treatment	Control condition	Reconsolidation procedure	Outcome (ES calculation)	Publication-reported outcomes (bold=result used for ES calculation)
Substance Use								
Das et al. (2015a) Dependent current smokers	20:19	50%	28.39	Memantine (10 mg/kg)	NRETRIEVAL+Tx	10 mg memantine → 210-min → retrieval (5-min)	Craving (QSU)	**No Δ craving (1 week)** No Δ cue-induced BP; SCR; HRV (1 week) No Δ smoking-related attentional bias (1 week) No Δ relapse latency (3 month) No Δ nicotine dependence (3 month)
Das et al. (2015b) Non-dependent current hazardous drinkers	19:20	50%	22.33	Counter-conditioning	NRETRIEVAL+Tx	Retrieval (5-min)→10-min→ Counter-conditioning	Craving (ACQ)	↓ **craving (expectancy; 1 week)** ↓ alcohol attentional bias (1 week) ↓ Cue liking (1 week) No Δ in self-reported drinking (1 week)
Das et al. (2018b)/*Chapter 3* Non-dependent current hazardous drinkers	20:21	66%	27.25	Nitrous Oxide	NRETRIEVAL+Tx	Retrieval (5-min) → 10-min → Nitrous Oxide (30-min)	Craving (ACQ)	**No Δ craving (10 days)** No Δ drinking behaviour, cue liking or cue-induced urge to drink (10 days) Following post-hoc group reassignment based on level of prediction error at retrieval (10 days): ↓ craving, drinking behaviour; cue-induced urge to drink in high PE - N_2O group (10 days) No Δ cue liking in high PE - N_2O group (10 days)
Chapter 4 Non-dependent current hazardous drinkers	19:17	61%	27.48	Ketamine (350 ng/dl)	NRETRIEVAL+Tx	Retrieval (5-min) → 10-min → ketamine (30-min)	Total alcohol consumption / week (units)	↓**Total alcohol units \week (9 months)** ↓N drinking days \week, AUDIT score (9 months) ↓Total alcohol units \week, N drinking days \week, AUDIT score, beer cue liking, beer cue wanting, enjoyment of beer, desire to drink more (9 months) No Δ in attentional bias (10-14 days)
Germeroth et al. (2017) Dependent current smokers	39:34	64%	47.50	Retrieval-extinction	NRETRIEVAL+Tx	Retrieval (5-min)→ 10-min → Cue exposure	Craving (QSU)	↓ # cigarettes/day (2 weeks) No Δ craving for novel and familiar cues (2 weeks) No Δ CO level (2 weeks) ↓ **craving for novel and familiar cues (1 month)** ↓ # cigarettes/day (1 month) ↓ expired CO level (1 month) No Δ cotinine (1 month) No Δ cue-induced BP; HR (1 month)

Study	N Cont:Tx	% Male	Mean Age	Retrieval-dependent treatment	Control condition	Reconsolidation procedure	Outcome (ES calculation)	Effects reported in publication (bold=result used for ES calculation)
Substance Use (continued)								
Hon et al. (2016) Non-dependent current hazardous drinkers	16:16	62%	27.00	Cognitive reappraisal	NRETRIEVAL+Tx	Retrieval (5-min)→ 10-min → Reappraisal	Craving (ACQ)	**↓ craving (purposefulness; 1 week)** ↓ verbal fluency for +ve alcohol words (1 week) No Δ drinking (1 week) No Δ attentional bias (1 week)
Pachas et al. (2015) Dependent current smokers	31:23	73%	42.05	Propranolol (0.67mg/kg)	RETRIEVAL+NTx	0.67 mg/kg propranolol (SA) → 90 min→1.0 mg/kg propranolol (LA)→ retrieval (duration not specified)	Craving (VAS)	**No Δ craving (1 week)** No Δ cue (smoking-script)-induced HR, SCR, EMG (1 week)
Saladin et al. (2013) Dependent current cocaine users	24:26	66%	39.95	Propranolol (40mg SA)	RETRIEVAL+NTx	Retrieval (20-min)→ 40 mg (SA) propranolol	Craving (CDMS)	↓ in craving, systolic and diastolic BP (1 day) No Δ in HR (1 day) **No Δ cue-induced craving, HR, or BP (1 week)**
Xue et al. (2012) Dependent, abstinent heroin users	22:22	100%	37.70	Retrieval-extinction	NRETRIEVAL+Tx	Retrieval (5-min) → 10-min → Cue exposure (60 min)	Craving (VAS)	↓ in heroin craving, Diastolic BP, Systolic BP, HR (4 days) No Δ in HR (4 days) ↓ in heroin craving, Systolic BP (34 days) No Δ in Diastolic BP (34 days) No Δ in HR (34 days) **↓ in heroin craving (184 days)** ↓ in Systolic BP (184 days) No Δ in HR, Diastolic BP (184 days)

Note for Pachas et al. (2015) and Wood et al. (2015) retrieval duration details were not provided but based on references to previous script-driven retrieval (Pitman et al., 1987; Brunet, et l., 2007). Note on control groups: Some studies used a three-group design. Only the control group used in the ES calculation is described in the table.NRETRIEVAL= no retrieval, Tx= treatment, NTx=no treatment, VAS=Visual analogue scale, QSU=Questionnaire on smoking Urges; ACQ=Alcohol Craving Questionnaire, CDSM = Craving/Distress/Mood States, CCQ= the 14-item Cocaine Craving Questionnaire, IES=Impact of Events Scale, FFI= Fear of Flying inventory, FSP=Fear of Spiders/Snakes Questionnaire, FSQ=Fear of Spiders Questionnaire, PE=prediction error VRET=Virtual reality exposure therapy; SA=short acting; LA=long acting, BDI=Beck Depression Inventory, STAI=State Trait Anxiety Inventory, SCR=skin conductance response, F-EMG=frontalis EMG, C-EMG=corrugator EMG

**=retrieval followed by a 6 hr delay, followed by treatment*

***=treatment preceded retrieval*

2.2.6 Statistical approach

2.2.6.1 Effect size determination

Data required for effect size (ES) determination were extracted. Random effects models (DerSimonian and Laird 1986) were selected and the generic inverse variance method used. ESs were calculated as between groups standardised mean differences (Hedge's g; Higgins & Green, 2011) using the Review Manager Software (version 5.3; The Cochrane Collaboration, 2014) and interpreted using the standards of Cohen (1988) and Sawilowsky (2009): ~0.1=very small, ~0.2=small; ~0.5=medium; ~0.8=large and ~1.2=very large. Intermediate descriptive labels (e.g. small-medium) were used to describe ESs, where appropriate.

ESs related to the primary 1 *df* comparison of interest, namely, Retrieval + Treatment (pharmacological or behavioural) versus a suitable control condition. A comparison with a No Retrieval + Treatment control was deemed to best represent the specific effect of a memory interfering/weakening treatment via reconsolidation. Where such a group was not used, ESs were calculated relative to a Retrieval + No Treatment condition. Other control groups are also suitable for testing reconsolidation effects. Unlike pharmacological studies, in which drug effects are likely to be present for several hours (i.e. during the period of memory lability) even if the treatment is administered prior to reactivation, retrieval-dependent memory-interfering behavioural treatment effects are theoretically constrained if the treatment occurs before retrieval. As such, treatment *followed by* retrieval is a suitable control condition in behavioural studies (however see Hutton-Bedbrook & McNally, 2013 for discussion of effects that are not consistent with a standard reconsolidation interpretation). Finally, comparison groups in which treatment is delivered after retrieval but outside of the 'reconsolidation window' are also suitable controls for retrieval-dependent memory effects, as destabilisation/reactivation is a time limited process (lasting <6 hr).

Given that reward- and threat-related disorders have distinct aetiologies and underlying learning processes, these disorder types were evaluated separately in meta-analyses. Given the aetiological similarity in terms of the proposed central role of classical conditioning in specific phobias and trauma-related disorders, these two classes of disorders were considered together as a single category (phobia/trauma). Further, using subgroup analysis, we examined whether treatment type (behavioural versus pharmacological) produced different population ES estimates within each broad

disorder type. Finally, we examined moderation by gender ratio, participant age and score on the methodological appraisal tool (based on number of positively endorsed desirable study characteristics as a proportion of the total number of items that could be positively endorsed) across *all studies* using these as continuous variables in meta-regressions. Note, although variation in retrieval parameters (especially retrieval trial duration and time between reactivation and treatment) could affect the extent to which memories are reactivated or weakened/over-written, insufficient variability in these parameters prevented exploration of these as moderators (cf Kredlow et al., 2016).

Subgroup analyses and forest plots were derived from RevMan. Heterogeneity across studies was assessed using the I^2 statistic and described qualitatively as: ~25% =low; ~50%=moderate, ~75% = high (Higgins et al. 2003). Sensitivity analysis was conducted when heterogeneity was high and involved testing the effects of sequentially removing individual studies to determine which had the greatest influential on heterogeneity. Alternative aggregate ESs are reported where removal of the most influential study resulted in a reduction of heterogeneity to moderate levels or below (i.e. $I^2<50\%$). Where insufficient information was available in publications to calculate ESs from means/SDs and these details were not available from authors (Pachas et al., 2015; Xue et al., 2012) estimates were obtained from figures in the relevant publications using Plot Digitizer software (Poisot, 2011). Publication bias (symmetry of funnel plots and trim and fill) was assessed using the MAVIS package Version 1.1.3 (Hamilton, Aydin, & Mizumoto, 2017) and moderation analyses were performed using custom SPSS syntax (Field & Gillett, 2010).

2.2.7 Terminology

'Reactivation' refers to the first stage of the reconsolidation process, as well as a memory state that is highly accessible and malleable (Gisquet-Verrier & Riccio, 2012). This term is also used to describe procedures intended to achieve this memory state. Since the terms 'reactivated' and 'destabilised' are both used to describe a labile, potentially modifiable state of long-term memories they were used interchangeably. 'Retrieval' is used here to refer to experimental procedures that are intended to reactivate/destabilise memories, but which may or may not be successful in this regard. This term is not intended to imply recall of a discrete memory trace (cf Telch et al., 2017), but rather, retrieval or reactivation of a more complete network of reward (substance use) or threat-related (phobic/trauma-related) associations.

2.3 Results

2.3.1 Study and sample characteristics

After exclusions, the literature search yielded 19 studies from 18 publications (n=809). Five were studies on specific phobias, five on trauma-related symptoms and nine on substance use. Of the phobia/trauma studies, five examined pre- or post-retrieval pharmacological interventions (Brunet et al., 2018; Soeter & Kindt, 2015a; Suris et al., 2013; Wood et al., 2015 studies 2 and 3) and five, post-retrieval behavioural strategies (Bjorkstrand et al., 2017; Kredlow et al., 2016; Maples-Keller et al., 2017; Shiban et al., 2015; Telch et al., 2017). The nine substance use studies also examined either pharmacological (k=5; Chapter 4; Das et al., 2015a; Das et al., 2018b/Chapter 3; Pachas et al., 2015; Saladin et al., 2013) or behavioural reconsolidation interference strategies (k=4; Das et al., 2015b; Germeroth et al., 2017; Hon et al., 2016; Xue et al., 2012).

Participant details (gender ratio; age) are presented in *Table 2.1*. There was considerable variation between studies in terms of gender ratio of participants. Among substance use studies, gender was generally balanced or there was a higher proportion of men, in line with the gender prevalence of SUD in epidemiological studies (Seedat et al., 2009). Xue et al. (2012) was an exception as it only included male participants (detoxified heroin users). In contrast, studies of phobia/trauma were generally skewed towards a higher representation of women, again, in line with epidemiological evidence (McLean, Asnaani, Litz, & Hofmann, 2011). An exception was the study by Surís et al. (2013), which only recruited men (combat veterans). Participant age varied widely across studies, although the mean age of participants was not statistically different ($F_{1,17}$=0.003, p=0.959) between phobia /trauma studies (M=33.06, SD=10.30) and substance use studies (M=33.29, SD=8.63).

2.3.2 General study methodologies

Key design features of studies and the presence/absence of specific desirable methodological study features are outlined in *Table 2.1* and *Table 2.2*. *Table 2.2* shows that studies generally contained many desirable methodological features. The most common methodological limitations across studies were a lack of comprehensive experimental conditions that controlled for the effects of simple retrieval or treatment alone. In addition, a lack of experimenter/assessor blinding was a virtually universal limitation of the behavioural studies, but uncommon in pharmacological studies.

Table 2.2. *Methodological/reporting features of studies*

Study Name	A	B	C	D	E	F	G	H	I	J	K	L	M
Brunet et al. (2018)	Y	Y	Y	Y	Y	Y	N	Y	N	Y	Y	Y	Y
Das et al. (2015a)	Y	Y	N	Y	Y	N	Y	Y	N/A	Y	Y	Y	Y
Das et al. (2015b)	Y	Y	Y	N	Y	N	N	Y	N/A	Y	Y	N/A	Y
Das et al. (2018a)	Y	Y	N	N	Y	N	N	Y	N/A	Y	Y	Y	Y
Chapter 4	Y	Y	Y	Y	Y	Y	Y	Y	N/A	Y	Y	Y	Y
Germeroth et al. (2017)	Y	Y	Y	N	Y	Y	N	Y	N/A	Y	Y	N/A	N
Hon et al. (2016)	Y	Y	N	N	Y	Y	N	Y	Y	Y	N	N/A	Y
Pachas et al. (2015)	Y	Y	N	Y	Y	Y	N	Y	N/A	N	Y	Y	N
Saladin et al. (2013)	Y	Y	Y	Y	Y	Y	N	Y	N/A	Y	Y	Y	N
Xue et al. (2012)	Y	Y	N	N	Y	Y	N	Y	N/A	Y	Y	N/A	Y
Soeter & Kindt (2015)	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	Y	N
Telch et al. (2017)	Y	Y	Y	N	Y	Y	Y	Y	N/A	Y	Y	N/A	Y
Björkstrand et al. (2017)	Y	N	N	N	Y	N	N	N	N/A	Y	Y	N/A	Y
Shiban et al. (2015)	Y	Y	Y	Y	Y	N	N	Y	N/A	Y	Y	N/A	N
Maples-Keller et al. (2017)	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N/A	Y
Kredlow & Otto (2015)	Y	N	Y	Y	Y	Y	N	Y	Y	Y	Y	N/A	N
Surís et al. (2013)	Y	Y	N	Y	Y	Y	N	Y	N	Y	Y	N	Y
Wood et al. (2015; Study 2)	Y	Y	N	Y	Y	Y	Y	Y	N/A	N	Y	Y	Y
Wood et al. (2015; Study 3)	Y	Y	N	Y	Y	Y	N	Y	N/A	N	Y	Y	Y

Note: Y=Desirable study characteristic present; N=desirable characteristic not present.
A) Is the study design (or paradigm) described? B) Detailed inclusion and exclusion criteria provided? C) Procedures for randomisation described? D) Procedures for blinding (if appropriate, i.e. if outcome is experimenter rated) described? E) Primary outcome(s) clearly specified? F) Relevant demographics for subjects provided? G) Sufficient experimental control (i.e. both a No Retrieval + treatment and a Retrieval + placebo group included)? H) Groups comparable at baseline? I) Inter-rater reliability achieved and evaluated where relevant? J) Duration of retrieval trial provided (or reference made to duration from previous published studies)? K) Treatment 'dose' provided? L) Timing of drug administration relative to reconsolidation clearly described? M) Where relevant, missing data (>20%) dealt with appropriately?

2.3.3 Retrieval procedures

Most studies used *in vivo* exposure to CSs (e.g. powder resembling crack cocaine; a live spider), other visual representations of the CS (e.g. video of cocaine use; a series of pictures of spiders), or both, to reactivate memories. All trauma-related studies encouraged autobiographical recall of the traumatic incident(s) to reactivate trauma memory. Other studies also incorporated instructions to recall specific relevant autobiographical episodes evoked by the CSs (e.g. *Chapter 4*; Das et al., 2015a; Das et al., 2015b; Das et al., 2018b/*Chapter 3*; Hon et al., 2016; Pachas et al., 2015; Telch et al.,

2017) and six studies explicitly included prediction error at retrieval (Chapter 4; Das et al., 2015a; Das et al., 2015b; Das et al., 2018b/Chapter 3 ; Hon et al., 2016; Soeter & Kindt, 2015a). The latter involved some form of expectation violation (e.g. generating an expectation that the participant will experience the US, and then violating this expectation; Das, Gale, Hennessy, & Kamboj, 2018a).

As outlined in *Table 2.1* and *Table 2.2*, most studies provided some details about the duration of the retrieval procedure. The modal duration in substance use studies was 5 min (used in seven of the eight studies specifying retrieval duration); one study used a longer retrieval procedure (2 x 10 min; Saladin et al., 2013). Eight of the 10 phobia/trauma studies specified the duration of the retrieval procedures, which varied more than the substance use studies. All phobia studies used ≤2 min retrievals, with most studies clustered in the 5-15s range. The three trauma-related studies that specified retrieval duration used 4 min, 10-20 min, and 30-75 min (the relevance of the length of the retrieval procedure is outlined in the discussion).

2.3.4 Pharmacological and behavioural reconsolidation interference procedures

Pharmacological studies most commonly used propranolol (*k*=2 substance use studies: Pachas et al. 2015; Saladin et al. 2013, and k=2 phobia/trauma studies: Brunet et al. 2018; Soeter and Kindt 2015a). The reconsolidation interfering effects of mifepristone (k=2 Wood et al., 2015 study 2 and 3) and sirolimus (rapamycin; k =1; Suris et al., 2013) on threat memory and memantine (k=1; Das et al., 2015a), ketamine (k=1, *Chapter 4*) and nitrous oxide (k=1; Das et al., 2018b/Chapter 3) on reward memory were also examined. Authors selected drugs based on their on their putative downstream protein synthesis inhibiting effects in all cases. In particular, their tendency to interfere with the protein synthesis-dependent restabilisation phase of reconsolidation.

Among behavioural studies, retrieval-extinction was the most commonly tested procedure, either using 'standard' *in vivo* and/or picture-stimulus exposure in specific phobia (Bjorkstrand et al., 2017; Telch et al., 2017) and substance use (Germeroth et al., 2017; Xue et al., 2012) or virtual reality exposure for specific phobia (Maples-Keller et al., 2017; Shiban et al., 2015). The remaining behavioural studies examined post-retrieval counter-conditioning (Das et al., 2015b) and cognitive reappraisal (Hon et al., 2016) in substance users (heavy alcohol drinkers), or prose interference (Kredlow & Otto, 2015) in sub-clinical, trauma-exposed individuals.

2.3.5 Study Outcomes

Across all studies, 15 of the 19 ESs were positive (favouring retrieval-dependent reconsolidation-interference). With the exception of Brunet et al. (2018), who reported high levels of attrition at 6-month follow-up, ESs are based on comparisons between the retrieval and control condition on the last assessed time-point for the relevant outcome (the ES for the Brunet et al., 2018 study was based on the penultimate follow-up). For the outcomes selected for the current meta-analysis, this ranged from one day (Shiban et al., 2015) to 12 months (Maples-Keller et al., 2017; McLean et al., 2011; Soeter & Kindt, 2015a). We deemed this relatively stringent longest time-point comparison to be appropriate given the claim for *permanent* memory modification following reconsolidation interference.

Among the phobia/trauma studies, other than outcomes from the BAT (phobia studies) and trauma symptom severity/trauma memory recall (trauma-related studies) used to calculate ESs, some of the reviewed publications reported additional outcomes showing significant retrieval-dependent benefits (*Table 2.1*). These included reduced skin conductance in response to fear-provoking stimuli (Maples-Keller et al. 2017), subjective fear/phobic symptoms (Soeter and Kindt 2015a) and neural activity in the amygdala (Bjorkstrand et al. 2016; 2017). Notably, reductions in subjective fear (of spiders) in the Soeter and Kindt (2015a) study only emerged at long-term follow-up, suggesting a lagged benefit for some outcomes following reconsolidation-interference treatments. Conversely, Maples-Keller et al. (2017) reported relatively *higher* physiological arousal (heart rate) at 3 month follow-up in the Retrieval + Treatment (exposure) group, in the absence of, as well as during, exposure to feared cues. However, this was interpreted as a relative benefit to the retrieval group (i.e. high levels of fear were thought to attenuate physiological reactivity in the no retrieval group, although there were no between group differences in fear ratings at this time-point).

In addition to craving, other statistically significant effects were also reported in a number of the substance use studies (*Table 2.1*). These included reductions in smoking (Germeroth et al., 2017), alcohol consumption (*Chapter 4*), frequency of alcohol consumption (*Chapter 4*), alcohol attentional bias (Das et al., 2015b), alcohol cue liking (*Chapter 4*) and cue wanting(*Chapter 4*), fluency for positively valenced alcohol words (Hon et al., 2016), and cocaine and heroin cue-evoked blood pressure changes (Saladin et al., 2013; Xue et al., 2012).

2.3.6 Control conditions

A control group that received treatment in the absence of putative reactivation (No Retrieval + Treatment) was considered the most appropriate comparison condition and was the most commonly employed. A number of pharmacological studies used a Retrieval + no Treatment (placebo) group (Brunet et al., 2018; Pachas et al., 2015; Saladin et al., 2013; Suris et al., 2013; Wood et al., 2015 study 3), as did Kredlow and Otto (2015), who compared negatively valenced interfering prose with a no prose condition.

2.3.7 Effect size for symptoms of phobia and trauma

The aggregate ES for phobia/trauma symptoms was medium (k=10; n=402; g=0.44, 95% CI [0.13, 0.74], p=0.005*; Figure 2.2.A*) and showed moderate heterogeneity (I^2 = 55%). It is clear from inspection of the forest plots however, that the Soeter and Kindt (2015a) study contributes disproportionately to the overall ES. A sensitivity analysis showed that exclusion of Soeter and Kindt (2015a) eliminated heterogeneity (I^2=0%), but also reduced the ES (g=0.33, 95% CI [0.12, 0.53]), although it remained significantly>0 (p=0.002).

The aggregate ES for all pharmacological studies was medium (k=5, g=0.59, 95% CI [0.07, 1.11], p=0.03; *Figure 2.2.B*) and heterogeneity was relatively high (I^2=67%). When Soeter and Kindt (2015a), was retained in the analysis, the population ES estimate had poor precision, with the true effect lying in the range from very small to large-very large. Exclusion of Soeter and Kindt (2015a) eliminated heterogeneity (I^2=0%) but also reduced the ES, although it remained significant (k=4, g=0.32, 95% CI [0.01, 0.62], p=0.040). A small, non-significant ES was found for behavioural studies of phobia/trauma (k=5, g=0.32, 95% CI [-0.07, 0.70], p=0.10), with a moderate degree of heterogeneity (I^2=46%). Subgroup analysis showed that pharmacological and behavioural studies did not significantly differ, regardless of the inclusion ($\chi^2(1)=0.69$, p=0.400) or exclusion ($\chi^2(1)=0.00$, p=1.000) of Soeter and Kindt (2015a).

2.3.8 Effect size for symptoms related to substance use

Across all substance use studies, the aggregate ES was small and non-significant (k=9; n=407; g=0.26, 95% CI [-0.08, 0.59], p=0.14; *Figure 2.2.B*), with relatively high levels of heterogeneity (I^2=65%). Sensitivity analysis identified a single study (Pachas et al., 2015) that appeared to be especially influential. Its removal reduced

heterogeneity to I^2=31% and increased the aggregate ES to a small but significant magnitude (*k*=8; *n*=354; *g*=0.38, 95% CI [0.12, 0.64], *p*=0.004).

Subgroup analysis of substance use studies indicated that pharmacological studies (including Pachas et al., 2015) were associated with a small, negative ES (*k*=4; *n*=184; *g*=-0.16, 95% CI [-0.60, 0.27], *p*=0.470), and moderate heterogeneity (I^2=53%). The four pharmacological studies exclusive of Pachas et al. (2015) had a negligible combined ES, which was non-significant (*g*=0.05, 95% CI [-0.30, 0.40], *p*=0.780; *Figure 2.3*). In contrast, behavioural studies yielded a significant, medium ES (*k*=4; *n*=188; *g*=0.60, 95% CI [0.29, 0.92], *p*<0.001), with low heterogeneity (I^2 = 11%). A moderator analysis including Pachas et al. (2015) suggested that the ESs of behavioural and pharmacological studies of substance use were significantly different ($\chi^2(1)=7.71$, *p*=0.005, *Figure 2.4)*, although removal of this statistically influential study brought the two effect sizes closer together ($\chi^2(1)=5.21$, *p*=0.020).

Figure 2.2. *Forest plot of all included studies and a comparison of overall ES and associated CI and for anxiety (a) and substance use (b) studies.*

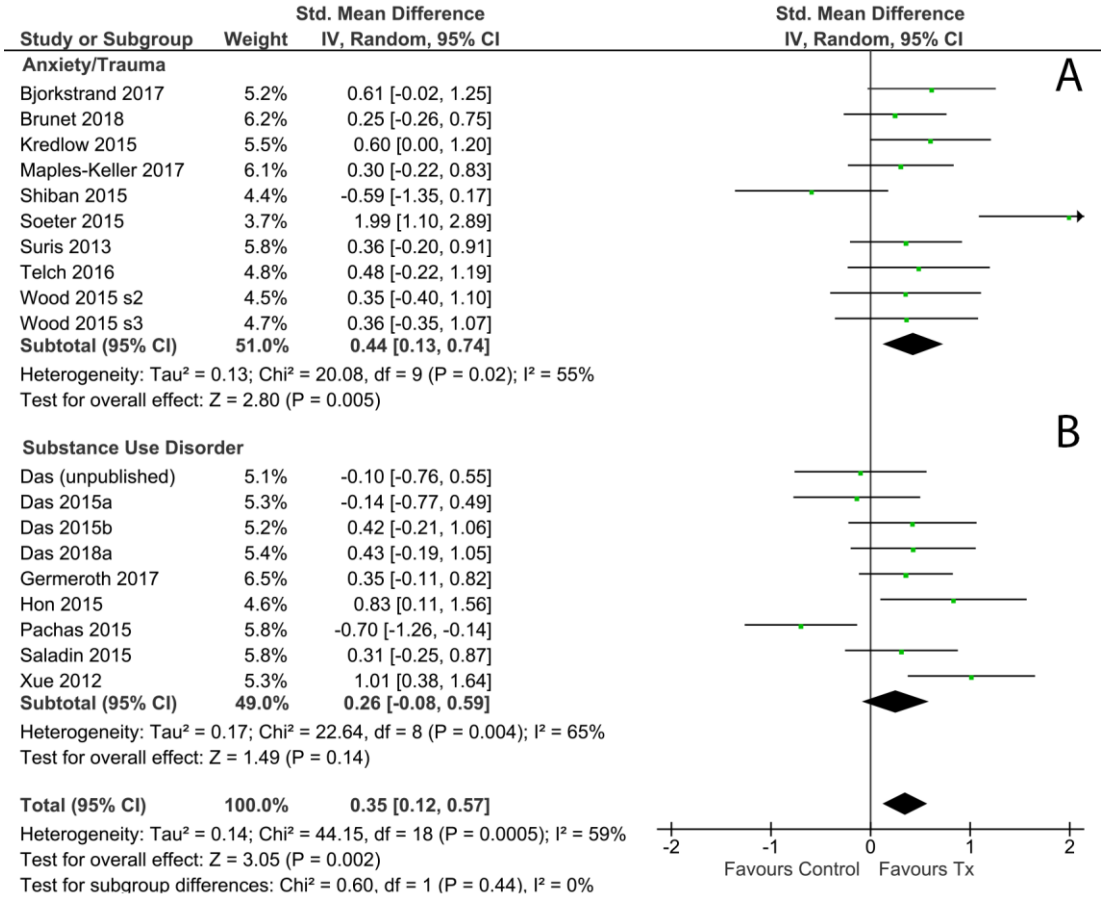


Figure 2.3. *Comparative forest plot for treatment type (behavioural vs. pharmacological) in studies of maladaptive threat memories (anxiety).*

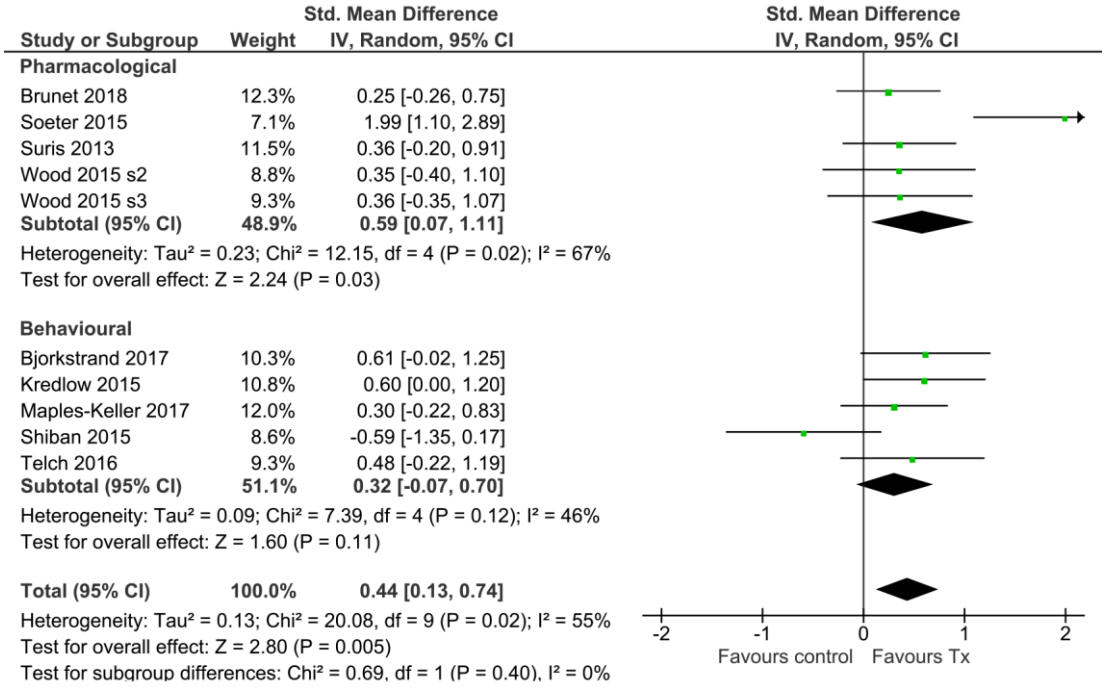
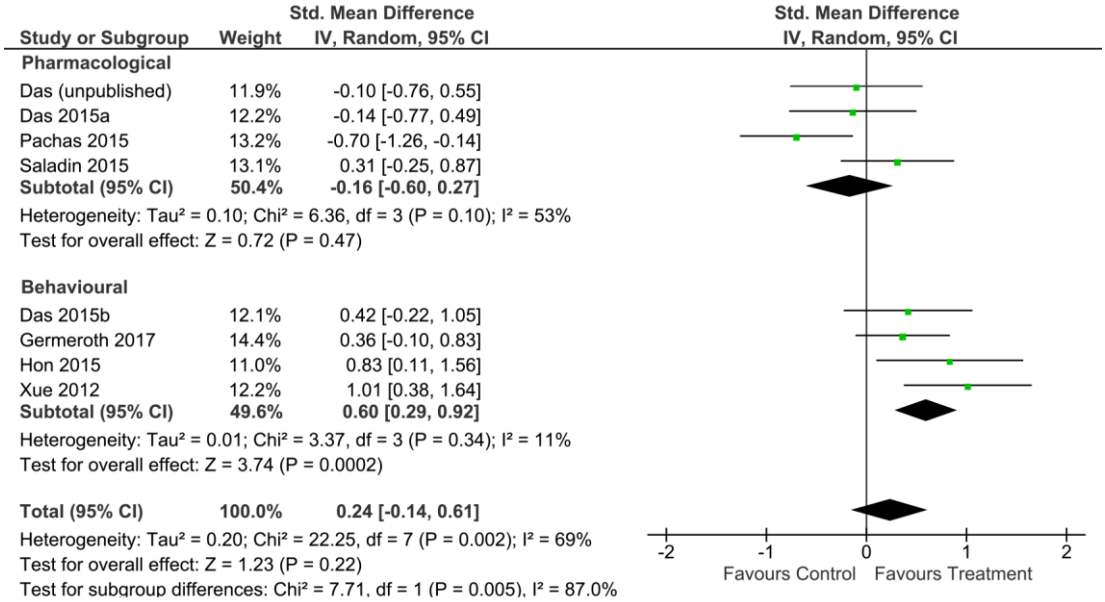


Figure 2.4. *Comparative forest plot for treatment type (behavioural vs. pharmacological) in studies of reward memories (substance use)*



2.3.9 Moderation

Across *all* studies, meta-regression suggested that none of the specified moderators (age, proportion of male participants or methodological appraisal score) were significant predictors of ES (*t* values <1.5, *p* values >0.1).

2.3.10 Publication bias

A funnel plot for the phobia/trauma studies did not indicate asymmetry (t_8=0.220, *p*=0.831; *Figure 2.5*). No adjustments to the effect of phobia/trauma studies were suggested by trim and fill (Duval & Tweedie, 2000a, 2000b). The funnel plot for studies of substance use similarly indicted a lack of asymmetry (t_7= 0.600, *p*=0.568; *Figure 2.6*), with trim and fill suggesting one study with a negative ES was absent. No adjustment to effect was observed following the exclusion of Pachas et al. (2015). Overall, these results suggest an absence of publication bias for phobia/trauma and substance use studies.

Figure 2.5. *Funnel plot of ES against standard error for studies of anxiety/trauma*

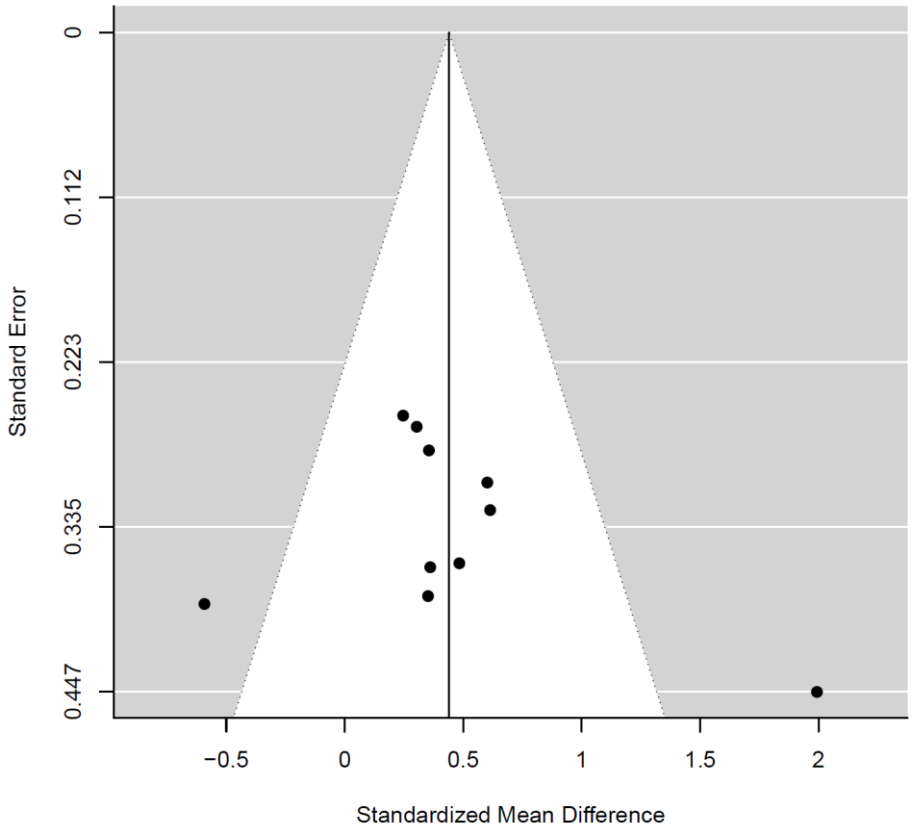
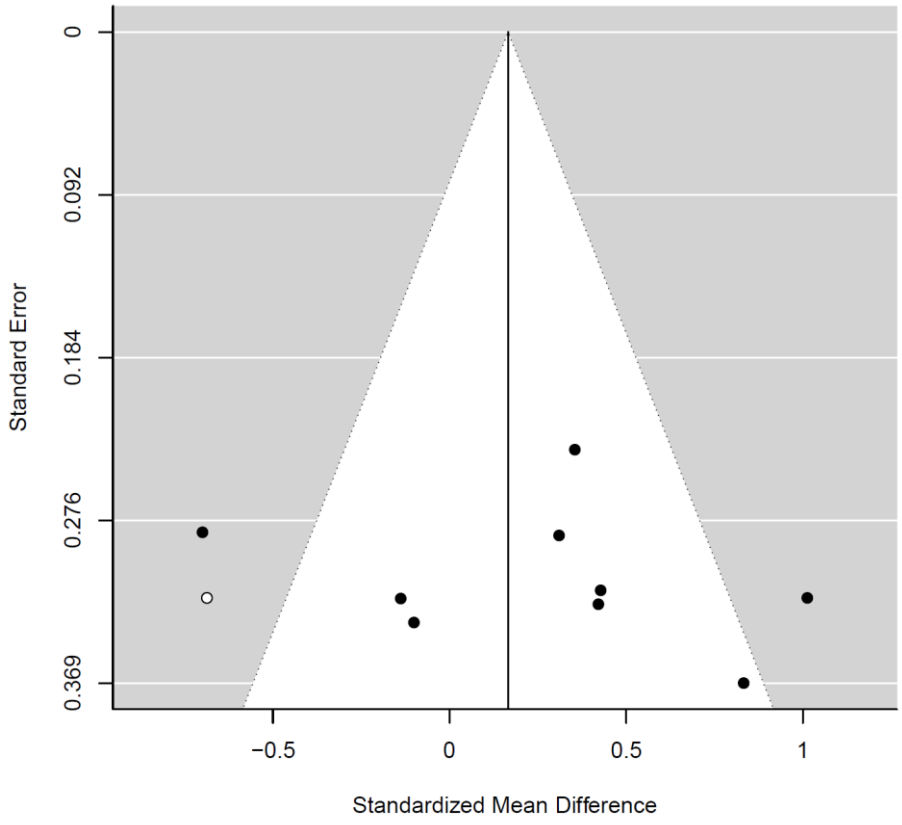


Figure 2.6. *Funnel plot of ES against standard error for studies of substance use disorder*



2.4 Discussion

This meta-analysis provides a synthesis and critical evaluation of research on reconsolidation of naturalistic maladaptive memories using pharmacological and behavioural memory-weakening/interference strategies in (sub)clinical samples. Extension of non-human and human experimental findings to clinically relevant populations is a relatively new area of translational research, with the oldest publication in this review dated 2012. As such, there are currently a small number of relevant studies, although findings across these were relatively consistent. In particular, 15 of 19 ESs were in the predicted direction (i.e. favouring a reconsolidation-modulation interpretation), but of the two broad disorder categories, only the population ES estimate for phobia/trauma (behavioural and pharmacological studies) was significant. Moderator analysis by intervention type (pharmacological versus behavioural) indicated larger effects in behavioural versus pharmacological studies in the case of substance use, while the opposite pattern was observed for phobia/trauma studies, with pharmacological treatments producing an almost two-fold larger ES than behavioural treatments (although the comparison was not statistically significant). However, these general findings need to be considered in the context of the finding that the ES for each 'disorder type' was substantially influenced by a single study that inflated heterogeneity and skewed the results towards either a larger (phobia/trauma) or smaller (substance use) ES.

2.4.1 General overview of studies

Across all substance use studies, the overall ES was small and non-significant, although removal of Pachas et al. (2015) reduced heterogeneity and increased the aggregate ES estimate, rendering it significant. Anxiety/trauma studies had a significant medium ES when all studies were considered, and a significant small-medium ES when Soeter and Kindt (2015a) was excluded. Overall, these findings support the idea that, despite their chronological age and strength relative to experimentally acquired memories, naturalistic maladaptive memories are capable of being destabilised and subsequently weakened/over-written using reconsolidation-modulating strategies. As such, the putative boundary conditions (memory remoteness and strength) that appear to limit the 'destabilisation potential' of experimentally trained memories in non-human animals (Milekic & Alberini, 2002; Suzuki et al., 2004; Vousden & Milton, 2017) do not necessarily preclude destabilisation of naturally acquired memories in humans,

although they might constrain the degree of destabilisation, and hence, the magnitude of intervention effects. This provisional conclusion is promising for the development of such strategies as therapeutic interventions for threat-related and substance use disorders.

ESs varied considerably across substance use and phobia/trauma studies. This might have reflected substantial variation in retrieval procedures (e.g. retrieval duration; timing of treatment relative to retrieval, number of treatment sessions), the nature of reconsolidation interference strategies (i.e. the use of different drug classes and behavioural interventions), and participant characteristics. As such, there continues to be uncertainty about optimal retrieval parameters and/or retrieval-dependent interference strategies required to interfere effectively with naturalistic maladaptive memories. Such memories are likely to constitute highly distributed traces, involve multiple memory systems (semantic, autobiographical-episodic, priming/implicit; varying in affective valence) and vary substantially in strength and remoteness from one individual to another. This is clearly very different to the situation in studies of laboratory-trained memories, in which learning across participants is uniform and usually involves a limited set of stimuli from well-defined categories (e.g. sets of simple sensory stimuli as CSs) within a single context. Moreover, effective retrieval (reactivating) cues in laboratory studies are simply those that were used during training, whereas the nature of *suitable* (i.e. optimal) retrieval cues for naturalistic memories is unclear. Given the uncertainty regarding suitable retrieval conditions for naturalistic memories, as well as the likelihood that such memories are more strongly entrained (over a long period) than experimentally acquired memories, it might be expected that ESs would differ between experimental and naturalistic memories. It is therefore instructive to compare our ES estimates with those obtained in previous meta-analyses of reconsolidation studies of experimentally acquired memories in humans. The results reported in two relevant meta-analyses on emotional (threat-related) memories are therefore considered (Kredlow et al., 2016; Lonergan et al., 2013) in relation to phobia/trauma studies.

2.4.2 Phobia/trauma studies

Kredlow et al. (2016) examined changes in conditioned fear in studies employing the prototypical behavioural reconsolidation procedure, retrieval-extinction (Monfils et al., 2009). Their reported aggregate ES, relating to tests of 'return of fear', is not dissimilar to that for behavioural strategies (also most commonly, retrieval-extinction)

employed in the phobia/trauma studies reviewed here. The aggregate ES reported here for phobia/trauma studies using pharmacological treatments (propranolol, mifepristone and sirolimus; *g*=0.59) is consistent with the ES reported in a meta-analysis of studies of the effects of propranolol on memory for negatively valenced words and cue-induced fear responding (g=0.56; Lonergan et al., 2013). Heterogeneity of ESs was broadly similar between the currently reviewed behavioural studies and those in Kredlow et al. (2016), as well as between the current pharmacological studies and those in Lonergan et al. (2013). These broad similarities underscore the potential for applying research findings on experimentally acquired memories to naturalistic memories in clinical disorders. However, they also highlight the need for further research to identify common sources of heterogeneity in ESs in reconsolidation research.

As noted above, a single influential study (Soeter and Kindt 2015a) contributed disproportionately to the medium ES of pharmacological studies summarised here and removal of this study substantially reduced the ES. However, given that this study produced the most pronounced, prolonged (one year), and generalised (across behavioural and subjective-evaluative indices of fear memory) effects, it is worth considering the study features that might have contributed to these particularly large and durable effects. It is noteworthy, for example, that Soeter and Kindt (2015a) used post-retrieval propranolol as a pharmacological interference strategy and was also the only pharmacological study on specific phobia rather than PTSD. The use of propranolol by these authors was based on their multiple previous demonstrations of reconsolidation impairing effects of propranolol on experimentally-acquired memories (conditioned fear; Kindt et al. 2009; Sevenster et al. 2012; 2013; 2014; Soeter and Kindt 2010; 2011; 2012a; b; 2015a, but see Bos et al. 2014; Schroyens et al. 2017). The use of *post-retrieval* propranolol may be particularly relevant to the large effects seen in this study. While specific NMDAR receptor subunits have repeatedly been shown to be involved in destabilisation (see below), the role of β-adrenergic signalling in this first phase of reconsolidation has been unclear. However, there is some recent indirect support for the idea that adrenoceptors might also be involved in destabilisation and that their premature blockade might therefore constrain reconsolidation-interference effects (Lim et al., 2018). If this is true, post-retrieval propranolol administration might be the optimal strategy for reconsolidation-interference-based therapies.

While fear conditioning reconsolidation studies that have used propranolol have demonstrated that relatively brief (<10 s) retrievals involving an unreinforced presentation of a CS appear sufficient to reactivate conditioned fear memories, Soeter and Kindt (2015a) used a longer retrieval duration (2 min), as apparently required for reactivation of chronologically remote memories (Suzuki et al. 2004a). This was substantially longer than the retrievals used in the other (behavioural) phobia studies reviewed here (Bjorkstrand et al., 2016; Maples-Keller et al., 2017; Shiban et al., 2015; Telch et al., 2017). The retrieval procedure used in Soeter and Kindt (2015a) also involved a PE (participants expected that they would touch the spider during retrieval, but in fact, this did not occur). Finally, the retrieval procedure involved *in vivo* exposure to a spider (cf. Bjorkstrand et al., 2016; Maples-Keller et al., 2017; Shiban et al., 2015), which, as a biological 'prepared' stimulus, might be considered to possess qualities of a US. Some researchers (Liu et al., 2014) have suggested that use of USs at retrieval results in a more generalised destabilisation of relevant associations (between all CSs and the US). This might also explain the more generalised effects on fear responding observed in Soeter and Kindt (2015a). Close replication is required to establish that this combination of factors (i.e. use of post-retrieval propranolol; medium duration retrieval and/or use of cues with US properties and/or incorporating a relevant prediction error at retrieval) reliably produces large and durable reconsolidation effects on naturalistic fear memories. Thereafter, studies might seek to determine if this combination is *required*.

The basic behavioural pharmacology of other neurotransmitter/neuromodulator systems in reconsolidation is less well developed relative to the noradrenergic system. Although a role for the glucocorticoid stress system in the disruption of memory has been implicated in psychological disorders (de Quervain, Schwabe, & Roozendaal, 2017), few basic behavioural studies have been conducted on the role of this system in reconsolidation in humans. Moreover, endogenous and exogenous corticosteroids have a variety of distinct and opposing effects on memory (e.g. impairment of retrieval versus enhancement of (re)consolidation), depending upon, for example, timing of the glucocorticoid surge relative to retrieval, the number (or duration) of CS exposure at retrieval (e.g. Cai, Blundell, Han, Greene, & Powell, 2006) and background levels of arousal (see Meir Drexler & Wolf, 2017). These varied glucocorticoid effects might explain the conflicting results reported in existing human and non-human animal studies of glucocorticoid modulation of reconsolidation (de Quervain et al., 2017). For

instance, the two Wood et al studies (2015 studies 2 and 3) showed no statistical effect of mifepristone, a glucocorticoid receptor antagonist (although effects were in the predicted direction). In Wood et al (2015, study 3), the authors additionally attempted to augment the impairing effects of mifepristone through pre-treatment with the NMDAR (glycine site) partial agonist, D-cycloserine (DCS). This strategy might be particularly relevant as prior stress can induce long-term allostatic processes (Espejo, Ortiz, Martijena, & Molina, 2016) which result in an enduring down-regulation of the NMDAR (NR2B) subunits required for memory destabilisation (Ben Mamou et al., 2006; Wang et al., 2009). However, as is evident from *Figure 2.2.A*, and *Figure 2.3*, DCS did not appear to affect mifepristone's ability to interfere with reconsolidation of trauma memory in this case.

The limited effects of mifepristone might also be attributable to specific procedural factors in the two studies described in Wood et al. (2015). For example, the use of individualised scripts likely introduced variability in retrieval duration across participants. Further, prolonged 'script preparation' procedures at retrieval (writing about two traumatic experiences from them same or different events and recalling subjective, visceral and muscular reactions associated with these experiences) might have engaged extinction rather than reconsolidation processes. Alternatively, *relatively* prolonged (intermediate) retrieval durations can also engage a so-called 'limbo state' (Merlo et al., 2014), in which neither extinction nor reconsolidation is engaged. These limitations in the extant research on drugs that downregulate glucocorticoid receptor activity do not allow firm conclusions to be drawn about their application as reconsolidation-interfering treatments at this stage.

Unlike the role of the noradrenergic and glucocorticoid systems, little is known about the effects of manipulating the mTOR pathway on any aspect of memory functioning in humans. While rapamycin blocks fear-related memory reconsolidation in rodents (e.g. Blundell et al., 2008) this capacity has not yet been established in experimental studies of emotional learning and memory in humans. This makes it difficult to interpret the limited efficacy of rapamycin reported in Suris et al. (2013). In addition to the long retrieval duration (up to 75 min) used in this study, the pharmacokinetic profile of rapamycin (i.e. its central bioavailability after a single 15 mg oral dose) relative to the timing of destabilisation (assuming this actually occurred) may not have been optimal.

In contrast to the ES estimate from pharmacological studies of phobia/trauma, the population ES from studies of post-retrieval behavioural strategies was small (approximately half that obtained from pharmacological studies) and not significantly different from zero. Despite four of the five ESs favouring the Retrieval + Treatment group, the effect was skewed towards a smaller value by the Shiban et al. (2015) study. The ES in that study was based on a spontaneous recovery test performed one day after retrieval-extinction. If there is a 'sleeper effect' on BAT performance, as found for declarative aspects of fear by Soeter and Kindt (2015a; note these authors tested behavioural approach for the first time 11 days after treatment with propranolol), retrieval-dependent effects might not have been evident after such a short interval. However, in contrast to Soeter and Kindt (2015a), Shiban et al. (2015) reported no evidence for enhanced effects of retrieval-extinction on declarative fear after a long follow-up period (6 month). In addition to differences in the retrieval procedures between these two studies of spider phobics, (non-significantly) higher baseline levels of fear, heart rate and skin conductance, as well as lower baseline approach behaviour in the retrieval-extinction group (relative to the control group), might have contributed to limited extinction in the retrieval-extinction group in the Shiban et al. (2015) study.

One notable finding among the behavioural phobia/trauma studies was that described by Telch et al. (2017), who reported a relatively immediate reduction in fear responding (expectancy and peak fear) in the Retrieval + Treatment (extinction) group relative to a Treatment + Retrieval control group. These authors consider a number of explanations for this unexpected early effect (e.g. the occurrence of prediction error or increased noradrenergic activity resulting from the retrieval trial). However, it should also be noted that the interval between retrieval and the first extinction trial was especially long in this study (30 min). While the use of a delay between retrieval and the interference strategy is a common feature of behavioural reconsolidation studies (although the interval is usually only 10 min), the rationale for employing such a delay is unclear and may simply be a carryover from the procedure used in the first studies of retrieval-extinction in rodents and humans (Monfils et al., 2009; Schiller et al., 2009). Indeed, without employing *high cognitive load tasks* between the end of retrieval and start of the interference task (e.g. Chapter 4; Das et al., 2015b; Das et al., 2018b/Chapter 3; Hon et al., 2016), there is the potential for ongoing cognitive engagement/rehearsal following exposure to the reminder cue, possibly initiating extinction. This might be an

alternative explanation for the apparent early retrieval dependent enhancement of extinction reported by Telch et al. (2017).

2.4.3 Substance use studies

Despite the small number of studies of laboratory-based reward-memory reconsolidation in humans (e.g. Xue et al., 2017; Zhao et al., 2011), there is substantial evidence for reconsolidation modulation in rodent models of maladaptive reward/addiction. Meta-analytic findings on appetitive-reward memory in non-human animals suggest that the ES associated with reconsolidation interference using NMDAR antagonism is substantially larger than that for β-adrenergic antagonists (Das et al., 2013). This might explain the relatively modest and short-lived effects of propranolol reported by Saladin et al. (2013). Alternatively, the retrieval procedure in Saladin et al. (2013) was relatively protracted (2 x 10 min of *in vivo* and video cue exposure to cocaine cues), which might have limited plasticity through activation of extinction or generating a limbo state. The other study that used propranolol (Pachas et al., 2015) showed a *negative* ES (the propranolol group showing *higher* levels of craving relative to Retrieval + No Treatment). It should be noted however, that the latter study used a script preparation protocol inspired by the same studies that informed the Wood et al. (2015) retrieval procedures. Again, while the duration of the retrieval procedure was not stated in Pachas et al., (2015), it is likely that it was also prolonged (and likely to vary between participants). As such, the potential for extinction /limbo state processes is again relevant. In addition, since craving was *higher* in the Retrieval + Treatment group, it is possible that (inadvertent) extinction consolidation was *impaired* by propranolol relative to the placebo group (Cahill, Pham, & Setlow, 2000).

Excluding Pachas et al. (2015), the remaining four pharmacological studies (Chapter 4; Das et al., 2015b; Das et al., 2018b/Chapter 3; Saladin et al., 2013) showed no evidence of a combined effect consistent with reconsolidation interference. Based on the larger effects of NMDAR- versus β-adrenergic antagonists on reward memory reconsolidation (Das et al., 2013), Das et al. (2015a) examined the NMDAR antagonist memantine, but found no evidence for an effect consistent with reconsolidation blockade. It is unclear whether the typical therapeutic dose (10 mg) and route of administration (oral) of memantine is suitable for blocking reconsolidation in humans. Memantine has slow absorption kinetics, and relatively low selectivity for the central NR2A NMDAR subunit (Ogden & Traynelis, 2011) implicated in restabilisation (Milton et al., 2013). It is therefore uncertain whether suitable reductions in NMDAR activity were achieved in

the post-retrieval period. Another putative NMDAR antagonist used in this group of studies, nitrous oxide (N_2O; Das et al., 2018b/Chapter 3), also did not show a reconsolidation blocking effect based on the planned statistical analysis. However, when the data was reanalysed to take account of whether participants in the retrieval groups experienced a prediction error at retrieval, significant retrieval-dependent effects of nitrous oxide were evident. Since the latter findings were not based on a pre-specified statistical analysis plan, the ES for this study used in the current meta-analysis was based on the non-significant findings. The final study in this group utilised ketamine, a potent, non-competitive NMDAR antagonist (*Chapter 4*). Significant reductions in measures of cue-alcohol memory strength, and reductions in alcohol consumption were observed at 10-14 days post-intervention. However, at 9 months, no difference between groups was observed, reflected by a negative ES in the current analysis.

In contrast to pharmacological strategies, behavioural methods for interfering with reconsolidation showed more promise in the case of substance use. Indeed, the ES associated with behavioural studies was significantly larger than that of pharmacological studies, although this statistical finding needs to be treated with caution given the small number of studies and lack of precision in the population ES estimate of pharmacological studies. Of the four behavioural substance use studies, two employed post-retrieval cue exposure ('retrieval-extinction'; Germeroth et al. 2017; Xue et al. 2012), one counter-conditioning (Das et al. 2015b), and one, cognitive reappraisal (Hon et al. 2016). All showed positive ESs on measures of craving, and the overall ES was moderate-large, with minimal heterogeneity. It is noteworthy that while the nature of the reactivating cues varied between studies (e.g. drug use video, *in vivo* drug cues, drug pictures or a combination of these) all used the same retrieval duration (5 min), along with a 10 min interval between termination of retrieval and start of the behavioural strategy. As such, these retrieval parameters can be recommended for future substance use reconsolidation studies, at least until optimal parameters are firmly established through studies that parametrically vary retrieval duration.

It is noteworthy that all the substance use studies that used behavioural reconsolidation-interference/updating strategies reported significant effects on more than one outcome. Das et al. (2015b), for instance, reported results consistent with a comprehensive rewriting of affective, attentional and cognitive aspects of alcohol-related memories in heavy drinkers. However, the follow-up period for this study –

along with Hon et al. (2015) was relatively short (1 week), whereas the other two studies tested participants at 1 month (Germeroth et al., 2017) and 6 months (Xue et al., 2012). Three of the studies examined changes in substance use behaviour (Das et al., 2015a; Germeroth et al., 2017; Hon et al., 2016), but only one of these showed significant changes in drug (cigarette) use at the final follow-up (Germeroth et al., 2017). Germeroth et al. (2017), along with the Xue et al. (2012) used a two-session treatment protocol (two Retrieval + Treatment session). As such, despite low levels of heterogeneity in ESs of these behavioural studies, there was considerable methodological variation. It remains to be determined whether counter-conditioning and cognitive reappraisal result in sustained effects on craving (and/or in attentional bias and effective responding to alcohol: Das et al., 2015b; and semantic memory for alcohol; Hon et al., 2016) and whether behavioural effects might also emerge after a longer delay.

Given time constraints and a lack of readily available data, the results from one study (Kaag et al., 2018) were excluded from the final analysis. This study used a behavioural working memory task following retrieval of cue-alcohol memories in a population of heavy drinkers. Participants in the study received the post-retrieval working memory task for three sessions, with a final follow-up measuring alcohol consumption and craving 33 days after the baseline session. At this time point, no effect of the post-retrieval working memory task was detected on craving for alcohol, although a decrease in craving was observed in the group that underwent retrieval *after* the working memory task. As the same PE inclusive retrieval procedure was used in all three sessions, it is likely that the expectancy that they would experience a surprising event prevented the retrieval procedure from reliably inducing PE and associated destabalisation of the memory (as observed in Das et al., 2018b). It is likely that inclusion of this study into the current analysis would have reduced the aggregate effect size for the behavioural treatments of SUD and increased heterogeneity.

2.4.4 Limitations

The effects reported here are based on a relatively small number of studies in each category of disorder (these were further reduced in the treatment-type moderator analyses). In addition, the studies themselves generally had small sample sizes. Moderation was only examined for a small number of covariates in the current analysis. However, assuming detailed methodological reporting in future studies, meta-analysis/regression based on a larger number of studies with greater variability in

retrieval variables might prove to be a particularly effective way of establishing the role of retrieval parameters (e.g. retrieval duration; use of prediction error at retrieval) in successful memory reactivation. The alternative (and preferable) approach would involve parametric variation of these retrieval parameters within individual studies, although this approach would require very large sample sizes due to the number of potential factors and levels of key retrieval variables that would be manipulated.

In contrast to the range of outcomes reported in the reviewed studies, the current analysis focused on a narrow set of pre-determined outcomes, primarily trauma symptoms in studies of trauma-exposed individuals, behavioural approach or subjective fear (based on BAT performance) in phobia studies and craving or substance consumption in the case of substance use studies. We opted to base our ES calculations on these outcomes rather than those reported as statistically significant in publications. However, it is possible that quite different results would be achieved if only the significant results reported by study authors (either singly or as composites of multiple significant outcomes) were used to determine ESs. This is not necessarily a limitation, as our intention was to reduce the potential for bias in cases where multiple outcomes were reported but none was pre-specified as primary.

2.4.5 Recommendations for future research

A strength of the reviewed studies was their tendency to recruit participants in line with the relative gender prevalence of disorders in question. However, it should be noted that recent evidence suggests that men and women may be differentially susceptible to some reconsolidation interfering treatments. In particular, Meir Drexler et al. (2016) showed that whereas men showed retrieval-dependent weakening following hydrocortisone, this effect was absent in women. It is currently unclear whether this finding is specific to glucocorticoid modulation of reconsolidation, rather than reflecting a general insensitivity to reconsolidation interference in women. Indeed, the latter seems highly unlikely given the very large effects seen with propranolol seen in Soeter and Kindt (2015a), whose sample consisted almost exclusively of women (91%). No individual study that we are aware of has yet examined gender moderation in (sub)clinical populations, although this seems a particularly important factor to consider if reconsolidation-interference is to be used clinically. It should be noted provisionally, that our moderation analysis did not suggest an effect of gender.

Among the pharmacological studies, there was no common use of a single reconsolidation-interfering drug. Although propranolol was most commonly studied, this amounted to only two studies in the substance use category and two studies in the phobia/trauma grouping. As such, it remains unclear whether one drug class category might be more effective in preventing restabilisation than others. Despite strong evidence from studies with non-human animals, only three of the reviewed studies examined an NMDAR antagonist. Given the central role of glutamatergic neurotransmission in learning and memory, further research on the effects of NMDAR antagonist effects on reconsolidation in humans seems to be a special priority. On the other hand, clinical studies should be preceded by more basic psychopharmacological studies in order to determine the importance of drug timing (relative to retrieval) and route of administration. This is particularly important given the potential for iatrogenic effects of NMDAR antagonists (i.e. the potential for paradoxical strengthening of maladaptive memories in some contexts; Honsberger, Taylor, & Corlett, 2015).

Despite its reported importance in memory destabilisation, only a minority of the reviewed studies examined the role of prediction error (cf. *Chapter 4*; Das et al., 2015a; Das et al., 2015b; Das et al., 2018b/*Chapter 3*; Hon et al., 2016; Soeter & Kindt, 2015a). As noted previously, if there is a requirement for an optimal learning signal at retrieval, those studies showing beneficial effects of Retrieval + Treatment in the absence of prediction error, might in fact represent the lower bound of efficacy that could be achieved during reconsolidation modulation. As such, tailoring retrieval procedures to maximise PE may bolster the likelihood that reconsolidation can be leveraged for clinical benefit.

Overall, our findings suggest that reconsolidation-interference is worth pursuing as a clinical strategy. However, before proceeding with costly and labour-intensive clinical trials, the multiple sources of uncertainty regarding determinants of efficacy of this approach should be more thoroughly investigated through basic experimental human and animal research to ensure that studies with clinical populations are optimised, and therefore as informative as possible.

Chapter 3.

Predictors of reconsolidation-interference: Interference with reconsolidation via N_2O is prediction-error dependent

3.1 Study 1

3.1.1 Introduction

Substance Use Disorders (SUD) are chronically relapsing disorders characterised by compulsive drug seeking and taking, loss of control, and the experience of a negative emotional state upon withdrawal (Koob & Volkow, 2010). SUDs can be conceptualised as disorders of maladaptive reward memories (MRM), in which environmental cues predictive of drug taking become associated with the rewarding effects of drugs (Hyman, 2005). Subsequent exposure to these cues, even in the absence of the drug, can promote craving and increase risk of relapse (Crombag & Shaham, 2002; Lu, Grimm, Hope, & Shaham, 2004). Given the poor long-term prognosis for SUDs following treatment (Dawson, Goldstein, & Grant, 2007), modification of the associative learning that underpins relapse is required if we are to prevent relapse after periods of successful abstinence or reduction in use.

Following retrieval, consolidated memories can sometimes be 'reactivated', returning to a transient, labile state in which they are susceptible to modification (e.g. Nader et al., 2000; Rodriguez, Horne, & Padilla, 1999; Schafe & LeDoux, 2000) before restabilising into long-term storage via the protein-synthesis dependent process of reconsolidation. Intervening in the 'reconsolidation window' of lability can therefore weaken or update memory traces. For instance, the administration of anisomycin (a protein synthesis inhibitor) following exposure to a fear-conditioned reminder cue disrupts conditioned fear memory (Nader et al., 2000). Reconsolidation has since been demonstrated as a fundamental memory process across species (Nader & Hardt, 2009), dependent on the molecular cascades downstream of the receptor targets of glutamate and noradrenaline neurotransmitters (Ben Mamou et al., 2006; Tronson & Taylor, 2007). Memory reconsolidation is typically demonstrated by reactivating memories prior to the administration of pharmacological agents with downstream protein synthesis inhibiting properties (e.g. Kida et al., 2002; Milekic & Alberini, 2002; Nader et al., 2000), such as N-methyl-D-aspartate receptor (NMDAR) antagonists (e.g. Ben Mamou et al., 2006; Flavell, Lambert, Winters, & Bredy, 2013; Milton, Lee, Butler, Gardner, & Everitt, 2008a; Pedreira et al., 2004; Przybyslawski & Sara, 1997; Sangha, Scheibenstock, & Lukowiak, 2003; Suzuki et al., 2004) and β-adrenoceptor (βAR) antagonists (Milton et al., 2008b).

Reconsolidation is putatively a mechanism for maintaining the relevance of memories through updating or integration of new information into existing (long-term) memory traces (Dudai, 2002; Lee, 2009; Sara, 2000). Thus, while extinction training produces a new memory trace which merely competes with the original memory (producing transient weakening of the memory; Quirk & Mueller, 2008; Suzuki et al., 2004), reconsolidation is thought to engender direct and potentially permanent memory modifications. The blockade of reconsolidation has been demonstrated several times in humans using βAR antagonists (e.g. Brunet et al., 2018; Kindt & Soeter, 2013; Schwabe, Nader, & Pruessner, 2013; Soeter & Kindt, 2011, 2012a, 2012b, 2015a, although see Bos et al., 2014; Pachas et al., 2015; Spring et al., 2015; Tollenaar, Elzinga, Spinhoven, & Everaerd, 2009a; 2009b for failures to replicate), where 40mg dose of Propranolol has consistently been associated with the reduced expression of fear memories when administered with memory reactivation (Lonergan et al., 2013). Despite the requirement for research into the modulation of maladaptive memories, far fewer studies have been conducted on the reconsolidation of appetitive reward memories relative to fear. However, consistent with a role for reconsolidation as a 'general purpose' memory maintenance mechanism, βAR and NMDAR antagonists are similarly associated with the impairment of reward memory reconsolidation in animal models (e.g. Lee & Everitt, 2008; Milton et al., 2008a; Milton et al., 2008b; Milton et al., 2012; Wouda et al., 2010). The clinical application of reconsolidation therefore provides a promising strategy for the treatment of disorders of maladaptive memory, including post-traumatic-stress-disorder (PTSD), phobic disorders, and addiction.

Meta-analysis of the animal literature has demonstrated a superior effect of NMDAR antagonists for disruption of MRM reconsolidation (Das et al., 2013), warranting further exploration of their use as a pharmacological blockade of memory reconsolidation in humans. However, options for tolerable, high-affinity NMDAR antagonists with a favourable pharmacokinetic profile for reconsolidation blockade (rapid onset and maintenance of receptor binding) are limited in humans. Ketamine, a potent non-competitive NMDAR antagonist, can be administered intravenously, allowing for tight control over administration and timings for peak plasma concentrations (relative to reactivation) when compared to orally administered drugs. Strong dissociative and psychotomimetic effects and controlled drug status in the UK limits administration to hospital settings however, restricting its clinical use. The

NMDAR antagonist nitrous oxide (N_2O), on the other hand, has favourable pharmacokinetics (rapid onset and offset; Marin, Hupbach, Maheu, Nader, & Lupien, 2011; Mennerick et al., 1998; Nagele, Metz, & Crowder, 2004; Richardson & Shelton, 2015), and an excellent tolerability and safety profile. As a gas, it does not require venepuncture and avoids inconsistency in achieved plasma levels due to metabolic variation among individuals, meaning it can easily be administered in a variety of clinical settings. While N_2O also acts on other receptor sites (namely dopaminergic and opioid), the high safety profile and ease of administration of this dissociative anaesthetic makes this a promising drug for use as a reconsolidation blocker.

There are however several 'boundary conditions' within which a memory can be rendered labile, and destabilisation does not occur each time a memory is retrieved. Indeed, older and more strongly encoded memories appear resistant to destabilisation (Milekic & Alberini, 2002) and require longer (Suzuki et al., 2004) or delayed (Wang et al., 2009) retrieval procedures. Given the proposed role of reconsolidation in updating memories, it is suggested that destabilisation occurs only when there is new, relevant information to be learnt (Finnie & Nader, 2012). As such, a mismatch between what is expected and what actually occurs (prediction error; PE) at retrieval is thought to signal that there is something new to be learnt, leading to destabilisation and allowing updating of the original memory trace. Since learning accrues by minimising PE, incidental PE during retrieval of well-learned memories (such as those underpinning relapse) is likely to be low and specific procedures may be required to artificially engender this PE.

In the current study, N_2O was administered following memory retrieval that incorporated a PE-generating procedure in a group of hazardous drinkers. It was predicted that following destabilisation, N_2O would block the reconsolidation of maladaptive reward memories, demonstrated by a reduction in alcohol consumption, craving, and attentional bias towards alcohol-related cues only in the group that underwent prior retrieval with PE.

3.1.2 Methods

3.1.2.1 Participants

Sixty beer-preferring hazardous drinkers (37 male), defined as consuming ≥3 alcoholic drinks on ≥3 days a week, with ≥30 units total/week for women, and ≥4 drinks, ≥3 days a week, with ≥40 units total/week for men, were recruited via convenience sampling and online advertising. Note: 'drinks' subsequently refers to typical UK measures of alcohol (1 drink or 'unit'=8g of pure alcohol). Beer consumption was measured in pints (568ml, equivalent to 18.18g alcohol), wine in glasses (175ml, 16.8g alcohol), and spirits as a single 25ml measures (8g alcohol). Additional inclusion criteria included: consumption of >5 pints of beer/week; a score ≥8 on the Alcohol Use Disorders Identification Test (AUDIT; Saunders, Aasland, Babor, de la Fuente, & Grant, 1993); normal, or corrected to normal colour vision; age >18<65: and fluent English. Exclusion criteria were: endorsing >3 items coded as "3" on the structured interview for alcohol (SCID) of the DSM-IV (Spitzer, Williams, Gibbon, & First, 1992b); current mental health problems (e.g. requiring treatment); current major physical illness; breathing difficulties; vitamin B12 deficiency (due to effects of N_2O on B12 metabolism); current pregnancy or breastfeeding; history of drug or alcohol abuse disorder; and recreational use of ketamine or N_2O (>2 time per month). All participants gave written informed consent prior the commencement of the study. Participants were reimbursed £35, with an additional £5 incentive to complete a one-month follow up. All procedures were approved by the University College London research ethics committee and were in accordance with the declaration of Helsinki.

3.1.2.2 Study Design

In a single-blind randomised controlled design, participants were randomly assigned to one of three groups: memory retrieval with PE (Ret-PE, N=21), retrieval without PE (Ret-NoPE, N=19), and no retrieval with PE (NoRet-PE, N=20). Unequal groups were the result of a technical error, which resulted in the deployment of the wrong task condition to a single participant. All participants received N_2O. Procedures were conducted over the course of three days (baseline; retrieval and drug administration; test) with measures repeated within-subjects, and memory retrieval procedures differing between groups.

3.1.2.3 Apparatus and Tasks

3.1.2.3.1 Self-report assessments

Alcohol related measures

Alcohol consumption in the two weeks prior to the baseline session (i.e. 'Day 1') and for the one week before the test session (7-10 days after Day 1) was assessed using the calendar-based Time Line Follow Back (TLFB; Sobell & Sobell, 1992). This requires participants to indicate the number of 'drinks' of beer, wine, and spirits they consumed on each day over the required period (i.e. 14 days before baseline and the 7-10 days between baseline and test). An infographic was provided to participants to orient them to alcohol quantities in typical drinks.

Alcohol cue-elicited liking and urge ratings were obtained using a standardised cue-reactivity task, in which participants provided ratings for 10 alcohol and non-alcohol images and an in vivo cue of 250ml glass of beer visible for the duration of the task. To maintain central eye fixation during cue rating, all participants were instructed to verbally rate the images presented on a computer screen. The image cues were presented in a pseudorandom order, and comprised of: seven beer images, four of which were also used during the retrieval procedure (nominated 'Beer Retrieval' cues); three presented during cue-rating at baseline and test only ('Beer Non-Retrieval' cues); three wine images to assess between category generalisation ('Wine' cues); four orange juice images ('OJ' cues) used in the retrieval procedure for the NoRet-PE group (in which cue-alcohol retrieval did not occur); and two soft drink images ('Soft Drink' cues), used in both the cue-alcohol and control retrieval procedures. For each image participants were firstly asked "how pleasant do you find the image from 0 (extremely unpleasant) to 10 (extremely pleasant)" and secondly, "How does the image affect your desire to drink the drink in front of you from 0 (greatly decreases) to 10 (greatly increases)". Each image was presented for a minimum of 10 seconds after which the 250ml glass of beer itself was rated. Tonic craving was assessed using the Alcohol Craving Questionnaire-Now (ACQ-NOW; Singleton, 1994), which generates subscales describing overall craving (General), urges and desires to drink in anticipation of the benefits of drinking (Compulsivity), urges and desires associated with intent to drink (Purposefulness), and urges and desires to drink in anticipation of relief from withdrawal or negative affect (Emotionality).

Hazardous and harmful patterns of alcohol use were identified using the Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993). Drinking concern and desire to change drinking behaviour were assessed by the Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES; Miller & Tonigan, 1996), which yields three subscales representing stage of behaviour change; Recognition, Ambivalence, and Steps. Participant's subjective response to alcohol was measured using the Comprehensive Effects of Alcohol Questionnaire (CEOA; Fromme, Stroot, & Kaplan, 1993) which yields six subscales: Sexuality, Tension reduction, Liquid courage, Cognitive and behavioural impairment, Risk and aggression, Self-perception, and Sociability.

Heritability of alcoholism was measured using a brief assessment of family history, (Family History of Alcoholism, FMA; Mann, Sobell, Sobell, & Pavan, 1985) in which participants indicated if any first and secondary degree relatives (maternal and paternal) had experienced alcohol-related problems. Participants were classified as FH^+ if either biological parent was identified having alcohol-related problems and FH^- otherwise. High levels of agreement between brief and full-scale measures of family history suggest such brief measures perform almost as well as their comprehensive counterparts (Prescott et al., 2005). Further, we have previously demonstrated that the current measure predicts subjective response to N_2O (Walsh, Das, & Kamboj, 2017).

A comprehensive history of personal alcohol use across the lifetime was measured using a Lifetime Drinking History Interview (LTDHI) devised for the current study (see *Appendix item 2*). The interview was researcher administered and identified drinking patterns across four periods of life: (i) period of first alcohol consumption; (ii) first period of regular alcohol consumption; (iii) period of heaviest use; and (iv) current drinking. Any significant periods of abstinence were also given. For each period participants identified the age at which the period began and ended, and the participant answered questions regarding: (1) estimated days per week alcohol was consumed; (2) average, minimum, and maximum number of drinks consumed per day; (3) average time spent drinking; (4) number of binge episodes per month (≥6 drinks in a single sitting); (5) average, minimum, and maximum number of drinks in a typical binge episode; (6) types of alcohol consumed (percentage consumed as wine, beer, and spirits); drinking speed assessed by (7) whether drinks were sipped or gulped; (8) estimated number of sips per drink; (9) estimated time to drink a glass of beer, wine,

and spirits; and (9) whether alcohol was typically consumed with or without other people (defined as a percentage of total drinking occasions).

Mood-related measures

Baseline anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) and the State-Trait Anxiety Inventory for adults (STAI; Spielberger, 1970). The Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988) measured drug-induced changes in affect.

Emotional distress tolerance was measured using the Distress Tolerance Questionnaire (DTS; Simons & Gaher, 2005), and reward responsivity on three activation subscales (Drive, Fun, and Reward) and one Inhibition subscale, assessed using the Behavioural Activation Scale (BIS/BAS; Carver & White, 1994).

Drug induced changes

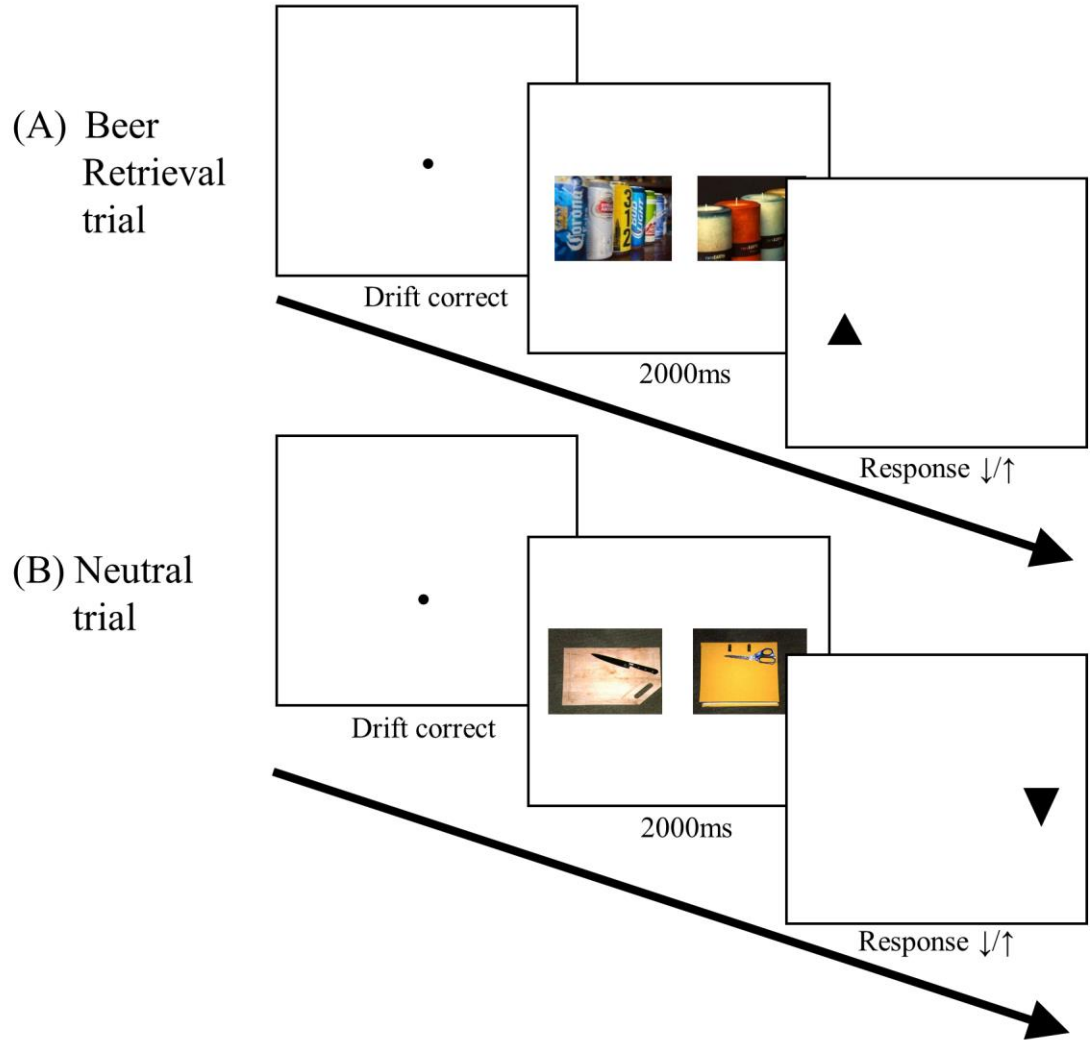
Subjective response to N_2O was assessed using the visual analogue scale-based Bodily Symptoms Scale (BSS; Bond & Lader, 1974) and Clinician-Administered Dissociative States Scale (CADSS; Bremner et al., 1998).

3.1.2.4 Behavioural assessment

The motivational salience of alcohol cues was assessed using an attentional bias measure (via tracking eye movements during a visual probe task*; Figure 3.1*). Composition matched colour images for this task were rendered at 300x300 pixels and sourced from an online database. Ten image pairs were presented to the participant for 2000ms, following which a triangular probe was revealed 'behind' one image. Participants were instructed to indicate whether this triangle probe was pointing up or down using the up and down keys on a keyboard as quickly and accurately as possible. Image pairs included an alcohol-containing ('Target') and a matched non-alcohol ('Non-Target') image presented simultaneously to the participant. Target images consisted of two Beer Non-Retrieval, four Beer Retrieval, two Wine, and two Neutral (non-alcohol related control) images. Non-Target cues were an additional set of neutral, non-alcohol images composition matched to the Target alcohol images. All image pairs were presented eight times, with order of presentation randomised and target image location (left or right), probe orientation (up or down), and probe location (behind Target or Non-Target image) balanced across the task. Eye movements were recorded using a desktop-mounted Eyelink 1000 eye tracker (SR Research, Ontario,

Canada) at a sampling rate of 1000Hz. Head position was stabilised using a chin rest 70cm from a 1024x768 monitor. The visual probe task yielded three measures of attentional bias: dwell time (total fixation time across image presentation), first fixation latency (the time elapsed prior to the first fixation), and first fixation duration. While reaction time (speed of response to probe orientation) was recorded, this is not a reliable measure of attentional bias when cues are presented for longer than 100ms (Cooper & Langton, 2006) and as such, this data was not analysed.

Figure 3.1. *Schematic of the visual probe task*



Following drift correction, participants were presented with an image pair consisting of a Target and Non-Target image. (A) represents a Beer Retrieval cue, paired with a composition matched non-alcohol neutral image. (B) represents a non-alcohol Neutral cue, with a non-alcohol neutral composition matched image. Following presentation of the image pair, a triangle probe is revealed 'behind' one of the images. Participants were required to indicate whether the triangle probe was pointing upwards or downwards as quickly and accurately as possible.

3.1.2.5 Memory Retrieval

Naturalistic cue-elicited alcohol memories were retrieved using a short form of the cue-reactivity task described above, followed by a negative prediction error. Participants were told they would consume the 250 ml in vivo drink in front of them (beer for Ret-PE and Ret-NoPE groups, orange juice for NoRet-PE group), according to a set of on-screen prompts, once the cue-reactivity task was complete.

Once the instructions had been understood, the short cue-reactivity task began. Those in the Ret-PE and Ret-NoPE groups rated four colour beer images ('Beer Retrieval' cues) presented in a pseudorandom order on a -5 (very unpleasant) to +5 scale (very pleasant; 0=neither pleasant nor unpleasant). The NoRet-PE group rated four images of orange juice ('OJ' cues) after a 250ml glass of orange juice was placed in front of them. All participants rated two further soft drink images ('Soft Drink' cues), to assess for generalisation of effects. Images were presented for a minimum of 10 seconds, with stimuli offset occurring once ratings were made.

Following image presentation participants viewed the on-screen drinking prompts. Prediction error in the Ret-PE and NoRet-PE groups was generated by instructing participants to "Pick up the drink", to "Prepare to drink", then, unexpectedly, to "STOP! Do not drink" and then "Put the drink down". The drink was then removed from sight (this procedure is outlined in detail in Das et al., 2018a). Participants in the Ret-NoPE group viewed "Pick up the drink", "Prepare to drink", and "Drink now", following which they consumed the beer as expected, replicating cue-reward contingency in a prototypical drinking episode. Each on-screen prompt was displayed for 3000ms. Participants were then asked to rate how surprising they found this procedure on a scale of 0 to 10, with 0 being completely unexpected, and 10 being completely expected. The value of the surprise ratings was reversed for analysis purposes (10=completely unexpected).

3.1.2.6 Distractor tasks

To ensure the retrieval task (which could potentially engender extinction if prolonged) was time-limited with a discrete offset, a series of distractor tasks lasting a total of 10 minutes were administered immediately following PE generation. These included forward and backward digit span, verbal fluency for alcohol words, category fluency for fruit, and trial-making A to B tasks.

3.1.2.7 Verification of recent abstinence

Blood alcohol concentration was measured using Lion Alcolmeter S D2 breathalyser. All participants were required to give a breath alcohol reading of 0.00 ng/dl prior to the commencement of the session.

3.1.2.8 Drug administration

A gaseous mixture of 50% nitrous and 50% oxygen (Entonox; British Oxygen Company, UK) was administered for a total of 30 minutes (10-minute equilibrium and habituation, 20-minute maintenance). Inhalation was via a demand valve (BOC, UK), which ensured N_2O is delivered only during inhalation and prevented re-inhalation of expired CO_2 /N_2O.

3.1.2.9 Procedure

The current study took place over the course of three sessions, with the memory retrieval procedure occurring during the second session. A schematic of the full procedure is presented in *Figure 3.2*.

Day 1 (baseline)

After providing written informed consent, all participants were breathalysed upon arrival. If estimated blood alcohol level (from breath alcohol) was 0.00 ng/dl (all participants gave this reading), basic information, including age, gender, weight, height, smoking status and education were obtained. Further assessments were carried out in the following order FHA, CEOA, TLFB (past two weeks), SOCRATES, BIS/BAS, DTS, STAI, PANAS, HADS, and the participant was interviewed using the LTDHI. Participants then completed the visual probe and cue-reactivity tasks. Upon leaving participants were reminded not to drink in the 12 hours prior to the drug administration session.

Day 2 (retrieval and drug administration; Day 1+48 hours)

Participants returned to the laboratory where they were again required to produce a breathalyser reading of 0.00 ng/dl before proceeding. The memory retrieval/no retrieval procedures were then administered depending on group. Distractor tasks were administered immediately following retrieval/no retrieval followed by baseline HADS, BSS, ACQ, and CADSS questionnaires.

N_2O administration began after a five-minute baseline period. 'On-gas' questionnaires (HADS, BSS, ACQ, and CADSS) were completed 10 minutes into drug

administration allowing for habitation to drug effects and the equilibrium of N_2O blood concentration. Inhalation continued for a further 20 minutes (total of 30 minutes). A two-minute re-equilibration period occurred following the commencement of drug inhalation, after which post-gas questionnaires were administered. Participants were invited to remain in the laboratory for a further 30 minutes to ensure cessation of all drug effects.

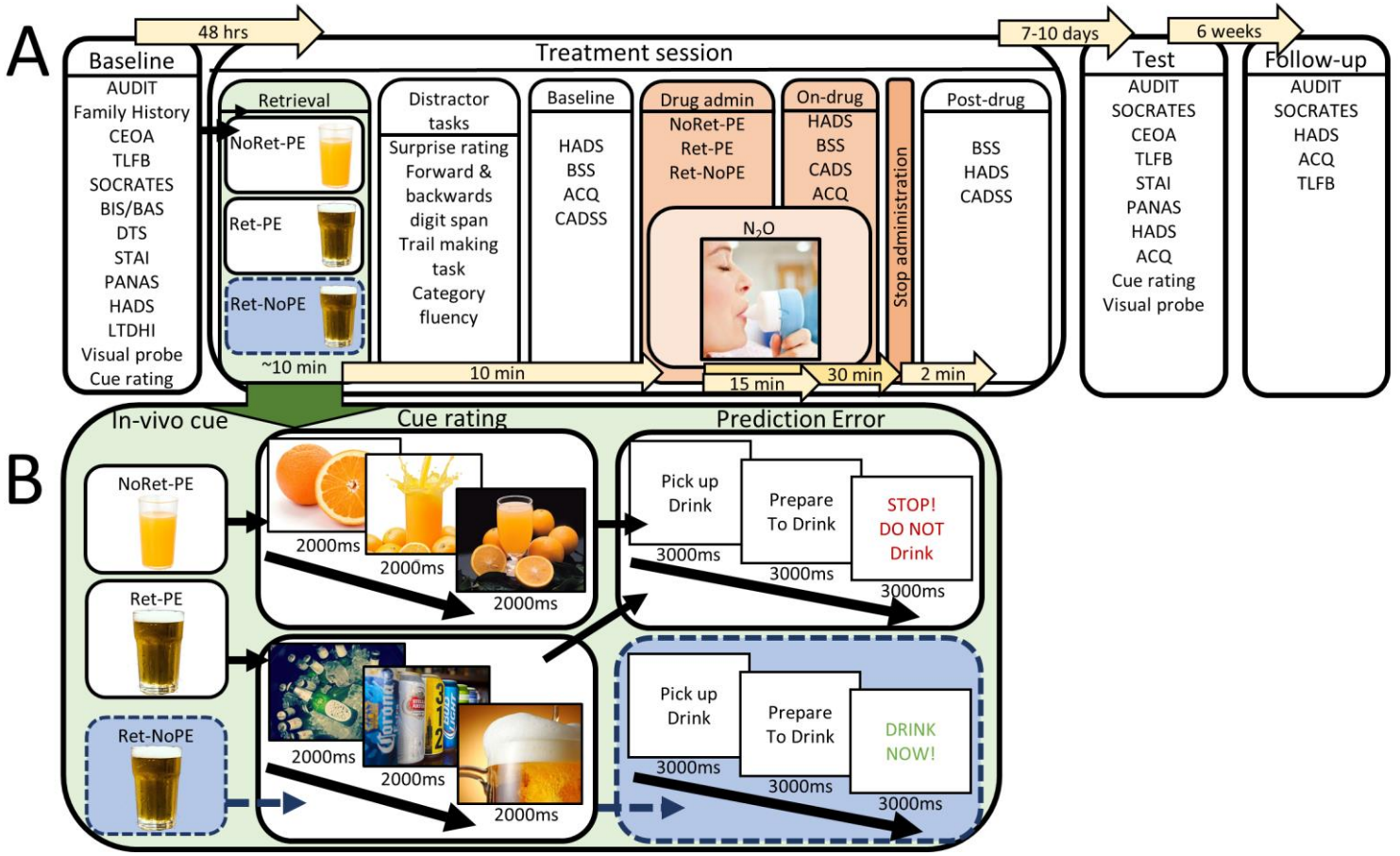
Day 3 (test; Day 1 + 7-10 days)

The assessments and order of assessment for the test session were identical to the baseline session. Participants were subsequently debriefed and compensated.

Follow-up (Day 3 + 6 weeks)

Participants completed the AUDIT, SOCRATES, HADS, ACQ, and a TLFB for the previous week over the phone.

Figure 3.2. *Schematic of the three-day protocol (A) and retrieval and drug administration procedure (B).*



During the retrieval procedure (B) participants rated four beer (if assigned to the Ret-PE and Ret-No PE groups) or four orange juice images (NoRet-PE), and two further soft drink images (all groups). Prediction error was then generated in the Ret-PE and NoRet-PE groups, after which all groups inhaled N_2O for a total of 30 minutes.

3.1.2.10 Statistical approach

All data were analysed in IBM Statistical Package for the Social Sciences (SPSS) v.25 for Windows. All data were checked for normality, homogeneity of variance and sphericity where appropriate. Non-normal data were transformed if skewed and Greenhouse-Geisser corrections were applied to *df* and *p* values where Sphericity was violated. Where Greenhouse-Geisser procedures estimated epsilon as <0.75, Wilks' lambda (λ) is reported. Welch's F test is reported where homogeneity of variance was violated in one-way ANOVA. Outliers more than 3 standard deviations away from the sample mean were replaced with a score at 3 standard deviations from the mean.

Eye tracking data were extracted and processed using SR Research's Dataviewer program. Trials with a latency to first fixation <100ms were removed as these trials reflect instances where subjects were looking at the cue location prior to cue presentation.

One-way ANOVA is reported for group differences on baseline variables. Owing to the comparison of multiple measures (> 20) a more conservative α=0.01 was selected for baseline comparisons. For all other analyses, the threshold for statistical significance was α=0.05. Mixed ANOVAs with Group (Ret-PE, Ret-NoPE, NoRet-PE*)* as a between subjects factor and Day (baseline, test) as a within-subjects factor, were used for the primary outcomes of alcohol consumption and craving. Additional within-subjects factors of cue type (Beer Retrieval, Beer Non-Retrieval, OJ, Soft Drink*)* were included for data taken from the cue-reactivity task, and Cue type (Beer Retrieval, Neutral, Beer Non-Retrieval, Wine) and Target (Target, Non-Target) were included for dwell time, first fixation duration, and first fixation latency data generated by the visual probe task. Bonferroni-corrected pairwise comparisons on marginal means were conducted where significant k>2 main effects and interactions were observed.

3.1.3 Results

3.1.3.1 Baseline alcohol and questionnaire data

Baseline demographics and measures of alcohol consumption, attitudes to alcohol, craving, mood, readiness to change, and behavioural inhibition/activation data are presented in *Table 3.1*. No significant differences between groups were observed at baseline (all $p>0.01$). Welch's ANOVA was used to compare baseline STAI and daily spirit consumption due to heterogeneity between groups within these variables.

Table 3.1. *Baseline demographic, drinking behaviour and questionnaire measures*

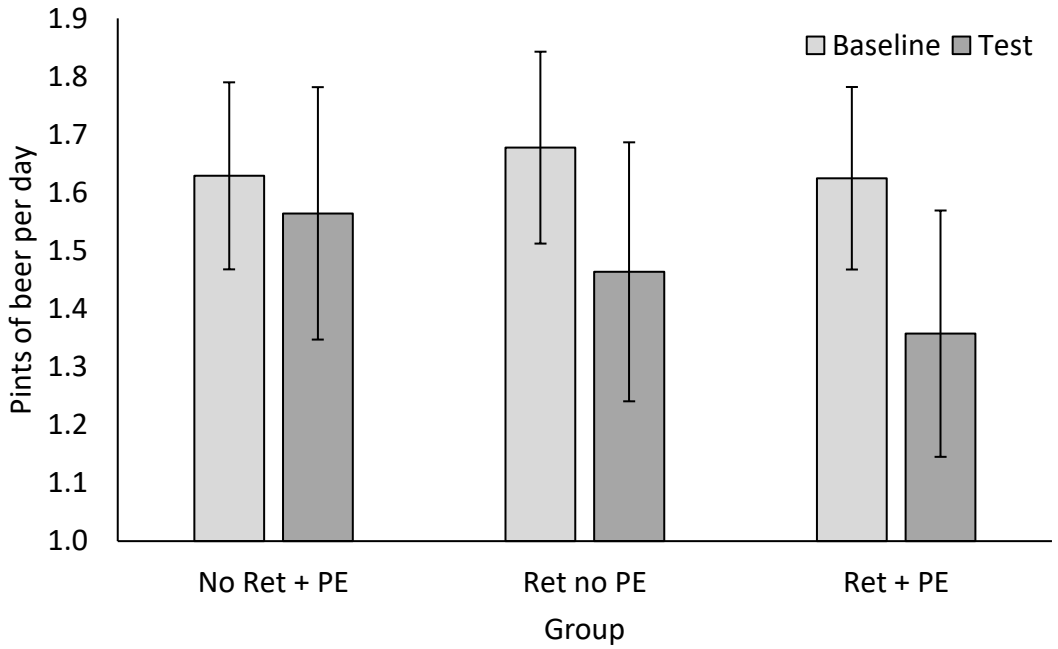
	NoRet-PE N=20	Ret-PE N=21	Ret-NoPE N=19	$F_{2,57}$	p	η^2
Gender (N of males)	15	13	10	0.603	0.550	0.02
Age	28.2±9.48	26.29±8.21	24.21±7.29	1.103	0.339	0.04
AUDIT	15.60±4.95	14.67±3.69	13.79±5.3	0.731	0.486	0.03
HADS *Depression*	2.96±2.48	2.81±2.48	2.42±2.36	0.244	0.785	0.01
HADS *Anxiety*	5.86±2.90	6.52±3.84	6.37±3.70	0.205	0.816	0.01
ACQ *General*	2.91±0.64	2.57±0.63	2.77±0.70	1.373	0.262	0.05
ACQ *Compulsivity*	1.13±0.88	0.80±0.88	0.95±0.80	0.919	0.405	0.03
ACQ *Expect*	2.73±0.92	2.14±0.78	2.41±1.29	1.725	0.187	0.06
ACQ *Purposefulness*	5.43±0.91	5.44±0.72	5.65±0.85	0.453	0.638	0.02
ACQ *Emotionality*	2.35±1.01	1.86±1.21	2.06±1.25	0.916	0.406	0.03
BAS *Drive*	24.85±5.12	22.24±5.84	26.11±5.043	2.735	0.073	0.09
BAS *Fun*	7.40±2.52	6.71±1.74	6.58±1.68	0.945	0.395	0.03
BAS *Reward*	12.30±3.54	12.19±3.01	12.42±2.97	0.026	0.974	0.00
BAS *BIS*	9.00±3.03	8.48±4.86	8.16±2.930	0.252	0.778	0.00
PANAS *Positive*	34.65±6.84	35.42±4.82	34.42±5.18	0.176	0.839	0.00
PANAS *Negative*	17.45±5.21	18.33±6.81	18.89±7.41	0.244	0.785	0.00
STAI $_W$	55.00±4.63	52.26±8.16	53.67±4.42	0.905	0.414	0.04
DTS	38.80±13.68	38.76±11.97	39.21±15.86	0.006	0.994	0.00
Daily Pints Beer	1.63±0.77	1.62±0.74	1.68±0.64	0.032	0.968	0.00
Daily Glasses Wine	0.49±0.69	0.69±0.58	0.83±0.90	1.050	0.357	0.04
Daily Spirits (25ml) $_W$	1.34±1.30	0.49±0.49	1.10±1.38	4.906	0.014	0.10
SOCRATES *Recognition*	18.15±5.83	16.45±4.25	16.05±5.88	0.850	0.433	0.03
SOCRATES *Ambivalence*	7.75±4.33	7.25±3.65	6.48±3.186	0.568	0.57	0.02
SOCRATES *Steps*	15.10±5.26	14.50±5.36	14.89±5.32	0.066	0.936	0.00
CEOA *Sociability*	23.70±5.08	23.85±4.51	26.74±5.45	2.266	0.113	0.07
CEOA *Tension reduction*	6.70±1.98	6.62±1.50	6.74±1.94	0.022	0.978	0.00
CEOA *Liquid courage*	12.00±2.92	11.86±2.82	12.32±3.46	0.115	0.891	0.00
CEOA *Sexuality*	8.10±2.51	7.71±2.67	8.53±2.80	0.465	0.63	0.02
CEOA *Impairment*	19.85±5.12	19.29±4.94	20.37±4.10	0.259	0.773	0.01
CEOA *Risk and aggression*	10.75±2.90	10.00±3.71	11.11±3.05	0.607	0.548	0.02
CEOA *Self-perception*	5.90±2.25	6.81±2.77	5.58±1.71	1.560	0.219	0.05

Values mean±SD. All tests were one-way ANOVA unless indicated with $_W$, denoting the use of Welch's ANOVA.

3.1.3.2 Changes in drinking behaviour

Mixed ANOVA with Group as a between-subjects factor and Day (baseline; test) as a within-subjects factor did not find any difference in mean daily beer consumption between baseline and test (main effect of Day: $F_{1,57}$=2.843, p=0.097, η_p^2=0.048) nor between groups (Day x Group interaction: $F_{2,57}$=0.321, p=0.726, η_p^2=0.011; *Figure 3.3*). Nonetheless, in absolute terms, those in the Ret-PE group reduced their beer consumption by 0.27 pints per day (1.88 pints per week), relative to 0.21 and 0.07 pints per day (1.50 and 0.46 pints per week respectively) in the Ret-NoPE and NoRet-PE groups.

Figure 3.3. *Mean daily beer consumption at baseline (two weeks preceding baseline session) and test (week preceding manipulation)*



Consumption did not significantly differ as a result of the manipulation (all p>0.05). Bars represent mean±SEM.

No interaction (Day x Group) was observed for Wine ($F_{2,57}$=0.011, p=0.370, η_p^2=0.034), nor a main effect of Day ($F_{1,57}$=1.107, p=0.297, η_p^2=0.019) or Group ($F_{2,57}$=1.799, p=0.175, η_p^2=0.059). Similarly, no interaction (Day x Group; $F_{2,57}$=2.023, p=0.142, η_p^2=0.066), nor a main effect of Day ($F_{1,57}$ =1.107, p =0.297, η_p^2=0.180) or Group ($F_{2,57}$ =2.433, p=0.097, η_p^2=0.471) was observed for Spirit consumption.

Total craving as assessed by the ACQ did not differ across Group or Day (Day x Group interaction: $F_{2,57}$=0.197, p=0.659, η_p^2=0.003). No main effects or interactions

were observed for any of the subscales of the ACQ, although a trend level increase in the subscale measuring the urges and desires associated with intention to drink was shown (main effect of Day: $F_{1,57}$=3.280, p=0.075, η_p^2=0.054).

3.1.3.3 Reactivity to alcohol cues

No significant interactions were observed for cue liking on the cue-reactivity task (Day x Group: $F_{2,57}$=2.985, p=0.086, η_p^2=0.082; Day x Cue x Group: $F_{6,176}$=0.771, p=0.598, η_p^2=0.026), although a large significant main effect of Cue was shown ($F_{4,54}$=50.743, p<0.001, λ=0.790). Pairwise comparisons revealed the main effect of Cue was driven by greater liking for OJ images relative to all other Cue types (all *ps*<0.010), greater liking of Soft Drink images relative to Beer Non-Retrieval and Wine images ($p<0.001$), and greater liking of Beer Retrieval images relative to Beer Non-Retrieval and Wine images (p<0.001).

Mixed ANOVA on urge to drink on the cue-reactivity task showed a significant main effect of Cue ($F_{4,54}$=35.679, p<0.001, λ=0.725) and a significant interaction between Day and Cue ($F_{4,54}$=3.025, p=0.025, λ=0.183). Bonferroni-corrected pairwise comparisons showed greater urge ratings for Beer Retrieval cues, relative to all other cues (all *ps*<0.001), and greater urge ratings for Beer Non-Retrieval relative to Wine, Soft Drink, and OJ cues (all *ps*≤0.001). Wine cues were rated significantly higher on urge than Soft Drink cues (p=0.049) and OJ and Soft Drink cues received equivalent urge ratings (p=1). The Day x Cue interaction was driven by a significant *decrease* in urge for Soft Drink cues from baseline to test only (p=0.006).

3.1.3.4 Motivational salience of cues

A mixed ANOVA on dwell time found a significant interaction between Target (Target; Non-Target neutral pair) and Cue (Beer Retrieval, Beer Non-Retrieval, OJ, Soft Drink; $F_{3,55}$=3.967, p=0.014, λ=0.175), driven by a significantly greater dwell times for Target Neutral images, relative to their Non-Target neutral pair (p=0.018). For Target images, participants spent a greater amount of time looking at Neutral cues relative to all other cues (all *ps*<0.05). A trend-level interaction between Day, Target, Cue, and Group ($F_{6,163}$=2.074, p=0.062, η_p^2=0.068) and a trend main effect of Cue ($F_{3,55}$=2.797, p=0.051, η_p^2=0.05) were observed.

A main effect of Cue ($F_{3,55}$=8.774, p<0.001, η_p^2=0.133), Target ($F_{1,57}$=11.183, p=0.001, η_p^2=0.164) and a Target x Cue ($F_{2,171}$=5.372, p=0.004, η_p^2=0.086) interaction was shown for first fixation latency in the visual dot probe task. Pairwise comparisons suggested

time to first fixation to Neutral cues was more rapid than those to Beer Retrieval, Beer Non-Retrieval, and Wine cues (all *ps*<0.05). Moreover, time to first fixation was faster to Target Beer Retrieval and Beer Non-Retrieval cues relative to their Non-Target composition-matched non-alcohol pair.

Mixed ANOVA on first fixation duration showed a significant main effect of Cue ($F_{3,171}$=2.938, p=0.035, η_p^2=0.049), and a Target x Cue ($F_{3,171}$=3.621, p=0.014, η_p^2=0.060), and a Day x Target x Cue interaction ($F_{2,142}$=3.527, p=0.023, η_p^2=0.058). The main effect of cue was driven by a trend to longer first fixations on Beer Non-Retrieval relative to Wine cues (p=0.056). The interaction between Target and Cue was driven by longer first fixations on Target Beer Non-Retrieval cues, relative to Target Beer Retrieval (p=0.036) and Target Wine cues (p=0.006), while the Day x Target x Cue interaction was driven by greater first fixation durations for Target Beer Non-Retrieval cues relative to Wine cues (p=0.006) at baseline.

3.1.4 Discussion

Study 1 failed to show an effect of post-retrieval N_2O on any measure of cue-alcohol memory strength. Whether N_2O failed to block reconsolidation, or if memories failed to destabilise initially, is unknown. Further exploration of the mechanisms by which null results occurred is therefore warranted. We conducted a series of exploratory analyses in *Study 2*, with the aim of elucidating the reasons why no such effect was observed and to gain an understanding of the interaction between potential predictors of reconsolidation.

3.2 Study 2

3.2.1 Introduction

Despite some promising findings using pharmacological and behavioural reconsolidation-interference procedures (see *Chapter 2*), the field is marked by inconsistent findings and frequent null results. For example, *Study 1* failed to show the predicted effect of N_2O on the reconsolidation of cue-alcohol memories in hazardous drinkers. This finding highlights a common interpretational issue of null findings in reconsolidation studies. Specifically, it is not clear whether the null finding was the result of N_2O failing to block memory reconsolidation (e.g. because it is an insufficiently effective NMDAR antagonist) or if certain 'boundary conditions' prevented memories from destabilising in the first place. As previously discussed, the destabilisation of older memories is contingent on the inclusion of surprising, relevant information at retrieval (e.g. Alfei et al., 2015; Exton-McGuinness et al., 2015; Sevenster et al., 2012, 2013, 2014). While the study outlined in *Study 1* intended to induce PE at retrieval by violating expectations of the availability of alcohol, at debrief some participants reported an expectation that "something was going to happen", potentially limiting the extent of intended surprise upon alcohol being withheld. That is, PE and subsequent destabilisation would only be proportional to the extent that participants *expected to actually drink* the beer given to them, questioning the efficacy of the manipulation.

Given these potential explanations for the null findings in *Study 1* (i.e. potentially insufficient NMDAR blockade and/or insufficient destabilisation), further analysis of the results was warranted. In particular, this study aimed to determine whether indices of (i) putative alcohol memory strength, (ii) sensitivity to N_2O's NMDAR antagonistic effects, and (iii) the degree of *experienced* PE (as an index of the degree of successful destabilisation) were examined as potential moderators of N_2O's reconsolidation blocking effect.

As noted above, it is possible the naturalistic maladaptive alcohol memories targeted in the current study were simply too old or strongly encoded to be reliably destabilised by the retrieval plus PE procedure. While naturalistic maladaptive reward memories have been successfully destabilised by a PE-generating procedure previously (e.g. Das et al., 2015b; Hon et al., 2016), without knowledge of the strength and age of the targeted maladaptive reward memories it was not possible *a priori* to know if the

current PE procedure was sufficient to destabilise alcohol memories in all participants (e.g. because these were simply too strong or old to be destabilised). The current study therefore re-analysed the data presented in *Study 1* using scores from the Lifetime Drinking History Interview (LTDHI; an index of alcohol memory strength) as a moderator. The LTDHI was intended to provide an approximation of the number of cue-alcohol exposures across the lifetime, and therefore a proxy for learning history. To the extent that memory age and strength constrains memory reconsolidation, scores on this measure might be expected to moderate the effect of retrieval condition on indices of memory strength (e.g. drinking behaviour) following N_2O treatment.

It is possible that dysregulated NMDAR function also influences the extent of N_2O's ability to block NMDARs. Chronic ethanol consumption can result in alterations in the glutamatergic system (Vengeliene, Bilbao, Molander, & Spanagel, 2008). Given the NMDAR's central role within reward pathways, such glutamatergic adaptations might alter sensitivity to NMDAR antagonists, as reflected in participants' subjective response to these drugs (Krystal et al., 2003b). For example, a greater ratio of reinforcing /stimulant effects to dysphoric/sedative effects, in response to NMDAR antagonists is seen in alcohol-dependent patients and in non-dependent individuals with a family history of alcohol use disorder (e.g. Walsh et al., 2017; Yoon, Pittman, Limoncelli, Krystal, & Petrakis, 2016). Inherited dysregulation of the NMDAR system may therefore increase the risk of an individual engaging in heavy drinking, driven by these greater reinforcing effects, tolerance to alcohol and a decreased sensitivity to the unpleasant responses to alcohol which typically trigger the cessation of alcohol consumption (Krystal et al., 2003a). Importantly, both the destabilisation and restabilisation phases of reconsolidation are dependent upon NMDAR signalling, meaning reconsolidation processes may specifically be limited in these individuals and this may directly contribute to the maintenance of addictive behaviour (Tronson & Taylor, 2013). Given that the current study aimed to block the reconsolidation of maladaptive reward memories with the NMDAR antagonist N_2O, it is feasible that responses to the manipulation could be moderated in those with a family history of alcohol use disorder, driven by an underlying dysfunction in the NMDAR system.

Alternatively, while it is hypothesised that N_2O's mechanism of action in the current study is via NMDAR-mediated blockade of reconsolidation, changes in behaviour may occur as a result of memory *updating*. Under this interpretation, N_2O's subjective psychological and interoceptive effects might be *integrated* into the labile memory,

subsequently rendering the accessibility of the memory *state dependent*. The eventual effect of this on memory expression (i.e. behaviour) would depend on the state of the individual during N_2O 'intoxication' (e.g. see Gisquet-Verrier et al., 2015; Gisquet-Verrier & Riccio, 2018). For instance, alcohol seeking behaviour can be reduced by pairing disgusting unconditioned stimuli (extremely bitter tastes; disgusting images) with alcohol cues during the reconsolidation window (Das et al., 2015b). We have demonstrated that family history of alcohol-use problems (representing a genetic vulnerability to alcohol addiction) is associated with an altered subjective response to N_2O (Walsh et al., 2017). Specifically, those with a family history of alcohol-use problems experience an increased stimulant, and a blunted sedative response to N_2O, relative to those with no such history. That is, the extent to which N_2O is experienced as reinforcing vs. dysphoric could positively reinforce or negatively counter-condition the labile memory, bidirectionally moderating the valence of memory updating and subsequent impact on behaviour. If changes in alcohol seeking behaviour are a result of memory updating, rather than NMDAR blockade, changes in measures of alcohol memory following N_2O might be moderated by an individual's subjective response to N_2O. If this is the case, those who experience greater euphoric effects may integrate the heightened positive subjective effects of N_2O into the existing memory trace, potentially *increasing* alcohol seeking, relative to those who experience greater dysphoric effects.

Given the frequent failures to demonstrate robust reconsolidation effects in the literature in humans, especially through use of drug treatments intended to reduce protein synthesis dependent restabilisation of reward memories (Walsh et al, 2018/*Chapter 2*), it is essential to explore potential moderating factors that might explain inconsistent or unexpected findings. Owing to a failure of the current manipulation to observe an effect of post-retrieval N_2O on measures of cue-alcohol strength, a detailed understanding of the interaction between important variables was undertaken using a moderation analysis.

It was therefore predicted that putative measures of alcohol memory strength (drinking behaviour, craving, and attentional bias to alcohol related cues), would reduce only when N_2O was administered after a retrieval and prediction error procedure which *successfully* induced surprise. It was further predicted that these changes would be moderated by measures of memory age and strength, subjective experience of side effects, and family history of alcohol-related problem. Specifically,

greater reductions in drinking behaviour would be observed in those with younger, weaker memories; in those who experienced more sedative effects, relative to stimulant side effects in response to N_2O; and in those without a family history of alcohol use problems.

3.2.2 Methods

Owing to the use of the same dataset as *Study 1*, the methods of the current study were identical to the first.

3.2.2.1 Measures of memory strength and age

A crude measure of memory age and strength was obtained from the LTDHI. Years of alcohol consumption as identified by the LTDHI was highly correlated with participant age (r^2=0.967), likely reflecting a lack of variability between participants concerning the age at which they first began to consume alcohol and a lack of periods of abstinence. Memory strength was thus calculated as the approximate number of cue-alcohol reinforcement pairings across the lifetime, calculated using the following equation:

$$Total\ lifetime\ drinks = \frac{(\sum Ia\ IIa\ IIIa\ IVa)\ \cdot (Z - \chi - B)}{\sum Ib\ IIb\ IIIb\ IVb)}$$

X=age of first drink
Z=current age
B=years of abstinence
Ia=approximate number of drinks consumed in period I (first alcohol consumption)
IIa=approximate number of drinks consumed in period II (regular alcohol consumption)
IIIa=approximate number of drinks consumed in period III (heaviest use)
IVa=approximate number of drinks consumed in period IV (current use)
Ib=years spent in period I (first alcohol consumption)
IIb=years spent in period II (regular alcohol consumption)
IIIb=years spent in period III (heaviest use)
IVb=years spent in period IV (current use)

An example illustrates the calculation of an individual's estimated total lifetime alcohol consumption: a participant who regularly consumed five drinks, once a week when they first started drinking (given as between the ages of 16 and 18), and 3 drinks a day, 4 times a week up until their current age (between the age of 19 and 25), with no periods of abstinence, had a calculated number of 4888 cue-alcohol exposures (number of drinks consumed) across the lifetime.

3.2.2.2 Statistical approach

To assess whether the retrieval and PE procedure reliably induced surprise, a one-way ANOVA on surprise was conducted with Group (Ret-PE, Ret-NoPE, NoRet-PE) as a between-subject's factor. In this study, participants were (re)allocated to groups according to the degree to which they *experienced* surprise. Those who indicated a ≥5

surprise rating were allocated to the new Ret-PE grouping, while those who gave a <5 were assigned to the new Ret-NoPE group. GLM mixed ANOVA was used to maintain consistency and allow for comparisons across *Study 1* and *2*. To assess whether level of PE (surprise) interacted with the retrieval procedure performed, a Group x Surprise interaction term was then added to models.

Where GLM repeated measures ANOVA observed a significant interaction between Day and Group (based on the new allocation), potential moderators of this relationship were explored. Number of cue-alcohol exposures (Total Lifetime Drinks on the LDHI), and family history of drinking problems were selected as potential moderators (M) of the relationship between Group (Ret-PE, Ret-NoPE, NoRet-PE) and beer consumption change (beer consumption at baseline–test; Y). Group was recoded into dummy variables (indicator coded; *Table 3.2*) due to independence between groups (Hayes & Montoya, 2017). In this coding, X_1 signifies the difference in beer consumption change (baseline–test) between the recoded Ret-PE group and the mean of the Ret-NoPE and NoRet-PE groups. X_2 signifies the difference between the NoRet-PE group and the mean of the Ret-PE and Ret-NoPE groups.

Table 3.2. *Indicator coding for moderation analysis with group as multi-categorical predictor variable*

Group \ Interaction term	X_1	X_2
Ret-PE	1	0
NoRet-PE	0	1
Ret-NoPE	0	0

A hierarchical regression was conducted independently for each moderator variable, with the moderator variable (M), predictor (Group; X_1, X_2) and covariate (Surprise) entered in to Step 1. The interaction terms (X_1*M; X_2*M) were added into Step 2. Post-hoc analysis and plots were constructed using the PROCESS macro (Hayes, 2018) and simple slopes represent the 25th (low), 50th (median), and 75th (high) percentiles (selected due to positive skew).

3.2.3 Results

3.2.3.1 Manipulation check

One-way ANOVA suggested surprise differed significantly between groups ($F_{2.57}$=4.455, p=0.016, η^2=0.135). As expected, those in the NoRet-PE group experienced significantly greater surprise during the retrieval procedure (M=7.7, SD=2.18) than those in the Ret-NoPE (M=5.26, SD=3.28; p=0.014) group. However, those in the Ret-PE group did not differ significantly in surprise ratings when compared to the NoRet-PE (p=0.902) or Ret-NoPE (p=0.169) groups, suggesting the manipulation did not reliably induce surprise as intended in the primary group of interest. To explore if *experienced* surprise was predictive of change in alcohol seeking behaviour, participants in the Ret-PE and Ret-NoPE groups were reallocated using participant's self-report post-retrieval surprise ratings. Specifically, those in the Ret-NoPE who reported surprise as >5 were reallocated to the Ret-PE group (N=29), and those in the Ret-PE group scoring <5 to the Ret-NoPE group (N=11).

3.2.3.2 Baseline alcohol and questionnaire data

Following reallocation, groups did not differ significantly on any baseline demographic, measures of drinking behaviour, or any potential moderators of drinking behaviour (*Table 3.3;* all p>0.01). Heterogeneity between groups was observed for the ACQ comprehensive, ACQ general, BAS/BIS, and spirit consumption. Welch's F is therefore reported for these variables.

Table 3.3. *Baseline characteristics of groups assigned according to experienced prediction error.*

	NoRet-PE N=20	Ret-PE N=29	Ret-NoPE N=11	$F_{2,57}$	*p*	η^2
Gender (N of males)	14	17	6	0.452	0.638	0.02
Age	28.20±9.48	25.31±7.73	25.27±8.19	0.789	0.459	0.03
AUDIT	15.60±4.95	14.62±4.76	13.27±3.72	0.892	0.416	0.03
HADS *Depression*	2.95±2.48	2.79±2.13	2.18±3.09	0.369	0.693	0.01
HADS *Anxiety*	5.85±2.90	6.76±3.80	5.64±3.59	0.611	0.546	0.02
ACQ *General*$_W$	2.91±0.64	2.69±0.76	2.60±0.31	1.534	0.229	0.03
ACQ *Compulsivity*$_W$	1.13±0.88	0.96±0.80	0.95±0.23	3.870	0.030	0.05
ACQ *Expect*	2.73±0.92	2.21±1.86	2.43±0.56	1.544	0.222	0.05
ACQ *Purposefulness*	5.43±0.91	5.41±0.87	5.85±0.36	1.248	0.295	0.04
ACQ *Emotionality*	2.35±1.01	2.16±1.25	1.42±0.99	2.530	0.089	0.08
BAS *Drive*	24.85±5.12	25.28±5.27	20.91±5.74	2.790	0.070	0.09
BAS *Fun*	7.40±2.52	6.90±1.45	6.00±2.15	1.758	0.182	0.06
BAS *Reward*	12.30±3.54	12.69±2.70	12.27±3.47	0.811	0.449	0.03
BAS/BIS$_W$	9.00±3.03	8.03±2.63	9.09±6.52	0.712	0.502	0.02
PANAS *Positive*	34.65±6.84	34.31±4.72	36.64±5.39	0.701	0.701	0.02
PANAS *Negative*	17.45±5.21	19.17±6.67	17.09±7.99	0.620	0.620	0.02
STAI	55.00±4.63	55.04±8.16	50.00±7.04	2.507	2.507	0.09
DTS	38.80±13.68	41.01±13.81	33.27±12.49	1.345	1.345	0.05
Daily Pints Beer	1.63±0.77	1.59±0.68	1.81±0.71	0.370	1.370	0.01
Daily Glasses Wine	0.49±0.69	0.65±0.56	1.00±1.09	1.776	1.776	0.06
Daily Spirits (25ml)$_W$	1.34±1.30	0.58±0.88	1.29±1.31	3.265	0.056	0.10
SOCRATES *Recognition*	18.15±5.83	16.79±4.43	14.91±6.39	1.328	0.273	0.05
SOCRATES *Ambivalence*	7.75±4.33	7.36±3.51	5.64±2.94	1.214	0.305	0.04
SOCRATES *Steps*	15.10±5.26	15.32±5.23	13.09±5.28	0.753	0.476	0.03
CEOA *Sociability*	23.70±5.08	25.72±4.96	23.91±5.56	1.098	0.341	0.04
CEOA *Tension*	6.70±1.98	6.76±1.68	6.45±1.81	0.114	0.892	0.00
CEOA *Courage*	12.00±2.92	12.69±2.79	10.45±3.45	2.280	0.112	0.07
CEOA *Sexuality*	8.10±2.51	8.38±2.80	7.36±2.80	0.584	0.561	0.02
CEOA *Impairment*	19.85±5.12	20.24±4.64	18.64±4.25	0.458	0.635	0.02
CEOA *Risk Aggression*	10.75±2.90	10.83±3.20	9.73±4.00	0.486	0.618	0.02
CEOA *Self Perception*	5.90±2.25	6.45±2.56	5.64±1.80	0.610	0.547	0.02
Total *Lifetime Drinks*	13.71±12.17	8.5± 83.24	10.55±11.66	1.476	0.237	0.05
Family History	0.45±0.51	0.31±0.47	0.45±0.52	0.614	0.545	0.02

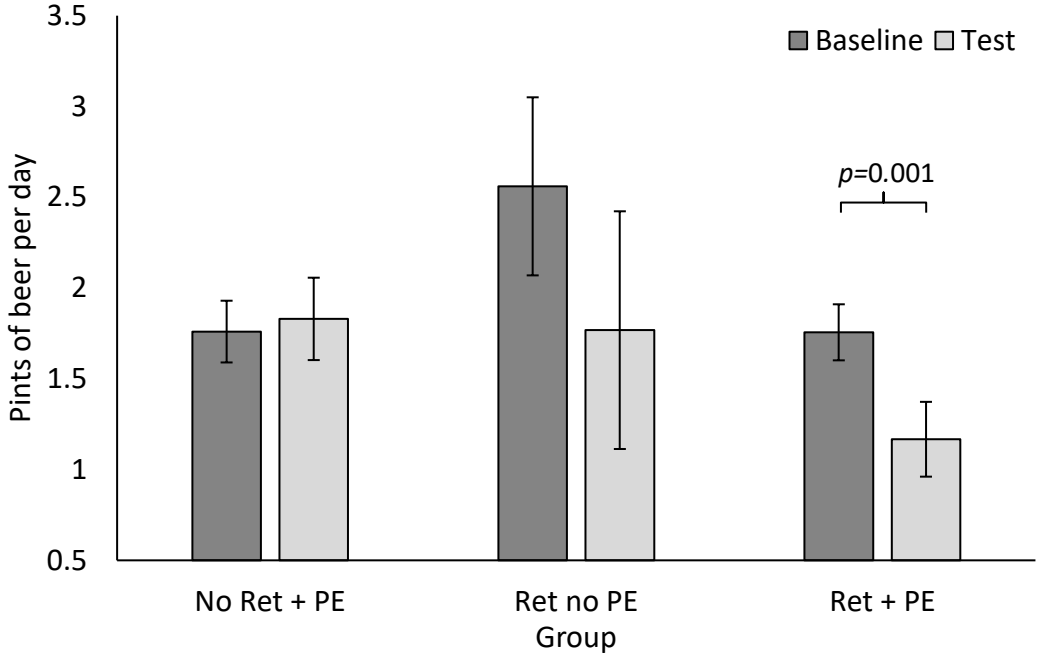
Note: Values mean±SD. All tests were one-way ANOVA unless indicated with '$_W$*' denoting the use of Welch's ANOVA.*

3.2.3.3 Changes in drinking behaviour

Mixed ANOVA using the reassigned Group factor (as a between-subjects factor) and Day (baseline; test) as a within-subjects factor on beer consumption, with surprise added as a covariate, revealed significant interactions between Day and Group ($F_{2,54}$=4.489, *p*=0.010, η_p^2=0.156) and between Day, Group and Surprise ($F_{2,54}$=4.737, *p=0.*013, η_p^2=0.149). Under the Day x Group interaction, the multivariate simple effect

of Day was significant only in the Ret-PE group ($F_{1,54}$=11.315, p=0.001, η_p^2=0.173), with a mean reduction of 0.6 pints/day, or 4.2 pints/week observed from baseline to test (*Figure 3.5*). While non-significant ($F_{1,54}$=2.029, p=0.160, η_p^2=0.036), an absolute reduction of 0.79 pints (5.53 pints a week) was observed in the Ret no PE group.

Figure 3.4. *Mean beer consumption at baseline (pre-treatment) and at test (1-week post treatment) for groups assigned using experienced prediction error*



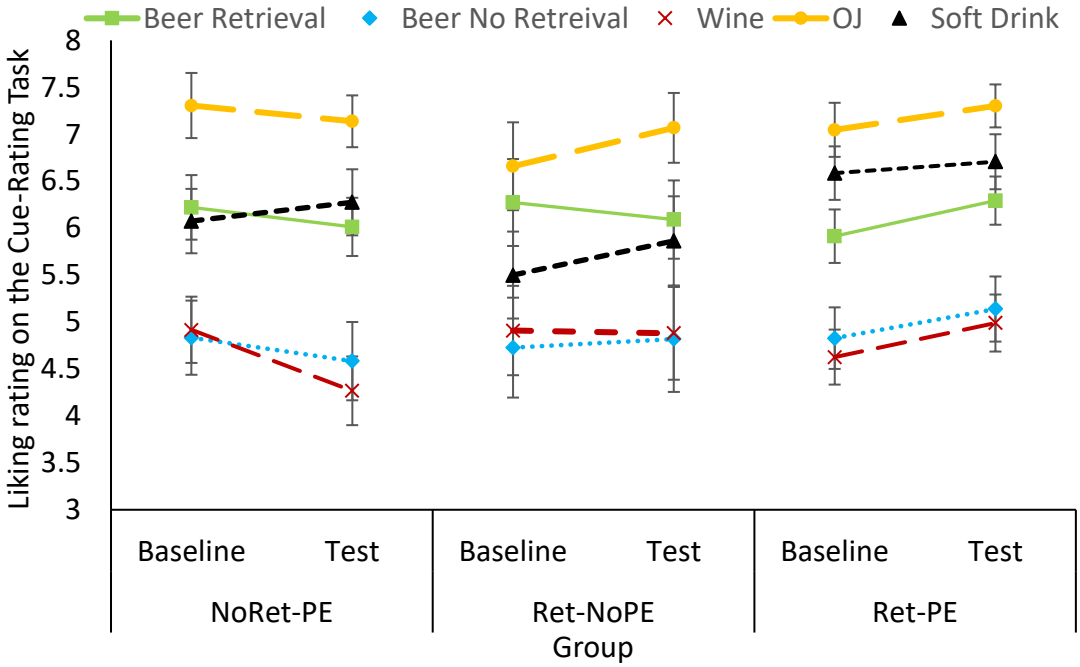
Note: Bars represent mean±SEM.

The same analysis for wine consumption showed a significant Day x Group x Surprise interaction ($F_{2,54}$=3.517, p=0.037, η_p^2 =0.115), driven by a significant *increase* in wine consumption from baseline to test in the Ret-PE ($F_{1,54}$=5.868, p=0.019, η_p^2=0.098) and Ret-NoPE groups ($F_{1,54}$=4.553, p=0.037, η_p^2=0.078). No significant main effects or interactions were found for Spirit consumption. Change in beer consumption and wine consumption (baseline-test) were weakly negatively correlated across groups (r=-0.113 p=0.558) and within the Ret-PE Group (r=-0.236, p=0.070). Momentary craving, as assessed by the ACQ-NOW did not differ across Day or Group.

3.2.3.4 Reactivity to alcohol cues

A significant main effect of Cue ($F_{4,51}$=3.573, p=0.012, λ=0.219), and significant interaction between Day x Cue x Group ($F_{6,3}$=2.5536, p=0.012, η_p^2 =0.095), and Day x Cue x Group x Surprise ($F_{6,162}$=2.208, p=0.027, η_p^2=0.083) were observed when a mixed ANOVA was conducted on cue liking ratings generated by the cue-reactivity task. Beer Retrieval images were rated significantly higher for liking than Beer Non-Retrieval, and Wine cues (p<0.001), Soft Drink images were rated significantly higher than Wine cues (p=0.020), and OJ images were rated significantly higher than Beer Non-Retrieval, Wine, and Soft Drink cues (p≤0.001). Under the interaction, the simple multivariate effect of Day suggested liking for Soft Drink images increased from baseline to test in the NoRet-PE group only ($F_{1.54}$=3.556, p=0.041, η_p^2=0.075; *Figure 3.5*). When anlayses were conducted within each group, the Day x Cue ($F_{1,15}$=3.860, p=0.024, η_p^2=0.507) and Day x Cue x Surprise ($F_{1,15}$=3.669, p=0.028, η_p^2=0.495) interactions reached significance in the NoRet-PE group only.

Figure 3.5. *Liking of image cues on the cue-reactivity task at baseline (pre-treatment) and test (1-week post-treatment)*



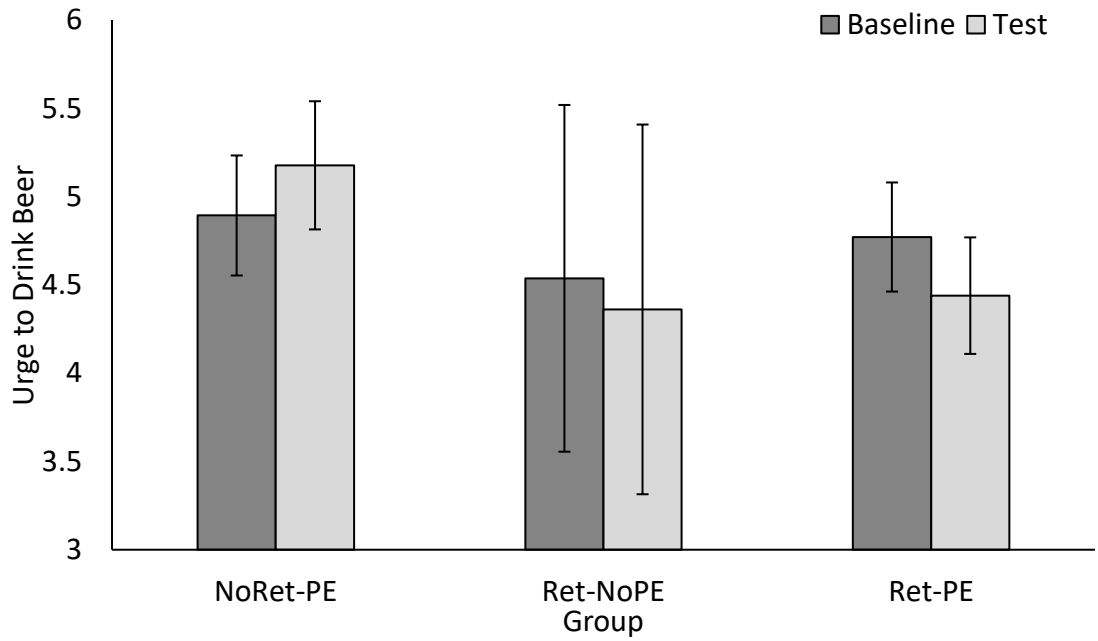
Note: data points represent mean±SEM. Beer Retrieval= beer cues used during retrieval, Beer Non-Retrieval= beer cues used only at baseline and test, Wine=wine cues seen only at baseline and test, OJ=orange juice cues used in the NoRet-PE group during retrieval.

For urge ratings on the cue-reactivity task, a significant Day x Group ($F_{2,54}$=3.752, p=0.030, η_p^2 =0.122) interaction was shown. The simple effect of Group within each Day, and the multivariate simple effect of Day within each group, each failed to reach significance (all p>0.05; *Figure 3.6*). However, simple inspection of the plots suggested

change in urge to drink beer followed a similar pattern to beer consumption, with reductions seen in the Ret-NoPE and Ret-PE groups, and an increase in the NoRet-PE group. Exploratory correlations showed change in urge to drink beer in response to beer cue images was significantly related to change in beer consumption only in the Ret-PE group (Beer Retrieval: r_{29}=0.411, p=0.027; Beer Non-Retrieval: r_{29}=0.39, p=0.036).

Conversely, urge to drink in response to Wine cues did not follow the same pattern as change in wine consumption. Specifically, a non-significant decrease in urge was observed from baseline to test in the Ret-PE group, suggesting urge was unrelated to the observed increase in wine consumption.

Figure 3.6. *Urge to drink 250ml of beer after exposure to cues at baseline (pre-treatment) and test (1-week post-treatment).*



Note: Data points represent mean±SEM. Groups did not differ.

3.2.3.5 Motivational salience of alcohol cues

Mixed ANOVA on dwell time, latency to first fixation, and first fixation duration failed to show an attentional bias towards alcohol cues (as evidenced by a lack of main effect of Target, nor a Cue x Target interaction). Results from the visual probe task will therefore not be analysed here.

3.2.3.6 Predictors of change in beer consumption

To explore the potential mechanisms through which beer seeking behaviour was reduced, bivariate correlations between potential moderators, predictors, and covariates are given in *Table 3.4.* As previously demonstrated (Walsh et al., 2017) on-drug stimulation-to-sedation ratio was significantly related to family history (r=0.320, p=0.013).

Table 3.4. *Means, standard deviations, and bivariate (unless otherwise indicated) correlations between study variables.*

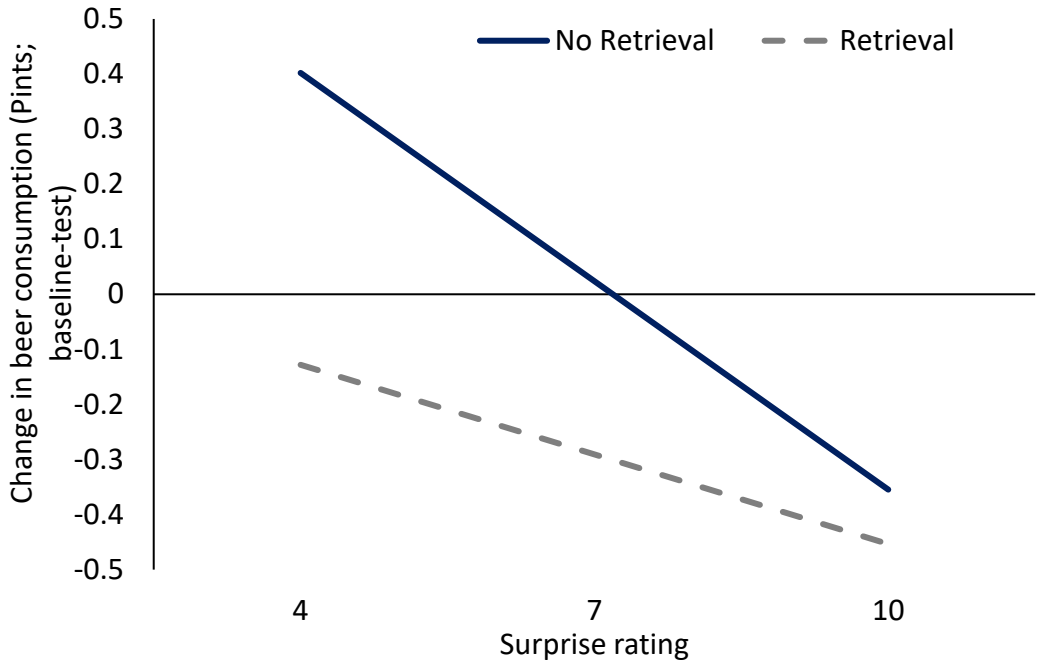
Variable	Beer Drinking Δ	Total Lifetime Drinks	Family History (r^{pb})	Stim-Sed ratio	Surprise
Beer Drinking Δ	1	-0.104	0.189	-0.022	-0.186
Total Lifetime Drinks		1	0.123	0.140	0.016
Family History			1	**0.320***	-0.134
Stim-Sed ratio				1	0.111
M	-0.183	10639.62	0.380	0.215	6.633
SD	0.826	10407.88	0.490	0.819	2.731

*Note: Stim-Sed: stimulation to sedation ratio, Beer Drinking Δ: change in beer consumption (baseline-test), * p<0.05 level (2-tailed), r^{pb} = point biserial correlation coefficient.*

3.2.3.7 Moderating role of surprise, drinking history and family history of drinking problems in the link between group and change in beer consumption

To explore the relationship between surprise and beer consumption change, the Ret-PE and Ret-NoPE groups were combined to form a 'retrieval' group, and a hierarchical multiple regression was conducted to explore the moderating effect of surprise on the relationship between Group (retrieval; no-retrieval) and beer consumption change. In Step 1, Group was not related to beer consumption change (p=0.217), although a trend towards a main effect of surprise was observed (b=-0.070, t=-1.737, p=0.088). In Step 2, addition of the interaction term Surprise*Group did not account for a significant proportion of the variance (ΔR^2=-0.009, $F_{1,56}$=0.542, p=0.465). Inspection of the plots (*Figure 3.7*) suggested that in the retrieval group, high self-reported surprise was associated with a reduction of 0.35 pints a day (3.17 pints per week), relative to 0.13 pints a day (0.90 pints a week) when self-reported surprise was rated as low. Interestingly, a greater discrepancy between high and low levels of surprise was seen in the no-retrieval group, with high self-reported surprise associated with a reduction of 0.35 pints a day (2.48 pints a week), and an increase of 0.40 pints a day (2.81 pints per week) when self-reported surprise was rated as low.

Figure 3.7. *Conditional effect of Surprise (X) on change in beer consumption (baseline-test; Y) following Retrieval or No Retrieval of cue-alcohol memories prior to N_2O inhalation.*



Hierarchical multiple regressions were conducted to test the effect of potential moderators on the relationship between group and beer consumption change (see *Appendix item 5*). Given that participant age and total lifetime drinks were highly correlated (and total lifetime drinks is a function of participant age), only the moderator effects of total lifetime drinks, stimulation-to-sedation ratio, and family history were entered into the regression. For ease of interpretation, total lifetime drinks and stimulation-to-sedation ratio were centred. In Step 1, beer consumption change was not significantly related to any of the potential moderators (M; total lifetime drinks, family history), Group (X_1; X_2), or covariates (surprise). At Step 2, addition of the interaction terms M* X_1 and M* X_2 did not account for a significant portion of the variability in beer consumption change when total lifetime drinks (ΔR^2=-0.096, $F_{2,53}$=0.325, p=0.724), stimulation-to-sedation ratio (ΔR^2=0.001, $F_{2,53}$=0.035, p=0.967), or Family History (ΔR^2=0.005, $F_{2,53}$=0.603, p=0.551) were selected as moderators. As the dummy coding did not allow for simple or main effects of the Ret-NoPE group to be modelled the hierarchical regression was re-run, with X_1 representing NoRet-PE vs Ret-PE and Ret-NoPE, and X_2 Ret-PE vs. NoRet-PE and Ret-NoPE. No significant simple effect or interaction terms were observed for any moderator variables (all p>0.05).

3.2.3.8 Exploratory analyses

To see if family history of alcohol use disorders influenced drinking behaviour at baseline, one-way ANOVA was conducted between those who identified an alcohol-related problem in either biological parent (FH^+) and those who did not (FH^-). Results are presented in *Table 3.5.*

Table 3.5. *Baseline drinking behaviour in those with (FH^+) and without (FH^-) a family history of alcohol problems.*

	FH- N=37	FH+ N=23	$F_{2,58}$	*p*	η_p^2
Gender (% male)	57	70	0.967	0.329	0.016
$AUDIT_W$	13.68±3.49	16.35±5.80	3.989	0.054	.
ACQ Total	32.14±8.65	34.11±6.81	0.862	0.862	0.360
Daily Pints Beer	1.55±0.73	1.80±0.66	1.758	0.190	0.847
Total Lifetime Drinks	9638±10499	12250±10282	0.892	0.349	0.015
Age of first $drink_W$	15.14±1.55	14.43±2.54	1.423	0.242	.

Note: Values mean±SD, ACQ: Alcohol Craving Questionnaire, AUDIT Alcohol Use Disorders Test,

Baseline and on-drug subjective responses to N_2O are presented in *Table 3.6*.

Table 3.6. *Subjective responses to N_2O across all groups at baseline pre-administration) and on-drug (15-minutes post administration onset)*

	Baseline	On-drug
BSS *Depression*	18±2.73	8.32±1.89
BSS *Impairment*	24.65±2.87	22.26±3.31
BSS *Palpitations*	19.00±2.67	23.75±3.72
BSS *Nausea*	11.32±1.87	16.12±3.17
BSS *Emotional numbness*	18.67±2.59	22.68±3.49
BSS *Euphoria*	25.73±38.14	38.14±4.16
BSS *Drowsy*	28.77±2.95	25.842±3.38
BSS *Tension reduction*	27.00±3.38	16.96±3.088
BSS *Headache*	15.60±2.56	10.28±1.85
BSS *Concentration*	24.28±2.82	34.46±3.97
BSS *Trembling*	12.29±2.34	13.24±2.50
BSS *Vertigo*	7.70±1.56	12.67±2.
BSS *Confusion*	11.49±1.86	25.74±3.39
BSS *Anxiety*	28.42±3.38	15.46±3.04
CADSS *Total*	3.58±0.79	19.03±1.72
CADSS *Amnesia*	0.72±1.65	2.13±0.28
CADSS *Depersonalisation*	0.80±0.28	4.78±0.55
CADSS *Derealisation*	2.07±0.44	12.12±1.08

Note: Values mean±SD, BSS: Bodily Symptoms Scale, CADSS: Clinician Administered Dissociative States Scale

3.2.4 Discussion

Study 1 did not show an effect of post-retrieval N_2O on the reconsolidation of cue-alcohol memories. The potential reasons for this null effect were explored in *Study 2*. Importantly, the preliminary analysis of surprise ratings in *Study 2* revealed an apparent failure to reliably induce the intended PE among participants in the Ret PE group, and a tendency to unintentionally induce PE in the Ret no PE group. As a result, reported surprise did not differ between the retrieval groups as intended.

While the results of *Study 1* did not provide support for the inclusion of a PE, it became clear during data collection that participants experienced expectancy violation effects during the 'no PE' procedure. Specifically, during debriefing many participants in RET+ PE were *not truly expecting* to drink and were thus not surprised when the beer was withheld. When participants were reallocated to groups according to *experienced* PE, a statistically significant reduction in beer consumption was observed only when participants experienced retrieval *and* PE prior to N_2O administration, relative to no retrieval or PE. This supports the notion that inclusion of PE at retrieval is necessary to destabilise of memories (Sevenster et al., 2013) and extends this to cue-alcohol memories.

While non-significant, a reduction in beer consumption from baseline to test was additionally observed in the group that had their cue-alcohol memories retrieved but did not experience PE. The small number of participants within the group yielded a high level of variability and consequently, it is not possible to conclude whether participants significantly reduced their beer consumption following retrieval alone. However, the reduction in beer consumption within the no PE group suggests that memory destabilisation may not be an all or nothing process, as has been previously suggested (Das et al., 2015b). When entered into a moderation analysis, with the retrieval groups combined, low levels of self-reported surprise conferred a small reduction in beer consumption (0.90 pints a week), relative to 3.17 pints a week when surprise was rated as high. The linear relationship between surprise and beer consumption change suggests that low levels of PE may trigger suboptimal destabilisation of the original maladaptive memory and that procedures maximising *experienced PE* may be critical to the efficacy of reconsolidation-based interventions.

Moderation analyses suggested that the greatest reduction in beer consumption was seen in those who were "extremely surprised" during the PE procedure. Given that

extinction, rather than destabilisation can occur when the mismatch between the original learning and the retrieval procedure is too discrepant (Osan, Tort, & Amaral, 2011), the linear relationship between surprise and beer consumption change (in which the highest surprise rating was associated with the greatest reduction in beer consumption) suggests the retrieval procedure used here did not promote new learning, despite inducing high levels of surprise. However, as level of mismatch is typically quantified as accumulation of prediction errors across multiple trials rather than magnitude of suprise within a single retrieval event, it is unclear whether single-trial learning (as used here) could produce new learning. Further, the boundaries governing the transition of memory states from simple retrieval (inactive) to destabalisation (active) to extinction are poorly understood, even in rodent models. Indeed, it is likely that the *optimal* length of time, number and type of cue shown to the participant to induce destabilsiation is dynamic and dependent on the individual's learning history. Understanding these factors will be critial to designing effective and reliable reconsoldiation therapies.

Interestingly, surprise was not found to moderate the relationship between retrieval and beer consumption change from baseline to test. A lack of moderation likely occurred due to the positive, linear relationship between change in beer consumption and self-reported surprise in both the retrieval *and* non-retrieval groups. Notably, those who reported low levels of surprise in the group that did *not* experience retrieval increased their consumption by 2.81 pints a week, relative to a decrease of 2.48 pints a week when surprise was high. High levels of PE may therefore produce changes in drinking behaviour independently of retrieval mechanisms, possibly via higher arousal potentiating any non-mnemonic therapeutic effects of N_2O.

Further analysis suggested the reduction in beer consumption seen when participants were administered N_2O following retrieval and PE may be driven by a reduction in urge to drink beer in response to alcohol-related cues. Indeed, self-rated urge to drink beer in response to new and previously seen beer images mirrored actual beer consumption across time and group, with cue-elicited craving (urge) declining in the Ret-PE and Ret-NoPE groups. Importantly, change in cue-elicited urge to drink was correlated with change in beer consumption only in the group that experienced retrieval *with* PE, suggesting that a reduction in beer consumption from baseline to test might be attributable to changes in cue-reactivity in this group. Interestingly, this was not the case for cue liking. This discrepancy between wanting (assessed through urge

ratings) and liking represents the dissociability of hedonic and motivational responding in addiction, and as individual's progress into addiction they report a *decrease* in liking of drugs, parallel to an *increase* in wanting (Anselme & Robinson, 2016; Berridge & Robinson, 2016). It is of note, however, that the higher cue liking ratings seen for orange juice images may reflect participants rating the specific image on elements such as composition and brightness, rather than liking of the depicted drink itself.

Mirroring the reduction in beer consumption observed in the Ret-PE group, an *increase* in wine consumption was observed in the Ret-PE group alone. Moreover, while change in wine consumption from baseline to test did not correlate with beer consumption across groups, a weak negative correlation *was* observed in the Ret-PE group. Those in the Ret-PE may therefore have replaced beer consumption with wine and if this is the case, then it is unlikely the retrieval procedure targeting beer memories produced generalised destabilisation of maladaptive alcohol memories. This is a potentially important consideration for the design of reconsolidation-based treatments for SUD disorders, for which it is essential that effects generalise across types of alcohol. Multiple treatment sessions, wherein each type of alcohol memory is reactivated may therefore be required to produce a universal decrease in alcohol consumption. It is of note that urge scores in response to Wine cues did not reflect an increase in wine consumption, suggesting that wine consumption did not increase because of greater cue-elicited craving.

Reponses to the pharmacological agent through which reconsolidation is blocked may also be influenced by individual differences in neurochemistry (e.g. the number or 'sensitivity' of NMDARs). We have previously demonstrated that a family history of alcohol problems is associated with a blunted dysphoric, and increased stimulant response to NMDAR antagonists (Walsh et al., 2017) and these findings are consistent with literature suggesting that this pattern of response may represent an underlying dysfunction of the NMDAR system (Krystal et al., 2003a; Krystal et al., 2003b; Yoon et al., 2016). Given that N_2O targets the NMDAR system, we explored whether those with a family history of alcohol use problems might have experienced greater (or reduced) reconsolidation blockade relative to those with no such history, reflecting underlying differences in the neuropsychopharmacology of NMDARs. However, beer consumption change did not differ between those with and without family history in the retrieval with PE group. While non-significant, an absolute difference of 3.8 pints a week was observed between those with a positive family history and no family history in the

group that experienced PE *without* retrieval. As all participants in the current study expressed an interest in reducing their intake of alcohol, this may reflect those with a vulnerability to alcohol use problems experiencing greater difficulties in reducing their consumption. Indeed, exploratory analyses in the current sample suggested that those with a positive family history may have more harmful patterns of alcohol use at baseline.

3.2.4.1 Limitations

The failure to reliably induce PE at a group level and the post-hoc allocation of participants to new groups represents the primary limitation of the current study. As a consequence of the reallocation of groups, participants were no longer randomly assigned, and group size differed significantly. This is particularly problematic for the Ret-NoPE group, which had just 11 participants following group reallocation. This likely adversely affected the precision with which the population mean was estimated in this group, as indicated by the large standard errors. Indeed, while beer consumption reduced significantly only in the Ret-PE group, an absolute reduction was observed in the Ret-NoPE group but failed to reach significance due to high levels of variation. It is therefore unknown whether this reduction in consumption would have reached significance had group size been larger, or if this result is spurious. As such, the results of the current study should be viewed as preliminary and exploratory, allowing us to generate hypotheses for future testing and highlighting the caution required when designing retrieval procedures for future studies. Indeed, in most published literature, no manipulation check regarding PE during retrieval is performed and incidental variation in PE may therefore explain the inconsistency in results. Replication using larger sample sizes and a priori criteria for allocating participants to groups would strengthen the preliminary conclusions of the current study.

Similarly, while it was assumed that beer consumption in the retrieval with PE group reduced as a consequence of memory weakening, a lack of biomarkers of destabilisation and reconsolidation blockade precludes any firm conclusions on actual memory weakening. However, if beer consumption reduced due to memory *updating,* it might be expected that those who experienced greater dysphoric (relative to stimulant) effects in response to N_2O would have greater reductions in consumption. In particular, those in the retrieval with PE group who experienced greater euphoric (stimulant) effects might be expected to *increase* their drinking. Since this was not observed, it is unlikely

change in beer consumption occurred as a result of an individual's subjective response to N_2O being integrated into the memory trace.

Despite a lack of moderation by stimulation-to-sedation ratio, it is still *possible* that a reduction in beer consumption occurred as a consequence of memory integration. Indeed, a reduction in beer consumption may represent a failure to retrieve the memory, rather than a weakening of the memory trace (Gisquet-Verrier & Riccio, 2018). Under this interpretation, integration of the subjective effects of N_2O into the original memory trace may have rendered the memory state-dependent. If this is the case, then the original cue-alcohol memory is still available but not accessible. As we did not re-assess memory strength under N_2O intoxication at test, we are not able to rule out a state dependent explanation as the mechanism through which beer consumption was reduced.

A lack of change in attentional bias data suggests a change in the motivation salience of alcohol related cues did not drive the observed reduction in beer consumption. However, as we did not observe any differences in response to alcohol-related cues (relative to a neutral, non-alcohol, composition matched pair) at baseline, we are unable to confirm whether any changes in attentional bias towards alcohol cues occurred. This was the case for both *Study 1* and *2*, suggesting the current study failed to capture an attentional bias for alcohol-related cues. Given that attentional bias has been reported for heavy drinkers (Field, Mogg, Zetteler, & Bradley, 2004), and a reduction in attentional bias has been previously observed following post-retrieval counter-conditioning in a similar population (Das et al., 2015b), the observation that participants did not look at alcohol cues faster, or for longer than Neutral cues may be the result of the specific image set used here. For instance, images of beer taps and a bar mat (as used here) may not accurately reflect a prototypical drinking episode for the current sample.

Finally, despite efforts to obtain follow-up data, too few participants responded to the follow-up for analysis of the data to be conducted, particularly given the already-small sample size in the re-assigned groups. As such, while a reduction in beer drinking was observed seven to ten days later at test, it is not possible to conclude that the current procedure led to long-term suppression of beer consumption. Given the potential of reconsolidation to *permanently* modify maladaptive memories, greater

efforts, perhaps with a greater incentive to complete the follow-up, should be made to obtain long-term data in future.

In sum, the results from the current analysis are mixed, although they do suggest a potential for N_2O to block the reconsolidation of maladaptive reward memories in hazardous drinkers. While initial analyses failed to observe an effect of post-retrieval N_2O on measures of reward memory strength, analysis of effect of N_2O on groups reassigned according the experienced PE revealed a reduction in beer consumption only when N_2O was administered after retrieval *and* PE. This effect does not appear to depend on how long an individual has been drinking for, subjective side-effects to N_2O, nor family history of drinking problems. Due to group reassignment, any conclusions made from the current analysis are speculative, and future studies should aim to replicate the current findings directly. The current analysis also reveals the importance of 1) obtaining an individual's learning history, 2) taking 'manipulation checks' during retrieval to ensure surprise is truly being generated and 3) the identification of procedures that can destabilise older, more robust memories if this treatment is to be used in clinical populations.

Chapter 4.

Post-retrieval ketamine attenuates cue-alcohol memories in a population of hazardous drinkers

4.1 Introduction

Alcohol use disorder (AUD) is associated with vast social, personal, and economic costs (e.g. Hill, Blow, Young, & Singer, 1994; Manning, Best, Faulkner, & Titherington, 2009; Thavorncharoensap, Teerawattananon, Yothasamut, Lertpitakpong, & Chaikledkaew, 2009; WHO, 2011). Patients undergoing current treatments for alcohol and substance use disorders (SUDs) suffer from high relapse rates, even after the physical withdrawal symptoms have passed and allostatic pressures are no longer motivating the individual to consume alcohol (Koob & Volkow, 2010). This is because said treatments do not directly target the long-term *learned* components underlying AUD: maladaptive reward memories (MRM; Milton & Everitt, 2012). Over the course of years and thousands of repeated exposures, associative memories between alcohol cues and the pleasurable, rewarding effects of drinking are formed. Even in the absence of alcohol and long after acute withdrawal, these cues can trigger craving, a significant predictor of relapse (e.g. Stohs, Schneekloth, Geske, Biernacka, & Karpyak, 2019). The weakening and elimination of these MRM is therefore a key target for the treatment of AUD/SUDs

As previously demonstrated in this thesis, under certain circumstances, consolidated memories return to a labile or 'active' state in which they are vulnerable to modification, thus allowing for memory updating and the integration of new and relevant information (Gisquet-Verrier & Riccio, 2018; Przybyslawski & Sara, 1997). Following this process of memory *reactivation,* memories subsequently require *reconsolidation* to return to a stable or 'inactive' state. This reconsolidation process is critically dependent on the N-Methyl D-Aspartate receptor (NMDAR) and the intracellular processes downstream of its targets (Tronson & Taylor, 2007). Indeed, post-retrieval blockade of the NMDAR is associated with the weakening of several memory types, including auditory fear (e.g. Ben Mamou et al., 2006; Milton et al., 2013), spatial (e.g. Liang et al., 1994) and Pavlovian and instrumental reward memory (for a review see Das et al., 2013).

While other compounds, such as β-blockers, may interfere with reconsolidation, their effects in reward memory reconsolidation generally seem to be weaker than NMDAR antagonists (Das et al., 2013). Problematically, few experimental NMDAR antagonists are safe for human use and thus few studies have explored their potential in humans (Das et al., 2018b/*Chapter 3*). One such study investigated the non-

competitive NMDAR antagonist Memantine, and failed to observe weakening of behavioural, attentional or declarative indices of smoking memories (Das et al., 2015a). It is not clear whether null effects were due to the unique and unusual receptor-level properties of memantine, a relatively low-affinity NMDAR antagonist, or due to slow peak-plasma latency requiring administration prior to reactivation (and thus potential interference with destabilisation). Indeed, as NMDARs are required for memory *destabilisation* in addition to *reconsolidation* (Milton, 2013), oral preparations of NMDAR antagonists may be unsuitable for reconsolidation blockade. Conversely, the rapid on/off kinetics of Nitrous Oxide (N_2O, an NMDAR antagonist; Jevtovic-Todorovic, Wozniak, Benshoff, & Olney, 2001) allows for rapid post-retrieval blockade of the NMDAR. In *Chapter 3*, exploratory analyses suggested weakening of human cue-alcohol memories occurred in a prediction error (PE) dependent fashion. Surprise (used as a measure of PE) was not, however, induced uniformly within groups, and significant effects were revealed only when groups were reassigned according to *experienced* PE (Das et al., 2018b/Chapter 3). Further exploration of the efficacy of NMDAR antagonists in humans is therefore warranted using optimised reactivation procedures (e.g. procedures that maximise PE at retrieval).

Ketamine is a potent, non-competitive NMDAR antagonist that, via intravenous administration, can be administered to rapidly reach active plasma concentrations post-retrieval. Due to its good safety profile, ketamine is used frequently as an anaesthetic in field medicine and paediatrics. Moreover, the animal literature has successfully demonstrated weakening of Pavlovian appetitive reward memories following post-retrieval ketamine administration (e.g. Suzuki et al., 2000; Zhai et al., 2008). Zhai et al. (2008), for example, intraperitoneally administered 60mg/kg of ketamine to rodents following re-exposure to a morphine conditioned context (conditioned place preference: CPP). While CPP was preserved following saline, ketamine administration attenuated CPP, which was maintained following a priming injection of morphine aiming to reinstate CPP. To date, no studies have explored ketamine's efficacy in attenuating reactivated alcohol MRM in a human reconsolidation protocol.

A significant limitation of the reconsolidation literature is that, in the presence of null findings, it is impossible to identify *why* a post-reactivation intervention failed to impact behavioural outcomes. While it is possible that null findings reflect failure to block reconsolidation following successful (but unverified/unverifiable) memory

destabilisation, it is also conceivable that the memory was not initially destabilised. Central biomarkers of the cellular and molecular processes underlying changes in synaptic plasticity may serve to *indirectly* identify when reconsolidation has (or has not) occurred in reconsolidation studies. Neurotrophins are proteins that promote neuronal differentiation and survival during development (e.g. Bolanos & Nestler, 2004; Castren et al., 1993; Maffei, 2002) and play an important role in mediating the synaptic plasticity associated with learning and memory in the nervous system (e.g. Arancio & Chao, 2007; Asthana et al., 2016; Bramham & Messaoudi, 2005; McAllister, Katz, & Lo, 1999; Schinder & Poo, 2000). Brain Derived Neurotropic factor (BDNF) secretion activates signalling pathways associated with cellular processes underlying memory consolidation in the hippocampus (e.g. Cunha, Brambilla, & Thomas, 2010; Yoshii & Constantine-Paton, 2007), resulting in modifications at the synapse (Murer, Yan, & Raisman-Vozari, 2001). Notably, blockade of memory consolidation via ketamine is associated with an attenuation of BDNF expression, such that BDNF may act as a marker of memory consolidation (Goulart et al., 2010).

A role for BDNF in *reconsolidation* has been also been suggested (e.g. Radiske et al., 2017; Radiske et al., 2015; Samartgis, Schachte, Hazi, & Crowe, 2012), however, the requirement for BDNF is unclear, and likely site specific. For instance, Lee, Everitt, and Thomas (2004) demonstrated a requirement for BDNF in the consolidation, but not reconsolidation, of a contextual fear memory. Similarly, Wang et al. (2012) observed expression of BDNF in rodents following the reconsolidation of a conditioned taste aversion memory in the insular cortex, but not the central nuclei of the amygdala. Given that serum BDNF in humans may serve as a biomarker of the anti-depressant effects of ketamine (Haile et al., 2014, although see; Machado-Vieira, Salvadore, Diazgranados, & Zarate, 2009) it might be speculated that the expression of BDNF following memory reconsolidation may also be detectable. Further, if ketamine successfully blocks reconsolidation, constrained expression of BDNF (as seen with blockade of consolidation via ketamine; Goulart et al., 2010) may also been observed when reconsolidation is blocked. Given the utility of a biomarker of reconsolidation in humans, measurement of peripheral BNDF following the demonstration of memory destabilisation is warranted.

While the current study aimed to examine the effect of ketamine within a reconsolidation paradigm, ketamine's effects on neurogenesis and synaptic plasticity mean the drug may have impacts on psychiatric symptoms independently of its effect

on a destabilised memory. Ketamine has recently received attention as a fast-acting pharmaco-therapy for treatment-resistant depression (e.g. Han et al., 2016; Kishimoto et al., 2016; Romeo, Choucha, Fossati, & Rotge, 2015; Serafini, Howland, Rovedi, Girardi, & Amore, 2014) and alcohol addiction (e.g. Kolp, Friedman, Young, & Krupitsky, 2006; McAndrew et al., 2017; Sabino, Narayan, Zeric, Steardo, & Cottone, 2013). In rodents, the administration of ketamine is associated with elevated BDNF (Choi, Lee, Park, Kim, & Son, 2017), suggesting ketamine may increase neurogenesis and synaptic plasticity via BDNF (Duman & Li, 2012) and other factors mediating synaptogenesis and remodelling, such as the mammalian target of rapamycin complex (mTOR). Indeed, ketamine induces increases in mTOR activity and ketamine-associated reductions in both alcohol consumption (Sabino et al., 2013) and depression-like behaviour (Li et al., 2010) are abolished following inhibition of the downstream (mTOR) processes. Consistent with this, the anti-depressant actions of ketamine are thought to be mediated by mTOR signalling, such that increased mTOR activity is coupled with a rapid increase in the elevation of synaptic proteins and spine formation in the pre-frontal cortex (Li et al., 2010). These synaptic changes are associated with elevated 5-HT transmission, potentially providing a mechanism through which the rapid anti-depressant actions of ketamine are observed (Duman & Li, 2012). The anti-alcohol effects of ketamine may therefore be mediated by a similar mechanism. Amongst other synaptic targets (Roberto & Varodayan, 2017), both chronic alcohol consumption (e.g. Nagy, 2008), and genetic predispositions to alcohol use disorder (e.g. Yoon et al., 2016) are associated with the dysregulation of the NMDAR system. Ketamine may therefore reduce alcohol consumption via increased synaptic plasticity within these systems, via a reconsolidation-independent mechanism. Under this interpretation, we might also expect ketamine-induced changes in alcohol consumption to be related to post-ketamine plasma BDNF levels in the absence of alcohol memory retrieval.

The current study administered intravenous ketamine following cue-alcohol maladaptive memory reactivation in hazardous/harmful beer drinkers. It was predicted that putative indices of alcohol MRM strength, including drinking behaviour, craving, and attentional bias towards alcohol cues, would reduce when ketamine was administered after MRM retrieval (and putative destabilisation) relative to placebo, due to reconsolidation blockade. A second control group examined the effects of ketamine in the absence of MRM retrieval. It was predicted that blood BDNF would be associated

with blood plasma ketamine following administration in this group, but that this association would be abolished when alcohol MRMs were retrieved prior to ketamine administration.

4.2 Methods

4.2.1 Participants

Ninety hazardous drinkers (55 male), defined as consumption of ≥40 units per week for men and ≥30 units per week for women, for ≥4 days in seven, were recruited via online advertisement. Additional inclusion criteria were: age >18<65; a desire to reduce drinking; preference for beer; a score of ≥8 on the Alcohol Use Disorders Test (AUDIT; Saunders et al., 1993); normal or corrected to normal colour vision; and native or fluent English. Exclusion criteria were; Alcohol or drug dependence as indicated by a score ≥3 on the structured clinical interview for DSM-IV (SCIDS; Spitzer et al., 1992b); past or current psychiatric disorders requiring treatment; personal or family history of psychosis; currently pregnant or breastfeeding; a fear of needles or blood; or major physical health issues or any conditions for which ketamine is contra-indicated. Participants were reimbursed £80 upon completion of the three-day procedure, with an additional £5 incentive to complete four follow-up questionnaires at two weeks, two months, six months, and nine months (for a total of up to £100). All procedures were approved by the UCL research ethics committee and were in accordance with the declaration of Helsinki.

4.2.2 Study Design

In a single blind, randomised, placebo-controlled design, participants were randomly assigned to one of three groups: intravenous ketamine following brief cue-driven alcohol memory reactivation (Ret-Ket, N=30), ketamine following control memory retrieval (NoRet-Ket, N=30), or placebo following cue-alcohol memory retrieval (Ret-PBO, N=30). Procedures were conducted over the course of three days (baseline; treatment; test) with measures repeated within-subjects, and retrieval and/or drug administration differing between groups.

4.2.3 Apparatus and Tasks

4.2.3.1 Self-Report assessments

Reactivity to alcohol cues

Cue-elicited 'liking' and induced 'urge to drink' ratings were acquired for 16 images: four beer images included in the MRM retrieval procedure (nominated 'Beer Retrieval' cues); three beer images seen only at baseline and test in the current task (nominated 'Beer Non-Retrieval' cues); three wine images ('Wine' cues); and four orange juice ('OJ'

cues) images. Participants were first poured a 150ml glass of beer that was placed in front of them for the duration of the task and told they would consume this following a rating of several images. They then rated each image on an 11-point scale according to 'how pleasant they thought the drink depicted in the image was' (-5 extremely unpleasant, +5 extremely pleasant) and 'how much the depicted image affected their desire to drink' (-5 greatly reduced desire, +5 greatly increased desire). Participants were then informed that they would drink the beer, but first were required to give a rating as to 'how much do you think you will enjoy the beer' (-5 not at all, +5 extremely). All participants actually consumed the beer after following on-screen prompts ('Pick up the drink', 'Prepare to drink', 'Drink now'). Following consumption of the entire beer participants were asked 'how much did you enjoy the beer you just drunk' and then 'how much would you like to drink more beer' (-5 not at all, +5 very much). Note: given the failure to reliably induce prediction error (PE) during the retrieval procedure in *Chapter 3*, this in vivo 'guided-drinking' cue-reactivity task on Day 1 also served as a method for 'expectation maximization', such that participants would genuinely anticipate drinking the drink during the MRM retrieval procedure. This was predicted to generate high levels of expectation violation/PE on Day 2. On Day 3 the guided drinking procedure served purely as a method for obtaining cue-reactivity ratings using the urge and enjoyment questions above.

Tonic craving was measured using the Alcohol Craving Questionnaire-Now (ACQ-NOW; Singleton, 1994), generating the subscales of overall craving (General*),* urges and desires to drink in anticipation of the benefits of drinking (Compulsivity*),* urges and desires associated with intent to drink (Purposefulness), and urges and desires to drink in anticipation of relief from withdrawal or negative affect (Emotionality*)*.

Harmful patterns of alcohol use were assessed using the Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993). Desire to reduce drinking was measured using the Stages of Change Readiness and Treatment Eagerness Scale for Alcohol (SOCRATES; Miller & Tonigan, 1996), while responses to alcohol were measured using the Subjective Response to Ethanol (SRE) questionnaire (Schuckit, Smith, & Tipp, 1997) and Alcohol Expectancy Questionnaire (AEQ; Fromme et al., 1993).

Mood-related measures

Measures of depression, anxiety and positive and negative mood were obtained respectively using the Beck Depression Inventory (Beck, Steer, & Brown, 1996), the Spielberger State-Trait Anxiety Inventory (STAI; Spielberger, 1970) and the Positive and Negative Affect Scale (PANAS; Watson et al., 1988). Responses and attitudes to distressing events were rated using the Distress Tolerance Scale (DTS; Simons & Gaher, 2005).

Drug induced changes

Subjective response to ketamine was assessed using the Bodily Symptoms Scale (BSS; Bond & Lader, 1974) and Clinician-Administered Dissociative States Scale (CADSS; Bremner et al., 1998).

4.2.3.2 Behavioural assessment

Drinking-related behaviour

Alcohol consumption in the two weeks prior to the 'baseline' session (Day 1), the week prior to the test session (10-14 days following Day 1), and in the two weeks prior to each follow-up (2 weeks; 2 months, 6 months; 9 months) was assessed using the timeline follow-back (TLFB; Sobell & Sobell, 1992) for alcohol.

Attentional bias

Attentional bias towards alcohol cues was assessed using a visual probe task (see *Figure 3.1* for full details and a schematic of the task; Mogg, Bradley, Field, & De Houwer, 2003). Ten alcohol and matched control images rendered at 300x300 pixels were presented simultaneously on the left and right side of a 1024x768 monitor. Following 2000ms, a single triangular probe replaced the image pairs and participants were required to indicate whether the probe was pointed upwards or downwards. Eye movements towards cues were recorded using a desktop-mounted Eyelink 1000 eye tracker (SR Research, Ontario, Canada) at a sampling rate of 1000Hz. Head position was stabilised 70cm from a 1024x768 monitor using a chin rest. Three measures of attentional bias were obtained: dwell time (total fixation time on each image across trial), first fixation latency (time elapsed prior to the first fixation on each image), and first fixation duration. To ensure alcohol images matched the brightness and composition of orange juice images, and to ensure all images were of beverages, new image pairs were sourced for the current study.

4.2.3.3 Memory Retrieval

A short-form of the cue-reactivity task (described above) followed by an omission-based prediction error was used to retrieve naturalistic cue-alcohol memories (see *Figure 4.1.B*). As with the cue-reactivity task, prior to rating, a bottle of beer was opened and 150ml poured into a cold glass in Ret-Ket and Ret-PBO groups. In NoRet-Ket an identical amount of orange juice was poured instead. In all groups, the glass was placed in front of the participant throughout the rating. Following the presentation and rating of four beer images (Ret-Ket, Ret-PBO groups), or four orange juice images (NoRet-Ket), alongside two further Soft Drink images (all groups), all participants experienced a negative omission PE. On screen prompts instructed the participant to "Pick up the drink", to "Prepare to drink", then, unexpectedly, to "STOP! Do not drink" and then "Put the drink down". The drink was then removed from sight (this procedure is outlined in detail in Das et al., 2018a). This served to generate negative PE in all groups.

4.2.3.4 Distractor tasks

Immediately following the retrieval/PE generation, forwards and backwards digit span, and prose recall (taken from the Rivermeade Behavioural Memory Test; Kurtz, 2011) tasks were administered to ensure participants disengaged with the retrieval procedure.

4.2.3.5 Verification of recent abstinence

All participants provided a breath alcohol reading of 0.00 ng/dl prior to the commencement of each session. Blood alcohol concentration was obtained using an ion Alcometer S D2 breathalyser (Lion 500 Breathalyser, Lion Instruments, UK).

4.2.3.6 Drug administration

Ret-Ket and NoRet-Ket groups received intravenous racemic ketamine hydrochloride with a target plasma concentration of 350ng/dl, controlled via syringe pump using a pharmacokinetic (domino) infusion model. PBO-Ret group received intravenous saline solution. Ketamine and saline concentrations were infused for 30 minutes. All infusions were performed via intravenous cannulation, with infusion speed controlled by a computer running STANMPUMP software to drive a Graseby 3400 syringe driver.

All participants took domperidone (10mg) two hours prior to ketamine/placebo infusion to prevent needle or ketamine-induced nausea, which was observed during piloting.

4.2.3.7 Blood sampling

Blood samples were obtained 15 minutes pre and post infusion, and at the final test session (10-14 days post treatment). Whole blood samples were collected in purple vacutainer tubes. Samples were spun out for 5 minutes at 3000RPM and plasma was pipetted out into labelled cyro-tubes for storage at -80 degrees until analysis. Gas chromatography was used to assay achieved plasma levels of ketamine and its metabolites, norketamine (NK) and dehydroxynorketamine (dhNK). Plasma BDNF levels were measured using enzyme-linked immunosorbent assay (ELISA; Human BDNF ELISA Kit, RAB0026, Sigma-Aldrich, now Merck, UK) according to the manufacturer's instructions.

4.2.3.8 Procedure

Following screening, participants attended three in-person sessions followed by four remote follow-up sessions. A schematic of the procedure is presented in *Figure 4.1.*

Day 1 (baseline)

The first session took place at the Clinical Psychopharmacology Unit, UCL. Informed consent was obtained and after providing a 0.00mg/ml breath-alcohol sample all participants provided basic demographic details including age, gender, weight, height, education level, and ethnicity. Lifetime drinking history was obtained via semi-structured interview (full details available in *Chapter 3*) and subsequent questionnaires (SRE, AEQ, SOCRATES, TLFB, BDI, STAI, PANAS, and DTS) were administered on paper and computer. Cue-reactivity and visual probe tasks were then administered. Upon completion of the baseline session participants were reminded to fast (no solid food or non-clear liquids) for 6 hours and to avoid clear liquids for 2 hours prior to the retrieval session. A 10mg domperidone tablet was given to participants with instructions to take it two hours prior the drug administration session.

Day 2 (retrieval and drug administration; Day 1 + 48-72 hours)

Participants attended a clinical room at University College London Hospital (UCLH). After breathalysing and confirming participants had correctly fasted and consumed the domperidone, the attending anaesthetist inserted the cannula and obtained the first blood sample. Participants completed baseline measures of the CADSS and BSS and then sat on a hospital trolley while baseline heart rate and blood pressure measures were obtained. Following a 10-minute resting period, the retrieval/control and PE procedure was administered. The glass of beer (Ret-Ket, PBO-

Ret) or orange juice (NoRet-Ket) was then removed from sight and participants rated their surprise in response to the PE procedure on an 11-point scale (-5: completely unexpected, to +5: completely expected). All participants completed distractor tasks for an approximate 5 minutes. Ketamine (Ret-Ket, NoRet-Ket) or saline solution (PBO-Ret) was then administered for a total of 30 minutes. Fifteen minutes into ketamine/saline administration participants were again asked to complete the CADSS and BSS. These questionnaires were either self-reported or administered with the aid of the experimenter if participants did not feel able to complete them by themselves. Heart rate was monitored throughout infusion and blood pressure was measured at 10-minute intervals. After the 30-minute infusion, participants were asked to remain on the trolley until they felt secure in standing up unassisted. A second blood sample was then obtained, and the cannula removed. Participants completed final BSS and CADSS, along with a delayed prose recall. After completing a series of competency checks, the attending anaesthetist confirmed the participant was safe to go home.

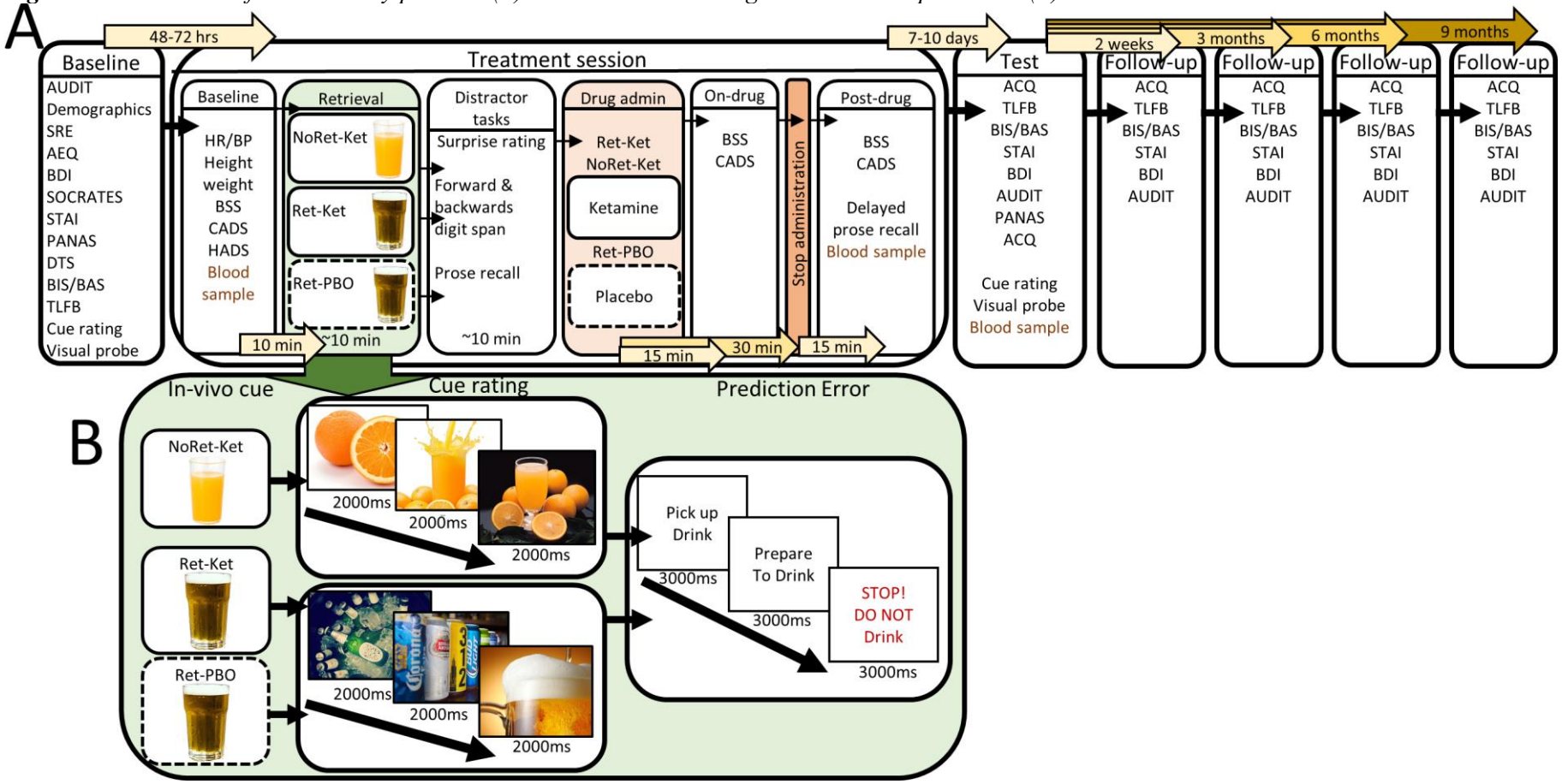
Day 3 (test; Day 1 + 7-10 days)

Session 3 was identical to the first (with the omission of demographic measures. Questionnaires and computer-based tasks were completed in the same order and a delayed prose recall was additionally completed. A final blood sample was taken after which participants were debriefed and reimbursed.

Follow-up (Day 3 + 2 weeks, 3 months, 6 months, 9 months)

Participants remotely completed online versions of the ACQ, TLFB, BIS/BAS, STAI, BDI, and AUDIT questionnaires.

Figure 4.1. *Schematic of the three-day protocol (A) and retrieval and drug administration procedure (B).*



During the retrieval procedure (B) participants rated four beer (if assigned to the Ret-Ket and Ret-PBO groups) or four orange juice images (NoRet-Ket), and two further soft drink images (all groups). Prediction Error was then generated in all groups, after which the NoRet-Ket and Ret-Ket groups received intravenous ketamine for 30 minutes. The Ret-PBO group received saline solution.

4.2.4 Statistical approach

Sample size was calculated in G*Power 3.1.9.2 for 1-β=0.95 to detect a minimum effect size of n_p^2=0.05 at α=0.05 for the interaction in 2 (baseline, post-manipulation) x 3 (Group) mixed ANOVA, assuming ρ of 0.5. This yielded a total required sample size of N=78 (26 per group). Anticipating minimal attrition and technical error, we randomised N=30/group.

Data analysis was performed using IBM SPSS Statistics version 25 for windows. All data were checked for normality, homogeneity of variance, and sphericity (for repeated measure with >2 comparisons). Greenhouse Geisser corrections were applied where sphericity was violated. One-way ANOVA was used to assess group differences at baseline, using a conservative α=0.01 due to multiple unhypothesised (>20) comparisons of group differences. A mixed Day (baseline, test) x Group (Ret-Ket, PBO-Ret, NoRet-Ket) ANOVA was used to test for group effects on the primary outcomes of alcohol consumption and cue-reactivity. Cue-reactivity tasks were assessed using Day (baseline, test) x Group (Ret-Ket, PBO-Ret, NoRet-Ket) x Cue (Beer Retrieval, Beer Non-Retrieval, Wine, Neutral), and visual probe data with Day (baseline, test) x Group (Ret-Ket, PBO-Ret, NoRet-Ket) x Cue type (Beer Retrieval, Beer Non-Retrieval, Wine, Neutral) x Target (Target, Non-Target) mixed ANOVA. Where significant main effects of Day or Group were observed for k>2 ANOVA, Bonferroni-corrected pairwise comparisons were applied (to control for Type I error). Day x Group interactions were followed up with an assessment of the simple effect of Group at baseline, to ensure no baseline differences were present. If this was not the case, the multivariate simple effect of Day (baseline, test) was analysed within each group. Follow-up analyses were conducted using mixed Day (baseline, test, 2-week follow-up, 2-month follow-up, 6-month follow-up, 9-months follow-up) x Group (Ret-Ket, PBO-Ret, NoRet-Ket) ANOVA. These analyses were conducted separately to the main analysis to maintain degrees of freedom following expected attrition. To assess the relationship between blood plasma ketamine and BDNF, Spearman's rank correlations (due to non-normal data of the blood markers) were run within each group between ketamine, NK, and dhNK blood plasma levels and blood plasma BDNF level post-ketamine administration.

4.3 Results

4.3.1 Baseline alcohol and questionnaire data

Baseline demographics and measures of alcohol consumption, attitudes to alcohol, craving, mood, readiness to change, and behavioural inhibition/activation data are presented in *Table 4.1*. Groups did not significantly differ at baseline (at corrected alpha; all *ps*>0.022). Due to heterogeneity in variance, Welch's ANOVA is presented for SCID, motivation to reduce alcohol consumption, and total weekly spirit consumption.

Table 4.1. Baseline demographic, drinking behaviour and questionnaire measures.

	PBO-Ret N=30	Ret-Ket N=30	NoRet-Ket N=30	$F_{2,86}$	*p*	η^2
Gender (N=male)χ	21	19	15	2.618	0.270	.
Age	27.70±8.334	26.50±6.252	28.48±9.573	0.354	0.703	0.008
AUDIT C	9.10±1.029	9.20±1.031	8.90±1.185	0.596	0.553	0.014
AUDIT Total	22.17±4.857	23.77±5.151	20.47±4.329	3.559	0.033	0.076
$SCID_W$	0.93±0.828	1.10±0.923	0.77±0.568	1.493	0.234	.
Motivation to $reduce_w$	3.17±0.592	3.13±0.346	3.17±0.379	0.054	0.947	.
BDI	15.47±9.694	14.03±8.584	11.30±8.703	1.657	0.197	0.037
BAS *Drive*	11.10±2.708	11.53±2.980	10.57±2.445	0.951	0.390	0.021
BAS *Fun*	13.27±2.753	14.33±1.605	13.77±1.716	1.957	0.147	0.043
BAS *Reward*	16.63±1.650	16.53±2.623	16.30±1.878	0.201	0.819	0.005
BAS *BIS*	20.87±2.688	19.57±2.812	19.97±2.566	1.837	0.165	0.041
PANAS *Positive*	32.07±7.909	33.47±7.248	31.93±8.111	0.359	0.699	0.008
PANAS *Negative*	20.90±6.381	21.10±7.439	19.47±6.907	0.497	0.610	0.011
STAI	45.82±11.646	46.32±11.232	44.32±12.217	0.221	0.802	0.005
DTS *Absorbance*	2.789±1.077	3.0111±1.1163	2.922±1.312	0.273	0.762	0.006
DTS *Tolerance*	2.922±0.878	3.022±1.0136	2.933±1.077	0.091	0.913	0.002
DTS *Appraisal*	3.117±0.955	3.550±0.829	3.133±1.091	1.944	0.149	0.043
DTS *Regulation*	3.100±1.021	2.700±1.080	2.878±1.015	1.115	0.332	0.025
DTS *Total*	2.982±.806	3.071±0.840	2.967±0.985	0.122	0.885	0.003
Days Drinking	5.600±1.329	5.633±1.273	5.700±1.466	0.042	0.959	0.001
Daily Alcoholic Drinks	4.542±2.054	5.622±2.853	4.221±1.711	3.170	0.047	0.068
Total Pints Beer	17.300±10.436	22.767±16.952	18.100±11.804	1.465	0.237	0.033
Total Glasses Wine	8.867±10.500	8.567±8.653	6.900±8.600	0.390	0.678	0.009
Total Spirits $(25ml)_W$	6.633±8.556	10.400±12.339	5.333±6.525	1.958	0.151	.
Total weekly units	69.347±32.214	86.727±45.751	66.193±32.078	2.645	0.077	0.057
Binge days	1.767±1.794	2.600±1.868	1.400±1.354	3.983	0.022	0.084
SOCRATES *Recognition*	24.87±3.137	23.83±3.425	23.33±4.213	1.400	0.252	0.031
SOCRATES *Ambivalence*	11.30±2.902	10.97±3.792	9.90±3.448	1.388	0.255	0.031
SOCRATES *Steps*	21.77±4.040	21.33±4.037	20.87±4.524	0.343	0.710	0.008
CEOA *Sociability*	25.77±3.431	26.30±4.162	24.43±3.821	1.904	0.155	0.042
CEOA *Tension reduction*	7.30±2.277	6.57±1.813	7.03±2.109	0.960	0.387	0.022
CEOA *Liquid courage*	12.93±2.477	12.63±2.977	3.081±0.563	0.453	0.637	0.010
CEOA *Sexuality*	9.07±2.803	8.30±2.667	8.07±2.434	1.179	0.313	0.026
CEOA *Impairment*	20.97±5.242	21.30±5.491	20.70±3.984	0.111	0.895	0.003
CEOA *Risk and aggression*	11.27±3.039	12.03±3.737	11.43±3.461	0.416	0.661	0.009
CEOA *Self-perception*	7.17±2.365	6.77±2.921	6.00±2.228	1.657	0.197	0.037
OCDS OBS	6.60±3.307	6.57±3.380	5.53±3.451	0.966	0.385	0.022
OCDS COMP	11.00±2.244	10.47±2.662	10.93±2.888	0.371	0.691	0.008
OCDS Total	17.60±5.130	17.03±5.524	16.47±5.906	0.315	0.731	0.007

Note: Values mean±SD. All tests were one-way ANOVA unless indicated with $_W$, denoting the use of Welch's ANOVA, or χ, denoting use of Chi Square. No significant differences ($p<0.01$) between groups were observed at baseline.

4.3.2 Changes in drinking behaviour

Mixed Group (*Ret-Ket, PBO-Ret, NoRet-Ket*) x Day (baseline, test) ANOVA was conducted for number of drinking days per week, total units consumed per week, pints of beer consumed per week, glasses of wine per week, spirits consumption per week, and AUDIT score. Mean pre- and post-treatment data, main and interaction effects are presented in *Table 4.2*.

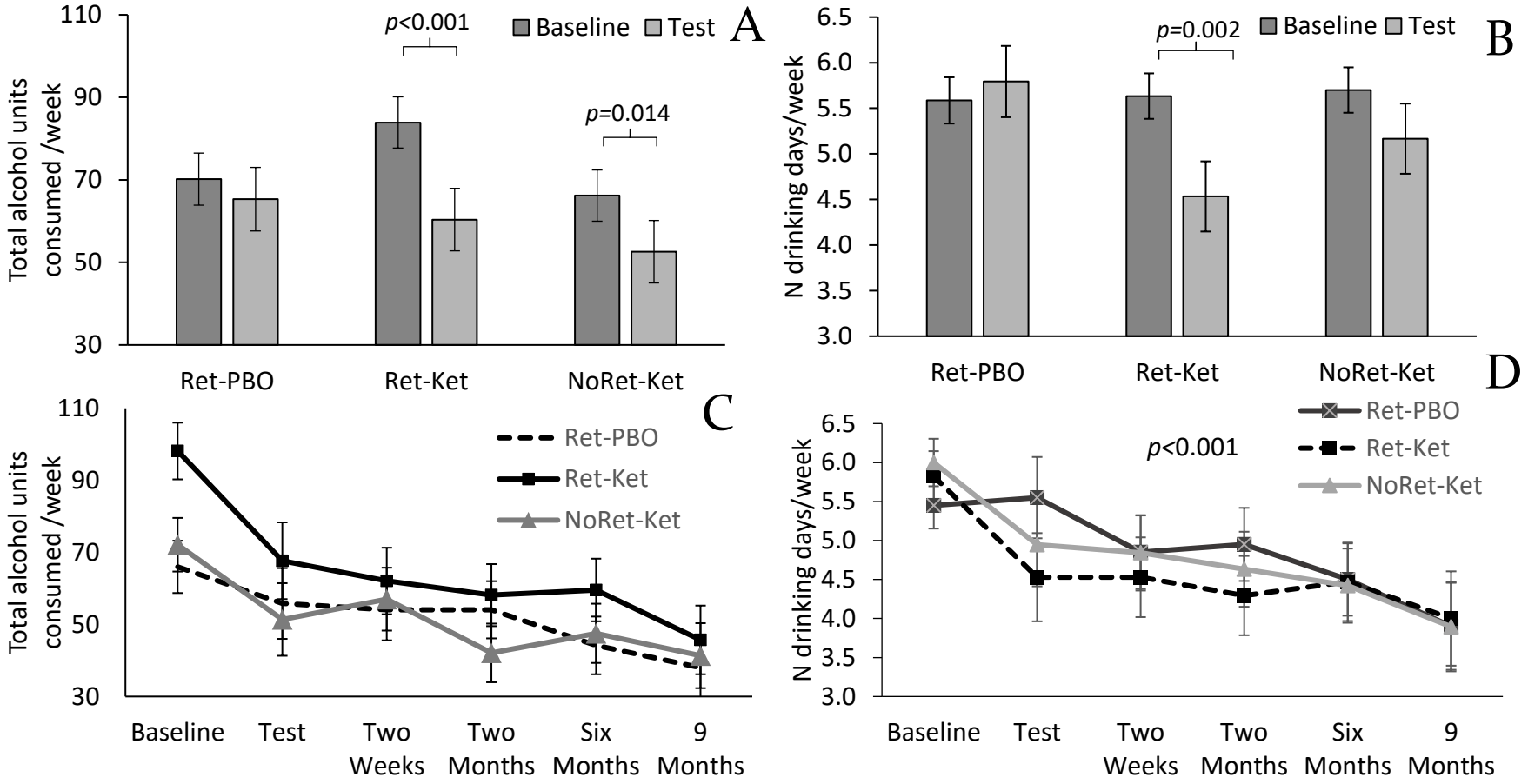
Table 4.2. *Outcomes for drinking-related behaviour at baseline (48 hours pre-manipulation) and at test (7-10 days post-manipulation) and associated main effects and interactions.*

Outcome		Ret-PBO N=29	Ret-Ket N=30	NoRet-Ket N=30	Main effects	Interactions
N drinking days /week	BL	5.59±1.35	5.63±1.27	5.70±1.47	**Day**: $F_{1,86}$=5.866, p=0.018, η_p^2=0.064	**Day x Group**: $F_{2,86}$=3.689, p=0.029, η_p^2=0.079
	Test	5.79±2.38	4.53±1.80	5.17±2.12		
Total units /week	BL	70.18±32.45	83.91±37.09	66.19±32.1	**Day**: $F_{1,86}$=19.800, p<0.001, η_p^2=0.187	**Day x Group**: $F_{2,86}$=2.929, p=0.059, η_p^2=0.064
	Test	65.32±42.61	60.37±45.25	52.60±36.0		
Pints beer /week	BL	17.48±10.57	21.97±14.50	18.10±11.8	**Day**: $F_{1,86}$=28.927, p<0.001, η_p^2=0.252	None¥
	Test	14.39±10.11	14.47±13.57	13.45±11.2		
Glasses wine /week	BL	8.97±10.67	8.57±8.65	6.90±8.60	None¥	None¥
	Test	8.59±11.32	7.27±8.99	5.90±6.59		
Drinks spirits /week	BL	6.79±8.66	10.40±12.34	5.33±6.52	None¥	None¥
	Test	5.45±7.758	8.10±10.47	4.70±5.76		
AUDIT	BL	22.17±4.86	23.77±5.15	20.47±4.33	**Day**: $F_{1,87}$= 35.005, p<0.001, η_p^2=0.187	None¥
	Test	19.47±5.49	19.90±6.70	18.03±5.67		

Note: Values mean±SD. BL: baseline. Only significant main effects and interactions are reported. Drinks spirits = 25ml. ¥ = only main effects and interactions where F≥2.5 are presented

Total weekly alcohol consumption reduced significantly in the Ret-Ket group ($F_{1,86}$=18.876, p<0.001, η_p^2=0.180; *Figure 4.2.A*), and in the NoRet-Ket group ($F_{1,29}$=6.301, p=0.014, η_p^2=0.068). For number of drinking days per week, a multivariate simple effect of Day showed that the number of days per week alcohol was consumed reduced in the Ret-Ket group only ($F_{1,86}$=10.585, p=0.002, η_p^2=0.110; *Figure 4.2.B*). These effects were maintained at 9 months follow up, with mixed Day (baseline, test, two-week, two-month, six-month, 9-month) x Group (Ret-Ket, Ret-PBO, NoRet-Ket) ANOVA observing a main effect of Day for total alcohol consumption (*Figure 4.2.C*; $F_{5,49}$=11.745, p<0.001, λ=0.545), number of drinking days per week ($F_{5,49}$=8.139, p<0.001, λ=0.454; *Figure 4.2.D)* and AUDIT score ($F_{5,231}$=62.884, p<0.001, η_p^2=0.529).

Figure 4.2. *Total alcohol units consumed (A) and number of drinking days per week (B) at baseline and at test (10 days post treatment). Data for all timepoints (including follow-ups) (C,D)*



Note: Bars represent mean±SE. Total alcohol consumption (A) and drinking days per week (B) reduced significantly in the group that received ketamine following cue alcohol retrieval. Follow-up data suggests these effects were maintained at 9 months (C, D).

4.3.3 Reactivity to alcohol cues

Results for liking of image cues and wanting of beer in response to image cues, and desire to consume (prior to drinking), post-consumption desire to "drink more", as well as anticipated enjoyment of, and actual enjoyment of the in vivo beer cue are presented in *Table 4.3.* Day x Group interactions were probed by analysing the simple effect of Day within each group. In almost all cases, scores reduced from baseline to test in the Ret-Ket group only. Namely, picture cue liking ($F_{1,87}$=15.379, p<0.001, η_p^2=0.150), in vivo beer cue liking ($F_{1,87}$=24.708, p<0.001, η_p^2=0.221; *Figure 4.3.A*), urge to consume beer in response to image cues ($F_{1,87}$=9.231, p=0.003, η_p^2=0.096), urge to consume the in vivo beer itself ($F_{1,87}$=19.703, p<0.001, η_p^2=0.185; *Figure 4.3.B*), anticipated enjoyment of the in vivo beer ($F_{1,87}$=20.273, p<0.001, η_p^2=0.189; *Figure 4.3.C*), actual enjoyment of the beer itself ($F_{1,87}$=8.670, p=0.004, η_p^2=0.091; *Figure 4.3.D*), and post-consumption desire to "drink more" of the beer ($F_{1,87}$=24.460, p<0.001, η_p^2=0.219; *Figure 4.3.E*). Significant reductions were also observed in the NoRet-Ket group for actual enjoyment of beer ($F_{1,87}$=4.294, p=0.041, η_p^2=0.047) and a trend for post-consumption desire to drink more of the beer ($F_{1,87}$=3.840, p=0.053, η_p^2=0.042).

A trend effect of Group was observed at baseline for liking the of in vivo beer ($F_{2,87}$=2.820, p=0.065, η_p^2=0.061), with greater scores observed in the Ret-Ket, relative to Ret-PBO group (p=0.067). For anticipated enjoyment of the beer, a simple effect of Group was observed at baseline ($F_{2,78}$=3.925, p=0.023, η_p^2=0.083), with Bonferroni-corrected pairwise comparisons suggesting the Ret-Ket group anticipated enjoying the beer more than the Ret-PBO group (p=0.020). Desire to "drink more" differed at test ($F_{2,87}$=3.106, p=0.050, η_p^2=0.067), with higher scores in the Ret-PBO, relative to Ret-Ket group (p=0.066).

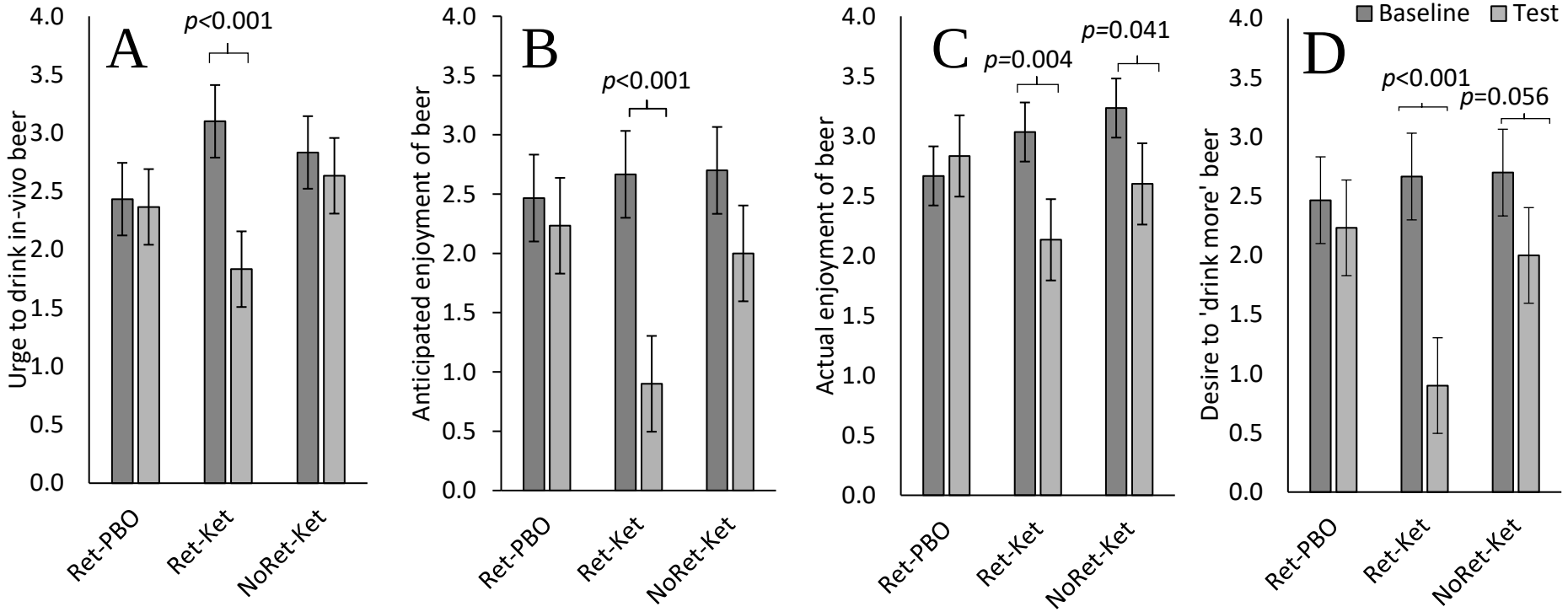
Bonferroni-corrected pairwise comparisons showed significantly higher liking ratings for OJ, Beer Retrieval (the beer cues used in the retrieval procedure), and Beer Non-Retrieval cues (newly presented beer cues not used in the retrieval procedure) relative to Soft Drink (ps<0.001) and Wine (ps≤0.001) cues. Greater urge to drink scores were similarly observed in response to Beer Retrieval and Beer Non-Retrieval cues, relative to Wine, OJ and Soft Drink cues (ps<0.001). Urge scores decreased from baseline to test for OJ (p=0.019), Beer Retrieval (p<0.001) and Beer Non-Retrieval (p<0.001) image cues.

Table 4.3. *Mean liking and urge scores for image and in vivo cues at baseline and test*

DV	Cue		Ret-PBO N=30	Ret-Ket N=30	NoRet-Ket N=30	Main effects	Interactions
Cue Liking	Beer Retrieval	BL	2.58±1.28	2.80±1.15	2.45±1.28	**Cue**: $F_{4,84}$=17.205, p<0.001, λ=0.450 OJ, Beer Retrieval, Beer Non-Retrieval > Wine, Soft Drink (ps<0.001) **Day:** $F_{1,87}$=11.163, p=0.001, η_p^2=0.114 baseline > test	**Day x Group**: $F_{2,87}$=2.159, p=0.047, η_p^2=0.068
		Test	2.19±1.32	2.23±1.16	2.33±1.17		
	Beer Non-Retrieval	BL	2.49±1.45	2.80±1.29	2.61±1.45		
		Test	2.43±1.28	2.28±1.12	2.38±1.30		
	Wine	BL	1.39±2.18	1.37±2.22	2.12±1.68		
		Test	1.36±1.88	1.00±2.11	1.74±1.44		
	Soft Drink	BL	1.48±1.42	0.85±2.05	1.50±1.52		
		Test	1.67±1.43	0.52±2.23	1.53±1.45		
	OJ	BL	2.34±1.50	2.77±.99	2.45±1.33		
		Test	2.35±1.43	2.38±1.40	2.39±1.36		
	In vivo beer	BL	2.07±1.72	3.00±1.46	2.70±1.47	**Day:** $F_{1,87}$=13.919, p<0.001, η_p^2=0.138	**Day x Group:** $F_{2,87}$=6.781, p=0.002, η_p^2=0.135
		Test	2.10±1.65	2.00±1.58	2.37±1.38		
Urge	Beer Retrieval	BL	2.61±1.28	3.08±1.23	2.68±1.37	**Cue**: $F_{4,84}$=55.063, p<0.001, λ=0.724 Beer Retrieval, Beer Non-Retrieval > Wine >OJ, Soft Drink (ps≤0.002) **Day:** $F_{1,87}$=4.789, p=0.031, η_p^2=0.052 baseline > test	**Day x Cue**: $F_{3,284}$=7.210, p<0.001, η_p^2=0.077 **Day x Group:** $F_{2,87}$=2.509, p=0.087, η_p^2=0.055
		Test	2.38±1.26	2.46±1.24	2.47±1.24		
	Beer Non-Retrieval	BL	2.60±1.35	2.77±1.13	2.63±1.33		
		Test	2.02±1.58	1.82±1.42	2.46±1.36		
	Wine	BL	1.09±1.66	1.12±1.53	1.52±1.40		
		Test	1.09±1.40	0.47±1.49	1.27±1.51		
	Soft Drink	BL	0.53±1.35	0.33±1.88	0.75±1.75		
		Test	0.57±1.57	0.02±1.74	0.95±1.69		
	OJ	BL	0.52±1.30	0.31±1.54	0.57±1.36		
		Test	0.65±1.58	0.27±1.62	1.02±1.34		
	In vivo beer	BL	2.43±1.92	3.10±1.54	2.83±1.62	**Day:** $F_{1,87}$=9.624, p=0.003, η_p^2=0.100	**Day x Group:** $F_{2,87}$=5.312, p=0.007, η_p^2=0.109
		Test	2.37±2.08	1.83±1.82	2.63±1.35		
Anticipated enjoyment	In vivo beer	BL	2.13±1.89	3.20±1.03	2.80±1.42	**Day:** $F_{1,87}$= 5.375, p=0.023, η_p^2=0.058	**Day x Group:** $F_{2,87}$=8.234, p=0.001, η_p^2=0.159
		Test	2.43±1.41	1.97±1.63	2.63±1.35		
Actual enjoyment	In vivo beer	BL	2.67±1.45	3.03±1.23	3.23±1.38	**Day:** $F_{1,87}$= 6.664, p=0.012, η_p^2=0.071	**Day x Group:** $F_{2,87}$= 3.298, p=0.042, η_p^2=0.070
		Test	2.83±1.42	2.13±2.06	2.60±2.03		
Post-consumption desire to "drink more"	In vivo beer	BL	2.47±1.24	2.67±1.90	2.70±2.23	**Day:** $F_{2,87}$=19.044, p<0.001, η_p^2=0.180)	**Day x Group:** $F_{1,87}$=4.842, p=0.100, η_p^2=0.100)
		Test	2.23±2.03	0.90±2.38	2.00±2.21		

Note: Values mean±SD. BL: baseline (48hrs pre-treatment), test (10-14 days post-treatment). Only main effects and interactions where F>2.0 are reported

Figure 4.3. *Self-rated urge to consume a glass of beer (A), anticipated enjoyment of the beer (B), Actual enjoyment of beer (C), and self-rated desire to 'drink more' following consumption of beer(D) at baseline (48 hours pre-retrieval/drug session) and test (~10 days post retrieval/drug session)*



Note: Data points represent mean±SEM. Pre-consumption wanting and anticipated enjoyment of beer, and post-consumption actual enjoyment of, and desire to consume more beer, reduced significantly in the group that received ketamine after cue-alcohol retrieval. Smaller reductions in actual enjoyment and desire to drink more beer were also observed in the NoRet-Ket group.

4.3.4 Motivational salience of cues

No interactions between Day and Group were observed on any measure of attentional bias. Main effects and interactions are presented in *Table 4.4*.

***Table 4.4**. Main effects and interactions for the visual probe task*

Outcome	Main effects	Interactions
Dwell time	**Target**: $F_{1,85}$=7.121, p=0.009, η_p^2=0.077 - Target > Non-Target **Day**: $F_{1,85}$=4.571, p=0.035, η_p^2=0.051 - baseline > test **Cue**: $F_{3,255}$=27.399, p<0.001, η_p^2=0.244 - Beer Retrieval, Beer Non-Retrieval > Wine	**Cue x Target**: $F_{2,200}$=19.094, p<0.001, η_p^2=0.183
First fixation duration	**Target:** $F_{1,85}$= 18.989, p<0.001, η_p^2=0.183 - Target > Non-Target **Cue**: $F_{3,255}$=39.836, p<0.001, η_p^2=0.319 - Beer Retrieval > Beer Non-Retrieval > Wine, Neutral	**Cue x Group:** $F_{6,255}$=2.331, p=0.033, η_p^2=0.052 **Cue x Target**: $F_{3,224}$=4.697, p=0.005, η_p^2=0.052
Latency to first fixation	**Day**: $F_{1,85}$=10.676, p=0.002, η_p^2=0.112 - baseline < test **Cue**: $F_{3,255}$=18.427, p<0.001, η_p^2=0.178 - Beer Retrieval, Beer Non-Retrieval < Wine, Neutral	**Cue x Group:** $F_{6,255}$= 2.154, p=0.048, η_p^2=0.048 **Cue x Target**: $F_{3,255}$=17.675, p<0.001, η_p^2=0.172

Note: only main effects and interactions where F>2.0 are presented.

For dwell time, multivariate simple effects of Target showed significantly longer dwell times on Target Beer Retrieval ($F_{1,85}$=5.535, p=0.021, η_p^2=0.061) and Target Beer Non-Retrieval image cues relative to their Non-Target composition matched neutral pair ($F_{1,85}$=, p<0.001, η_p^2=0.346). A trend towards longer dwell times for Non-Target, relative to Target, Wine cues was also observed ($F_{1,85}$=3.668, p=0.059, η_p^2=0.041).

Longer first fixations were observed for Beer Retrieval cues relative to all other cues (ps<0.001), and for Beer Non-Retrieval relative to Wine (p=0.038) and Neutral (p<0.001) cues. The simple effect of Target within each Cue showed longer first fixations were observed for Target, relative to Non-Target cues, for Beer Retrieval ($F_{1,85}$=6.128, p=0.015, η_p^2=0.067), Beer Non-Retrieval ($F_{1,85}$=31.579, p<0.001, η_p^2=0.271) and Neutral ($F_{1,87}$=4.030, p=0.048, η_p^2=0.045) cues. Overall, these findings are consistent with an initial general attentional bias towards beer-related cues.

Bonferroni-corrected pairwise comparisons showed latency to first fixations were more rapid towards Beer Retrieval and Beer Non-Retrieval cues than to Wine and Neutral cues (ps≤0.001). The multivariate effect of Cue was significant in the Ret-Ket

($F_{3,83}$=8.836, p<0.001, η_p^2=0.253) and NoRet-Ket groups ($F_{3,83}$=9.393, p<0.001, η_p^2=0.253), with Bonferroni-corrected pairwise comparisons suggesting more rapid fixations were observed only in the Ret-Ket and NoRet-Ket groups towards Beer Retrieval relative to Wine (ps≤0.049) and Neutral (ps<0.001), and Beer Non-Retrieval relative to Neutral (ps≤0.001). The simple effect of Target within each Cue showed faster fixations were observed towards Target, relative to Non-Target cues, for Beer Non-Retrieval cues ($F_{1,87}$=32.088, p<0.001, η_p^2=0.274), with faster latencies towards Non-Target cues for Neutral ($F_{1,85}$=6.106, p=0.015, η_p^2=0.067), and Wine ($F_{1,87}$=9.898, p=0.002, η_p^2=0.104) cues.

4.3.5 Metabolites

Blood plasma concentrations (ng/ml) for ketamine, norketamine, dehydroxynorketamine, and BDNF are presented in *Table 4.5*.

Table 4.5. *Blood plasma concentration of metabolites (ng/ml)*

	Ret-PBO		Ret-Ket		NoRet-Ket	
	Baseline	On-drug	Baseline	On-drug	Baseline	On-drug
BDNF	21.27± 11.18	29.45±41.36	14.86±8.47	60.85±144.79	18.39±11.21	17.83±19.50
Ketamine	0.00±0.00	3.75±12.36	76.38±381.89	475.66±1319.39	00.00±0.00	353.22±910.00
NK	0.19±0.99	1.57±3.86	0.20±1.02	7.70±5.65	0.18±0.97	6.82±4.35
dhNK	0.00±0.00	0.32±1.18	0.00±0.00	1.01±1.96	0.00±0.00	0.80±1.78

NK: Norketamine, dhNK: Dehydroxynorketmaine

To explore potential mechanistic biomarkers of ketamine's effects, where Group x Day interactions were observed, the association between craving at test, and measures of drinking behaviour were assessed using Spearman's rho with blood plasma concentration of ketamine (ng/ml) and its metabolites, and BDNF blood plasma concentration (ng/ml) following ketamine administration (*Table 4.6*).

Table 4.6. *Spearman's rho correlations between blood plasma concentration ketamine, ketamine metabolites, and BDNF at 45 minutes post-ketamine and putative indices of cue-alcohol memory strength at test (10-14 days post-treatment)*

	Ret-Ket group				NoRet-Ket group			
	plasma BDNF N=27	plasma ketamine N=28	plasma NK N=28	plasma dhNK N=28	plasma BDNF N=30	plasma ketamine N=29	plasma NK N=29	plasma dhNK N=29
Total Units	-0.020	**-0.543****	-0.290	-0.080	0.175	0.177	0.053	-0.02
N drinking days	-0.170	**-0.465***	-0.200	-0.050	-0.050	-0.060	-0.020	0.152
Anticipated enjoyment	-0.051	-0.335	-0.053	-0.132	-0.095	-0.172	-0.048	-0.117
Actual enjoyment	0.020	-0.205	-0.272	-0.084	-0.158	-0.068	0.095	-0.052
Want more	-0.134	-0.362	-0.204	-0.177	-0.197	-0.125	-0.160	-0.133
ACQ-T	**-0.393***	0.190	0.115	-0.114	0.098	0.277	0.302	0.014
AUDIT	0.044	-0.222	**-0.441***	**-0.446***	-0.048	0.108	0.010	0.1370

*Note: N drinking days: number of days alcohol was consumed in a week, NK: norketamine, dhNK: dehydroxynorketmaine, * = $p<0.05$, **$p<0.01$*

Spearman's rho correlations showed blood plasma ketamine level was positively related to BDNF in the NoRet-KET group (ρ=0.444, p=0.016) only. Post-administration blood plasma ketamine correlated with total alcohol consumption (ρ=-0.543, p=0.003), and number of drinking days (ρ=-0.465, p=0.013) in the Ret-Ket group only. AUDIT scores at test similarly correlated with post-administration norketamine (ρ=-0.441, p=0.019) and dhNK (ρ=-0.446, p=0.017) blood plasma levels in the Ret-Ket group only.

Mixed Time (baseline, 45-min post-ketamine, test) x Group (Ret-PBO, Ret-Ket, NoRet-Ket) ANOVA did not show a main effect of Group ($F_{2,66}$=1.151, p=0.323, λ=0.034), nor a Time x Group interaction ($F_{4.132}$=1.012, p=0.404, λ=0.030) for serum BDNF levels.

4.3.6 Correlates

To assess the relationships between measures of drinking related behaviour, cue-reactivity, and on-ketamine subjective side effects, Pearson's correlations were conducted on change scores. Correlation coefficients are presented in *Table 4.7*.

Table 4.7. *Pearson's correlation coefficients between change (test – baseline scores) in alcohol consumption and on-drug subjective side effects*

	Ret-PBO			Ret-Ket			NoRet-Ket		
	Total Units	N drinking days	AUDIT	Total Units	N drinking days	AUDIT	Total Units	N drinking days	AUDIT
	N=29	N=29	N=29	N=30	N=30	N=30	N=30	N=30	N=30
Stimulation (euphoria)	0.238	0.329	0.097	-0.093	0.053	**0.402***	0.194	0.184	-0.053
Sedation (drowsiness)	-0.103	0.006	0.017	0.234	-0.297	0.012	-0.186	-0.259	-0.001
DEQ (liking)	0.131	0.003	0.243	0.038	-0.073	0.029	0.320	0.203	-0.310
DEQ (dislike)	-0.145	0.124	-0.002	0.004	0.002	0.072	-0.214	-0.060	**0.538****

*Note: N drinking days: number of days alcohol was consumed in a week, DEQ: Drug effects questionnaire, * = p<0.05, **p<0.01*

4.3.7 Subjective responses to ketamine

Table 4.8. *Subjective responses to N_2O at baseline (pre-administration) and on-drug (15-minutes post administration onset)*

	Ret-PBO		Ret-Ket		NoRet-Ket	
	Baseline	On-drug	Baseline	On-drug	Baseline	On-drug
BSS *Anxiety*	29.43±22.26	15.57±18.34	24.63±21.55	16.30±22.66	28.03±23.48	26.79±30.43
BSS *Depression*	20.53±18.82	12.63±16.22	14.73±21.14	10.37±18.12	17.62±21.30	14.38±20.93
BSS *Memory*	17.40±17.49	8.50±11.20	14.27±18.26	40.57±29.78	13.46±15.65	37.75±28.71
BSS *Heart palp*	12.43±15.10	8.43±10.92	7.86±7.58	32.62±33.15	11.68±16.14	29.82±24.28
BSS *Nausea*	9.10±11.62	7.07±11.44	6.60±10.43	11.27±16.75	10.07±14.99	13.14±18.01
BSS *Emot numb*	18.40±19.70	10.50±10.35	14.90±15.69	29.50±28.06	14.00±17.14	38.71±31.32
BSS *Euphoria*	7.20±7.45	5.97±7.18	3.70±5.00	50.27±33.50	9.00±13.25	44.00±27.68
BSS *Drowsy*	16.53±20.10	16.47±18.47	15.67±17.01	39.60±32.09	14.18±16.78	41.21±28.19
BSS *Tension*	9.10±9.87	9.77±14.25	11.24±13.91	17.69±25.98	17.04±19.33	12.04±20.61
BSS *Headache*	9.47±13.34	7.27±9.45	6.47±8.28	6.13±12.00	11.59±18.28	8.19±14.60
BSS *Concentr*	15.70±17.68	13.07±14.93	15.47±18.74	59.70±36.43	14.68±18.29	51.18±28.63
BSS *Trembling*	10.57±16.41	6.93±10.03	11.73±19.83	31.10±28.58	12.77±20.06	26.71±26.75
BSS *Vertigo*	10.57±12.82	4.37±5.20	5.00±10.03	33.47±33.85	4.47±5.54	22.71±26.81
BSS *Confusion*	6.07±5.84	9.77±12.50	5.43±6.21	55.27±32.79	3.97±3.93	41.14±32.70
CADSS *Total*	03.10±4.59	3.70±5.48	2.10±2.86	34.33±14.82	3.40±4.17	25.57±14.03
CADSS *Amnesia*	0.50±0.90	0.43±0.77	0.40±0.67	4.50±2.43	0.33±0.66	3.90±2.59
CADSS *Depers*	0.67±1.03	0.70±1.12	0.50±0.94	9.87±5.10	1.07±1.72	5.80±4.49
CADSS *Dereal*	1.93±3.36	2.57±4.11	1.20±1.85	19.97±8.43	2.00±2.61	15.87±8.44

Note: Values mean±SD, BSS: Bodily Symptoms Scale, CADSS: Clinician Administered Dissociative States Scale, Palp: palpitations, Depers: Depersonalisation, Dereal:Derealisationn, Concentr: Concentration

4.3.8 Mood

Results for a mixed Day (baseline, test) x Group (Ret-Ket, PBO-Ret, NoRet-Ket) ANOVA conducted for BDI, STAI, PANAS, and DTS scores are given in *Table 4.9*.

Table 4.9. *Measures of mood pre and post (10-14 days) treatment, main effects and interactions*

DV		Ret-PBO N=30	Ret-Ket N=30	NoRet-Ket N=30	Main effects	Interactions
BDI	BL	15.47±9.69	14.03±8.58	11.30±8.70	**Day:** F1,87=18.423, p<0.001, ηp2=0.174	None¥
	Test	11.17±8.41	10.30±9.00	9.07±8.82		
PANAS+	BL	32.07±7.91	33.47±7.25	31.93±8.11	None¥	None¥
	Test	31.53±9.25	32.67±7.28	31.668.61		
PANAS-	BL	20.90±6.38	21.10±7.44	19.47±6.91	**Day**: F1,87=5.274, *p*=0.024, ηp2=0.057	**Day x Group**: F2,87=3.427, *p*=0.037, ηp2=0.073
	Test	19.33±6.29	18.17±7.45	20.10±8.32		
STAI	BL	48.60±3.18	49.03±2.54	48.60±2.99	None¥	None¥
	Test	49.03±2.50	49.70±2.82	48.77±3.62		

Note: values mean±SD. BL: baseline (48hrs pre-treatment), test (10-14 days post-treatment).
¥ = only main effects and interactions where F≥2.5 are presented

The negative affect subscale of the PANAS significantly reduced only in the Ret-Ket group, ($F_{1,87}$=9.106, *p*=0.003, η_p^2=0.095). Self-reported depression, as assessed by the BDI, showed a reduction across time ($F_{2,172}$=11.154, *p*<0.001, ηp2=0.115). No group effects were observed.

4.3.9 Drug guess

Chi-square analysis showed significant differences between groups ($X^2_{(2)}$=77.143, *p*<0.001) for drug guess. All participants in the ketamine groups (Ret-Ket; N=30, NoRet-Ket; N=30) correctly guessed that they received ketamine. Three participants in the Ret-PBO guessed that they received ketamine, relative to 27 who correctly guessed they received placebo.

4.4 Discussion

The current study administered ketamine following alcohol MRM retrieval. Reductions specific to the post-retrieval ketamine group were observed across putative measures of cue-alcohol memory strength. Notably, participants in the Ret-Ket group more than halved their alcohol consumption and frequency of drinking. These reductions were maintained nine months after treatment, although it should be noted that groups showed roughly equivalent levels of consumption at 9 months. That is, the majority of reduction in these measures occurred rapidly following manipulation in Ret-Ket. As such, the current study suggests ketamine is an effective blocker of cue-alcohol memory reconsolidation and this is a strategy worth pursuing for future treatment development.

The current study is the first to administer ketamine after retrieval in a human reconsolidation protocol (for a study of pre-retreival ketamine see Corlett et al., 2013). Consistent with the animal literature (e.g. Suzuki et al., 2000; Zhai et al., 2008), post-retrieval ketamine produced significant reductions in measures of cue-alcohol memory strength. Indeed, reduced cue liking, and a reduced desire to consume beer were observed only in the group that received ketamine after cue-alcohol memory retrieval. Participants in the Ret-Ket group similarly anticipated enjoying the beer less, also actually enjoyed the beer less, and reported a reduction in "wanting more" of the beer after drinking. Importantly, these reductions in cue-reactivity translated to reduced alcohol consumption, wherein the Ret-Ket group significantly reduced both the frequency of, and total volume of alcohol consumed per week.

These findings are consistent with previous chapters of the current thesis, as well as the existing literature suggesting that naturalistic maladaptive reward memories, such as those underlying addiction, can undergo destabilisation (Das et al., 2018a; Germeroth et al., 2017; Hon et al., 2016; Lonergan et al., 2013; Saladin et al., 2013; Xue et al., 2012) and require NMDA-dependent *restabilisation* to persist in the long-term (Das et al., 2018b). Blockade of the NMDAR is therefore a promising target for the treatment of alcohol and substance use disorders. These finding are contrary to studies which failed to demonstrate blockade of naturalistic reward-memory reconsolidation in humans via βAR (e.g. Jobes et al., 2015; Pachas et al., 2015) and NMDAR receptor antagonists (e.g. Das et al., 2015a). These discrepancies likely reflect differences in retrieval procedures, which are highly inconsistent across the reconsolidation

literature. As demonstrated in *Chapter 3*, inclusion of prediction error (PE) at retrieval is thought to be necessary to destabilise older, more strongly conditioned naturalistic memories. In contrast to Jobes et al. (2015) and Pachas et al. (2015), the current retrieval procedure was inclusive of PE, and replicated the positive results of studies in which the same retrieval procedure was used (e.g. Das et al., 2018a; Das et al., 2015b; Hon et al., 2016). Further to this, the current study presented participants with an in vivo beer cue, relative to a script-driven retrieval procedure as used in Jobes et al. (2015) and Pachas et al. (2015). This is consistent with a requirement for the retrieval procedure to sufficiently replicate the original learning for destabilisation of the original memory trace to occur. These discrepancies in findings highlight the clear need for a focus on understanding sufficient retrieval procedures and standardising these across the human literature, to better identify why null results are observed.

Given the more limited differences in the retrieval procedure in the current study and the study in which memantine failed to block memory reconsolidation (Das et al., 2015a), it is possible that these discrepant results reflect pharmacokinetic differences between the two drugs. Firstly, ketamine has greater affinity at the NMDAR relative to memantine (Gideons, Kavalali, & Monteggia, 2014), which has relatively low selectivity for the NR2A subunit (Ogden & Traynelis, 2011), which is most strongly involved in restabilisation of destabilised memory. Secondly, ketamine has more favourable pharmacokinetics and the intravenous route of administration for ketamine in the current study meant administration could occur *after* retrieval. The NMDA receptor is implicated in both the destabilisation *and* restabilisation of memory, with differing subunits thought to mediate these mechanistic processes (Milton et al., 2013). Thus, the slow pharmacokinetics of oral preparations of NMDAR antagonists, which require pre-retrieval administration for peak-plasma to coincide with restabilisation, means they may prevent initial destabilisation from occurring. Outcomes consistent with reconsolidation-blockade in the current study therefore highlight the importance of administering NMDAR antagonists *after* retrieval, such that interference with destabilisation does not occur. This is important for continued clinical translation, as there are only limited NMDA antagonists that can be administered in this manner (i.e. N_2O, or intranasal or intravenous ketamine).

Despite the visual probe task capturing an attentional bias towards new and retrieved beer cues in the current study, a reduction in attentional bias specific to the Ret-Ket group was not observed. Rather, across groups, a reduction in dwell time and

longer latency to first fixations were observed pre- to post-treatment. As such, it is unlikely the reductions in alcohol consumption reflect a change in attentional bias. Exploratory analyses showed only one measure of drinking behaviour change (number of drinking days) was related to anticipated enjoyment of the in vivo beer in the group that received ketamine after retrieval. In the group that did not undergo retrieval, anticipated enjoyment and total alcohol consumption, and actual enjoyment of the in vivo beer and AUDIT score correlated, suggesting that while alcohol cues retained motivational salience (as assessed by the measure of attentional bias used here), reduced liking and desire to consume beer (both actual and anticipated) was related to reduced consumption.

Despite significant reductions in total alcohol consumption, specific reductions were not, however, observed in spirit and wine consumption. This may simply reflect the chosen population, as the current sample was selected to consume little wine and spirits relative to beer in order to make retrieval cues more drink-relevant.

While the long-lasting, relapse-resistant nature of interventions of memory reconsolidation-interference is touted as a strength of this approach, few studies incorporate follow-up sessions beyond days or weeks (see *Chapter 2* for details). A strength of the current study is therefore its use of follow-up for 9-months after treatment. Importantly, the significant reductions in alcohol consumption observed ~10 days post-treatment were maintained at 9-months. It is notable, however, that at 9-months, the difference between groups was no longer significant and all groups significantly reduced their total alcohol consumption and frequency of use over the course of the study. Cross-group reductions likely reflect a Hawthorne effect (McCambridge, Witton, & Elbourne, 2014), and a desire to reduce alcohol consumption was a pre-requisite of study participation. Given the relatively young sample, this may further represent natural medium-long term situational and maturational factors. It is also notable that there was a moderate level of attrition (roughly 62% of participants completed the 9-month follow up), and it is likely that those who complete follow-up were either more motivated to reduce their drinking, or more motivated to complete follow-up having successfully reduced their drinking. Attrition was roughly equal across groups, and the lack of group differences at 9-months may therefore reveal a requirement for multiple treatment sessions, wherein multiple reactivation sessions may confer greater memory weakening (e.g. Brunet et al., 2011). Alternatively, this may reveal a requirement for adjunctive forms of psycho- or pharmacotherapy. Participants

in the current study did not receive any other forms of intervention into drinking. Thus, it is likely that upon completion of the study they reengaged in the situations that precipitated alcohol consumption prior to study participation.

An alternative mechanism for the current effect is memory integration or ketamine-induced state dependency. Rather than blocking reconsolidation, the subjective, psychoactive effects of ketamine may instead have been integrated into the original memory trace. Under a similar interpretation, the cue-alcohol memory is no longer accessible when the individual is not experiencing the effects of ketamine (Gisquet-Verrier & Riccio, 2018). While we cannot discount the latter possibility, an alternative for the former is suggested by the associations between blood-plasma ketamine and drinking-related outcomes. Measures of blood plasma ketamine correlated with drinking behaviour outcomes only in the retrieved ketamine group, suggesting greater blood-ketamine levels were associated with lower levels of drinking at test. The lack of correlation between sedative effects and change in alcohol consumption behaviour, and between disliking of drug effects and change in alcohol consumption behaviour further supports a reconsolidation-dependent explanation, as it would be expected that an aversive on-drug experience would produce a greater reduction in drinking behaviour under a state integration hypothesis. Interestingly, greater euphoric effects of ketamine were associated with a greater reduction in harmful patterns of alcohol use. Had these positive effects been integrated into the memory trace then we would speculate this would confer an *increase* in consumption. Importantly, while an integration account or reconsolidation-blockade account are both feasible, they are neither mutually exclusive, nor do they differ in the requirement for ongoing retrieval-induced memory plasticity. Thus, while mechanisms may be incompletely elucidated, the ability to interfere with established maladaptive memories remains, something that is extremely encouraging the future of treatments for AUDs.

In line with current evidence (e.g. Kolp et al., 2006; McAndrew et al., 2017; Sabino et al., 2013), ketamine was associated with a reduction in alcohol consumption even in the absence of reactivation. In this case, it is possible that enhancement of synaptogenesis (e.g. Li et al., 2010) or neurogenesis (e.g. Chambers, 2013) was responsible for a reduction in alcohol consumption in the NoRet-Ket group. Both the anti-depressant (Li et al., 2010) and anti-alcohol (Sabino et al., 2013) effects of ketamine are linked to mTOR functioning in pre-clinical models, suggesting a common neuroplasticity mechanism. However, while associations between the anti-depressant

effects of ketamine and serum levels of the neuroplasticity marker BDNF have previously been observed in humans (Haile et al., 2014, although see; Machado-Vieira et al., 2009), the current study did not observe an association between alcohol consumption and serum BDNF in the NoRet-Ket group. It is possible that associations would have been detected later, as the current study measured serum BDNF at 45-minutes post-administration, relative to 240-minutes as in Haile et al. (2014). Alternatively, as an increase in BDNF was detected only in those who responded to ketamine (experienced a reduction in depression symptoms), we might speculate that individual differences could also moderate BDNF's utility as a biomarker of anti-alcohol response. However, as BDNF was not related to any outcomes here, it is likely that peripheral BDNF is simply not sensitive enough as a measure of central variability to act as a valid biomarker.

It was posited that potential markers of neuroplasticity might also act as markers of memory reconsolidation. In rodent models, expression of BDNF is associated with both the consolidation of object recognition (Goulart et al., 2010) and the reconsolidation of conditioned taste aversion *(*Wang et al., 2012*)*, object recognition (Radiske et al., 2017), and fear extinction memory (Radiske et al., 2015). However, serum BDNF in the current sample did not differ between groups, suggesting a rise in BDNF was not detected in the group that putatively underwent reconsolidation (the Ret-PBO group). Again, it is possible that the current study simply did not detect an increase in BDNF as measures were taken too early. Alternatively, where an increase in BDNF was limited to the insular cortex following the reconsolidation of a conditioned taste aversion memory in rodents (Wang et al., 2012), plasma BDNF cannot reflect expression specific to isolated brain areas. The current results are, however, consistent with Lee, Everitt, and Thomas (2004), who observed a role for Zif268, but not BDNF, in the reconsolidation of a contextual fear memory, and vice versa for memory consolidation. Thus, the role of BDNF in reconsolidation remains unclear, although the results presented here suggest it is not a viable biomarker of the occurrence of human reconsolidation.

Despite the large literature on the rapid antidepressant actions of ketamine (e.g. Han et al., 2016; Kishimoto et al., 2016; Romeo et al., 2015; Serafini et al., 2014), the current study observed a reduction in negative affect in the Ret-Ket group only, suggesting this was not an effect of ketamine generally. Rather, this likely reflects changes in drinking behaviour, as withdrawal following alcohol consumption itself is associated with negative affect (Marsh et al., 2019; McKinney & Coyle, 2006). No other

group-specific changes in mood were observed, although this may be due to floor effects, as despite high co-morbidity between AUD and depression (e.g. Gilman & Abraham, 2001; Grant & Harford, 1995; Stohs et al., 2019), mean BDI scores in the current sample indicated minimal levels of depression. Alternatively, ketamine's effect on depression tends to be rapid and short acting, with meta-analysis suggesting anti-depressant effects peak one day following ketamine infusion, and are comparable to placebo 10-12 days later (Kishimoto et al., 2016). Given the test session occurred 10-14 days post-treatment in the current study, it possible that acute antidepressant responses were missed. While on- and post-drug measures of depression also failed to detect a group-specific reduction in depression, as these used a simple VAS, it is likely they were not sensitive enough to detect a change in mood.

4.4.1 Limitations

While the current findings suggest that retrieval followed by ketamine is a potentially effective way of enhancing the efficacy of ketamine as a treatment for AUD, it is important to consider the cost-benefit ratio of such a procedure. A significant barrier to clinical implementation is the current legal status, highly psychomimetic nature of ketamine and the requirement of highly qualified medical personnel to administer the drug. Ketamine currently needs to be administered by an anaesthetist within a hospital setting and consequently, is resource intensive. This is particularly apparent when compared to other pharmacological reconsolidation blockades including propranolol and N_2O, both of which have excellent safely and side effect profiles, allowing prescribed treatments to be either self-administered or administered by healthcare staff with more limited training. However, given that the current treatment was administered only once and was associated with a halving of alcohol consumption at 9-months, the costs relative to the alcohol-related harms that may accrue over that period are likely to be small.

At the time of writing an intranasal preparation of one of ketamine's isomers (s-ketamine) has been approved by the FDA for use in treatment-resistant depression (Johnson & Johnson, 2019), and is currently in phase 3 trials in the UK (NIHR, 2019). In-vitro, s-ketamine is associated with greater NMDAR inhibition relative to the r-ketamine enantiomer (Molero et al., 2018; Ulrich Zeilhofer, Swandulla, Geisslinger, & Brune, 1992) and has greater affinity for the NMDAR relative to ketamine (Persson et al., 2002). Thus, in the current study with racemic ketamine, we may have used a 'suboptimal' version of the drug. Further, although the antidepressant effects of s-

ketamine and racemic ketamine are comparable (Paul, Schaaff, Padberg, Moller, & Frodl, 2009), s-ketamine may be associated with fewer psychoactive side effects *(*Molero et al., 2018*).* This improved tolerability, coupled with an intranasal route of administration means treatment could take place outside of a hospital setting. It will be important to assess equivalency or otherwise of these different isomers of ketamine in future research. Whether a single administration of intranasal ketamine would produce comparable effects to a 30-minute, continuous intravenous infusion as used in the current protocol is unclear, as intranasal administration is associated with half of the bioavailability of that during intravenous administration (Trimmel et al., 2018). An 84-mg intranasal dose of s-ketamine did, however, reach comparable blood-plasma levels to 0.2mg/kg of intravenous s-ketamine (Daly et al., 2018). As such, intra-nasal s-ketamine may potentially be an easy to administer analogue for ketamine as used in the current protocol. Identifying the optimal dose for reconsolidation blockade would therefore be a key consideration for the clinical development of this approach.

While the current study incorporated a placebo group and infusions were intended to be single blind, all participants in the ketamine groups correctly identified that they had received the drug, while only two in the placebo group guessed that they received ketamine. Consequently, participants were not blind to drug condition. The reductions in drinking behaviour unique to the Ret-Ket group, and associations between markers of plasticity and blood plasma ketamine level suggest reconsolidation blockade as the method through which memory strength was attenuated in the Ret-Ket group. However, it is possible that the smaller effects of ketamine in drinking behaviour in the NoRet-Ket group will be the result of a non-mnemonic effect. Indeed, greater disliking of ketamine effects was predictive of a greater reduction in harmful drinking patterns in the NoRet-Ket group, suggesting a non-mnemonic effect may underlie the behavioural change in this group. Active placebos are frequently used when drugs have a strong-side effect profile, although their use is not well suited to reconsolidation research. Experience of aversive side effects following retrieval may result in the integration of these effects into the original memory trace, meaning any effects on memory weakening may be attributable to an aversion/ counter-conditioning mechanism, rather than a placebo effect. As in the case of the current study, use of biomarkers such as blood-ketamine levels may elucidate the mechanisms by which an effect was observed. The lack of a relationship between behavioural outcomes and

markers of neural plasticity in the current sample, for example, may indicate that reductions in drinking in the NoRet-Ket group are attributable to a placebo effect.

The current study utilised a population of hazardous beer drinkers, at risk of developing alcohol-use disorders. There may be fundamental differences between hazardous drinkers (such as those in the current study), and those with SUD. Firstly, although the current sample consumed very significant amounts of alcohol (a mean average of 74 units per week) and were 2-3 times over the AUDIT threshold for hazardous drinking, it is possible that the maladaptive cue-alcohol memories formed in a clinical population will be stronger, having undergone a greater number of conditioning trials. Secondly, a learning model of human addiction ignores additional factors that can contribute to the development of alcohol addiction, including genetic factors such as those discussed in *Chapter 3* (Walsh et al., 2017). Given the significant reduction in drinking produced by the current protocol, clinical trials within a population of individuals with clinical alcohol use disorders is a clear next step.

In sum, intravenous ketamine, when administered after cue-alcohol memory retrieval, resulted in significant reductions of alcohol consumption in a population at risk of developing alcohol dependency. Importantly, reduced alcohol consumption was maintained at nine-month follow-up, suggesting the current procedure was resistant to relapse. Clinical trials are warranted to explore the efficacy of the current protocol in a clinical population.

Chapter 5.

Pre-retrieval rapamycin fails to block the reconsolidation of natural appetitive reward memories in individuals with a sub-clinical propensity to binge or overeat chocolate

5.1 Introduction

Chapters 3 and 4 of the current thesis have focused primarily on reconsolidation interference as a method to attenuate the maladaptive appetitive memories associated with alcohol addiction and abuse. However, just as drugs of abuse become associated with cues that predict their effects, we also form associations between environmental cues and the consumption of non-drug rewards such as food. The current chapter extends the examination of reconsolidation mechanisms from *Chapter 3* and *4* to the non-drug related associative memories that may underlie a propensity to binge or overeat highly palatable foods (HPFs).

Binge Eating Disorder (BED) is characterised by recurrent engagement in food binges, in which a large quantity of food is consumed in a discrete period of time, with no accompanying compensatory behaviour, such as purging or excessive exercising (American Psychiatric Association, 2013). Commonly, these binges are coupled with a feeling of loss of control and are followed by feelings of distress or guilt that drive a subsequent period of restriction. BED is the most commonly diagnosed eating disorder, with lifetime prevalence estimates at 3.5% in women and 2% in men (Hudson, Hiripi, Pope, & Kessler, 2007). In those seeking weight control treatment the prevalence rate may be as high as 30% (Spitzer et al., 1992a). Unsurprisingly, between 40-70% of those with BED are obese (Dingemans & van Furth, 2012; Hudson et al., 2007; Kessler et al., 2013).

While there is considerable debate about the existence of 'food addiction' or, less commonly 'eating addiction' (e.g. Avena et al., 2008; Benton, 2010; Bruinsma & Taren, 1999; Davis & Carter, 2009; Hebebrand et al., 2014; Schulte et al., 2015; Wilson, 1991, 2010; Ziauddeen & Fletcher, 2013), eating is a necessary consummatory behaviour and food consumption is naturally rewarded via the dopaminergic motivational system (Kelley & Berridge, 2002). These characteristics of food distinguish it from addictive drugs, although both food and drugs are capable of 'hijacking' the brain systems responsible for natural rewards (Kelley & Berridge, 2002; Robbins & Everitt, 2002). This is particularly the case for 'hyper-palatable foods' (HPF): foods high in fat and sugar (Schulte et al., 2015). Chocolate, for instance, represents the most frequently craved food (Hetherington & MacDiarmid, 1993; Hill & Heaton-Brown, 1994; Pelchat, 1997; Rozin, Levine, & Stoess, 1991), particularly among women. As with the maladaptive memories underlying substance use disorders (SUD), the hedonic

properties of food can become associated with cues that reliably predict these rewarding effects. Subsequent exposure to these cues can therefore trigger conditioned responses, such as craving (Volkow & Wise, 2005; Wang, Volkow, Thanos, & Fowler, 2004), hedonic hunger (eating in the absence of hunger), and the overconsumption of highly palatable and energy dense foods (Tuomisto et al., 1999).

Food cravings are particularly high in those with BED (Mussell et al., 1996), and exposure to food-related cues can trigger a bingeing episode (Legenbauer, Vogele, & Ruddel, 2004; Sobik, Hutchison, & Craighead, 2005). While avoidance of 'trigger foods' may temporally alleviate the symptoms of BED, given the ubiquity of food (cues) in the modern environment, avoidance serves only to perpetuate the 'binge-restrict' cycle that typifies BED. Indeed, restrained eaters show higher sensitivity to visual and olfactory cues, and report higher liking and craving for HPFs relative to non-restrained eaters (Brunstrom, Yates, & Witcomb, 2004; Fedoroff, Polivy, & Herman, 2003; Fedoroff, Polivy, & Herman, 1997; Legoff & Spigelman, 1987; Papies, Stroebe, & Aarts, 2007). Similarly, higher levels of dietary restraint are predictive of relapse following treatment for BED (Safer, Lively, Telch, & Agras, 2002).

Given the pivotal role of maladaptive reward memories in disorders of reward as outlined in *Chapter 1*, weakening of the associative memories that underlie binge eating may therefore serve to reduce HPF consumption and binging frequency without overtly increasing restriction in those with BED. Disruption of the reconsolidation of naturalistic cue-reward memories has previously been demonstrated in rodents (Milton et al., 2008b), suggesting the reconsolidation of both natural and addictive drug reinforcers is dependent on the similar neurobiological mechanisms. In the study by Milton et al (2008b), sucrose was paired with the US as a part of a conditioned-reinforcement procedure. Following retrieval of the conditioned appetitive memories, systemic propranolol administration resulted in attenuated sucrose seeking, relative to rats who received saline after retrieval, or received propranolol without retrieval. Memory reconsolidation is therefore proposed as a memory plasticity mechanism through which cue-food memories and induced food cravings may be weakened.

5.1.1 Role of mTOR pathway in memory reconsolidation

While the pharmacological agents used in *Chapters 3* and *4* to block reconsolidation targeted the NMDAR, animal studies have additionally demonstrated reconsolidation interference via blockade of cellular processes downstream of glutamatergic receptor

sites. The mammalian target of rapamycin (mTOR) is a serine/threonine kinase associated with protein synthesis and degradation (Bockaert & Marin, 2015; Guertin & Sabatini, 2009; Hoeffer & Klann, 2010). As such, the mTOR pathway is critically involved in synaptic plasticity and regulates the strengthening and weakening of the neural connections underlying memory (Helmstetter, Parsons, & Gafford, 2008; Roesler, 2017). Animal models suggest post-retrieval administration of sirolimus – also known as rapamycin (an inhibitor of mTOR1 used clinically as an immunosuppressant) - is associated the disruption of consolidation and reconsolidation of cued auditory fear memory (Mac Callum, Hebert, Adamec, & Blundell, 2014; Parsons, Gafford, & Helmstetter, 2006b), contextual fear memory (Blundell et al., 2008; Gafford et al., 2011; Glover, Ressler, & Davis, 2010; Jobim et al., 2012a; Pedroso et al., 2013; Stoica et al., 2011), and long-term retention of trace fear memory object recognition memory (Jobim et al., 2012b; Myskiw et al., 2008). However, the effects of rapamycin on the reconsolidation of fear memories may not be observed across all forms of cue-fear associations. Glover et al. (2010), for instance, showed that rapamycin had no retrieval-dependent effect on fear (startle response) when an odour cue was used.

Few studies in rodents have assessed the impact of rapamycin on the reconsolidation of reward memories. However, activation of the mTOR pathway in the prelimbic and orbitofrontal region of the prefrontal cortex is associated with the reactivation of alcohol-associated memories in rats (Barak et al., 2013). Post-retrieval infusion of rapamycin to this area is associated with reduced alcohol seeking and consumption as assessed by reduced lever press in rapamycin relative to vehicle treated rats (Barak et al., 2013). Lin et al. (2014) similarly demonstrated an attenuation of morphine, alcohol, and cocaine conditioned-place preference following systematic post-retrieval rapamycin treatment.

To date, only one clinical study has explored the efficacy of rapamycin as a blocker of reconsolidation in humans (Suris et al., 2013). In this study, veterans with PTSD were given rapamycin (or placebo) prior to the retrieval of trauma memories (administration occurred prior to retrieval to ensure adequate plasma levels during the reconsolidation window, as rapamycin achieves peak levels slowly). While no overall effect was observed, PTSD symptoms as assessed by the Clinician Administered Diagnostic scale reduced in Veterans who more recently experienced trauma, relative to those who developed PTSD many years prior. The authors suggest that newer memories may therefore be more susceptible to disruption via rapamycin, although it is notable that

these reductions in symptomology were not maintained at three-month follow-up. The long and variable retrieval procedure means it difficult to ascertain whether null results were due to a lack of reconsolidation blockade by rapamycin, or if the retrieval procedure was not effective in destabilising trauma memories (owing to its length, it may have triggered extinction, rather than reconsolidation).

Given the promising effects of rapamycin on reconsolidation in the animal literature, and the current lack of studies in humans, exploration of the efficacy of rapamycin as a blockade of reconsolidation in humans is warranted and timely. The current study explored reconsolidation of non-drug appetitive reward memories in healthy participants with a non-clinical propensity to overconsume or binge on chocolate. Following retrieval (and putative destabilisation) of associative chocolate reward memory, 10mg of sirolimus was expected to attenuate indices of reward memory strength, namely: attentional bias towards chocolate-related cues, chocolate craving, chocolate consumption, and motivation to earn chocolate. It was expected that those in the control conditions (chocolate reward memory retrieval paired with placebo, and non-chocolate control memory retrieval paired with 10mg of rapamycin) would not experience any weakening of associative reward memory strength.

5.2 Methods

5.2.1 Participants

Seventy-five regular (>3x /month) over-consumers of chocolate (21 male), defined as >20 such overconsumption episodes over their lifetime, were recruited using online advertisements and convenience sampling. Additional inclusion criteria included: a score ≥45 on the Food Cravings Questionnaire-chocolate-trait (FCQ-chocolate-t; Meule, 2018); normal, or corrected to normal colour vision; age <18>45; blood pressure <145/90 mmHg; and fluent spoken English. Participants were informed that they would be required to consume chocolate and strawberries; fast for four hours prior to each session; and provide blood samples via finger-prick. Exclusion criteria consisted of: undergoing current treatment for an eating disorder or other psychiatric problem; binging ≥ 3/month; compensatory behaviours for binging (e.g. vomiting, laxative use, thyroxin or dieting pills); highly restrictive dietary requirements (veganism, Jainism, food allergies); BMI<18.5>45; major physical health problems/ contraindications to rapamycin including diabetes, cancer, heart, autoimmune or liver disease; recent cold of flu (<3 weeks); family history of long QT syndrome; use of blood-sugar-control medication (e.g. insulin, or metformin) or statins; an allergy to Macrolides; recreational use of drugs (>1x /week); weekly consumption of >30 UK units of alcohol (240g); and pregnancy or breastfeeding. Participants were reimbursed £60 upon completion of the study and a further £10 following completion of the follow-up questionnaire. All procedures were approved by the UCL Research Ethics Committee and were in accordance with the Declaration of Helsinki and UK General Data Protection Regulation.

5.2.2 Study Design

In a double blind between groups design, participants were randomly assigned to one of three groups via block randomisation: retrieval with rapamycin (RAP-Ret, N=25), retrieval with placebo (PBO-Ret, N=25), and no retrieval with rapamycin (RAP-NoRet=25). Laboratory procedures were conducted over the course of three sessions (baseline: Day 1, retrieval: Day 1 + 48 hours, test: Day 1 + 10-14 days) with measures repeated within-subjects, and memory retrieval procedures/drug treatment differing between groups. Additional outcomes were assessed at a long-term follow-up time-point (Day 1 + 1 month)

5.2.3 Apparatus and Tasks

5.2.3.1 Self-Report Assessments

Chocolate consumption and binging

Chocolate consumption in the two weeks preceding the first session (‘*baseline’*), one week prior to the third session (‘*test’*), and the two weeks prior to the one-month follow up, were recorded using the Time Line Follow Back (TLFB; Sobell & Sobell, 1992). A graphical depiction of typical quantities of chocolate was provided to help participants gauge quantity (in grams) of chocolate consumed. Daily chocolate consumption was additionally recorded using a daily diary, which participants were prompted to complete after their final meal or snack at the end of the day via text and email. The diary was completed daily for one week preceding their first session, and for two weeks following the first session. For the purposes of analysis, diary data was collapsed into the week prior to the retrieval and drug administration session (1-week pre), the first week immediately following the retrieval and drug administration session (1-week post), and the second week following the administration session (2-weeks post). In addition to quantity of chocolate, participants were instructed to indicate their highest level of chocolate craving, whether they overconsumed or binged on chocolate, or if they overconsumed or binged any other foods on the given day.

Chocolate craving was assessed using the chocolate version of the trait and state Food Cravings Questionnaire (FCQ-chocolate-t/s; Meule, 2018). Attitudes towards chocolate consumption were measured using the Attitudes to Chocolate Questionnaire (ACQ; Benton, Greenfield, & Morgan) which is comprised of three subscales; Craving, Guilt, and Functional Approach (the latter subscale describes the ‘functional’ use of chocolate as an energy source when a meal is missed or exercise is undertaken).

Cue-reactivity

The cue-reactivity task used in *Chapter 3* and *4* was adapted to include food-related images, consisting of nine chocolate images (nominated ‘Chocolate’ cues) and five images of low palatability food (‘Neutral’ cues). Participants selected a 30-gram bar of chocolate prior to rating, which was then present within the eye line of the participant for the duration of the task. For each image, participants responded to the following questions on a 101-point on-screen sliding visual analogue scale: ‘how pleasant does the food depicted in the image look’ (0=extremely unpleasant, 50=neither pleasant or unpleasant, 100=extremely pleasant); referring to the in vivo chocolate bar: ‘thinking

back to a time when you have eaten the food in the image, how does that memory effect your desire to eat the chocolate in front of you' (0=greatly reduces desire, 50=no effect on desire, 100=greatly increases desire); and finally, 'how likely would you be to overeat the food depicted in the image' (0=extremely unlikely, 50=neither unlikely or likely, 100=extremely likely). After completing the image rating, participants provided pleasantness, wanting, and likelihood of overeating ratings for the in vivo chocolate bar itself (rated in the same manner as the on-screen images). Following guided consumption of the in vivo chocolate cue, participants rated 'how pleasant they found the chocolate they just ate' and 'how much would they like more of what they just ate'.

Disordered-Eating-related measures

Specific binge eating symptomology was assessed using the Binge Eating Scale (BES; Gormally, Black, Daston, & Rardin, 1982). The Three-Factor Eating Questionnaire (TFEQ; Karlsson, Persson, Sjostrom, & Sullivan, 2000) assessed eating behaviour across three constructs; 1) Dietary restraint, which describes a tendency to constantly and consciously restrict food intake; 2) Uncontrolled eating, which describes a tendency to overeat; and 3) Emotional eating, which refers to eating in response to negative emotions.

The Revised Restraint Scale (RRS; Herman & Mack, 1975; Herman & Polivy, 1980): identified restrained eaters across three factors: Weight fluctuation, Concern with dieting, and Food consciousness.

The Intuitive Eating Scale (IES; Tylka & Kroon Van Diest, 2013) assesses an individual's ability to follow internal hunger and satiety cues when deciding what and how much to eat across three subscales: eating for physical rather than emotional reasons (Physical); unconditional permission to eat (Permission); reliance on internal hunger and satiety cues (Internal); and Body-food choice congruence (Choice), which describes an individual's ability to match their food choice to their bodies' need. The psychological influence of food-environments was assessed using the Power of Food Scale (POF; Lowe et al., 2009).

Heritability of eating disorders was measured using a brief assessment of family history, in which participants indicated if any first- or second-degree relatives had experienced an eating disorder or disordered eating behaviour.

Drug-related psychological changes

Subjective response to rapamycin was assessed using a visual analogue scale-based Bodily Symptoms Scale (BSS; Bond & Lader, 1974).

5.2.3.2 Behavioural assessment

Attentional Bias

The motivational salience of chocolate cues was assessed via eye movement metrics during a visual probe task. Behavioural responses were collected to ensure task engagement but not analysed. On each trial, one of eight colour chocolate images (nominated 'Chocolate' cues) were presented side by side, with one of eight low palatability food images ('Neutral' cues) rendered at 300x300 pixels. As described in *Chapter 3* and 4 (see *Figure 3.1* on page 97 for a graphical depiction of the task) each dot probe trial involved simultaneously presenting each image type for 2000ms after a fixation cross (to correct drift, trial began when eye was fixed on cross). After image offset, a triangular probe appeared in the location of one of the images. Each Chocolate image was paired with each Neutral image twice across the task, with image location (left/right) counterbalanced across the 128 trials (64 trail combinations x 2). Order of trial presentation was randomised. Participants were instructed to indicate the direction of a triangular probe (up/down) as quickly and accurately as possible. Eye-movements were recorded using a Tobii T-60 eye tracker (Tobii AB; Danderyd, Sweden) at a sampling rate of 60Hz. Head position was stabilised using a chin-rest 60cm from the 1024x764 monitor. The cue-reactivity task yielded three measures for Chocolate and Neutral cues: latency to first fixation, first fixation duration, and dwell time (total time gaze was within the bounds of the image within trial).

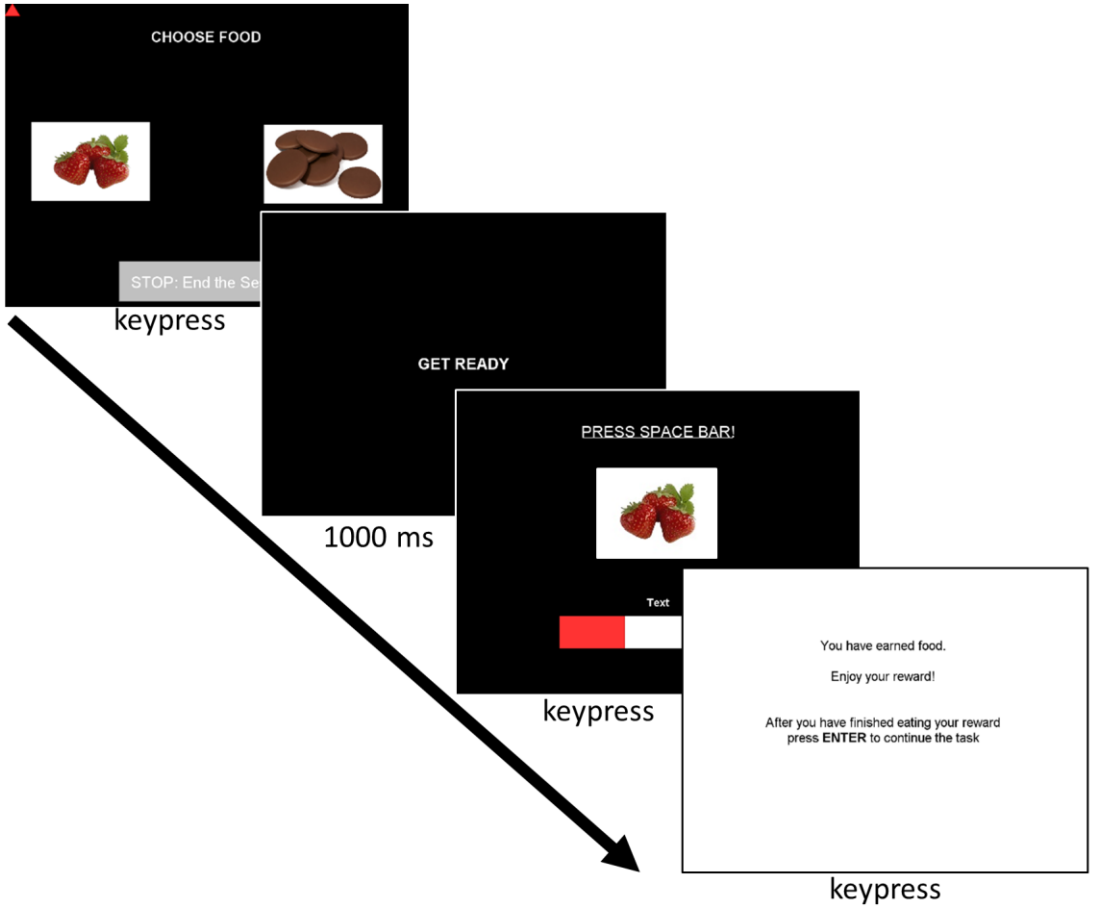
Motivation to earn chocolate

Motivation to acquire chocolate reward was assessed using a progressive ratio task (*Figure 5.1*). In each trial, participants could choose to earn either chocolate (a single chocolate button; Cadbury Diary Milk buttons, Bournville UK) or a slice of freeze-dried strawberry of approximately equal weight to a chocolate button (MSK Ingredients Ltd.). A small bowl of each food-type was present for the duration of the task and regularly topped up throughout the task. Following selection of food type, participants were required to tap the spacebar until an onscreen bar was filled. Each trial was time limited, with the limit increasing as the number of presses increased (equivalent to three key presses per second, i.e. trials where 30 presses were required had a time limit

of 10 seconds). Participants only 'won' the food if they completed the required number of presses in time. A progressive ratio schedule was used, requiring 20 additional key presses upon successful completion (i.e. reinforcement) of the preceding trial. Number of presses remained the same as the previous trial if that trial was not reinforced. At the end of each trial participants rated their current hunger level and, if they were reinforced, also rated pleasantness of the reinforce ("how pleasant was the food you just ate") on a 101-point on-screen visual analogue scale as an index of the hedonic value of the food consumed. Trial number was not fixed, and participants could choose to end the task at any point. The progressive-ratio task generated two main outcome variables each for Food Type (chocolate and strawberry): 1) the breakpoint, i.e. the trial number at which a participant ended the task; and 2) a motivation index of that cue, calculated as a function of reaction time for each trial (RT) and total number of trials (N):

$$MI = \frac{1}{RT * N}$$

Figure 5.1. *Schematic of the progressive ratio task*



At the beginning of each trial participants could choose to play for a strawberry or chocolate reward. Following selection and a screen instructing participants to 'get ready', participants were required to press the spacebar within a select amount of time in order to fill the red bar. If participants completed the required number of presses within the set time limit, they could eat their chosen reward. Participants then rated how pleasant they thought the food was, and then how hungry they were. The number of presses required increased after every successful trial on the progressive ratio schedule. The task was self-timed, such that participants could choose to end the task at any time.

5.2.3.3 Memory Retrieval

Cue-elicited chocolate memories were retrieved using a short-form of the chocolate cue-reactivity task described above, followed by negative prediction error-generating procedure. Those in the RAP-Ret and PBO-Ret groups again selected a 30g bar of chocolate to consume, and then rated six chocolate images ('Chocolate' cues). Those in the RAP-NoRet group were given an equivalent weight of dried strawberry slices and rated six, low palatability images (i.e. fruit, salad, vegetables; nominated 'Neutral' cues). All images were presented for a minimum of 10 seconds, and required participants to rate 'liking', 'wanting', and 'likelihood of overeating' the depicted food. Participants then rated the 30g in vivo chocolate (or a slice of freeze-dried strawberry for the RAP-

NoRet group) cue, present for the duration of the cue-reactivity task, on liking, wanting, and likelihood of overeating. Prediction error (PE) was elicited in the retrieval groups (RAP-Ret and PBO-Ret) via on-screen instructions telling participants to "Pick up food", "Prepare to eat food", and then unexpectedly "STOP! Do not eat food". The in the RAP-NoRet group viewed screens instructing them to "Pick up food", "Prepare to eat food", and "Eat food now". Immediately following the PE / no PE procedure participants were asked "how surprising did you find what just happened" using a 101-point onscreen visual analogue scale between "not at all" and "extremely surprising".

5.2.3.4 Distraction Tasks

To disengage participants with the retrieval procedure, retrieval was immediately followed by forward and backwards digit span, prose recall (Rivermead Behavioural Memory Test), and trail-making tasks. These tasks lasted an approximate 10 minutes but were not analysed, as they served only to ensure working memory disengagement from the retrieval procedure.

5.2.3.5 Physiological measures

Blood pressure (BP) and heart rate (HR) was measured using an Omron blood pressure monitor. Blood glucose was assessed using a finger-prick test (SDCheck, SD Biosensor, Republic of Korea) and BMI was calculated in lab following recording of height and weight.

5.2.3.6 Drug Administration

All drug manipulations were double blind, such that the experimenter collecting and analysing data and participants themselves were unaware of the condition to which they were assigned. A 10mg oral rapamycin tablet (Rapamune; Pifter Limited) or closely matched placebo (multivitamin, Boots, UK) was administered 50 minutes prior to the initiation of the retrieval procedure. Administration was timed to allow peak plasma concentrations to coincide with the prediction error procedure (at one hour; Brattstrom et al., 2000), such that drug administration occurred 50-minutes prior to the cue-reactivity task and 1-hour prior to the prediction-error procedure (see *Figure 5.2* for full details on protocol timings). Due to difficulties obtaining an exactly matched placebo, participants were blindfolded, and a researcher not involved in data collection (only present for drug/placebo administration) administered the rapamycin or placebo.

Rapamycin (known also as sirolimus) is typically prescribed for its immunosuppressant effects and can have side effects including (but not limited to)

headaches, an increased susceptibility to infection, inflammation, weakness, rapid heart rate, nausea, and increased blood pressure. These side effects are typically associated with the accumulation of drug in the bloodstream, and no side effects have been reported in studies where a single dose was administered (Shi et al., 2009; Suris et al., 2013). Nevertheless, participants were informed of all potential side effects and acute heart rate and blood pressure of each participant were monitored following administration. The medical supervisor was available for consultation and participants were provided with a list of potential side effects and the attending doctor and experimenter's details before leaving. They were instructed to contact the medical doctor if they experienced any symptoms (see *Appendix item 11*).

5.2.4 Procedure

Procedures took place over the course of three in-person sessions, with one remote follow-up. A full schematic of the procedure is presented in *Figure 5.2*.

Screening (minimum 7 days prior to Day 1)

To assess eligibility, potential participants completed an online questionnaire (hosted on Qualtrics, Provo, Utah). Questions regarding health problems, contraindications to rapamycin, frequency of chocolate consumption, frequency of overeating (defined as "more than planned or till uncomfortably full") and frequency of bingeing (defined as "eating very large portions of food all at once until you feel uncomfortably full, and then often feeling upset of guilty. Binges are often planned in advance and may involve "special" binge foods. Binges normally involve a feeling of loss of control, where people stop eating only once they run out of food or feel physically sick or unwell"). The FCQ-chocolate was additionally completed at screening.

Those who met study criteria based on the online screening were additionally telephone screened to ensure eligibility. Prior to attending the first in-lab session, participants were required to complete a food diary for one week. Reminder emails and texts were sent at 8pm each day with instructions to complete the questionnaire after their final meal or snack. Participants were instructed to fast for four hours (only clear liquids) and not consume alcohol or other substances in the 12 hours prior to each testing session. The current study took place over the course of three sessions, with follow up occurring one month after the test session.

Day 1 (baseline)

After providing written informed consent, participant's weight, height, blood pressure, and blood glucose levels were obtained. Basic information, including education level, age and smoking status were then recorded. Participants completed computer-based questionnaires in the following order: current hunger level, Family History of Eating Disorders, Revised Restraint Scale, Binge Eating Scale, Power of Food Scale, Intuitive Eating Scale, Three Factor Eating Scale, Beck's Depression Inventory, Behavioural Inhibition and Activation Scale, State and Trait Anxiety, Attitudes to Eating Chocolate, and Timeline Follow Back questionnaires. Cue-reactivity, visual probe, and break point tasks were then completed.

Day 2 (retrieval and drug administration/manipulation session; Day 1+48 hours)

Following measurement of HR and BP, rapamycin (RAP-Ret and RAP-NoRet) or placebo (PBO-Ret) was administered in a double-blind fashion. The baseline Bodily Symptoms Scale (BSS) was then administered. On completion, participants could engage in quiet activities, e.g. watch a video or read a book. BP, HR, and subjective symptomology were measured at 20-minute intervals.

50 minutes after rapamycin/placebo administration, the retrieval and PE procedures were administered. Immediately following the retrieval and PE procedure a series of distractor tasks lasting approximately 10 minutes were then administered. On-drug BSS and drug effects were administered, and HR, BP, and subjective symptoms were checked. Participants were again allowed to engage in quiet activities for one hour, to ensure drug response measures could be monitored. Before leaving, participants completed a final BSS, and a final BP and HR check was conducted.

Day 3 (test; Day 1 + 10-14 days)

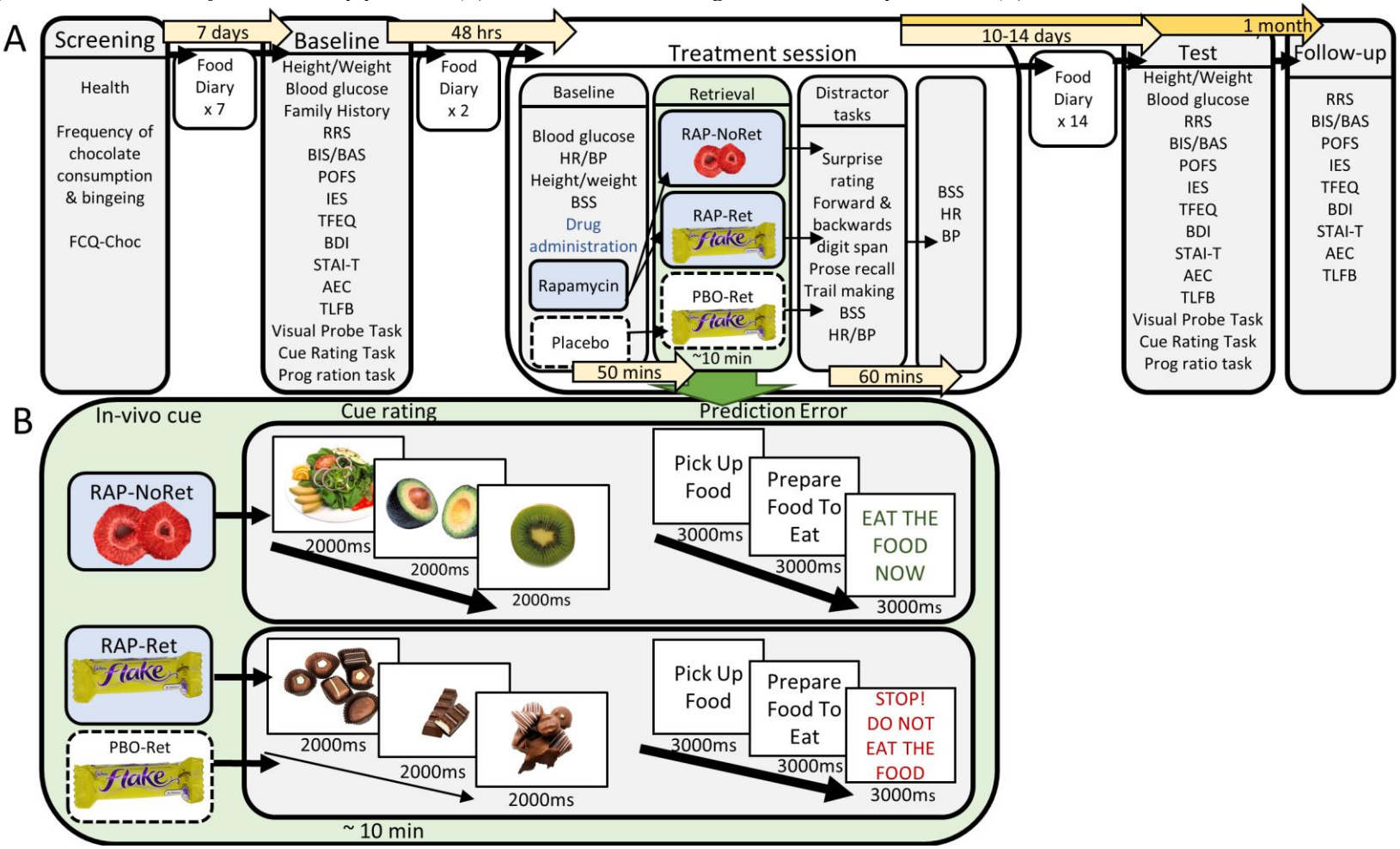
Participants returned to the laboratory and completed identical procedures to baseline. Participants were required to guess which drug condition they received, after which they were debriefed and reimbursed.

Follow-up (Day 3 + 1 month)

One month after the test session, participants completed the Attitudes to Eating Chocolate, State and Trait Anxiety, Behavioural Inhibition and Activation Scale, Beck's Depression Inventory, Timeline Follow Back, Intuitive Eating Scale, Power of Food Scale, and Revised Restraint Scale via online questionnaire (hosted on Qualtrics).

Participants were additionally asked “How much chocolate have you been eating compared to normal?” and “How much have you felt the urge to eat chocolate compared to normal?”, each answered on a 1 (a lot less than normal) to 6 (a lot more than normal) scale.

Figure 5.2. *Schematic of the three-day protocol (A) and retrieval and drug administration procedure (B).*



During the retrieval procedure (B) participants rated four six (if assigned to the RAP-Ret and PBO-Ret groups) or low palatability image cues (RAP-NoRet). Prediction Error was then generated in the RAP-Ret and PBO-Ret groups

5.2.5 Statistical Approach

G*Power 3.1.9 (Faul, Erdfelder, Lang, & Buchner, 2007) was used for the sample size calculation which was based on detecting a minimally meaningful effect size of f=0.2 in reducing chocolate craving and overconsumption. Based on 90% power to observe this effect in a mixed 3 (baseline period, test period, follow-up period) x 3 (Group) ANOVA, with α =0.05. This calculation assumed a repeated measures correlation of ρ=0.5, assuming sphericity. This yielded a total required N of 69 (23 per group) for 0.906 power. Expecting minimal attrition, we aimed to recruit 75 total participants (25 per group). Data were analysed using IBM Statistical Package for the Social Sciences (SPSS) v.25 for Windows. All data were checked for normality, homogeneity of variance and sphericity where appropriate. Outliers (>3 SD from the group mean) were winzorised. Eye tracking data were extracted and processed using Matlab R2018a. Trials were discarded if a blink was recorded during recording (identified as >25% of the trial), and when latency to first fixation data was <100ms.

Researchers were blind to groups during data analysis. One-way ANOVA is reported for group differences at baseline. A conservative threshold of α=0.01 was selected due to multiple comparisons (k>20) and a threshold of α=0.05 was selected for all other analyses. Where Greenhouse-Geisser procedures estimated epsilon as <0.75, Pillai's trace is reported (donated by the use of 'V' following the test statistic).

Factorial mixed ANOVAs, with Group (RAP-Ret, PBO-Ret, RAP-NoRet) as a between subjects factor and Day (baseline; test; follow-up) as a within subjects factor were used for primary (chocolate consumption and craving) and secondary outcomes (attentional bias, cue-reactivity, motivation to earn chocolate, and binge eating disorder symptomology). Specifically, cue-elicited liking, wanting, and overeating ratings were analysed using 2 (Day) x 3 (Group) x 2 (Cue type) mixed ANOVA, and ratings for the in vivo chocolate cue itself with 2 (Day) x 3 (Group) ANOVA. Dwell, first fixation latency, and first fixation duration data generated by the dot probe task were analysed using 2 (Day) x 3 (Group) x 2 (Cue type) factorial ANOVA, while questionnaire data were assessed using 3 (Day) x 3 (Group) ANOVA owing to follow-up data. Where significant main effects of Group were observed, post-hoc pair-wise tests were applied with Bonferroni-corrections. Main effects of Day were followed up with simple contrasts with baseline as the reference point. Group x Day interactions were followed up with an analysis of group differences at baseline and if none were observed, an

analysis of the simple effects of Day within each group was conducted. All data collection and analytic procedures as detailed here were pre-specified and pre-registered on the Open Science Framework database (*http://osf.io/tqxdb*) prior to data collection and all primary analysis were conducted with the analyst blind to condition.

5.3 Results

5.3.1 Baseline chocolate and demographic data

Groups did not differ on any baseline demographic, chocolate consumption, craving, disordered eating symptomology, attitudes to chocolate or mood-related measures (*Table* 5.1). All *ps*>0.122 (*ps* based on an adjusted α=0.01 to account for multiple comparisons).

Table 5.1. *Baseline demographic, chocolate consumption, disordered eating symptomology, and questionnaire measures*

	PBO-Ret N=25	RAP-NoRet N=25	RAP-Ret N=25	$F_{1,73}$	*p*	η^2
Gender (N of males)	7	6	8	0.191	0.826	0.005
Age	26.00±5.36	26.08±5.46	25.20±5.51	0.200	0.937	0.006
FCQ-chocolate-trait	61.00±9.44	55.84±9.38	59.04±12.04	1.579	0.213	0.042
RS *Concern*	5.92±2.25	6.28±2.834	5.96±2.54	0.149	0.862	0.004
RS *Weight fluctuation*	5.20±3.37	4.32±2.32	4.77±2.86	0.585	0.560	0.016
RS *Total*	11.12±4.95	10.60±4.28	10.76±5.04	0.078	0.925	0.002
BAS *Drive*	8.48±2.22	9.04±2.42	8.84±2.50	0.355	0.703	0.010
BAS *Fun*	7.48±2.38	7.76±2.52	7.40±2.10	0.163	0.850	0.005
BAS *Reward*	6.96±1.51	7.96±2.03	7.32±1.57	2.164	0.122	0.057
BAS *BIS*	13.56±2.50	14.52±2.28	13.88±2.37	1.051	0.355	0.028
Power of Food	3.40±0.75	3.25±0.64	3.46±0.82	0.537	0.587	0.015
IES *Permission*	3.52±0.56	3.33±0.68	3.51±0.57	0.795	0.456	0.022
IES *Physical*	2.87±0.93	2.55±0.72	2.62±0.67	1.188	0.311	0.032
IES *Internal*	3.47±0.95	3.22±0.97	3.28±0.93	0.595	0.554	0.016
IES *Choice*	3.53±1.00	3.59±0.69	3.20±0.91	1.433	0.245	0.038
IES *Total*	3.32±0.58	3.08±0.59	3.12±0.54	1.224	0.300	0.033
Binge Eating Scale	31.60±8.73	31.48±7.60	31.80±7.61	0.012	0.988	0.000
BDI	5.40±5.61	5.96±5.47	5.36±5.18	0.095	0.909	0.003
BIS	70.92±8.35	69.40±6.98	70.44±5.37	0.308	0.736	0.008
TFEQ *Restraint*	36.44±19.72	37.85±20.70	40.79±17.47	0.362	0.698	0.010
TFEQ *Uncontrolled*	56.44±17.54	54.75±12.18	58.07±16.69	0.328	0.721	0.009
TFEQ *Emotion*	49.33±31.77	57.49±23.37	54.67±24.62	0.577	0.564	0.016
TFEQ *Total*	48.59±13.59	49.57±12.75	51.75±12.12	0.418	0.660	0.011
STAI *Trait*	46.96±4.35	46.20±3.70	46.76±4.07	0.237	0.790	0.007
ACQ *Craving*	61.54±17.92	67.08±18.50	65.92±16.55	0.683	0.508	0.019
ACQ *Guilt*	44.48±19.98	47.24±19.53	45.39±19.68	0.127	0.881	0.004
ACQ *Functional*	55.99±14.83	55.72±15.44	55.39±20.24	0.008	0.992	0.000
TLFB *Consumption*	50.87± 33.79	51.00±24.30	49.68±33.18	0.026	0.974	0.001
Diary *Consumption*	69.16±47.77	65.40±32.90	71.80±55.48	0.120	0.887	0.003
Diary *binge risk*	0.15±0.17	0.25±0.26	0.15±0.25	1.564	0.215	0.042
Diary *Craving*	57.31±16.62	58.90±14.07	56.72±16.27	0.029	0.879	0.004
BMI	24.90±7.34	22.77±3.68	23.23±4.55	1.073	0.347	0.029

Note: Values mean±SD. All tests were one-way ANOVA

5.3.2 Attentional Bias

Dwell time and first fixation duration on the visual probe task were analysed with a Group (RAP-Ret, PBO-Ret, RAP-NoRet) x Day (baseline, test) x Cue (Chocolate, Neutral) mixed ANOVA. Significant main effects of cue were found consistently for all indices, with Day x Cue x Group and interactions on dwell time and first fixation duration. Statistics are presented in *Table 5.2*.

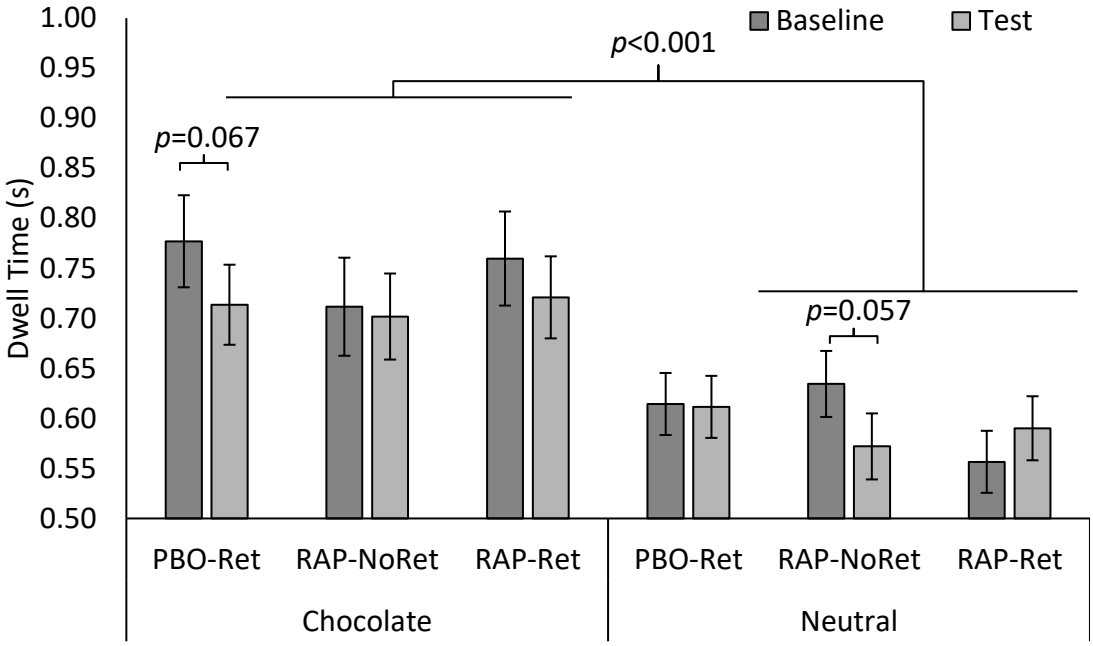
Table 5.2. *Main effects and interactions for the visual probe task*

Measure	Image Type	Time	PBO-Ret N=25	Rap-NoRet N=22	Rap-Ret N=24	Main effects	Interactions
Dwell time	HPF	BL	0.78±0.20	0.71±0.14	0.76±0.27	**Cue**: $F_{1,68}$=62.169, p<0.001, η_p^2=0.478 Chocolate > Neutral	**Day x Cue x Group**: $F_{2,68}$=3.433, p=0.038, η_p^2=0.092
		Test	0.71±0.22	0.70±0.19	0.72±0.20		
	LPF	BL	0.61±0.18	0.63±0.13	0.56±0.15		
		Test	0.61±0.14	0.57±0.17	0.59±0.16		
Latency to first fixation	HPF	BL	0.35±0.07	0.38±0.15	0.38±0.12	**Cue**: $F_{1,68}$=6.284, p=0.015, η_p^2=0.085 Chocolate > Neutral	**Day x Group:** $F_{2,68}$= 3.539, p=0.034, η_p^2=0.094 **Day x Cue**: $F_{1,68}$=10.263, p=0.002, η^2_p =0.131
		Test	0.35±0.09	0.41±0.14	0.35±0		
	LPF	BL	0.36±0.10	0.37±0.14	0.38±0.16		
		Test	0.40±0.15	0.44±0.19	0.37±0.11		
First Fixation duration	HPF	BL	0.55±0.17	0.49±0.13	0.57±0.27	**Cue:** $F_{1,68}$=55.212, p<0.001, η_p^2=0.448 Chocolate > Neutral	**Day x Cue:** $F_{2,68}$=3.244, p=0.076, η_p^2=0.046 **Day x Cue x Group:** $F_{2,68}$= 2.702, p=0.074, η_p^2=0.074
		Test	0.51±0.21	0.50±0.19	0.52±0.20		
	LPF	Test	0.39±0.18	0.42±0.13	0.35±0.17		
		BL	0.42±0.17	0.38±0.19	0.40±0.17		

Note: only main effects and interactions where F>2.5 are presented. Values represent mean±SD.

Multivariate simple effects showed that dwell time was greater on Chocolate than Neutral cues in all cases except for in the Rap-NoRet group, where only a marginal difference was observed at baseline ($F_{1,68}$=3.852, p=0.054, η_p^2=0.054). A trend-level reduction from baseline to test in dwell time was observed for Chocolate cues in the PBO-Ret group ($F_{1,68}$=3.452, p=0.067, η_p^2=0.048), and for Neutral cues in the RAP-NoRet group ($F_{1,68}$=0.057, p=0.057, η_p^2=0.052; *Figure 5.3*).

Figure 5.3. *Dwell Time on the visual probe task at baseline and test (~10 days post manipulation session*



Bars represent mean±SE. Total dwell time on Chocolate cues marginally reduced from baseline to test in the PBO-Ret, and for Neutral cues in the RAP-NoRet group.

Latency to first fixation across cues (Day x Group interaction) increased from baseline to test in the Rap-NoRet group only ($F_{1,68}$=6.790, p=0.011, η_p^2=0.091). The Day x Cue interaction was driven by an increase in first fixation latency from baseline to test for Neutral images only ($F_{1,68}$=8.622, p=0.005, η_p^2=0.113), with no change for chocolate images (p=0.902). Duration of the first fixation (Day x Cue x Group) was higher for Chocolate relative to Neutral cues in all cases except for at baseline in the Rap-NoRet group ($F_{1,68}$=3.659, p=0.06, η_p^2=0.051). Together these indicate only a very mild attentional bias to chocolate at baseline in Rap-NoRet.

5.3.3 Chocolate consumption and craving

Descriptive statistics and mixed ANOVA results on chocolate consumption and craving are shown in *Table 5.3*. Main effects of Day were found for all measures, with additional main effects of Group on chocolate and food binge frequency.

Table 5.3. *Means, main effects, and interactions for measures of consumption and craving*

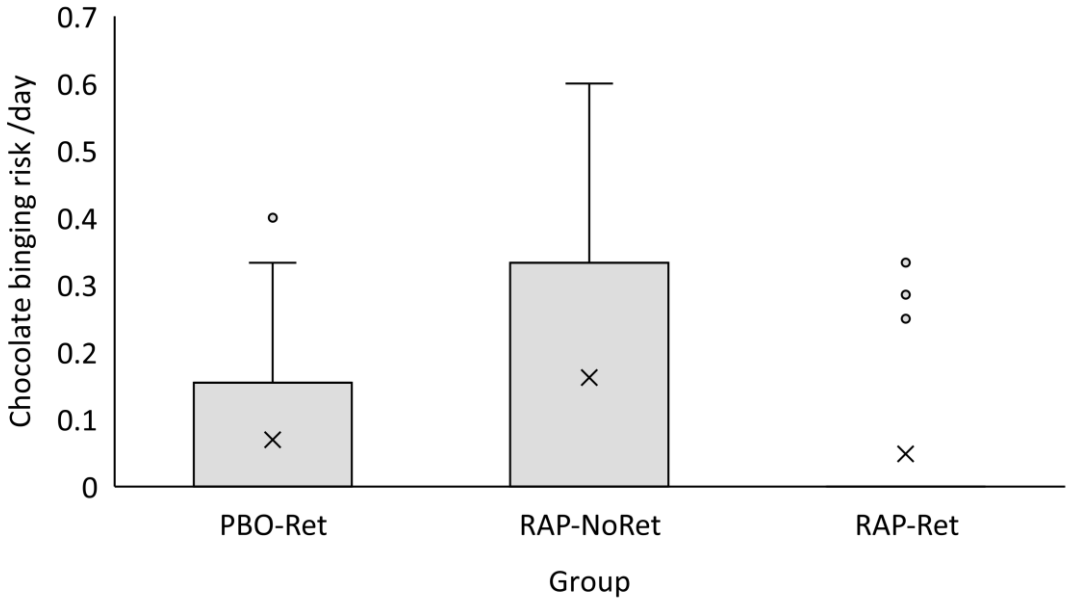
Measure	Outcome /subscale	Day/ time	PBO-Ret N=25	Rap-NoRet N=22	Rap-Ret N=25	Main effects
TLFB	Chocolate consumption /day (g)	BL	50.87±33.79	52.59±19.52	47.96±28.10	**Day:** $F_{2,138}$=14.714, p<0.001, η_p^2=0.176
		Test	48.29±41.01	43.40±25.40	43.30±25.60	
		FU	38.33±28.47	31.15±14.81	34.08±20.53	
Food Diary	Chocolate consumption /day (g)	Pre	69.16±47.77	63.40±30.17	65.83±46.06	**Day:** $F_{2,144}$=15.466, p<0.001, η_p^2=0.188
		1post	51.59±39.72	47.25±37.34	40.08±23.94	
		2post	52.93±36.81	42.28±35.66	40.08±16.44	
	Chocolate overeat risk/day	BL	0.29±0.23	0.34±0.25	0.29±0.25	**Day:** $F_{2,134}$=13.081, p<0.001, η_p^2=0.163
		1post	0.19±0.21	0.22±0.24	0.15±0.24	
		2post	0.17±0.17	0.17±0.20	0.20±0.24	
	Chocolate binge risk /day	BL	0.15±0.17	0.22±0.22	0.13±0.22	**Day:** $F_{2,112}$=5.438, p=0.009, η_p^2=0.075 **Group**: $F_{1,67}$=4.674, p=0.013, η_p^2=0.122
		1post	0.10±0.15	0.17±0.23	0.03±0.07	
		2post	0.07±0.12	0.17±0.21	0.05±0.11	
	Food binge risk/day	BL	0.09±0.13	0.13±0.17	0.06±0.11	**Day**: $F_{2,112}$=7.790, p=0.001, η_p^2=0.186 **Group**: $F_{1,69}$=3.193, p=0.047, η_p^2=0.085
		1post	0.04±0.08	0.06±0.13	0.03±0.06	
		2post	0.05±0.12	0.06±0.09	0.02±0.05	
	Chocolate craving	BL	57.31±16.62	57.59±13.09	56.86±14.07	**Day:** $F_{2,115}$=25.534, p<0.001, η_p^2=0.276
		1post	46.44±18.84	45.32±17.47	43.07±16.43	
		2post	44.78±20.74	43.95±20.05	47.28±17.33	
AECQ	Total	BL	54.00±12.60	56.99±11.14	55.57±11.95	**Day:** $F_{2,113}$=4.532, p=0.019, η_p^2=0.061
		Test	50.69±15.00	56.32±15.10	59.05±11.59	
		FU	46.44±14.42	54.99±14.77	52.99±13.58	
	Craving	BL	61.54±17.93	69.37±15.53	65.92±16.55	**Day**: $F_{2,112}$=5.677, p=0.020, η_p^2=0.075
		Test	58.49±17.35	65.22±16.43	68.43±16.08	
		FU	50.78±20.70	63.75±18.80	62.05±20.02	
	Guilt	BL	44.48±19.98	45.32±19.18	45.39±19.68	**Day:** $F_{2,129}$=2.764, p=0.071, η_p^2=0.038
		Test	39.48±20.34	46.87±22.41	50.13±21.34	
		FU	36.06±18.42	45.47±22.04	41.47±21.51	
	Function	BL	55.99±14.83	56.27±15.01	55.39±20.24	None¥
		Test	54.11±18.36	57.60±15.71	58.58±18.59	
		FU	52.49±14.90	55.76±16.29	55.44±17.17	
FCQ-c-t	Chocolate craving	BL	61.00±9.44	56.52±9.59	59.04±12.04	**Day**: $F_{2,120}$=17.931, p<0.001, η_p^2=0.209
		Test	49.44±12.89	53.00±14.21	49.88±15.56	
		FU	46.32±14.13	50.95±14.05	48.72±17.57	

Note: Values mean±SD. BL: baseline, FU: follow-up, 1post: 1-week post-manipulation, 2post: 2 weeks post-treatment. FCQ: Food Craving Questionnaire – chocolate - trait, AECQ: attitudes to eating chocolate questionnaire. ¥= No main effects and interactions where F>2.5 present

Simple contrasts showed chocolate consumption, as assessed by the TLFB, reduced non-significantly between baseline and test ($F_{1,69}$=3.695, p=0.60, η_p^2=0.050), and significantly between test and follow-up ($F_{1,69}$=11.161, p=0.001, η_p^2=0.139). The food diary indicated a reduction in chocolate consumption from 1-week pre, to 1-week post manipulation (t_{71}=3.341, p<0.001, r=0.369), with no further reduction from 1-week to 2-weeks post-manipulation (t_{71}=4.333, p=0.983, r=0.457). Cronbach's alpha for chocolate consumption in the week prior to manipulation as measured by the TLFB (M=57.71, SD=33.44) and the food diary (M=65.195, SD=44.906) was 0.860, suggesting a high level of agreement between the two measures.

Data from the food diary suggested 65% of participants (N=49) binged on chocolate over the course of the study. Mean consumption of chocolate during a binge was 146.36 grams (SD=74.415). Across all groups, binging risk (calculated as % chance of binging per day) reduced from baseline to test ($F_{1,67}$=7.023, p=0.01, η_p^2=0.095), with no further reduction from test to follow-up ($F_{1,67}$=0.097, p=0.757, η_p^2=0.001). The RAP-NoRet group binged on chocolate more frequently than the RAP-Ret group (t_{44}=3.000, p=0.012, r=0.412), but this was present from baseline. Repeated contrasts suggested likelihood of overeating chocolate reduced from baseline to test ($F_{1,67}$=18.246, p<0.001, η_p^2=0.214), with no further reduction from test to follow-up ($F_{1,67}$=0.16, p=0.900, η_p^2=0.001). While no Group x Day interaction was observed for chocolate binging, inspection of the plots suggested that in the RAP-Ret group alone, an abolition of chocolate binging was observed for all but four participants (see *Figure 5.4*).

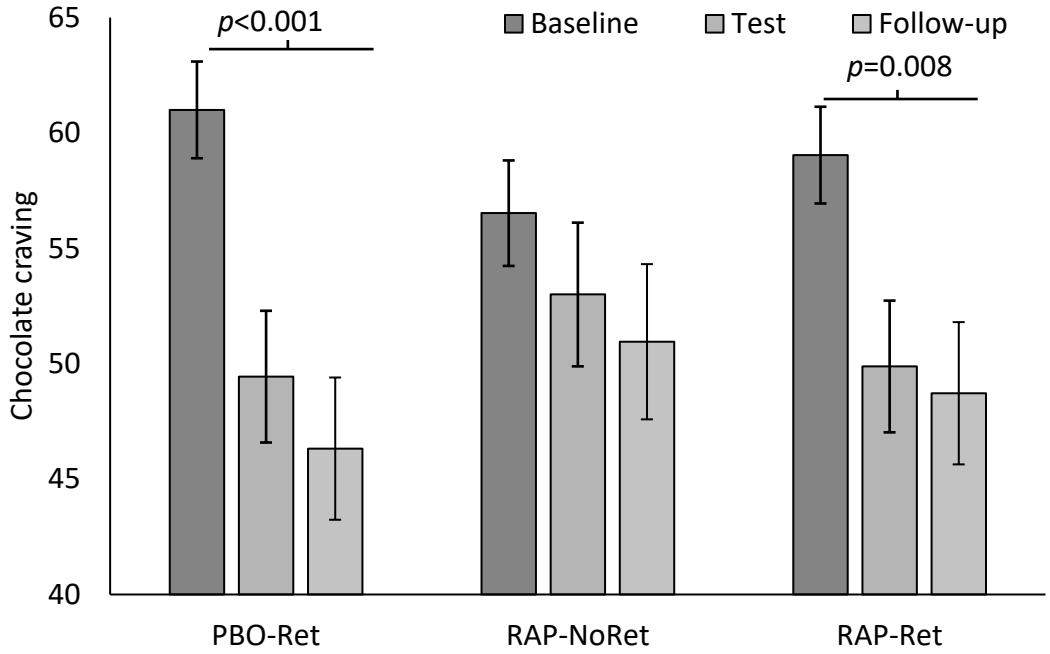
Figure 5.4*.Box and whisker plot for binging on chocolate at 2-weeks post retrieval session*



Note: crosses represent mean binging frequency. The Group x Time interaction was non-significant; however, plots suggest all but 4 participants did not binge at 2-weeks post in the RAP-Ret group.

Likelihood of binging on other foods similarly decreased between baseline and test ($F_{1,69}$=13.982, p<0.001, η_p^2=0.168), with no further reductions between test and follow-up ($F_{1,69}$=0.021, p=0.886, η_p^2<0.001). Repeated contrasts suggested the RAP-NoRet group binged on other foods more frequently than the RAP-Ret group (t_{71}=0.328, p=0.041, r=0.039), with exploratory analysis suggesting this difference was observed only at follow-up (t_{71}=2.471, p=0.005, r=0.281). Food diary-rated daily chocolate craving (Day main effect) reduced from 1-week pre- to 1-week-post manipulation ($F_{1, 67}$=36.588, p<0.001, η_p^2=0.353), with no further reduction from 1-week post to 2-weeks-post manipulation ($F_{1, 67}$=0.065 p=0.800, η_p^2=0.001). Chocolate craving, as assessed by the FCQ-chocolate, reduced significantly from baseline to test (t_{70}=4.104, p<0.001, r=0.44) and from test to follow-up (t_{70}=5.247, p<0.001, r=0.531). No Group x Day interaction was observed, however, visual inspection of the plots (*Figure 5.2*) suggested the largest reduction was observed in the PBO-Ret group (simple effect of Day within each group: $F_{2,67}$=10.021, p<0.001, η_p^2=0.230), followed by the RAP-Ret Group ($F_{2,67}$=5.147, p=0.008, η_p^2=0.133). No significant reduction was observed in the RAP-NoRet Group ($F_{2,67}$=1.239, p=0.296, η_p^2=0.036).

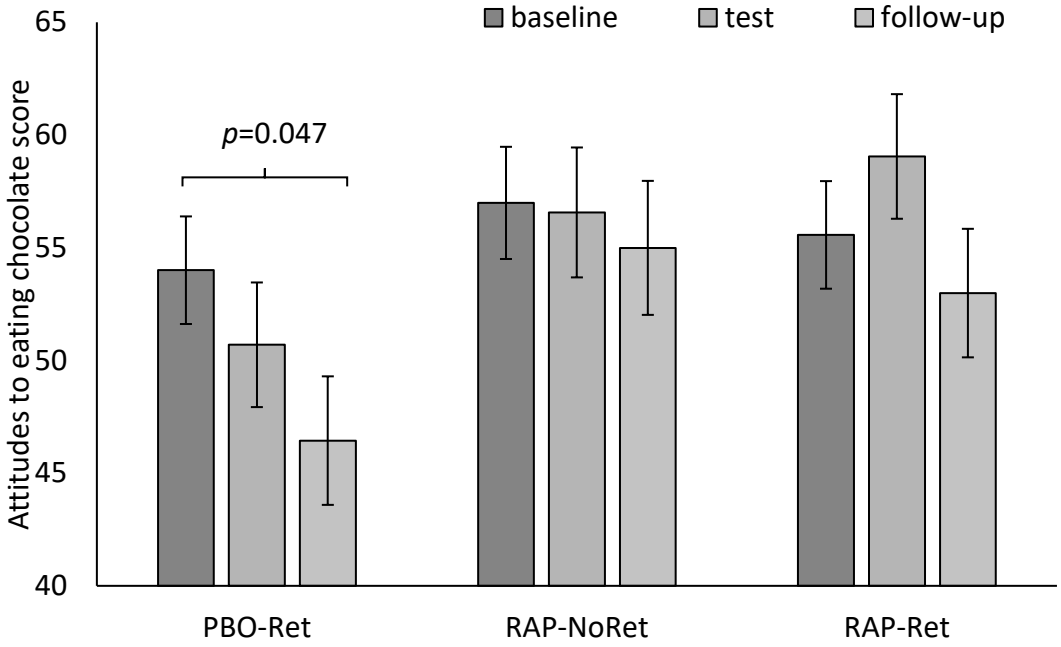
Figure 5.5. *Scores on the trait Food Craving Questionnaire – chocolate (FCQ-chocolate-t) at baseline (~48h pre-manipulation), test (10-14 days post-manipulation), and follow-up (1-month post-manipulation)*



Bars represent mean±SE. Although the Day x Group interaction failed to reach significance ($F_{4,120}$=1.288, p=0.280, η_p^2=0.036), significant reductions in craving were observed in the PBO-Ret and RAP-Ret groups only

Scores on total Attitudes to Eating Chocolate Questionnaire (AECQ) reduced from test to follow-up ($F_{1,70}$=6.052, p=0.016, η_p^2=0.080) with virtually no change between baseline and test ($F_{1,70}$=0.022, p=0.840, η_p^2<0.001). A significant Day x Group interaction emerged when comparing baseline and test scores only ($F_{1,70}$=3.243, p=0.045, η_p^2=0.085). Exploratory analysis of the simple effects of time (across all time points) showed these were only significant in the PBO-Ret ($F_{1,69}$=3.199, p=0.047, η_p^2=0.085) and RAP-Ret groups ($F_{1,69}$=3.574, p=0.033, η_p^2=0.094), with no change in the RAP-NoRet group ($F_{1,69}$=0.195, p=0.824, η_p^2=0.006; see *Figure 5.6*).

Figure 5.6. *Scores on the Attitudes to Eating Chocolate Questionniare (AECQ) at baseline, test (~10 days post-manipulation), follow-up (1-month post-manipulation)*



Bars represent mean±SE. A significant linear effect of time was observed in the PBO-Ret group only.

5.3.4 Cue-Reactivity

Reactivity to chocolate cues was assessed using Day (baseline, test) x Group (RAP-Ret, PBO-Ret, RAP-NoRet) x Cue (Chocolate, Neutral) mixed ANOVA. A Day (baseline, test) x Group (RAP-Ret, PBO-Ret, RAP-NoRet) ANOVA was additionally conducted for self-reported liking, desire to consume, likelihood of overeating, post-consumption liking and desire to consume more of the *in vivo* chocolate cue. A significant Day x Cue x Group effect emerged for cue liking ratings, otherwise only Day and Cue effects were observed. Results are presented in *Table 5.4*.

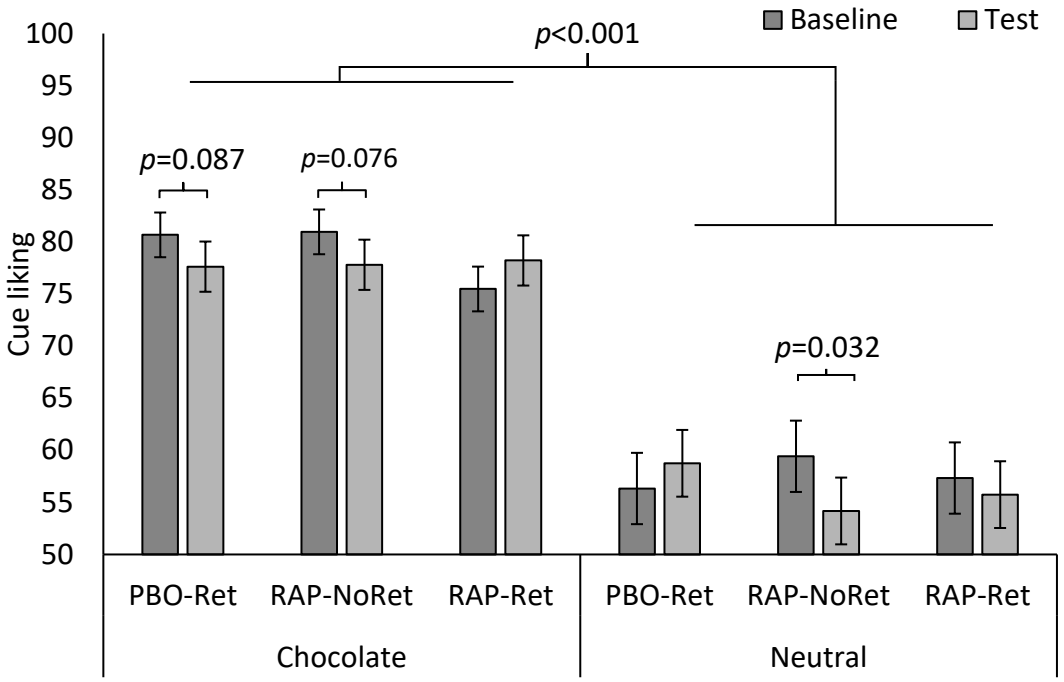
Table 5.4. *Mean liking, urge to consume, and likelihood of overeating scores for image and in vivo cues at baseline and test* for the cue-reactivity task

DV	Cue		PBO-Ret N=25	Rap-NoRet N=25	Rap-Ret N=25	Main effects	Interactions
Cue Liking	Choc	BL	80.68±10.81	80.96±8.78	75.48±12.30	**Cue:** $F_{2,72}$=95.125, p<0.001, η_p^2=0.569 Chocolate>Neutral	**Day x Group**: $F_{2,72}$= 2.648, p=0.078, η_p^2=0.069 **Day x Cue x Group**: $F_{2,72}$= 3.338, p=0.041, η_p^2=0.085
		Test	77.63±12.75	77.80±10.14	78.22±13.06		
	Neutral	BL	56.33±18.29	59.42±16.61	57.33±16.37		
		Test	58.75±15.09	54.17±14.39	55.74±18.30		
	In vivo Choc	BL	84.93±14.28	83.45±13.48	78.37±15.11	None¥	None¥
		Test	81.19±19.10	81.30±12.96	80.82±16.92		
Urge	Choc	BL	47.92±11.03	48.68±13.57	47.30±14.53	**Day:** $F_{1,71}$=13.356, p<0.001, η_p^2=0.156; BL>test **Cue:** $F_{1,71}$=120.551, p<0.001, η_p^2=0.626; Chocolate>Neutral	**Day x Cue**: $F_{1,72}$= 33.492, p<0.001, η_p^2=0.317
		Test	39.68±13.72	38.57±13.21	40.59±13.93		
	Neutral	BL	28.40±17.82	32.99±16.91	31.01±15.83		
		Test	27.79±17.68	27.48±14.02	32.13±16.97		
	In vivo Choc	BL	77.51±22.93	75.73±22.92	72.43±23.21	None¥	None¥
		Test	70.29±29.85	77.41±17.93	63.36±29.51		
Overeat	Choc new	BL	74.44±16.57	78.83±12.37	73.28±13.91	**Day:** $F_{1,72}$= 12.212, p=0.001, η_p^2=0.145 BL>test **Cue:** $F_{1,72}$=173.259, p<0.001, η_p^2=0.706; Chocolate>Neutral	None¥
		Test	71.97±20.65	74.51±12.71	73.74±13.58		
	Neutral	BL	47.64±15.47	46.26±15.77	44.79±19.28		
		Test	41.03±21.19	42.61±20.27	39.30±18.75		
	In vivo Choc	BL	83.90±16.04	88.12±11.56	80.18±19.71	None¥	None¥
		Test	83.62±17.49	80.30±12.28	79.07±17.67		
Post-consumption ratings of in vivo chocolate	Actual enjoyment	BL	91.05±10.80	83.62±18.25	85.66±15.06	None¥	None¥
		Test	90.03±13.76	81.18±22.91	84.11±20.78		
	Urge eat more	BL	86.82±16.43	76.44±21.48	78.58±18.34	**Day:** $F_{1,72}$=4.605, p=0.035, η_p^2=0.060 BL>test	None¥
		Test	76.72±25.21	78.02±21.85	70.55±30.60		

Values mean±SD. BL: baseline (48hrs pre-manipulation), test (10-14 days post-manipulation). ^{S}*=indicates where surprise was included as a main effect and covariate in the model.* $^{¥}$*= no effects present where F >2.5. Only main effects and interactions where F>2.5 included*

A multivariate simple effect of Day suggested a reduction in liking of all cues in the NoRet-Rap group only ($F_{1,72}$=7.251, p=0.009, η_p^2=0.091). Multivariate analyses under Group and Cue type showed the reduction across Day was significant only for Neutral cues in the NoRet-Rap group ($F_{1,72}$=4.797, p=0.032, η_p^2=0.062), while a trend reduction in liking of Chocolate cues was observed in the PBO-Ret ($F_{1,72}$=3.018, p=0.087, η_p^2=0.040) and RAP-NoRet groups ($F_{1,72}$=3.239, p=0.076, η_p^2=0.043; see *Figure 5.7*)

Figure 5.7. *Liking of image cues in cue-reactivity task*



Bars represent mean±SE. Cue liking reduced from baseline to test for Chocolate cues in the PBO-Ret group, and for Neutral cues in the RAP-NoRet group.

For desire to eat chocolate in response to image cues, a multivariate simple effect of Day ($F_{1,72}$=36.390, p<0.001, η_p^2=0.336) suggested greater wanting of chocolate in response to chocolate images at baseline, relative to test.

5.3.4.1 Progressive ratio task

Significant main effects and interactions for mixed Day (baseline, test) x Group (RAP-Ret, PBO-Ret, RAP-NoRet) x Food Type (chocolate, strawberry) ANOVA for break point and motivation index data on the progressive ratio task are presented in *Table 5.5*. Cue x Group interactions were observed, but no Food Type x Group x Day interactions emerged.

***Table 5.5**. Means, main effects, interactions for the progressive ratio task*

Measure	Food Type	ay	PBO-Ret N=25	Rap-NoRet N=23	Rap-Ret N=25	Main effects	Interactions
Break point	Choc	BL	5.120±2.877	5.087±2.877	6.040±2.850	**Day:** $F_{1\ 70}$=7.751, p =0.007, η_p^2=0.100 BL>test **Food Type:** $F_{1,70}$=38.06, p<0.001, η_p^2=0.352 Chocolate>Neutral	**Food Type x Group:** $F_{2,70}$=5.522, p=0.006, η_p^2=0.136
		Test	4.800±3.122	4.261±2.378	5.440±4.312		
	Neutral	BL	2.880±2.713	4.261±2.767	2.120±2.128		
		Test	2.840±2.641	3.435±1.727	1.800±1.633		
Motivational Index	Choc	BL	0.027±0.016	0.026±0.014	0.029±0.014	**Day:** $F_{1,70}$=5.31, p=0.024, η^2_p=0.071 BL>Test **Food Type:** $F_{1,70}$=41.151, p<0.001, η^2_p=0.370 Chocolate>Neutral	**Food Type x Group:** $F_{2,70}$=4.349, p=0.017, η_p^2=0.111
		Test	0.026±0.018	0.023±0.013	0.027±0.021		
	Neutral	BL	0.015±0.014	0.021±0.013	0.010±0.012		
		Test	0.015±0.016	0.017±0.009	0.008±0.008		

Values represent mean±SD. BL: baseline. Only main effects and interactions where F>2.5 included

Univariate tests on break points by Group suggested significant Group effects for strawberry rewards ($F_{2,70}$=4.663, p=0.013, η_p^2=0.118), with pairwise comparisons suggesting the RAP-NoRet group had higher break points than the RAP-Ret group (t_{47}=2.842, p=0.010, r=0.383). Multivariate tests by Food Type suggested high break points for Chocolate, relative to Strawberry rewards in the PBO-Ret ($F_{1,70}$=11.521, p=0.001, η_p^2=0.141) and RAP-Ret ($F_{1,70}$=37.329, p<0.001, η_p^2=0.348) groups only.

Higher motivation to earn Chocolate, relative to Strawberry, was observed in the RAP-Ret, and PBO-Ret groups, but not in the RAP-NoRet group ($F_{1,70}$=2.768, p=0.101, η_p^2=0.038), with further analysis suggesting this lack of difference was also present at baseline. The simple effect of Group was significant only for Strawberry rewards ($F_{1,70}$=4.380, p=0.016, η_p^2=0.111).

5.3.5 Eating Disorder Symptomology

Mixed Day (baseline, test, follow up) x Group (RAP-Ret, PBO-Ret, RAP-NoRet) ANOVA was conducted on the Binge Eating Scale (BES). A Day (baseline, test, follow up) x Group (RAP-Ret, PBO-Ret, RAP-NoRet) x Subscale mixed ANOVA was additionally conducted on the Three Factor Eating Questionnaire (TFEQ), Power of Food (POF), Restraint Scale (RS), and Intuitive Eating Scale (IES). Means and main effects are presented in *Table 5.6.* No interactions reached significance.

***Table 5.6**. Means, main effects and interactions for eating disorder symptomology*

Measure		Day/ time	PBO-Ret N=25	Rap-NoRet N=23	Rap-Ret N=25	Main effects
BES		BL	31.60±7.25	31.57±7.69	31.80±7.61	**Day:** $F_{2,140}$=28.677, p<0.001, η_p^2=0.291
		Test	26.72±5.60	27.87±7.14	28.16±7.26	
		FU	28.04±7.60	28.48±7.89	26.88±9.01	
TFEQ	Total	BL	48.59±13.59	49.57±12.75	51.75±12.12	**Day:** $F_{2,128}$=5.776, p=0.005, η_p^2=0.076
		Test	43.09±14.90	47.64±15.97	50.76±13.19	
		FU	42.92±14.89	46.84±13.81	48.87±17.04	
	Cognitive restraint	BL	36.44±19.72	37.85±20.70	40.79±17.47	None¥
		Test	39.05±20.25	39.30±23.14	42.73±22.84	
		FU	35.65±21.46	38.10±23.32	44.38±16.69	
	Uncontrolled eating	BL	56.44±17.54	54.75±12.18	58.07±16.69	**Day:** $F_{2,140}$=12.207, p<0.001, η_p^2=0.148
		Test	46.67±20.14	49.92±15.10	53.93±19.40	
		FU	48.74±19.32	50.89±14.00	51.11±23.57	
	Emotional Eating	BL	49.33±31.77	57.49±23.37	54.67±24.62	**Day:** $F_{2,140}$=12.207, p=0.021, η_p^2=0.054
		Test	40.44±28.85	57.49±27.14	57.33±26.97	
		FU	40.00±24.85	52.17±24.26	51.11±28.87	
Power of Food		BL	3.40±0.75	3.25±0.65	3.46±0.82	**Day:** $F_{2,142}$=13.277, p<0.001, η_p^2=0.158
		Test	3.21±0.78	3.11±0.83	3.31±1.06	
		FU	2.95±0.84	2.89±0.76	3.18±0.97	
RS	Dieting concern	BL	5.92±2.25	6.29±2.90	5.96±2.54	**Day:** $F_{2,142}$=4.122, p=0.018, η_p^2=0.055
		Test	5.76±2.40	6.13±3.13	5.48±1.90	
		FU	5.360±1.85	5.67±2.53	5.12±2.15	
	Weight fluctuation	BL	5.20±3.37	4.46±2.26	4.80±2.86	**Subscale:** $F_{1,71}$=17.405, p<0.001, η_p^2=0.197
		Test	5.04±2.81	4.42±2.24	4.52±2.83	
		FU	5.00±2.84	4.46±2.45	4.16±3.17	
Intuitive Eating scale		BL	3.32±0.58	3.09±0.60	3.12±0.54	**Day:** $F_{2,142}$=10.745, p<0.001, η_p^2=0.131
		Test	3.45±0.48	3.28±0.57	3.25±0.54	
		FU	3.43±0.57	3.28±0.56	3.40±0.56	

Note: Values mean±SD. BL: baseline, FU: follow-up. BES: Binge eating scale, TFEQ: Three factor eating questionnaire, RS: Restraint scale, POF: Power of food, IES: Intuitive eating scale. ¥= no effects present where F >2.5, only main effects and interactions where F>2.0 included

Scores on the BES (Day main effect) reduced from baseline to test (t_{72}=7.187, p<0.001, r=0.649), with no further reduction from test to follow-up (t_{72}=0.986, p=0.986, r=0.116).

Total score on the Three Factor Eating Questionnaire reduced from baseline to test ($F_{1,70}$=7.221, p=0.009, η_p^2=0.094) with no further reductions from test to follow-up ($F_{1,70}$=0.778, p=0.378, η_p^2=0.011). Scores on the Uncontrolled eating subscale similarly reduced from baseline to test ($F_{1,70}$=18.658, p<0.001, η_p^2=0.210), while scores on the

Emotional eating subscale reduced only from test to follow-up ($F_{1,70}$=4.149, p=0.045, η_p^2=0.056).

Power of Food scale score reduced from baseline to test ($F_{1,71}$=5.234, p=0.025, η_p^2=0.069), and from test to follow-up ($F_{1,71}$=8.488, p=0.005, η_p^2=0.107) in all groups (Day main effect).

Dietary restraint, as assessed by the Restraint Scale, did not differ between baseline and test ($F_{1,72}$=1.322, p=0.254, η_p^2=0.018), but a trend reduction was observed from test to follow-up ($F_{1,72}$=3.117, p=0.082, η_p^2=0.042).

5.3.6 Mood-related measures

Table 5.7. *Means, main effects, and interactions for mood-related measures*

Measure	Day	PBO-Ret N=25	Rap-NoRet N=23	Rap-Ret N=25	Main effects
BDI	BL	5.40±5.61	5.70±5.65	5.36±5.18	**Day:** $F_{2,117}$= 4.574, p=0.017, η_p^2=0.061
	Test	5.20±4.71	4.96±4.72	4.88±5.90	
	FU	4.96±4.17	4.30±5.62	2.88±4.06	
STAI	BL	46.96±4.35	46.20±3.70	46.76±4.06	**Day:** $F_{2,125}$=8.075, p=0.001, η_p^2=0.103
	Test	45.56±4.26	43.36±5.52	44.84±5.09	
	FU	47.84±3.76	41.92±13.67	47.64±4.66	

Note: Values mean±SD. BL: baseline, FU: follow-up, BDI: Beck's depression inventory, STAI: State and Trait Anxiety Index. Only main effects and interactions where F>2.5 included.

Depression as rated via the Beck Depression Inventory (BDI) reduced significantly from test to follow-up ($F_{2,70}$=4.296, p=0.042, η_p^2=0.042), with no difference between baseline and test ($F_{1,70}$=1.433, p=0.235, η_p^2=0.020). Reductions in trait-anxiety (STAI-T) scores were observed from baseline to test ($F_{2,72}$= 16.133, p<0.001, η_p^2=0.183), returning to baseline levels at follow-up ($F_{2,70}$=11.281, p=0.001, η_p^2=0.139).

5.3.7 Biological and physiological measures

Mixed Day (baseline, test) x Group (RAP-Ret, PBO-Ret, RAP-NoRet) ANOVA was conducted for BMI, blood glucose, blood pressure, and heart rate. Means, main effects and interactions are presented in *Table 5.8.*

Table 5.8. *Means, main effects, and interactions for biological and physiological measures*

	Day/ time	PBO-Ret N=25	Rap-NoRet N=25	Rap-Ret N=24	Main effects
BMI	BL	24.90±7.34	22.77±3.68	23.23±4.55	None¥
	Test	25.16±7.42	22.79±3.78	23.21±4.52	
Blood glucose	BL	5.23±1.04	5.05±0.89	5.24±0.75	None¥
	Test	5.25±0.47	5.26±0.65	5.34±0.47	
Heart rate	BL	82.74±12.61	69.46 ±9.34	74.75±12.58	**Group:** $F_{2,71}$= 7.216, p=0.001, η_p^2=0.169
	Test	81.20±9.34	72.12±11.54	76.06±13.99	
Systolic BP	BL	113.72±14.16	111.34±11.67	109.10±10.24	None¥
	Test	110.20±11.31	109.10±10.24	108.40±10.27	
Diastolic BP	BL	75.84±7.84	74.86±7.91	72.90±5.80	**Day:** $F_{1,72}$=3.405, p=0.069, η_p^2=0.045 BL>Test
	Test	74.28±7.36	72.80±9.40	70.52±9.41	
Fullness	BL	3.88±1.60	3.67±1.18	3.32±1.07	**Day:** $F_{1,70}$=5.559, p=0.021, η_p^2=0.074 Test>BL
	Test	4.17±1.66	4.50±1.94	3.96±1.77	
Hours since last meal	BL	8.42±4.49	7.80±4.34	9.06±5.25	None¥
	Test	8.33±4.97	8.29±4.54	7.08±4.42	

Note: Values mean±SD. BL: baseline, BP: blood pressure, HR: heart rate. ¥*= no effects present where F >2.5, only main effects where F>2.5 included*

The heart rate Group main effect represented higher heart rate in the PBO-Ret group, relative to the RAP-NoRet group (t_{71}=3.781, p=0.001, r=0.409), with pairwise comparisons within Day indicating this difference was present at baseline (t_{71}=4.048, p<0.001, r=0.433) and was thus unrelated to manipulation.

Chi square analysis of drug condition (placebo, rapamycin) x adverse effect experience during drug session (yes/no) was non-significant (χ^2_2=0.951, p=0.330), suggesting participants who received rapamycin did not report a greater number of acute side effects than those who received placebo. Chi square analysis of drug condition (placebo, rapamycin) x adverse effect experience *after* drug session (yes/no) was similarly non-significant (χ^2_2=0.865, p=0.865).

5.3.8 Drug guess

Chi square analysis of Group x drug guess (RAP-Ret, PBO-Ret, RAP-NoRet) found a significant effect of group (χ^2_2=7.721, p=0.021), with participants in the RAP-Ret group guessing they received rapamycin with greater frequency than the other two groups. Drug guess Ns per group were: PBO-Ret = 4; RAP-NoRet=7; RAP-Ret=13.

5.3.9 Exploratory analyses

5.3.9.1 Moderating effect of binging risk on the relationship between group and primary outcome variables.

Under the hypothesis that null results may reflect low strength of the 'maladaptive' memories conferring a limited impact on clinically-relevant behavioural outcomes, the moderating effect of binging risk at baseline (calculated as the percentage of days participants engaged in a chocolate binge in the week prior to the retrieval and drug administration session, as assessed by the food diary) on the relationship between group (PBO-Ret, RAP-NoRet, RAP-Ret) and change (test-baseline) in chocolate consumption, and wanting of the in vivo chocolate, was assessed. Group was recoded into dummy variables (indicator coded; *Table 3.2*) due to independence between groups (Hayes & Montoya, 2017). In this coding, X_1 signifies the difference in outcome (baseline–test) between the RAP-NoRet group and the mean of the PBO-Ret and RAP-Ret groups. X_2 signifies the difference between the RAP-Ret group and the mean of the PBO-Ret and RAP-NoRet groups.

Table 5.9. *Indicator coding for moderation analysis with group as multi-categorical predictor variable*

Group \ Interaction term	X_1	X_2
PBO-Ret	0	0
RAP-NoRet	1	0
RAP-Ret	0	1

A hierarchical regression was conducted for each outcome variable (chocolate consumption, in vivo chocolate wanting), with binge risk (M) and predictor (Group; X_1, X_2) entered into Step 1. Binge risk was centred for ease of interpretation. The interaction terms (X_1*M; X_2*M) were added into the second step. Post-hoc analysis and plots were constructed using the PROCESS macro (Hayes, 2018) and simple slopes represent -1 SD (low), mean, and +1 SD (high). These data are presented in *Table 5.10*.

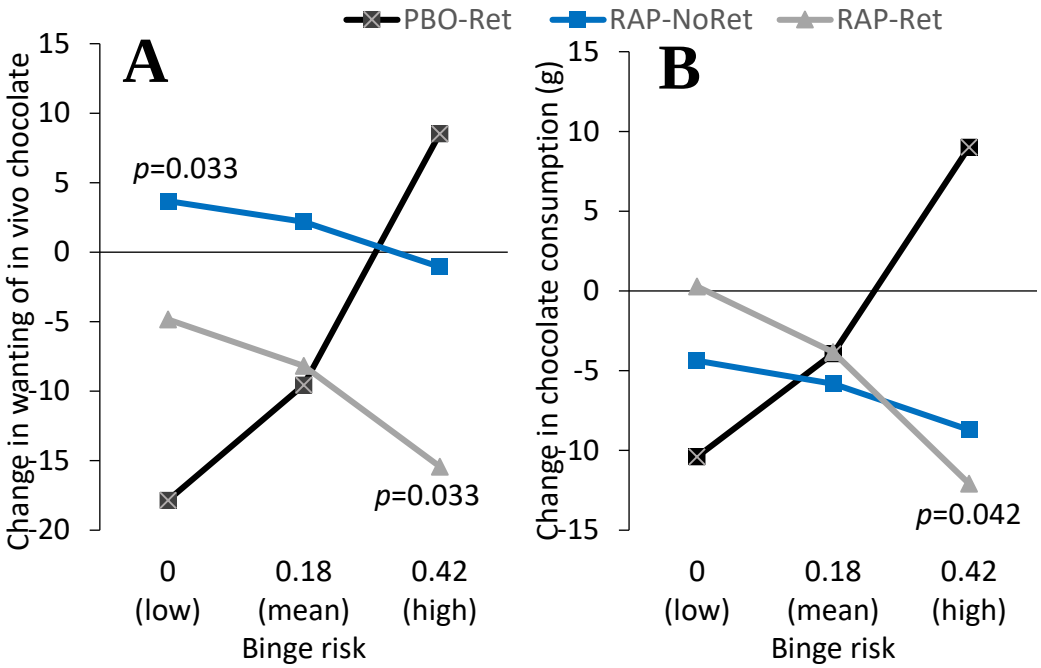
Table 5.10. *Moderating effect of daily binge risk at baseline on the relationship between Group and chocolate consumption, and wanting of in vivo chocolate (O)*

	O: Chocolate consumption Δ			O: wanting of in vivo chocolate Δ		
Moderator $_{df}$	*b*	R^2	*F*	*b*	R^2	*F*
Step $1_{3,71}$		0.014	0.328		0.127	0.644
Binge risk	-8.812			-4.109		
X_1 (RAP-NoRet)	-3.814			8.103		
X_2 (RAP-Ret)	-2.096			-1.853		
Step $2_{2,69}$		0.094	1.427		0.124	1.921
Binge risk	51.740			**70.366***		
X_1 (RAP-NoRet)	-5.611			6.214		
X_2 (RAP-Ret)	-4.890			-5.179		
Binge risk * X_1	-63.289			**-83.000***		
Binge risk * X_2	**-84.694***			**-98.590****		

*Note: * p<0.05 **p<0.01. O=outcome variable, X_1=RAP-NoRet group vs. RAP-Ret & PBO-Ret group, X_2=RAP-Ret vs RAP-NoRet & PBO-Ret groups.*

In Step 1, binge risk was not significantly related to any group outcomes (O; chocolate consumption change, wanting of in vivo chocolate change). At Step 2, addition of the interaction terms $M*X_1$ and $M*X_2$ accounted for a significant portion of the variability for change in wanting of the in vivo chocolate (ΔR^2=0.097, $F_{2,68}$=3.761, p=0.028), and a trend of the variability for change in chocolate consumption (ΔR^2=0.080, $F_{2,69}$=3.047, p=0.054).

Figure 5.8. *Moderating effect of binge risk at baseline on the relationship between group (PBO-Ret, RAP-NoRet, RAP-Ret) and change (baseline-test) in wanting of the in vivo chocolate (A) and chocolate consumption (B)*



Note: simple slopes represent binge risk at high (+1 SD), mean, and low (-1 SD) levels of binge risk, calculated as the percentage of days participants binged on chocolate in the week prior to the retrieval and drug administration session.

Simple slopes suggested that for change in wanting of the in vivo chocolate, the RAP-NoRet group (X_2) differed from mean of the PBO-Ret and RAP-Ret groups (X_1) at low (0%) levels of binge risk (*b*=-21.535, 95% CI [1.829, 41.240], t=2.181, *p*=0.0327). The RAP-Ret group (X_1) differed from the mean of the PBO-Ret and RAP-NoRet groups (X_2) at high (42%) levels of binge risk (*b*=-23.952, 95% CI [-54.632, -2.272], t=2.121, *p*=0.031). For daily chocolate consumption, the RAP-Ret group (X_1) differed from the mean of the PBO-Ret and RAP-NoRet groups (X_2) at high (42%) levels of binge risk only (*b*=-21.084, 95% CI [-41.376, -0.793], t=-2.073, *p*=0.042).

5.3.9.2 Effect of rapamycin on cue-reactivity

To see if rapamycin impacted the expression of craving following cue exposure at retrieval (e.g. see Shi et al., 2009), the Rapa-Ret and PBO-Ret group's liking, wanting, and overeating ratings for the Chocolate cues seen at retrieval and in vivo chocolate itself were entered in a mixed Time (baseline, retrieval, test) x Group (Rapa-Ret, PBO-Ret) ANOVA.

Table 5.11. *Chocolate cue-reactivity at retrieval*

group	Day	PBO-Ret N=25	Rap-Ret N=25	Main effects and interactions
Image cue liking	BL	81.76±10.58	76.42±13.73	**Day x Group**: $F_{2,82}$=5.562, p=0.008, η_p^2=0.104
	Retrieval	82.22±11.11	75.21±15.37	
	Test	75.39±18.77	79.00±13.43	
Image cue urge	BL	75.99±12.03	72.09±17.05	**Day:** $F_{2,96}$=4.816, p=0.010, η_p^2=0.091
	Retrieval	71.66±17.15	65.85±21.80	
	Test	66.30±23.94	68.65±18.51	
Image cue overeat	BL	76.52±15.46	74.06±14.93	**Day:** $F_{1,48}$=3.302, p=0.049, η_p^2=0.064
	Retrieval	71.66±17.15	65.85±21.80	
	Test	71.58±22.13	74.42±14.74	
In vivo cue liking	BL	84.93±14.28	78.37±15.11	None¥
	Retrieval	84.03±17.49	75.29±16.34	
	Test	81.19±19.10	80.82±16.92	
In vivo cue urge	BL	77.51±22.93	72.43±23.21	None¥
	Retrieval	74.57±27.22	71.25±18.97	
	Test	70.29±29.85	63.36±29.51	
In vivo cue overeat	BL	83.90±16.04	80.18±19.71	**Day:** $F_{2,47}$=4.167, p=0.022, η_p^2=0.151
	Retrieval	71.58±27.93	66.06±26.64	
	Test	83.62±17.49	79.07±17.67	

Note: Values represent mean±SD. ¥: No main effects or interactions where F>2.5 present. Only main effects and interacts where F>2 present

The Day x Group interaction was driven by decreased liking of cues from baseline to test in the PBO-Ret group ($F_{1,24}$=4.735, p=0.040, η_p^2=0.165), with no specific effects at retrieval. Likelihood of overeating the chocolate in response to the chocolate image cues (Day main effect) reduced from baseline to retrieval ($F_{1,48}$=5.404, p=0.024, η_p^2=0.101) with no further change from retrieval to FU ($F_{1,48}$=2.207, p=0.144, η_p^2=0.044). For the in vivo chocolate itself, likelihood of overeating was lower at retrieval relative to baseline ($F_{1,48}$=8.078, p=0.007, η_p^2=0.144) and test ($F_{1,48}$=7.718, p=0.008, η_p^2=0.139).

State chocolate craving during the retrieval session, as assessed by the FCQ-chocolate-s, was assessed with a Time (baseline, post-drug administration) x Group (Rapa-Ret, PBO-Ret, Rapa-NoRet) x Subscale (Craving, Hunger) mixed ANOVA. Total score increased across Time ($F_{2,72}$=6.204, p=0.015, η_p^2=0.080), with a Time x Subscale interaction ($F_{1,71}$=5.782, p=0.019, η_p^2=0.075) suggesting only hunger increased during the retrieval session (p=0.003). When craving alone was entered into a mixed Time (baseline, post-drug) x Group (Rapa-Ret, PBO-Ret, Rapa-NoRet) ANOVA, a trend Day x Group interaction was observed ($F_{2,72}$=2.993, p=0.056, η_p^2=0.077), with an increase in craving was observed only in the PBO-Ret group ($F_{1,72}$=5.471, p=0.022, η_p^2=0.071).

5.3.9.3 Gender effects

Where a higher proportion of female participants (72%) were recruited in the current study, gender differences in the severity of eating disorders and more general eating behaviours may have influenced results. Consistent with the eating disorder literature (Hallam, Boswell, DeVito, & Kober, 2016), females in the current study reported greater chocolate craving than men at baseline as assessed by the FCQ-chocolate-t ($F_{1,73}$=5.334, p=0.024, η_p^2=0.68), and the ACQ ($F_{1,73}$=5.855, p=0.018, η_p^2=0.074). Females also reported greater emotional eating relative to men (on the TFEQ; $F_{1,73}$=5.722, p=0.019, η_p^2=0.073), and higher levels of Concern for dieting on the Restraint Scale ($F_{1,73}$=11.468, p=0.001, η_p^2=0.136). However, results did not change when the main analyses were repeated in females only, with no additional Day x Group interactions emerging. The numbers of males and females did not differ between groups.

5.4 Discussion

The current study investigated the effects of 10mg of oral rapamycin in combination with retrieval of chocolate associative memories on a variety of eating behaviour and food-attitude and –motivation indices in a population of chocolate over-eaters. No retrieval-dependent (or retrieval independent) effects of rapamycin were observed, with no specific reductions in attentional bias, cue-reactivity, chocolate consumption, or craving observed in the group that received rapamycin prior to cue-chocolate memory retrieval. These results suggest that overall, pre-retrieval rapamycin failed to block the reconsolidation of chocolate-related cue associative memories in those with a tendency to 'overeat' chocolate. Alternatively, exploratory moderation analyses of the effect of baseline binge risk on the relationship between group and chocolate wanting, and chocolate consumption, suggested an effect of rapamycin with retrieval when participants were at a high risk of binging on chocolate.

A reduction in dwell time from baseline to test on chocolate cues on the visual dot probe task and liking of chocolate cues on the cue-reactivity-rating task was observed only in the group that underwent chocolate associative memory retrieval after receiving placebo (PBO-Ret). While small (non-significant) reductions in total dwell time and first fixation duration were also observed in the RAP-Ret group, these were not specific to this group and reflected generalised reductions in chocolate consumption, craving, wanting of the in vivo chocolate, desire to eat more of the chocolate, motivation to consume chocolate, eating disorder symptomology, and improved mood. These effects across all three groups likely reflect the Hawthorne effect (McCambridge et al., 2014), or beneficial impact of recording chocolate consumption and introspective analysis of food attitudes through answering questionnaires on this subject. Further, given that a desire to reduce chocolate consumption was a prerequisite for participation, a beneficial effect of recording daily chocolate consumption (Burke, Wang, & Sevick, 2011) may have produced the observed results *per se*. An exception was the lack of change in restrictive eating. Overall, the hypothesised retrieval-dependent (reconsolidation-based) effects of rapamycin were not observed.

Moderation analyses suggest this may be a result of the 'maladaptive' memories assessed here being of lower strength and therefore of lower impact on clinically relevant behavioural outcomes than those assessed previously in studies of heavy/hazardous drinkers. Indeed, the mean BMI of participants in the current study

was on the high end of normal and participants were not significantly overweight or obese, nor were they approaching clinical criteria for BED. The scope to see improvement in this sample on the back of memory interference may have therefore been limited *a priori*. Given that destabilisation likelihood at retrieval is a function of memory strength, this may lend credence to the possibility that new learning or extinction was engaged by retrieval procedures in the current study, when similar procedures were at the right level to produce destabilisation, previously. Indeed, moderation analyses in which the moderating effect of binge risk at baseline on the relationship between group and change in wanting, and between group and consumption of chocolate suggests this may have been the case. Those in the RAP-Ret group reduced their wanting of chocolate significantly more than those in the PBO-Ret and RAP-NoRet groups. Similarly, those in the RAP-Ret group who were at a high risk of binging (binged approximately 3 times a week) reduced their chocolate consumption by approximately 15g a day, relative to no change in those at a low risk (reported no previous binges). For the PBO-Ret group, the opposite pattern of results was observed, wherein an increase in wanting and consumption of chocolate was observed at high levels of binge risk, and a reduction was observed at no binge risk. These results may reflect a perpetuation in consumption following an attempt to reduce chocolate consumption in those with a propensity to binge, while those with a less 'maladaptive' relationship with chocolate were successfully able to cut down.

In line with this, with the exception of four participants almost a complete abolition of chocolate bingeing was observed in the group that received rapamycin after cue-chocolate memory retrieval. Interestingly, participants in the RAP-Ret group did not report an acute reduced likelihood of binging on chocolate at test during the cue-reactivity task. It is possible this reflects a lagged effect of reconsolidation treatment on the declarative component of the memory, mirroring the results of Soeter and Kindt (2015a), who demonstrated reductions in subjective fear only at follow-up. However, as subjective binge risk was not recorded at follow-up, we are unable to say whether this was the case here. Tentatively, these exploratory analyses suggest that rapamycin has the potential to interfere with reconsolidation in those with more clinically meaningful eating behaviours. This might be supported by the mild evidence for a reduction in *Neutral* cue liking in the RAP-NoRet group. Given the specificity of effect to the reactivated low palatable food (Neutral) cues, it might be argued that this reflects a reconsolidation-interference mechanism.

The null findings from the planned analyses fails to extend the extant literature in animal models of maladaptive associative memories, in which rapamycin has been shown to block the reconsolidation of fear (Blundell et al., 2008; Gafford et al., 2011; Glover et al., 2010; Jobim et al., 2012a; Mac Callum et al., 2014; Parsons et al., 2006b; Pedroso et al., 2013; Stoica et al., 2011), appetitive (Barak et al., 2013; Lin et al., 2014) and object recognition memory (Jobim et al., 2012b). In these animal studies, rapamycin is administered in liquid preparation intraperitoneally or intracerebroventricularly immediately following retrieval. Consistent with Suris et al. (2013), who also failed to observe a long-term effect of rapamycin on trauma memory reconsolidation, the current study used an oral preparation of rapamycin administered ~ 1 hr *prior to* retrieval. This pre-retrieval dosing strategy was used because of the pharmacokinetic profile of rapamycin, which reaches peak plasma levels slowly (Brattstrom et al., 2000).

Given that that there was some mild evidence of a reduction in chocolate craving and attitudes towards chocolate in the *placebo* group in the current study, an alternative explanation for the null result in the planned analysis is that rapamycin interfered with the initial destabilisation or retrieval of the cue-chocolate memory. The target of rapamycin (the mTOR1 pathway) is implicated in the retrieval of auditory fear memory, and infusion of rapamycin 10 minutes prior to retrieval is associated with the transient impairment of fear memory expression in rats (Lopez et al., 2015; Pereyra et al., 2018). Rapamycin is similarly associated with impaired cue elicited drug craving in humans. In a group of abstinent heroin addicts, administration of 2.5 or 5mg of rapamycin two-and-a-half hours prior to the exposure of a heroin-cue video resulted in dose-dependent blunting of craving (Shi et al., 2009). Exploratory analyses in the current study suggested a similar blunting effect when state craving was measured by the FCQ-chocolate, but not when measured with the cue-reactivity task at retrieval. It is notable that these measures were taken at different times, with on-drug cue-reactivity measured approximately one hour after rapamycin administration, and state craving at approximately 2 hours post-administration. It is therefore feasible that plasma rapamycin concentrations had not reached a sufficient level to produce craving reduction or memory effects by the time the retrieval/control memory procedures were performed.

However, blockade of the expression of craving (i.e. memory retrieval) does not preclude a blockade of memory reactivation (i.e. destabilisation). Memory retrieval and

destabilisation are biochemically separable (for a review see Delorenzi et al., 2014), and blockade of reconsolidation has been demonstrated even after attenuation of memory expression (e.g. Barreiro, Suarez, Lynch, Molina, & Delorenzi, 2013; Ben Mamou et al., 2006; Rodriguez-Ortiz et al., 2012). More specifically, blockade of reconsolidation has been observed when rapamycin was systemically infused 30 minutes prior to the retrieval of an auditory fear conditioned memory (Gafford et al., 2011; Mac Callum et al., 2014), suggesting rapamycin did not interfere with destabilisation in these cases. It is important to note that extending this literature to humans is difficult. There are discrepancies in the pre-clinical literature for the requirement of particular receptor sites in the reactivation of memory, which likely vary due to differences in memory type and brain structure. Given that oral rapamycin would have resulted in systemic mTOR inhibition in the current study, the impact of reduced memory expression can only be speculated.

An alternative explanation for the modest changes observed in the PBO-Ret group, is that the retrieval procedure induced new *extinction learning* that supressed the original associative memory at test. As extinction represents new learning, withholding of the chocolate following cue presentation acted as an extinction trial, which was successfully consolidated in the placebo group. The lack of effect in the experimental (RAP-Ret) group may therefore be the result of rapamycin interfering with the consolidation of this new learning. Extinction, rather than reconsolidation, can be induced if the retrieval procedure is too long, or involves too much prediction error. For example, administration of protein synthesis inhibitors is associated with the blockade of reconsolidation when delivered after a short (3 minute) retrieval trial, and with the blockade of extinction when lengthy (30 minute) reactivations are used (Suzuki et al., 2004).

Alternatively, reminder procedures that are too discrepant from the original may induce new learning, rather than updating of the original. Forcato et al. (2009), for instance, observed a blockade of declarative memory reconsolidation only when the retrieval procedure included the learned cue. The environment within which retrieval occurs is also important, and a retrieval procedure may fail to induce memory destabilisation if environment does not match that of the original conditioning (Misanin et al., 1968). It is possible that the chocolate images used in the current study were not closely associated enough with the type of chocolate participants typically eat.

Alternatively, the laboratory environment was too distinct from that during prototypical chocolate consumption.

The retrieval procedures used here were adapted from previous research showing reconsolidation-interference effects with such retrievals in heavy drinkers. One might therefore argue against the likelihood that these procedures instead produced extinction in the current study. In line with the studies described in *Chapters 3* and *4*, in Das et al. (2018a), and in Das et al. (2015b), the current study administered distractor tasks immediately following the retrieval procedure with the aim of disengaging the participant with the retrieved cue-chocolate memory. However, there were potentially important differences between the current and above-mentioned studies. Previously, following retrieval and distractor tasks, participants underwent the key reconsolidation-targeting manipulation (drug or counter-conditioning) while participants in the current study had already received the drug were required remain in the lab and engage in quiet activities, giving them the opportunity to re-engage with the retrieved memory. Both ketamine and N_20, as used in prior chapters, have much greater subjective and dissociative effects than rapamycin and elicit acute subjective drug states that are likely to prevent rehearsal and rumination upon the retrieval procedure. It is further possible that the subjective drug states themselves contributed to the previously observed reconsolidation effects, through incorporation of state-dependency into the reactivated memory traces that rendered them less retrievable outside of that subjective state currently (Gisquet-Verrier et al, 2018).

Fundamental differences between food rewards and drug-related appetitive reward memory may also provide an explanation as to why rapamycin failed to block the *reconsolidation* of associative chocolate memories. Consumption of both highly palatable food and drugs of abuse stimulate the same reward circuitry in the brain (Kelley, Schiltz, & Landry, 2005) and the rewarding effects of both food and substances of abuse can become associated with cues which reliably predict their use or consumption (Carter & Tiffany, 1999; Robinson & Berridge, 1993; Sobik et al., 2005). Like drugs of abuse (e.g. Diergaarde, Schoffelmeer, & De Vries, 2008; Milton et al., 2008a), the reconsolidation of Pavlovian food-cue appetitive food memories is dependent on de-novo protein synthesis (Hernandez & Kelley, 2004), NMDAR transmission (Lee & Everitt, 2008) and β-adrenergic signalling (Milton et al., 2008b). However, there is little evidence for requirement of mTOR in the reconsolidation of non-drug appetitive memories. For instance, while Barak et al. (2013) demonstrated an

impairing effect of rapamycin for the reconsolidation for alcohol-associated memories, rapamycin did not impair the reconsolidation of conditioned sucrose memories. Unpublished data has similarly shown no effect of rapamycin on reconsolidation-interference, where the study was stopped prematurely as rats receiving rapamycin lost too much body weight (Milton et al., Unpublished). Dissociation between non-drug and drug-associated rewards has also been demonstrated outside of the reconsolidation literature. The modulation of glutamate receptors in the nucleus accumbens (e.g. Besheer et al., 2010; Chiamulera et al., 2001; Hopf et al., 2010), enhanced nucleus accumbens activity (Hopf et al., 2010) and lesions to the nucleus accumbens (Caine & Koob, 1994) are associated with inhibition of cocaine and alcohol seeking, but not sucrose.

5.4.1 Limitations

As in all similar reconsolidation studies in which null effects are reported (e.g. Bos et al., 2014; Das et al., 2015a; Jobes et al., 2015; Pachas et al., 2015; Schroyens et al., 2017; Spring et al., 2015; Wood et al., 2015), the current study was unable to *conclusively* parse the factor(s) responsible for a lack of a retrieval-dependent effect of rapamycin. This highlights a significant limitation of the human reconsolidation literature. Specifically, in the current study we are unable to identify whether the reactivation procedure failed to destabilise or retrieve chocolate-cue memories, or if rapamycin did not sufficiently block memory reconsolidation. To identify whether rapamycin prevented memory reactivation a known reconsolidation blockade would also need to be applied *after* retrieval (e.g. ketamine as in *Chapter 4*). If rapamycin did indeed prevent reactivation when administered pre-retrieval, the known post-retrieval blockade of reconsolidation would not attenuate the memory (when compared to pre-retrieval placebo). Alternatively, replication of the current study within a proven protocol (for instance, retrieval of cue-alcohol memories as achieved in *Chapter 4*, and in Das et al. (2015b), or retrieval of conditioned fear memory as discussed in Lonergan et al. (2013) may aid in elucidating why no effect was observed here.

It is also unknown whether 10mg was the optimal dosage to block reconsolidation. The current study is only the second to administer rapamycin to humans within a reconsolidation paradigm. As such, drug dosing was based primarily on available safety information in humans (Brattstrom et al., 2000) and it is possible null effects were the result of insufficient levels of rapamycin. In mice, a dose-response curve suggested 40mg/kg of rapamycin did not produce significantly different levels of memory

impairment (via reconsolidation blockade) when compared to 20mg/kg (Blundell et al., 2008). Thus, while the first in-human study by Surís et al (in PTSD patients) administered a 50% higher dose (15mg; Suris et al., 2013), the pharmacokinetics of rapamycin do not suggest a linear relationship between dose and reconsolidation blockade (Blundell et al., 2008). Whether this is the case in a humans has yet to be confirmed. Moreover, the dose-response data from animal studies that used direct infusion of rapamycin into specific brain regions has no relevance to human subjects, where the only available routes of administration are systemic. Further research is therefore required to identify optimal dosage in a human population.

Oral administration of rapamycin similarly meant drug timing was based on projected peak plasma levels (Brattstrom et al., 2000) being reached during the reconsolidation window. Again, whether this is the optimal timing of administration is currently unknown. Shi et al. (2009), for instance, administered rapamycin two and a half hours prior to retrieval, in which a blunting of craving was observed. Further to this, the pharmacokinetic data from which the timings of the current study were derived used a sample of men. Given the greater number of females within the current study, it is possible that gender differences influenced optimal timing and dose strategy (Soldin & Mattison, 2009). As we did not assay blood levels of rapamycin or metabolites following retrieval, we are currently unable to disentangle these possibilities.

Unlike *Chapter 4* in which all groups underwent prediction error, the non-retrieval group in the current study did not experience a PE. As such, we are unable to separate out effects of PE itself, or other components of the retrieval procedure, on changes in memory strength. Firstly, it is possible arousal from PE itself confers some effects on memory weakening. As in *Chapter 3*, higher surprise ratings for PE were associated with greater reductions in alcohol consumption in the group that did *not* undergo retrieval. Secondly, as participants in the RAP-NoRet group consumed the strawberry (whereas those in the RAP-Ret and PBO-Ret did not), this amounted to an additional learning trial, theoretically leading to an increase in memory strength for low-palatability cues in this group. Liking data does not, however, support this assumption as a *reduction* in liking of low-palatability cues was seen. Finally, as the lack of PE likely prevented the memory from destabilising (Das et al., 2018b), we are potentially unable to assess whether rapamycin interfered with the reconsolidation of low-palatability food cue-memories in this group. It is notable that there was some mild evidence of a decrease low-palatability food liking in the RAP-NoRet group. If this occurred via a

reconsolidation- interference mechanism, it is possible that a greater effect would have been observed had a PE been included in the no-retrieval procedure. Thus, in future, studies should ideally include a PE in a non-retrieval control group.

In conclusion, the current study failed to show a convincing effect of rapamycin on the reconsolidation of natural appetitive reward memories in a population with a sub-clinical propensity to overeat or binge on chocolate. Moderation analyses suggest this may be due to the selected sample, as reductions in consumption and wanting of chocolate were observed when analyses were limited to those with more clinically relevant patterns of chocolate consumption. Given the well-tolerated nature of single-dose rapamycin and its demonstrated potential to interfere with reconsolidation, further studies are warranted to identify whether mTOR blockade is a viable avenue for weakening the associative memories underlying BED in a human population. Similarly, exploration of rapamycin as a blockade non-natural reward memory in humans is warranted, particularly within clinically relevant samples.

Chapter 6.
Discussion

6.1 Introduction

Despite the substantial literature on maladaptive appetitive memory reconsolidation in rodents, relatively few studies have been conducted within humans. At the outset, meta-analysis and systematic review (detailed in *Chapter 2*) identified just eight published studies on naturalistic maladaptive reward memory (MRM) reconsolidation (Das et al., 2015a; Das et al., 2015b; Das et al., 2018b; Germeroth et al., 2017; Hon et al., 2016; Pachas et al., 2015; Saladin et al., 2013; Xue et al., 2012), with just three additional studies that did not meet inclusion criteria (Lonergan et al., 2016; Xue et al., 2017; Zhao et al., 2011). An additional search of the literature has identified just one study on appetitive and reward memory reconsolidation (Kaag et al., 2018) which was published during the course of the current PhD. The three studies contained within the current thesis therefore make a significant contribution to the research on human maladaptive appetitive reward memory. Together, it is hoped that the empirical chapters described here adequately address the question posited at the outset of this PhD:

> *"Can we use pharmacological reconsolidation-interference strategies to attenuate maladaptive appetitive reward memories?"*

Using both meta-analytic and experimental methodologies, the current thesis has specifically addressed the following research questions:

1) Given putative boundary conditions of memory strength and age, is reconsolidation interference a viable treatment strategy in clinically relevant populations where memories are more strongly conditioned?

2) Prediction error (mismatch between expected and actual events) is considered necessary for the destabilisation of older, stronger memories. In a clinically relevant population, is prediction error required for the destabilisation of strongly conditioned naturalistic cue-alcohol memories?

3) Is ketamine (an NMDAR antagonist) a viable blocker of the reconsolidation of naturalistic cue-alcohol memories in a sample of hazardous drinkers?

4) Is rapamycin (an mTOR inhibitor) an effective blocker of natural (non-drug) appetitive reward memory reconsolidation in those with a tendency to overeat chocolate?

This final chapter will summarise the findings associated with the above research questions. Following this, the current findings will be integrated into the existing literature, and considerations for the potential implication and limitations of the current studies, along with potential areas of development, will be discussed.

6.2 Summary of findings

Chapter 2 details a meta-analysis and systematic review of behavioural and pharmacological studies of naturalistic reward or fear-related memory in clinical or subclinical human samples. An original search of the literature conducted on the 03/10/2017, and one study conducted as a part of the current thesis, yielded 19 studies from 18 publications (n=809) for the final analyses. Five were studies on specific phobia, five trauma-related, and nine on substance use disorder (SUD). Overall, study effect sizes were in the predicted direction, with nine out of ten trauma or fear, and six out of nine SUD studies favouring the retrieval + treatment group, relative to a non-retrieval or non-treatment control. Calculated effect sizes were moderate and depended on the nature of the treatment. For phobia/trauma studies, pharmacological treatments conferred a medium effect size (g=0.59), while behavioural treatments produced a small, non-significant effect (g=0.32). Conversely, in SUD, pharmacological treatments produced a small, negative effect (g=-0.16), relative to a medium effect of behavioural treatments (g=0.60), with treatment type being a statistically significant moderator. Overall, these results support the idea that naturalistic memories can undergo destabilisation and be subsequently weakened or modified via reconsolidation-modulating strategies.

The highly variable effect sizes, even within each disorder type, might have reflected the varied retrieval procedures used to destabilise the memory trace (i.e. retrieval duration, cue type, timing of treatment relative to retrieval, inclusion of prediction error) treatment types (i.e. differing drug-types and behavioural techniques), participant characteristics (particularly, diagnosis) or some combination of these. The relatively limited number of studies within each disorder category precluded any moderator analyses on these specific study characteristics, warranting further studies on both naturalistic memory reconsolidation in general, and on *optimal* retrieval procedures. *Chapter 3* therefore focused on elucidating the conditions for naturalistic memory destabilisation. Existing literature suggests prediction error (PE; the mismatch between what is expected to and what actually occurs) is required to induce the

reactivation (and destabilisation) of older and more strongly conditioned memories such as those that underlie SUD (e.g. Eisenberg & Dudai, 2004; Eisenberg et al., 2003; Milekic & Alberini, 2002; Robinson & Franklin, 2010; Winters, Tucci, & DaCosta-Furtado, 2009). Too few studies were present in the meta-analysis in *Chapter 2* (particularly those that explicitly included a PE) to appropriately assess the requirement for PE at retrieval. As such, *Chapter 3* examined the specific role of prediction error in destabilising strongly entrained alcohol-memories in heavy drinkers. The chapter describes a single blind randomised controlled study in which nitrous oxide (N_2O, an NMDAR antagonist and hypothesised blocker of reconsolidation) was administered following a memory retrieval procedure that incorporated a PE in a group of hazardous drinkers. Analyses initially failed to indicate greater memory weakening in the group that received N_2O after retrieval with PE (N=20), relative to controls that experienced PE without retrieval (N=20), or retrieval without PE (N=20; no Day x Group interaction on beer consumption). However, a manipulation check revealed a failure of the PE procedure to reliably induce surprise (a putative indirect marker of PE). Reassignment of groups according to *experienced* PE revealed a significant Day x Group interaction, with beer consumption reducing pre to post manipulation only in the group that underwent retrieval *with* PE. Putatively, this interaction was not moderated by memory strength (estimated using a measure of drinking history), suggesting more strongly conditioned memories are still able to destabilise. Tentatively, this study suggests a potential for N_2O to block maladaptive reward memories in a group of hazardous drinkers, although due to post-hoc group reassignment, any conclusions are currently speculative and replication is required.

Using a different sample of heavy drinkers, *Chapter 4* built on the results of *Chapter 3* by adapting the retrieval procedure to maximise PE, and administering intravenous ketamine (a non-competitive, potent NMDAR antagonist) following the retrieval of maladaptive beer-related memories. In a single-blind randomised controlled design, participants were randomly assigned to one of three groups: intravenous ketamine following cue-driven alcohol memory reactivation (N=30); ketamine following control memory retrieval (N=30); and placebo following cue-alcohol memory retrieval (N=30). Reductions across measures of maladaptive reward memory strength were seen, with significant Day x Group interactions observed for frequency of consumption and cue-reactivity to image and in vivo beer, and a trend interaction for total alcohol consumption. In all cases, reductions were observed within the group that received

ketamine after retrieval. Importantly, reductions in alcohol consumption were maintained at 9-months follow-up, although group differences were not observed at this time-point.

Together, *Chapters 3 and 4* support the idea that the maladaptive reward memories underlying alcohol use disorder (AUD) may be attenuated by reconsolidation-based treatments. Whether natural (non-drug) reward memories are also susceptible to reconsolidation-interference in humans remains unknown. Pre-clinical data has consistently demonstrated reconsolidation blockade via mTOR antagonism, however, to date just one human study has explored the potential of the mTOR antagonist rapamycin in a reconsolidation protocol. *Chapter 5* adapted the retrieval procedures used in *Chapters 3 and 4* to a group of chocolate 'over-eaters' and administered rapamycin prior to the retrieval of chocolate reward memories. This study suggested that 10mg of rapamycin in combination with chocolate associative memory retrieval failed to show any retrieval-dependent effects on measures of eating behaviour. It is unclear why this was the case; however, moderation analyses suggested that a reduction in chocolate wanting and consumption was seen in the retrieval + rapamycin group, specifically in those who binged on chocolate at baseline. As such, the overall main lack of effect may reflect limited scope to see an improvement on disordered eating behaviour *a priori*. Alternative explanations for the lack of effect include: prior administration of rapamycin leading to interference with memory destabilisation, insufficient plasma levels of rapamycin preventing reconsolidation blockade, or idiosyncratic features of the sample of chocolate eaters.

6.3 Synthesising the current results into the existing literature

As demonstrated in *Chapter 5*, and described in *Chapter 1*, inconsistent findings and frequent null results mark the reconsolidation field (e.g. Pachas et al., 2015; Wood et al., 2015). Disentangling the reasons why a manipulation may fail to produce memory weakening is challenging. However, careful evaluation of the results from the current thesis, in addition to studies in the wider literature may aid in elucidating the mechanisms thorough which null results are observed.

As described in the opening chapter, reconsolidation likely exists as a mechanism for memory updating, allowing memories to remain relevant and guide future

behaviour (e.g. Lee, 2009). As such, it makes sense that 'boundary conditions' exist which prevent adaptive memories from constantly and indiscriminately reconsolidating (Zuccolo & Hunziker, 2019). Given the target of sub-clinical, maladaptive appetitive reward memory within the current thesis, the boundary conditions of memory age and strength (e.g. Eisenberg et al., 2003; Suzuki et al., 2004; Wang et al., 2009; Winters et al., 2009) are of particular relevance. The existing literature has demonstrated a requirement for PE in the destabilisation of older or stronger memories (for a review see Exton-McGuinness et al., 2015). Indeed, a requirement for PE has been shown for pre-clinical appetitive (Gotthard et al., 2018; Wang, 2018), contextual (Pedreira et al., 2004), spatial (Morris et al., 2006), and fear memory (Diaz-Mataix et al., 2013), as well as human episodic (Sinclair & Barense, 2018), declarative (Forcato et al., 2009) and fear memory (Sevenster et al., 2012, 2013, 2014). In line with this existing evidence, the current thesis suggests a requirement for PE in the destabilisation of memories underlying maladaptive alcohol consumption. Post-hoc, exploratory analysis in *Chapter 3* observed reductions in behavioural indices of cue-alcohol memory strength only when N_2O was administered after a PE-inclusive retrieval procedure. Although PE was not directly manipulated in *Chapter 4*, it is notable that reductions in cue-alcohol memory strength were also observed in the retrieval + ketamine group, where retrieval was inclusive of PE. Here, relative to placebo and no-reactivation controls, marked reductions in frequency and total consumption of alcohol were observed, alongside reductions in wanting, liking and desire to drink more of an in vivo beer cue. Importantly, biological assays of blood-ketamine levels correlated with behavioural outcomes only in the group that received ketamine following a PE-inclusive retrieval procedure, suggesting a reconsolidation-interference mechanism. Taken together, these results suggest PE is an essential requirement for naturalistic reward-memory destabilisation.

It should be noted that despite adopting the retrieval and PE protocol described in *Chapter 4*, the study detailed in *Chapter 5* (in which the protein synthesis inhibitor rapamycin was administered prior to the retrieval of associative chocolate reward memory) failed to observe an effect consistent with reconsolidation interference. Like *Chapter 3* (in which memory weakening consistent with reconsolidation-interference was observed), self-reported surprise following the PE-generating procedure was uniformly induced within groups, suggesting PE was induced. How accurately self-reported surprise reflects experienced PE, however, is unclear. In pre-clinical models,

the release of dopamine and the activation of dopaminergic neurons correlates with PE (Schultz et al., 1997), and the destabilisation of appetitive (Merlo et al., 2015) and fear (Cahill et al., 2019) memories is dependent on dopamine receptors. As such, dopaminergic signalling may provide a better index of actual experienced PE, yet assaying such a signal would be challenging in human subjects using the described reactivation procedure.

A behavioural index of PE has previously been described by Sevenster et al. (2013). Here, fear was conditioned using a 100% reinforcement schedule, with explicit instructions of the contingency between the CS and US. During retrieval on Day 2, PE was induced by presenting the CS in the absence of the US. Relative to no PE controls, who experienced a reinforced retrieval procedure (CS with US); the PE group experienced a reduction in fear responding, indicative of a blockade of reconsolidation by propranolol. An additional PE group that were conditioned using a 33% reinforcement schedule and experienced the CS *with* the US at retrieval on Day 2, similarly reduced fear responding relative to the non-PE control group. Retrieval-induced changes to expectancy of the US (i.e. difference in expectancy from the last trial of fear conditioning on Day 1, and first trial of extinction at Day 3) were predictive of these changes in fear response, suggesting US-expectancy may act as a behavioural measure of PE. However, the utility of this measure in clinical samples may be limited for two reasons. Firstly, this index is calculated using explicit knowledge of US-expectancy during the conditioning procedure, which is not known for naturalistic memories. Secondly, while it may be possible to assess expectancy experimentally, in clinical samples this would involve either an ethically dubious additional conditioning trial (potentially increasing symptomology), or risk alerting the participants to the subsequent occurrence of the PE. While beyond the scope of the current thesis, EEG was recorded during the retrieval procedure in *Chapters 3 and 4*. Neural correlates of PE, for example a large negative EEG deflection known as error-related negativity (as seen in Dehaene, Posner, & Tucker, 1994) may provide a better index of PE than self-reported surprise and should be investigated in future, although there are clearly challenges related to signal-to-noise ratios as PEs are generated using single trial procedures.

However, were we to infer the occurrence of PE (and subsequent destabilisation) from memory weakening as observed in *Chapter 4*, Das et al. (2015b), Das et al. (2018b), and Hon et al. (2016), it may seem unlikely that the retrieval procedure itself was not

sufficient to produce PE. Rather, other factors may have prevented destabilisation in *Chapter 5*, for example, pharmacological interference with memory destabilisation. Oral administration of pharmacological substances is associated with a slow latency to peak plasma. As such, we decided to administer rapamycin prior to retrieval. In comparison, both ketamine and N_2O can be administered *after* retrieval and rapidly achieve peak plasma/central concentrations, meaning interference with memory reactivation and destabilisation of the memory trace was avoided in these experiments. Typically, pharmacological interference with the destabilisation of a memory trace is inferred via the administration of the pharmacological probe *prior* to retrieval, and a known blockade of reconsolidation *after* retrieval (e.g. Lee, Amorim, Cassini, & Amaral, 2019; Lee et al., 2008; Lim et al., 2018; Milton et al., 2013; Popik, Crestani, Silva, Quillfeldt, & de Oliveira Alvares, 2018). If memory weakening does not occur (relative to control group), one possibility is that the pharmacological probe prevented initial destabilisation. To the author's knowledge, no pre-clinical or human studies have co-administered rapamycin with a reconsolidation blocker prior to retrieval. However, pre-retrieval administration rapamycin is associated with the blunting of memory expression (a process that is molecularly independent from destabilisation; Balderas, Rodriguez-Ortiz, & Bermudez-Rattoni, 2013; Delorenzi et al., 2014; Rodriguez-Ortiz et al., 2012; Shi et al., 2009), and memory weakening has been demonstrated in pre-clinical models where rapamycin was administered prior to retrieval memory *(*Gafford et al., 2011*;* Mac Callum et al., 2014*)*. In *Chapter 5*, exploratory analyses revealed a moderation by binge risk at baseline, such that participants in the retrieval + rapamycin who engaged in a chocolate binge before treatment *did* appear to reduce their consumption and wanting of chocolate, relative to those who did not binge. Given that the sample in this study had a mean BMI on the high end of normal, and did not approach the clinical criteria for BED, there may have been limited scope to see improvements on measures of disordered eating. Thus, in those with more clinically relevant, 'maladaptive' memories, rapamycin *may* have blocked reconsolidation. Replication of *Chapter 5* with a more clinically relevant sample, such as hazardous drinkers as used in *Chapters 3* and *4*, or a sample that binge eats, is therefore warranted.

Taken together, the results from the experimental chapters of the current thesis highlight how both ketamine and N_2O represent particularly promising substances for interfering with the reconsolidation of human appetitive memory. Meta-analysis of the animal literature has previously determined a superior effect of post-retrieval NMDA,

relative to βAR blockade for appetitive memory reconsolidation (Das et al., 2013). However, at present, ketamine and N_2O are the only NMDAR antagonists available for human use that can be rapidly administered *after* reactivation. This is particularly important as the NMDAR is required for both the destabilisation and restabilisation of memory (Milton et al., 2013), and NMDAR antagonists safe for use in humans are not specific enough to target only restabilisation. A double dissociation between the Glu-NR2B and Glu-NR2A subunits of the NMDAR means that in animal models, selective disruption of the destabilisation *or* restabilisation of a memory can be achieved (Milton et al., 2013). Except for perhaps Ifenprodil (a selective blocker of destabilisation/the Glu-NR2B receptor), drugs with this level of specificity are not safe for human use. Thus, until novel NMDAR antagonists are developed, human reconsolidation protocols should continue to administer NMDAR antagonists *after* retrieval.

Although potentially less effective than NMDAR antagonism (Das et al., 2013), βAR blockade does represent an alternative pharmacological probe for appetitive reconsolidation interference in humans. The βAR antagonist propranolol has consistently demonstrated interference with the reconsolidation of human fear memory (for a review see Elsey et al., 2018). Moreover, unlike non-specific NMDAR antagonists, propranolol does not appear to block memory destabilisation when administered prior to reactivation (e.g. Kindt & Soeter, 2018; Thomas et al., 2017). However, the efficacy of propranolol as a blockade of appetitive and reward memory reconsolidation is less clear. In rodents, post-retrieval administration of propranolol is associated with the disruption of cocaine conditioned place preference (Bernardi et al., 2006; Otis et al., 2013) and cocaine and non-drug (sucrose) instrumental conditioned reinforcement (Milton et al., 2008b). More recently, Xue et al. (2017) described a translational study in which post-retrieval propranolol reduced a nicotine conditioned place preference in rats. Extending this to humans, the authors observed similar reductions in craving and cue-reactivity to conditioned and new nicotine cues, suggesting propranolol may be a useful blockade of appetitive reward memory reconsolidation. Similar results have been observed for cocaine (Saladin et al., 2013) and other varied drug (Lonergan et al., 2016) memories in human samples. However, as described in *Chapter 1*, other studies of βAR antagonism on naturalistic reward memory reconsolidation have been less positive. Both Jobes et al. (2015) and Pachas et al. (2015), for instance, failed to observe appetitive memory weakening as a result of post-retrieval propranolol administration.

It is important to highlight how varied retrieval procedures may account for discrepancies in reconsolidation findings. A large proportion of the research on propranolol and human fear memory reconsolidation is derived from a single lab, in which retrieval procedures are highly consistent. By contrast, retrieval procedures across appetitive studies vary widely, potentially explaining the discrepancies described here. It would therefore be interesting to directly compare the effects of post-retrieval propranolol with ketamine or N_2O to determine the optimal blockade of human reward memory reconsolidation.

Alternatively, the meta-analysis in *Chapter 2* determined that behavioural methods of appetitive memory reconsolidation interference might be more efficacious than pharmacological blockades of reconsolidation. This would certainly avoid the previously discussed issues associated with interference in the destabilisation of memory, as behavioural treatments can be administered immediately after retrieval. The most frequently used behavioural treatment within the analysis in *Chapter 2* was retrieval-extinction, in which extinction delivered within the reconsolidation window is thought to lead to memory updating, rather than new extinction learning (Monfils et al., 2009). While results were generally in the positive direction in *Chapter 2*, whether effects on measures of maladaptive memory strength occur as a consequence of memory overwriting has recently been challenged. Cahill et al. (2019) investigated retrieval-extinction's dependence on memory destabilisation, demonstrating that retrieval-extinction reduced cued-fear in both the presence and absence of dopamine-dependent memory destabilisation, and with and without the induction of PE. As an effect was not observed when rats were simply exposed to the conditioning context, it appears that relapse-resistance conferred by retrieval-extinction is dependent on CS exposure. Retrieval-extinction may therefore reflect an enhancement of the consolidation of the extinction memory, rather than a reconsolidation-dependent process. This is consistent with the pre-clinical literature demonstrating comparative effects when extinction was administered both immediately before *and* after retrieval (Millan et al., 2013), and the idea that D-cycloserine-enhanced fear extinction may also render extinction memory resistant to reinstatement or renewal (e.g. Ledgerwood, Richardson, & Cranney, 2004).

An alternative behavioural-interference reconsolidation strategy is counter-conditioning, in which formally rewarding cues are paired with disgusting or aversive outcomes during the reconsolidation window (e.g. Das et al., 2015b; Goltseker et al.,

2017). Das et al. (2015b) observed a reduction in attentional bias towards alcohol-related cues in the group that received retrieval paired with PE, but not in the group that did not undergo PE after retrieval. Thus, unlike retrieval-extinction (in which absence of PE did not reduce treatment efficacy; Cahill et al., 2019), counter-conditioning does not appear to merely rely on the enhancing effects of a CS exposure on the consolidation of counter-conditioning. This may be particularly promising given that behavioural interference strategies allow for reconsolidation interference or updating without administering pharmacological substances. This opens up this technique to pharmacological potentiation of destabilisation, which may amplify the effect of a behavioural treatment. In rodents, administration of the NMDAR agonist D-cycloserine is associated with the enhancement of fear memory destabilisation (e.g. Bustos et al., 2010; Gazarini, Stern, Piornedo, Takahashi, & Bertoglio, 2014; Ortiz, Giachero, Espejo, Molina, & Martijena, 2015; Ortiz, Molina, & Martijena, 2016), and may be used to overcome a resistance to destabilisation induced by ethanol withdrawal (Ortiz, Espejo, Molina, & Martijena, 2019). Whether D-cycloserine confers the same potentiation of reward memory destabilisation is less clear. However, D-cycloserine does appear to promote reward memory reconsolidation. Administration after a brief cue-drug retrieval was associated with an *increase* in drug seeking, suggesting that rather than strengthening retrieval-extinction as expected, reconsolidation was promoted (Lee, Gardner, Butler, & Everitt, 2009). Given this potential to *increase* symptomology, caution must be applied before attempting to potentiate destabilisation, especially if there is a possibility that conditioned responding could increase during post-reactivation treatment (for example in cases of 'unsuccessful' extinction).

At this stage, the relative efficacy of behavioural and pharmacological interference techniques can only be speculated. Even in the current thesis where similar retrieval procedures were utilised in *Chapters 3 and 4,* a direct comparison of the efficacy of N_2O and ketamine is not possible due to differences in other procedures and sample characteristics. Tentatively, reductions in alcohol consumption following post-retrieval ketamine were associated with greater effect sizes (relative to N_2O) and were consistent across multiple putative measures of cue-alcohol memory strength. However, while it might be speculated that this reflects ketamine's greater potency as an NMDAR antagonist relative to N_2O, we must be considerate that PE was suboptimal in the *Chapter 3* study. Thus, identification of the optimal conditions for blockade of

reconsolidation will require studies in which different methods of reconsolidation-interference are directly compared, whilst ensuring all other retrieval procedures, sample characteristics, and targeted memory type are kept as consistent as possible.

Models of visual salience suggest that shifts in eye movements are made towards the point of highest salience. As learning models of AUD (e.g. Robinson & Berridge, 2003) posit that repeated use of alcohol imbues environmental cues predictive of alcohol consumption with motivational properties, it might be assumed that we can capture these 'bottom-up' processes using measures of attentional bias. Indeed, an attentional bias towards alcohol-related cues is observed in heavy drinking samples, which correlates with measures of craving (Field, Munafo, & Franken, 2009). Using the visual dot probe, the current thesis demonstrated an attentional bias towards alcohol cues in *Chapter 4* but not in *Chapter 3*. It is possible this reflects the greater levels of hazardous drinking in the sample in *Chapter 4* (AUDIT score=22.14), which more closely mirrored an AUD population than the sample used in *Chapter 3* (AUDIT score=14.69). Attentional bias towards alcohol cues in a non-clinical sample (social drinkers) has previously been captured when cues were specific to the participant's preferred alcoholic drink (Christiansen, Mansfield, Duckworth, Field, & Jones, 2015), however, even when analyses in *Chapter 3* were limited to beer cues, an attentional bias was still not observed on the visual dot probe task. Following a lack of attentional bias in *Chapter 3*, the image cues were updated with those that more closely resembled the US (e.g. images of beer mats were replaced with images of beer itself). It is therefore possible this change to the task itself led to the detection of an attentional bias in this study.

It is notable that despite showing reductions in arguably more clinically meaningful outcomes (i.e. reductions in alcohol consumption and frequency of use), *Chapter 4* did not observe a change in attentional bias towards alcohol cues. What exactly attentional bias is indexing within the visual probe task is therefore unclear. In a previous study of human appetitive reconsolidation, reductions in attentional bias were observed and associated with reduced cue liking (Das et al., 2015b). Conversely, despite no change in attentional bias, *Chapter 4* showed significant reductions in self-reported liking and desire to consume in response to image cues. Thus, it appears that in this chapter attentional bias and cue-reactivity reflected distinct processes, whereby cues continued to capture attention but did not elicit a behavioural response (i.e. alcohol or seeking). Why this occurred in *Chapter 4* but not in Das et al. (2015b) is not clear, however it

may reflect differences in reconsolidation interference strategy. While ketamine was hypothesised to weaken the original maladaptive reward memory (MRM) trace by preventing its reconsolidation, counter-conditioning aimed to update the original memory by repeatedly pairing image cues with aversive images and bitter tasting drinks. It is possible that imbuing the cues themselves with aversive properties led to a greater reduction in attentional bias relative to more general memory weakening.

Alternatively, a lag in subjective, relative to implicit fear has previously been demonstrated (Soeter & Kindt, 2015a). Given that we only measured attentional bias at 10-14 days post-manipulation, it is possible that we would have seen a reduction had the visual probe task been re-administered at a later follow-up as changes in attentional bias may occur secondarily to changes in drinking behaviour (i.e. alcohol consumption). However, it is not possible to know if this is the case due to lack of follow-up data. Current data therefore suggest that the visual probe task may not be a useful measure of memory strength.

6.4 Challenges associated with human reconsolidation research

Research on humans inherently entails less control and greater heterogeneity (e.g. among participants) than animal research. One challenge within the human reconsolidation literature is identification of conditions under which a retrieval-interference procedure is associated with subsequent memory impairment. Even in animals, which can be genetically identical and trained with a uniform number of conditioning trials, there can be individual differences in the conditioning and subsequent susceptibility to reconsolidation-interference (e.g. Gillis & Morrison, 2019; Morrison, Bamkole, & Nicola, 2015). Extrapolating this out to humans with highly variable individual learning histories and genetic profiles means it can be particularly difficult to identify why a treatment failed to produce an expected effect. With this in mind, the studies in the current thesis were careful to record and assess potential mechanisms through which null effects might be interpreted. *Chapter 3*, for example, initially failed to observe an effect of post-retrieval N_2O, with exploratory analyses identifying the potential of N_2O only because manipulation checks were conducted, and experienced surprise was recorded. Similarly, in *Chapter 5*, probing potential individual differences in pre-existing memory strength suggested that those with a

propensity to binge eat *may* have experienced a reduction in memory strength because of pre-retrieval rapamycin. The need to conduct such manipulation checks is therefore clear. In all cases however, these analyses were post-hoc and exploratory in nature. Studies in which key variables indexing, for example, existing memory strength, age, and individual learning history, are directly manipulated would be required to have confidence in a moderating effect of these variables.

Thus, identifying a way to detect when or if reconsolidation has occurred should be a focus of future studies. *Chapter 4* attempted to identify a potential biomarker of human reconsolidation. In animals, the ability to administer pharmacological probes directly to the brain means not only can specific brain areas associated with reconsolidation be identified, but drugs can also be administered immediately before or after retrieval. These studies are beginning to refine our understanding of the neuropharmacology and neuroanatomy of reconsolidation in a way that is not possible in human participants. For example, CB1 receptors in the rodent hippocampus and basolateral amygdala (BLA) are differentially associated with memory destabilisation, such that infusions of CB1 antagonists prior to reactivation prevent destabilisation only when administered into the BLA. Conversely, post-reactivation infusions prevented destabilisation only when applied to the hippocampus (Lee et al., 2019). Similarly, other methodologies including protein expression analysis (e.g. Stoica et al., 2011) and the use of genetically identical, or knockout mice for the identification of specific molecular and cellular processes associated with reconsolidation (e.g. Asthana et al., 2016; Cestari et al., 2006) are, of course, limited to animals. Methods for independently (of behavioural outcomes) inferring the occurrence of reconsolidation in humans are therefore required. In *Chapter 4*, it was tentatively hypothesised that BDNF (a biomarker of neuroplasticity and potential biomarker of consolidation; Goulart et al., 2010) may act as a marker of reconsolidation. However, this was not the case, and post-retrieval plasma BDNF was not predictive of any behavioural outcomes. Although it is possible that BDNF was not measured at an appropriate time for the detection of destabilisation or restabilisation of the MRM, it is likely that peripheral measures of BDNF are simply not sensitive enough to detect individual variability.

Identification of biomarkers for destabilisation and restabilisation of the memory trace during reconsolidation is therefore ongoing. A limited number of neuroimaging studies of reconsolidation have been conducted, with fMRI demonstrating engagement of the amygdala and hippocampus during the reactivation of an emotional memory,

and reduced activation in these areas following reconsolidation-interference with propranolol (Schwabe, Nader, Wolf, Beaudry, & Pruessner, 2012). Attenuation of amygdala activation was similarly observed following retrieval-extinction of spider phobia memories, with this effect persisting for six months after treatment (Bjorkstrand et al., 2017). Although these neural correlates are suggestive of memory weakening, fMRI studies do not demonstrate reactivation (and thus destabilisation) *per se*. Rather, these studies demonstrate memory expression during reconsolidation, which is technically more tractable than identifying markers of destabilisation, and interference with reconsolidation is still assumed based on behavioural outcomes. These findings do, however, appear consistent with the pre-clinical literature, and weakening of fear memory (or at least, expression of that memory) seems to be detectable with fMRI. Whether the same brain areas would be recruited following reward memory reactivation is unclear, and if it is consistent with pre-clinical literature we might similarly expect recruitment of the amygdala (e.g. Ben Mamou et al., 2006; Olshavsky et al., 2013) as well as the reward-associated mesolimbic areas (Robbins & Everitt, 2002). However, these studies are yet to be conducted in humans.

Thus, neuroimaging with greater temporal specificity will be required to identify when and if memory reactivation and destabilisation has occurred. Zhu, Wang, Jia, and Wu (2019), for instance, used MEG to identify a decrease in beta-band power during episodic memory retrieval, and a decrease in alpha-band power during post-retrieval interference. By directly comparing MEG output, the authors were able to dissociate between post-retrieval memory integration and restabilisation interference. Whether similar neural processes underlie the destabilisation of appetitive memory restabilisation would be interesting to explore. Further, if a replicable neural correlate of destabilisation was identified using EEG, it may act as a suitable biomarker for memory destabilisation with potential application in clinical settings (though more sensitive, MEG remains a less accessible option for clinical application due to cost).

However, behavioural outcomes in the current thesis did correlate with blood plasma ketamine in the retrieval + ketamine group but not in the no retrieval + ketamine group. This lends support to the conclusion that results in *Chapter 4* were due to a reconsolidation-interference effect. The importance of using biological assays is therefore evident. Given that EEG was recorded throughout both ketamine and N_2O administration, future analyses or studies could look at the neural correlates of NMDAR antagonism (e.g. Ehrlichman et al., 2009; Shaw et al., 2015), to see if these also predict

behavioural outcomes when memories are reactivated prior to pharmacological antagonism.

The utility of blood-plasma drug levels (which unfortunately, were not available) was demonstrated in *Chapter 5*, where no effect of rapamycin was observed. Rapamycin dosage was selected based on safety and tolerability, and it is possible that this dose was simply not potent enough to sufficiently block reconsolidation. As cost restraints precluded the measurement of biological assays of rapamycin in *Chapter 5*, it was not possible to retrospectively identify if null effects were due to an insufficient dosage of rapamycin. In all, this highlights to merit of obtaining biological assays within human reconsolidation studies.

6.5 Optimising reconsolidation-interference procedures

Despite the extensive pre-clinical literature demonstrating the existence of appetitive reward memory reconsolidation, few studies have been conducted in human samples (see *Chapter 2* for details). Thus, replication studies are primarily required. Before expensive and time-consuming clinical trials are conducted, identification of the *optimal* reconsolidation procedures for attenuating MRM should be sought.

All studies described in the current thesis utilised (or adapted) the retrieval procedure outlined in Das et al. (2018a). This procedure has successfully produced destabilisation in both behavioural (Das et al., 2015b; Hon et al., 2016), and the pharmacological protocols described in *Chapter 4*, and putatively, in *Chapter 3* of the current thesis. It is not known, however, whether this is the *optimal* procedure to induce destabilisation of appetitive reward memory. For instance, in the current thesis memories were reactivated using six images and a single in vivo cue. In all cases, participants were asked to recall an instance in which they had consumed the depicted beverage or food, and rate how that influenced their desire to consume the in vivo cue. The inclusion of an in vivo cue may be an important feature of a retrieval procedure, as retrieval procedures in which the in vivo cue is reproduced using virtual reality (e.g. Maples-Keller et al., 2017; Shiban et al., 2015) have generally been less effective than those which use the feared object itself (e.g. Soeter & Kindt, 2015a; Telch et al., 2017). It might be speculated that retrieval procedures that closely replicate the original learning confer greater destabilisation of the memory trace. If this is the case,

administering the reconsolidation procedure within a prototypical drinking environment (rather than a laboratory setting as used in studies in the current thesis) may confer greater destabilisation of the memory trace, albeit difficult if pharmacological reconsolidation blockades are used.

An alternative consideration may be that in vivo cues are simply closer replicates of the US than image or virtual reality cues. Luo et al. (2015) compared retrieval procedures where either the CS or US were presented during a retrieval-extinction procedure. Comparable inhibitory effects were observed on cocaine-priming-induced reinstatement, spontaneous recovery, and renewal of the MRM when treatment was applied 1 day after acquisition. However, when retrieval-extinction procedures were applied after a 28-day cocaine withdrawal period, US, but not CS, retrieval procedures inhibited renewal and reinstatement of cocaine seeking. Furthermore, US retrieval procedures produced more generalisable effects, whereby reduced cocaine seeking in response to new drug-associated cues was observed following retrieval with US, but not CS, cues. Rating of the US, rather than images (as used in the current retrieval procedure), may therefore confer greater destabilisation and generalisation of effects. However, given that the above findings were observed pre-clinically, these results would need to be replicated in humans. Ideally, future studies should focus on identifying these optimal procedures, employing direct comparisons between cue types.

It is similarly unknown whether we used optimal doses of the tested drugs. A single dose of each drug was used in all the studies described in the current thesis, in each case based primarily on tolerability and safety profile rather than a knowledge of receptor level concentrations likely to be achieved. A positive relationship between blood-plasma ketamine and behavioural outcomes in *Chapter 4* suggests that a higher dose may confer a greater benefit. Alternatively, pre-clinical models of reconsolidation tentatively suggest a non-linear relationship between NMDAR antagonist concentration and behavioural outcomes. Indeed, meta-analysis demonstrated a U-shaped dose-response curve between MK801 dose and effect size in the pre-clinical reconsolidation literature. Ketamine dosage for the study described in *Chapter 4* was selected based on tolerability in a small number of non-dependent, heavy drinking participants during piloting. Given the shared target of the NMDAR, it is possible that those who are verifiably *dependent* on alcohol also have a higher tolerance for ketamine. If this is the case, there is a rationale for using even larger doses within such

participants. Under this interpretation, the dose that confers the greatest reconsolidation blockade may also vary depending on individual drinking history and/or family history, such that dose-response research may need to be conducted while being mindful of this.

In all cases within the current thesis, outcomes were based on a single retrieval followed by putative reconsolidation blockade. It is possible that improved efficacy will be observed following multiple treatments. For instance, greater reductions in fear memory have been demonstrated when reconsolidation-blockade is delivered over the course of multiple sessions. For instance, Brunet et al. (2018), Lonergan et al. (2016), and Brunet et al. (2011) observed a continuous reduction in Post-Traumatic Stress Disorder (PTSD) symptoms over six sessions. However, as these studies did not directly compare memory weakening from multiple sessions with a single session it is not possible to rule out that this reduction would occur regardless. Given the possibility of an additive effect of reconsolidation-treatment, the efficacy of multiple treatments should be explored.

One methodological issue associated with multiple retrieval sessions is the induction of multiple PEs. Once participants have experienced one PE during their first session, they will be an expectation of subterfuge in later sessions. As suggested by the results in *Chapter 3*, this expectation may be enough to prevent destabilisation of the memory trace. Identifying ways to increase the likelihood of PE either behaviourally or pharmacologically would therefore be worth pursuing. Given that PE induction and associated destabilisation of a memory trace is mediated by dopaminergic transmission (e.g. Exton-McGuinness et al., 2015; Merlo et al., 2015), pharmacological modulation of dopamine may be a viable target for inducing PE. Alternatively, pharmacological enhancement of other components of destabilisation, such as CB1 receptor agonism (Lee & Flavell, 2014; Lee et al., 2019), may also potentiate destabilisation and should be a consideration for future research. Moreover, activation of the GluNR2B NMDAR subunit (Lee et al., 2009; Torregrossa, Sanchez, & Taylor), might increase the tendency for memories to destabilise, although it is not clear whether PE-dependence would be reduced using such an NMDAR specific approach.

An additional consideration with multiple treatments is potential side effects or impacts of the drug used as a blocker of reconsolidation. Unlike propranolol, which is not psychoactive and can be administered safely multiple times, a more considered

approach would be necessary with the drugs used in the current thesis. Ketamine, for instance, is highly psychoactive, requiring hospital space and medical personal to administer intravenously. Although, as ketamine has safely been administered over multiple sessions for the treatment of depression (for a review see Serafini et al., 2014) this does not preclude its potential use. Similarly, N_2O appears hazardous to health only when used beyond the frequency likely for reconsolidation-treatment (Collado, Nicolas, Faulks, & Hennequin, 2007). Conversely, although rapamycin was not associated with any subjective side effects in *Chapter 5*, its safety profile limits its potential use for multiple treatment sessions. As an immune suppressor, repeated administration can produce side effects such as inflammation and an increased susceptibility to infection (Brattstrom et al., 2000). In all cases, these potential side effects should be weighed against the potential benefits of treatment.

6.6 Limitations

It is hoped that the limitations associated with specific chapters have already been sufficiently addressed and will not be repeated here. Rather, general limitations of reconsolidation-based treatments and the approach used within this thesis will now be discussed.

The present thesis has focused primarily on a learning model of addiction, wherein Pavlovian associations between previously neutral cues and the rewarding properties of drugs (or foods) are considered a risk factor for relapse. Thus, attenuation or amelioration of these MRM via reconsolidation is assumed a viable, long-lasting treatment for disorders such as addiction. However, a focus only on a learning model of addiction is reductive and treating addiction as a whole should be considerate of additional psychological risk factors that contribute to the progression into, persistence of, and relapse back to addiction. Indeed, unless risk factors such as stress (e.g. Johnston, Linden, & van den Bree, 2016; Koob, 2008), individual differences in risk and impulsivity (e.g. Kreek, Nielsen, Butelman, & LaForge, 2005), emotion regulation (e.g. Li & Sinha, 2008) and comorbidity with other psychiatric disorders (e.g. Conway, Compton, Stinson, & Grant, 2006) are considered, it is likely that any treatment of addiction will be insufficient. After all, if an individual drinks to relieve himself or herself of a negative emotional state, they are not drinking simply in response to craving. Failure to address that negative state will therefore lead to continued engagement in the addiction. However, it should be noted that reconsolidation and

other currently available behavioural and pharmacological treatments are not mutually exclusive. Rather, it is likely that the most effective treatment for disorders of appetitive memory will integrate these approaches. For instance, an optimal treatment for AUD may address the implicit maladaptive memories underlying craving and motivation to consume alcohol using reconsolidation, while addressing an individual's explicit thoughts and feelings towards alcohol via cognitive behavioural therapy (e.g. McHugh, Hearon, & Otto, 2010). Given that reconsolidation-based treatment can be delivered quickly (i.e. the retrieval procedures and drug administration sessions described in *Chapters 3 and 4* totalled less than one hour) and may be deliverable in a single session, this procedure may easily be integrated into existing comprehensive treatments that target the range of biopsychosocial factors.

It is also notable that translational reconsolidation studies, such as those contained within the current thesis, are based on rodent models of addiction and reward. Thus, whether animal models are sufficiently representative of the nuanced nature of human addiction is an important consideration. Instrumental and Pavlovian conditioning within rodents typically involves the association between a single cue and reward during conditioning. However, humans consume alcohol and other rewards in a wide variety of contexts and in the presence of multiple environmental stimuli. Thus, whether attenuation of memory for a single stimuli-response via reconsolidation-interference results in more generalised attenuation of a behavioural response is of considerable clinical relevance. As discussed, pre-clinical evidence has demonstrated generalised resistance to cocaine-seeking reinstatement when the US is used during reactivation, but not when the CS was used (Luo et al., 2015). This might suggest that exposure to the US results in destabilisation of the wider memory trace. In *Chapters 3* and *4*, reductions in alcohol consumption were largely constrained to beer, with limited reductions in wine and spirit consumption. While it is possible this is reflective of the selected sample (beer drinkers) and associated floor effects, it is important to consider that this may reflect a failure of the retrieval procedure to destabilise the wider alcohol memory network. Clinical populations will likely consume different types of alcohol in multiple contexts, and it will be important that retrieval procedures are individualised to optimally destabilise the MRM underlying their personal consumption behaviours. Again, this might involve identifying optimal (e.g. US-inclusive) retrieval procedures.

It was assumed that the memories underlying maladaptive consumption of highly palatable foods are close replicates of drug use and consumption. Natural (non-drug

rewards) are inherently rewarding, and activate the same mesolimbic reward pathways associated with drugs of abuse (Johnson & Kenny, 2010; Kelley & Berridge, 2002; Robbins & Everitt, 2002; Volkow & Wise, 2005). However, whether someone can develop 'food addiction' (Hebebrand et al., 2014) is a topic of considerable debate (e.g. Hebebrand et al., 2014; Ziauddeen & Fletcher, 2013). Exploratory results in *Chapter 5*, in which chocolate consumption and cue-reactivity change was moderated by binge-risk at baseline, suggested the effect of retrieval + rapamycin may be limited to those who binged at baseline. As a reduction in the retrieval + rapamycin group was not observed in those who did not previously binge on chocolate, it might be suggested that individuals who regularly 'over-consume' chocolate are not analogous to heavy drinkers. The food addiction literature proposes that highly palatable foods (i.e. those high in sugar and fat) may have addictive potential (Avena et al., 2008; Gearhardt, Davis, Kuschner, & Brownell, 2011; Johnson & Kenny, 2010). However, there are important distinguishing factors between natural (non-drug) and drug rewards. The incentive-salience model proposes that addiction occurs as a result of initially goal-directed behaviour becoming habitual and compulsive (Everitt & Robbins, 2005). As already stated, both natural and drug rewards are associated with the release of dopamine in the mesolimbic system. However, whereas the dopamine response to highly palatable food habituates following a single exposure (e.g. Bassareo, De Luca, & Di Chiara, 2002), drugs of abuse sensitise the dopaminergic system and are resistant to habituation (e.g. Di Chiara, 2005). Thus, the phasic dopamine responses that originally occurred in response to the drug itself eventually occur in response to the predictive cue, meaning drug seeking and taking may become habitual in a way that food consumption does not.

One important caveat to this may explain why a moderation by binge-risk at baseline was observed on the relationship between group and change in chocolate consumption, and cue-induced wanting of chocolate. In *Chapter 5*, those who engaged in binge eating prior to study participation did appear to reduce their consumption of chocolate following retrieval and rapamycin. In pre-clinical models, BED is modelled by giving rodents intermittent access to highly palatable foods (Avena et al., 2008; Corwin, 2006). Whereas rats with ad-libitum access to foods experience an attenuation of dopamine release following repeated consumption, this does not occur when access to sucrose is intermittent (Bassareo & Di Chiara, 1997). Similarly in humans with bulimia nervosa (in which binging is following by compensatory behaviour), repeated

palatable food exposure does not lead to a reduction in salivation (a measure of habituation), as it does in healthy controls (Wisniewski, Epstein, Marcus, & Kaye, 1997). Further, high levels of restriction are observed in those with BED, where recurrent engagement in a binge-restrict cycle is typical (e.g. Marcus, Wing, & Lamparski, 1985). While in this restricted state, cues can elicit greater craving and foods themselves can be more rewarding (Fedoroff et al., 2003; Fedoroff et al., 1997). These differential reward responses to food may explain why no group effects were initially observed on measures of disordered eating in *Chapter 5*, as an effect of rapamycin + retrieval was only observed in those who binged, with no such effect in those who were merely 'over eaters' of chocolate. Together, this suggests that this sample may not be a good sub-clinical model for binge eating, and future studies in sub-clinical samples should ensure that participants do engage in binges.

One potential limitation of the protocol used in the current studies may come from interaction effects from the tasks used to disengage participants with the retrieval procedure. As discussed in *Chapter 1*, long retrieval sessions can engage extinction, rather than reactivation of a memory trace. Thus, to keep the retrieval sessions brief, and induce a discrete offset time for the retrieval procedure, studies in the current thesis used distraction tasks such as working memory, or trail making tasks (Das et al., 2018a). One study has investigated the impact of high working memory load task after multiple retrievals (across five sessions) as a potential means for interfering with reconsolidation of alcohol memories (Kaag et al., 2018). This study did not demonstrate an effect of post-retrieval working memory load. It is possible that null effects were observed due to insufficient induction of PE, as the same PE procedure was used across sessions. However, if a high working memory load blocked memory reconsolidation, we might expect the placebo group in *Chapter 4* (who underwent alcohol retrieval followed by distractor tasks inclusive of a digit-span task), to have reduced their drinking. As this was the only group that did not display any significant reductions in measures of memory strength or drinking behaviour, it seems unlikely the working memory task interfered with reconsolidation here. Although this does not suggest any confounding effects of the distraction tasks used, it does highlight the importance of having a retrieval + no treatment control group.

In hindsight, being as consistent as possible throughout the empirical chapters of this thesis would have yielded the most informative results. Had *Chapter 5* used a sample of hazardous drinkers, rather than a sample of chocolate overeaters, the number

of factors that varied from *Chapter 4* (in which we did observe reconsolidation-interference) would have been reduced. As it stands, the different sample means it is possible that a lack of effect reflected floor effects on measures of disordered eating, and that the chosen sample simply did not reflect a clinical population (indeed, the moderation analyses suggest this may be the case). Had we tested with efficacy of rapamycin as a blocker of alcohol-associated MRM, and selected from the same population as *Chapter 4*, we might better ascribe null effects to a drug related mechanism (e.g. pre-retrieval administration, or incorrect dosage). Replication within a hazardous drinking sample is therefore suggested.

6.7 Ethical considerations of reconsolidation

It is worth considering the potential ethical implications of memory modulation. Philosophical accounts propose that our personal identity is comprised of our memories, and certainly, the loss of memory over the course of dementia might represent this. It is therefore unsurprising that people may express caution about using a treatment that potentially modifies our memories. Differentiating between explicit, autobiographical memories and the Pavlovian and instrumental motivational memories that underlie disorders associated with maladaptive memory is therefore important. While it appears that it may be possible to impair (or potentiate) declarative memories when they are selectively targeted (e.g. Forcato et al., 2007; Rodriguez et al., 2013), this tends to be memory for word lists or information, rather than autobiographical memories. Further, these studies tend to show effects with between subjects designs, which failed to replicate when between subjects designs were used (Levy, Mika, Radzyminski, Ben-Zvi, & Tibon, 2018).

Furthermore, when used in more clinically relevant memories, reconsolidation appears to selectively impair the emotional component of the memory. To illustrate, Soeter and Kindt (2015a) demonstrated selective attenuation of the emotional component of a spider-phobic memory. When asked to touch the spider after the reconsolidation-treatment, participants expressed surprise as they expected to feel fear when touching the spider but did not. As such, their explicit memories of past interactions with spiders were retained, while the emotional valence attributed to the spider was selectively abolished. It might be argued that a fearful response to danger is adaptive; however, as subjects retain explicit knowledge, it seems unlikely that reconsolidation-treatments would result in an individual engaging with a potential

danger following treatment. It is particularly difficult to argue for an adaptive role for the memories that underlie drug seeking and taking, such as those addressed in the current thesis.

An alternative ethical consideration is the use of pharmacological substances themselves. While all drugs used in the current thesis were deemed safe for use in humans, the potential side effects of each of these drugs should of course be carefully considered. However, their potential risks must be balanced against the potential gains of treatment. The reconsolidation treatments outlined in the current thesis each utilised a single dose of the drug. By comparison, addiction is associated with the chronic use of alcohol and drugs, which is associated with significant personal, economic, and societal harms. It might be considered that behavioural interference during reconsolidation will not be associated with pharmacological side effects. Certainly, if behavioural and pharmacological interference strategies confer comparable memory weakening, behaviour treatments may be considered superior. However, studies in which the efficacy of pharmacological and behavioural reconsolidation-strategies are directly compared are required to determine if this the case.

6.8 Alternative explanations for reconsolidation-interference

The current thesis has largely argued in favour of a memory weakening effect of memory reconsolidation-interference. However, alternative explanations for behaviour change following reconsolidation procedures have been proposed. Gisquet-Verrier and Riccio (2018), for instance, suggest that memory *updating,* rather than weakening, may occur following reactivation. Under this interpretation, the change in subjective and physiological state induced by the pharmacological manipulation is integrated into the original memory trace following destabilisation. Thus, when the individual is no longer in the drug-state, the memories are inaccessible. In line with this argument, studies have demonstrated recovery of memories after protein-synthesis reconsolidation-interference (Gisquet-Verrier et al., 2015; Gisquet-Verrier & Riccio, 2018). As none of the studies in the current thesis re-measured behavioural outcomes under the influence of the pharmacological probe, we are unable to refute this hypothesis. However, the lack of relationship between the aversive effects of ketamine and N_2O, and behavioural

outcomes, is not supportive of an integration explanation. Similarly, no moderating effect of stimulation-to-sedation ratio was observed in *Chapter 3*. This alternative explanation for reconsolidation also fails to explain animal studies where an altered drug-state induced using yohimbine within the reconsolidation window results in memory strengthening, rather than attenuation (e.g. Gazarini, Stern, Carobrez, & Bertoglio, 2013), nor why peripheral βAR blockers such as nadolol (which induces a similar drug-state to centrally acting propranolol), are not associated with reconsolidation interference (van Stegeren, Everaerd, Cahill, McGaugh, & Gooren, 1998).

It is possible that a state integration effect, such as that observed during counter-conditioning (e.g. Das et al., 2018a; Goltseker et al., 2017) had an additive effect within the current thesis. Indeed, the two more promising reconsolidation blockades within the current thesis (ketamine and potentially, N_2O) were psychoactive, and administered within the reconsolidation window. By comparison, participants were unable to differentiate between rapamycin and placebo. State-integration does not, however, explain why a more euphoric effect of ketamine was positively related to change in AUDIT in the retrieved ketamine group. If this effect of ketamine had been integrated into the memory trace, we might expect an *increase* in consumption, yet a greater reduction hazardous drinking was observed.

Under a similar alternative hypothesis, it is possible that reconsolidation-interference reflects a transient failure to retrieve a memory, rather than interference with the original memory trace itself. This hypothesis may explain why some pre-clinical studies have demonstrated a rebound of memory. Lattal and Abel (2004), for instance, administered a protein-synthesis inhibitor after the retrieval of a contextual fear memory in mice. While impaired freezing, consistent with reconsolidation interference, was observed the next day, a return of fear was observed at 21 days post-manipulation. The authors suggest this occurred as the original memory was not impaired, rather, it was rendered temporarily inaccessible. Memory rebound has been demonstrated elsewhere (e.g. Anokhin, Tiunova, & Rose, 2002; Power, Berlau, McGaugh, & Steward, 2006; Prado-Alcala et al., 2006) and may reflect a partially impaired memory, or insufficient original conditioning (Gold, Haycock, Marri, & McGaugh, 1973; Nader & Wang, 2006). This suggested potential for a memory to rebound highlights the requirement for long-term follow up (as used in *Chapter 4*). Despite the proposed use of reconsolidation-interference as a long-lasting, relapse

resistant treatment for disorders of maladaptive memory, relatively few studies (see *Chapter 2*) include follow-ups beyond two-weeks. Indeed, even in the current thesis only *Chapter 4* included follow-ups beyond one-month. In the case of *Chapter 3*, this was due to a lack of response, likely due to a low incentive to complete the follow-up. Although *Chapter 5* had much lower attrition (only two of 75 participants declined to complete follow-up), due to time and cost restraints follow-up was only completed at one-month. In *Chapter 4*, 9-month follow-up suggested that the reductions in consumption observed at test were maintained at follow-up, however, differences between groups were no longer significant. While it is possible this reflects a rebound of memory, it seems unlikely that the naturalistic memories used here were insufficiently conditioned (although it may still reflect greater memory strength or partially impaired memory). Furthermore, elsewhere in the literature reconsolidation-interference has produced attenuation of phobic memories that have persisted even at 1-year (Soeter & Kindt, 2015a)

6.9 Future directions

Given the inconsistent and frequent null results across the reconsolidation literature, replication of results is perhaps the most pressing requirement of the human reconsolidation field. This is particularly the case within studies of SUD and reward learning. One feature of the phobia reconsolidation literature is its high replication rate, in which propranolol has consistently been demonstrated to interfere with reconsolidation (e.g. Kindt & Soeter, 2018; Kindt et al., 2009; Sevenster et al., 2012, 2013, 2014; Soeter & Kindt, 2010, 2011; Soeter & Kindt, 2012a; Soeter & Kindt, 2012b, 2015a, 2015b, although see Schroyens et al., 2017 for a failure to replicate). As demonstrated in *Chapter 2*, where a lack of studies precluded moderation analysis on some variables (e.g. inclusion of PE), a greater number of studies allows for the more sensitive identification of why studies using reconsolidation-strategies might fail to observe effects. As previously discussed, retrieval methods within the SUD reconsolidation literature are much more discrepant, meaning more studies; with consistent retrieval, procedures are required in *general*.

More specifically, the promising results observed in *Chapter 4* warrants study within a clinical population. This is particularly critical given the older and more strongly conditioned nature of clinical memories, which tend to be more resistant to destabilisation (Milekic & Alberini, 2002; Robinson & Franklin, 2010; Suzuki et al.,

2004). Although the sample used *Chapter 4* were heavy drinkers, consuming a mean average of 74 units a week, they were selected on the basis that they were *at risk* of developing alcohol use disorder. As such, no participants reached the clinical threshold for alcohol use disorder as defined by the structured clinical interview for the DSM (SCID). It is likely that those who are verifiably dependent on alcohol and seeking treatment will have consumed more, started drinking at a younger age, and will have different responses to alcohol relative to the sample used here (Bujarski, Hutchison, Prause, & Ray, 2017; Dawson et al., 2007; DeWit et al., 2000).

Thus, these differences between a clinical population and heavy drinkers may result in different outcomes from those observed in the current thesis. For instance, there is considerable overlap between the neuro and cellular pathways involved in memory reconsolidation, and those which are dysregulated following excessive ethanol consumption (Kelley, 2004). Chronic ethanol administration is associated with dysregulation of the glutamatergic and dopaminergic systems that underlie neural plasticity (e.g. Blackwell et al., 2019; Heinz et al., 2004), and this is particularly pertinent given the target of the NMDAR in the current thesis (namely in *Chapters 3 and 4*). While no moderating effect of family history (a known associate of NMDAR dysregulation), nor drinking history (an approximate measure of memory strength and age) was observed in *Chapter 3*, it would be useful to replicate these analyses on the data in *Chapter 4*. Similarly, the dopaminergic system's role in the mediation of PE means it is possible chronic ethanol may influence the likelihood of a memory undergoing destabilisation. In all, these potential constraints highlight the importance of replicating *Chapter 4* within a clinical, dependent population.

It is important to note that these potentially limiting effects of reconsolidation in a clinical population are hypothetical, and do not guarantee that reconsolidation-interference will be *less* effective in a sample with alcohol use disorders. Indeed, reconsolidation blockade has previously been demonstrated in dependent samples (e.g. Xue et al., 2017; Xue et al., 2012). Although we should be cautious when directly comparing results between different samples, it is notable that despite having higher AUDIT scores and average baseline alcohol consumption, participants in *Chapter 4* experienced a greater reduction in alcohol consumption relative to *Chapter 3*. It might be speculated that a greater reduction is attributable to a greater potency of ketamine as an NMDAR antagonist (relative to N_2O), or to the retrieval procedure more reliably inducing PE. However, this does suggest that despite this sample having stronger

memories they were still able to undergo destabilisation. This also supports the moderation analyses in *Chapter 3*, which failed to observe a moderating effect of memory strength on treatment effect. Other differences in the samples may have also contributed to differences between these two studies, for example participants in *Chapter 4* also scored higher on measures of motivation to change drinking-related behaviour (potentially due to experiencing more negative effects from drinking, as suggested by high AUDIT scores). Extrapolating this to a dependent sample, it might tentatively be assumed that those seeking treatment for alcohol dependency will be more motivated to stop drinking than the samples used here.

It is notable that the current thesis has focused primary on the attenuation of the maladaptive motivational memories underlying disorders of reward. However, if reconsolidation exists as a way in which memories can be updated and strengthened (Lee, 2008, 2009), it seems there would be utility in using reconsolidation to strengthen *adaptive* memories. For instance, increasing the strength of the top-down, goal-directed memories that control behaviour may serve as a useful treatment for addiction and BED. Alternatively, strengthening habituation in BED may reduce binging frequency, and delivering cue-exposure treatments (e.g. Jansen, Broekmate, & Heymans, 1992) within the reconsolidation window may amplify their effectiveness and susceptibility to relapse.

6.10 Concluding remarks

In summary, the mixed results of the studies outlined in the current thesis suggests that overall, reconsolidation is a promising treatment for disorders of appetitive reward memory. In short, the major contributions of this thesis include:

1. Reconsolidation may be an effective clinical strategy for disrupting the maladaptive memories underlying addiction and fear. However, further basic human and pre-clinical studies are required to ensure that reconsolidation procedures are as effective as possible.
2. Prediction error is likely required for the destabilisation of naturalistic maladaptive reward memories, and should be incorporated in future studies of reconsolidation
3. Post-retrieval NMDA receptor antagonism may be an effective means of reducing cue-alcohol memory strength and associated alcohol consumption within a naturalistic sample.

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Appendix

Appendix item 1 *Study quality assessment tool for Chapter 2*

Study Quality Assessment Tool

Is the study design (or paradigm) described? *(1 = yes, 0= no)*

Are there clear inclusion and exclusion criteria? (*1 = criteria given, 1 = no exclusions and no need for criteria, 0 = exclusions without criteria definition)*

Are the procedures for randomization described and appropriate? *(1 = described, 0 = described but not appropriate, 0=not described)*

Are the procedures for blinding (if appropriate, i.e. if outcome is experimenter rated) described? *(1 = appropriate & described, 1 =not appropriate, 0 = appropriate and not described)*

Where outcome measure was experimenter rated, was inter-rater reliability achieved and evaluated? *(1 = No outcomes rated by experimenter, experimenter rated and inter-rater reliability was assessed, 0 = experimenter rated but no inter-rating or reliability testing)*

Are the outcome measures clearly defined, including methods of measurement? *(1 = yes, 0 = no)*

Are the outcome measures appropriate to assess the pharmacological/behavioural intervention in reconsolidation? E.g. measures of craving, skin conductance etc. *(1 = yes, 0 = no)*

Are all demographics (i.e. age/gender) for subjects included? *(1 = yes, 0 = no)*

Is there sufficient control i.e. a drug/no reactivation group and a placebo/reactivation group? *(1 = yes, 0 = no)*

In a multi-group study, were the groups comparable at baseline? (Just key pre-experiment variables) *(1 = yes, 0 = no)*

Is length of reactivation trial given? *(1 = yes, 0 = no)*

Was the reactivation trial sufficient to destabilise the memory? i.e. was a prediction error used (1=yes, 0 = no)

Was drug/behavioural treatment given before reactivation or after? *0 = before, 1 = after)*

Is drug dose given? *(1 = yes, 0 = no)*

Is timing of drug administration relative to reconsolidation clearly described? *(1 = yes, 0 = no)*

Are the analytic methods clearly described and appropriate for the data and study design? *(1 = given and appropriate, 0 =given and inappropriate, 0 = not given)*

Was there a significant amount of missing data and if so, was this dealt with sufficiently? *(1 = no missing data, 1 = missing data but dealt with appropriately, 0 = missing data not dealt with appropriately)*

Appendix item 2 *Lifetime drinking history interview used in Chapters 3 and 4*

	Age	Frequency (per week)	Drinks per day	drinks consumed per sitting	Binge episodes per month (>6 drinks)	Drinks per binge episode	Time spent drinking (per sitting/hrs)	Drink type (%)	Drinking style	Number of sips per drink (drinking speed)	Time to drink a glass/pint (minutes)	Context of drinking (%)
Period in which you first began to drink	_____ To _____		Average _____ Minimum_____ Maximum_____	Average _____ Minimum_____ Maximum_____			Average_____ Max_____	Wine _____ Beer/cider_____ Liquor_____	Sip Gulp	Average_____ Max_____	Wine_____ Pint_____ Spirit+mixer_____	Alone_____ With others____
Period in which you began to drink regularly	_____ To _____		Average _____ Minimum_____ Maximum_____	Average _____ Minimum_____ Maximum_____			Average_____ Max_____	Wine _____ Beer/cider_____ Liquor_____	Sip Gulp	Average_____ Max_____	Wine_____ Pint_____ Spirit+mixer_____	Alone_____ With others____
Period in which drinking peaked	_____ To _____		Average _____ Minimum_____ Maximum_____	Average _____ Minimum_____ Maximum_____			Average_____ Max_____	Wine _____ Beer_____ Liquor_____	Sip Gulp	Average_____ Max_____	Wine_____ Pint_____ Spirit+mixer_____	Alone_____ With others___
Current drinking levels	_____ To _____		Average _____ Minimum_____ Maximum_____	Average _____ Minimum_____ Maximum_____			Average_____ Max_____	Wine _____ Beer/cider_____ Liquor_____	Sip Gulp	Average_____ Max_____	Wine_____ Pint_____ Spirit+mixer_____	Alone_____ With others_____

Appendix item 3 *Ethics approval for study in Chapter 3*

UCL RESEARCH ETHICS COMMITTEE

Need MHRA confirmation that this is not a clinical trial comparing N_2O with memantine

UCL

Amendment Approval Request Form [redacted] 14/10/13

1	**ID Number:** Re: 3901/001	**Name and Address of Principal Investigator:** Dr Sunjeev Kamboj, Research Dept Clinical, Educational and Health Psychology
2	**Project Title**: Understanding Destabilisation and Updating of Drug Memories	
3	**Information about the amendment:**	
	(a) Is the amendment purely administrative?	☐ Yes ☒ No ☐ N/A
	(b) Has the Participant Information Sheet/Consent Form been changed as a result of the amendment?	
	If yes, please enclose a copy.	☒ Yes ☐ No ☐ N/A
		(changes to info sheet are in **bold**)
4	**Summarise the issues contained in the amendment:**	

1) Inclusion of an assay of inhaled Nitrous Oxide (N_2O) to probe the role of neural Nitric Oxide at NMDA receptors in the reconsolidation of drug memories.

In a parallel arm of the study, we plan to use inhaled Nitrous Oxide (N_2O ; provided by and overseen by the UCL Pharmacology department), a putative antagonist at NMDA receptors, to further assess the contribution of NMDA receptors to the restabilisation and updating of drug memories. This will complement the study by using an alternative to the current NMDA receptor antagonist we are using (memantine). N_2O has the advantage of being faster acting (and much faster to leave the body) and has known amnestic properties. Nitrous Oxide is widely used as an anaesthetic in dentistry and obstetrics and is administered to all medical undergraduate students and BSc students studying the 'Drugs and the Mind' course as part of a practical teaching session run by the UCL Pharmacology department in the labs in the Cruciform building.

Adverse events from N_2O are rare because it has rapid and easily controlled onset and offset and is generally very well-tolerated. We therefore envision minimal risk of adverse events from the inclusion of this assay, and certainly no greater than the use of memantine or propranolol, which we already have ethical approval to use as part of this study. The N_2O will be administered via dedicated respirator located within the Cruciform labs. Medical consultation and oversight for the study will be available to researchers and participants of the study if required. Training in the use of the N_2O/ oxygen (40:60% mixture) administration system will be provided by Mr Nick Hayes, lab manager in the Cruciform teaching laboratories.

We will test 40 participants using N_2O (n=20 without memory reactivation and n=20 with memory reactivation, as per previous protocol). An additional condition with memory reactivation without N_2O (n=20 breathing normal air, i.e. N_2O/oxygen ventilation switched off) will also be used.

In line with the N_2O administration, the following exclusion criteria will be added to the study: 1) Current bowel obstruction 2) Middle ear or sinus disease 3)Pneumothorax 4) Pregnancy 5) immunosuppressant medication 6) dihydropteridine reductase (DHPR) or B12 deficiency 7) Compromised respiration.

5	**Please give any other information you feel may be necessary:**

An updated information sheet has been prepared detailing information on this arm of the study and this

Signature of Principal Investigator: [redacted] **Date of Submission: 07/10/2013**

FOR OFFICE USE ONLY:
Amendments to the proposed protocol have been approved by the Research Ethics Committee.

Chair's Signatur[redacted] Date: 7/11/2013

Please return completed form to:

Secretary of the UCL Research Ethics Committee
Graduate School, North Cloisters, Wilkins Building
Gower Street, London WC1E 6BT

Amendment Approval Request Form

1	**Project ID Number:** 3901/001	**Name and Address of Principal Investigator:** Dr Sunjeev Kamboj, Research Dept Clinical, Educational and Health Psychology
2	**Project Title:** Understanding Destabilisation and Updating of Drug Memories	
3	**Type of Amendment/s (tick as appropriate)** ☒ Research procedure/protocol (including research instruments) ☐ Participant group ☐ Sponsorship/collaborators ☒ Extension to approval needed (extensions are given for one year) ☐ Information Sheet/s ☐ Consent form/s ☐ Other recruitment documents ☐ Principal researcher/medical supervisor* ☐ Other * *Additions to the research team other than the principal researcher, student supervisor and medical supervisor do not need to be submitted as amendments but a complete list should be available upon request.	
4	**Justification** (give the reasons why the amendment/s are needed) We request that the dose of nitrous oxide we use is increased from 40% to 50%. Standardised, pre-mixed and pre-verified doses of nitrous oxide are preferred to mixing gases (nitrous oxide and oxygen) ourselves using two separate cylinders. BOC provides premixed oxygen and nitrous oxide in a 50:50 ratio (entonox) which will prove more accurate, practical and economical to use. As such we request a change in the dose of nitrous oxide from 40% nitrous oxide:60% oxygen to 50% nitrous oxide:50 oxygen s supplied by BOC. Given that our aim is to temporarily produce as dense a state of amnesia as possible, this small increase in dose will be favourable for our aims.	
5	**Details of Amendments** (provide full details of each amendment requested, state where the changes have been made and attach all amended and new documentation) No other aspect of the study is changed.	
6	**Ethical Considerations** (insert details of any ethical issues raised by the proposed amendment/s) The slightly higher dose - which is safe and consistent with what is used in clinical setting - will produce slightly greater subjective and physiological effects on participants. However, these will be managed as previously outlined (ensuring effects are tolerable to participants during the experiment, ensuring participants are aware that they can withdraw without giving a reason, ensuring effects have reversed completely before participants leave the session)	
7	**Other Information** (provide any other information which you believe should be taken into account during ethical review of the proposed changes)	
	Declaration (to be signed by the Principal Researcher) • I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it. • I consider that it would be reasonable for the proposed amendments to be implemented. • For student projects I confirm that my supervisor has approved my proposed modifications.	

Signature:

Date: 23/01/2015

FOR OFFICE USE ONLY:

Amendments to the proposed protocol have been ...approved... by the Research Ethics Committee.

Signature of the REC Chair, Professor John Foreman: .....

Date: 27/1/2015.

Appendix item 4 *Information sheet for study in Chapter 3*

Information Sheet for Drinkers Involved in Memory and Cognitive Research Studies

Who are we recruiting?

We would like to invite heavy drinkers, defined as people who drink the governmental daily recommendation of alcohol at least 3 days out of every 7. The governmental daily guidelines are 2-3 units per day for women and 3-4 units for men. This is equivalent to a large glass of wine (250ml) or one/two normal strength beers (440ml, 4.5%).

Details of Study:

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or you would like more information. This study is being conducted by researchers from the Clinical Psychopharmacology Unit at UCL.

Why are we doing this study?

The way people learn about contingencies is thought to be important in psychiatric illnesses such as drug addiction and Post-Traumatic Stress Disorder (PTSD). Certain brain chemicals are thought to be important in the way learned associations are stored, recalled and control behaviour. We are interested in the role of a specific brain receptor in the recall of learned information. This receptor is blocked by Nitrous Oxide. Participants will therefore be required to breathe Nitrous Oxide (N_2O) gas or normal air when recalling previously learned information. By taking part in this study you will contribute to the scientific knowledge of the brain basis of memory and recall and inform potential future treatments for psychiatric disorders like addiction and PTSD. If you would like to receive an overview of the study's results once it has been completed, please ask the investigator and this will be arranged.

Advertisers are interested in the factors that determine how much people enjoy their products, particularly how people learn to like or dislike the taste of certain products and how these tastes can be changed. This study aims to test a specific theory about how rewarding people find beer. Certain brain chemicals are thought to be important in the way learned values are stored, recalled and control behaviour. We are interested in the role of a specific brain receptor in these processes. This receptor is blocked by Nitrous Oxide. To test our theory, on the second day of the study you will be required to consume different drinks

and rate how much you like the taste of them after viewing certain pictures. After this you will be required to inhale either the anaesthetic gas Nitrous Oxide or normal air. You will also complete some questionnaires and simple psychological tests. By taking part in this study you will contribute to the scientific knowledge of how tastes and valuation are affected by heavy drinking. If you would like to receive an overview of the study's results once it has been completed, please ask the investigator and this will be arranged.

What are these drugs and are they safe?

Nitrous Oxide, also known as 'laughing gas' is an inhalable gas that has analgesic (pain-killing) properties. It is a very safe drug that is widely used in dentists and in hospitals during birth as a painkiller. The effects of N2O are quite similar to being drunk, in that it can make people quite giggly, uncoordinated or dissociated. There will be a standard dose of N2O used in the study that will be inhaled through a mask that covers your nose and mouth. After you take off the mask, the effects of the N2O very quickly disappear and you will feel normal again within a few minutes.

What will I have to do?

If you agree to participate in this study you must contact the experimenter by email with contact information and a convenient time to call. You will then receive a call from us and we will ask you a series of questions about your use alcohol, physical and mental health history. Please note that we have strict inclusion criteria for the study and, based on you answers to these questions, you may not be eligible to take part in the study. If you are eligible to take part, you will be asked to come to the Clinical Psychopharmacology Unit (CPU; 1-19 Torrington Place, London WC1E 7HB) at UCL on three occasions each a few days apart, at times convenient for you. You will be required to abstain from drinking alcohol or using any psychoactive drugs (aside from caffeine) for the 24 hours before each testing session. This will be verified with a breathalyser test. If this shows that you have been drinking, testing will be arranged or you will be excluded from the study.

An overview of what will happen on each day of the study is as follows:

Day 1:

On the first day, after giving informed consent and a breathalyser reading to check you have not drunk in the past 24 hours, you will go through some questionnaires that assess your levels of drinking, general mood and personality factors and family history of alcohol abuse. You will then complete some computer tasks that will

Follow-Up

After Day 3, you will not be required to return to the study centre, but we would like to contact you to ask some follow-up questions about your mood and drinking habits. This follow-up information is extremely important for the study, so you will be entered in a prize draw to win £100 if you complete the follow-up. The follow up can be conducted entirely by telephone and will only take a few minutes.

How will I be paid?

You will receive payment for your participation upon completion of the final day. You will be reimbursed at the rate of £7.50 per hour. Unfortunately we cannot reimburse extra travel expenses. The total pay for the study will be around £35. If you complete the follow-up you will also receive an extra £5.

How will my data be stored?

All information which is collected about you during the course of the research will be kept strictly confidential and will be securely stored electronically, using a numbered code so that you cannot be identified. Only researchers directly involved in the study will have access to the data. All data will be stored in accordance with the Data Protection Act 1998. The data will be used only for informing the research question in this study and the results of the research will be disseminated in peer-reviewed scientific journals, but you will in no way be identifiable from such publications.

Note – if you have any further questions regarding this study please do not hesitate to contact any of the researchers above.

This study has been approved by the UCL ethics committee

It is up to you to decide whether or not to take part. If you choose not to participate it will involve no penalty or loss of benefits to which you are otherwise entitled. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part **you are still free to withdraw at any time and without giving a reason.**

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

All data will be collected and stored in accordance with the Data Protection Act 1998. This study has been registered with UCL data Protection; Number Z6364106/2013/05/27

This study has been approved by the UCL Research Ethics Committee (Project ID Number): **3901/001**

If you have any questions regarding the study please contact the experimenter:

Name	Ravi Das
Work Address	Clinical Psychopharmacology Unit, UCL, 1-19 Torrington Place, London WC1E 7HB.
Contact Details	Email: CPUexperiments@gmail.com
	Telephone [redacted]

Appendix item 5 *Hierarchical regression analyses for centred total lifetime drinks, stimulation-to-sedation ratio, and family history of alcohol problems conducted in Chapter 3*

	M: Total lifetime drinks			M: Stim -Sed ratio			M: Family History		
Moderators (df)	b	R2	F	b	R2	F	b	R2	F
Step 1 4,55		0.085	1.275	0.270	0.073	1.073		0.086	1.288
M	-0.000			0.005			0.250		
X1	-0.373			-0.332			-0.277		
X2	0.128			-0.019			-0.048		
Surprise	-0.047			-0.040			-0.039		
Step 2 6,53		0.096	0.937	0.272	0.074	0.001		0.106	1.047
M	0.000			-0.085			0.549		
X1 (Ret-PE)	-0.391			-0.316			-0.122		
X2 (NoRet-PE)	0.095			-0.002			-0.316		
Surprise	-0.036			-0.041			-0.027		
M * X1	-0.000			-0.102			-0.362		
M * X2	-0.000			0.111			-0.670		

Note: M=moderator variable, X_1=Ret-PE group vs. Ret-NoPE & NoRet-PE group, X_2=NoRet-PE vs Ret-PE & Ret-NoPE groups. Stim-Sed: stimulation to sedation ratio. No significant simple effects or interactions observed (all p>0.05).

Appendix item 6 ethics approval for study in Chapter 4

UCL RESEARCH ETHICS COMMITTEE

30/4/19

UCL

Amending an Approved Application

Should you wish to make an amendment to an approved study, you will need to submit an 'amendment request' for the consideration of the Chair of the UCL Research Ethics Committee. Applications can only be amended **after** ethical approval has been granted.

You will need to apply for an amendment approval if you wish to:

1. Add a new participant group;
2. Add a new research method;
3. Ask for additional data from your existing participants;
4. Remove a group of participants or a research method from the project, and have not yet commenced that part of the project;
5. Apply for an extension to your current ethical approval.

If you need to apply for an amendment approval, please complete the Amendment Approval Request Form on the next page.

When completing the form, please ensure you do the following:

- Clearly explain what the amendment you wish to make is, and the justification for making the change.
- Insert details of any ethical issues raised by the proposed amendments.
- Include all relevant information regarding the change so that the Chair can make an informed decision, and submit a copy of the sections of your application that have changed with all changes highlighted/underlined for clarity.
- You do not need to submit your original application in full again. However, if the changes you wish to make alters several sections of your application form, you are advised to submit this.

One signed hard copy of the form (and any amended documents), as well as an electronic copy of these same documents must be submitted to the REC Administrator to the address detailed below:

Administrator of the UCL Research Ethics Committee
Graduate School, UCL
North Cloisters
Wilkins Building
Gower Street
London WC1E 6BT

Email: ethics@ucl.ac.uk

Amendment requests are generally considered within 5-7 days of submission.

Amendment Approval Request Form

1	**Project ID Number:** 0760/004	**Name and Address of Principal Investigator:** Dr Sunjeev Kamboj, Research Dept Clinical, Educational and Health Psychology
2	**Project Title:** Examining the role of NMDA receptors in recall of learned information	
3	**Type of Amendment/s (tick as appropriate)** ☒ Research procedure/protocol (including research instruments) ☒ Participant group ☐ Sponsorship/collaborators ☒ Extension to approval needed (extensions are given for one year) – To 30/4/2019 ☒ Information Sheet/s ☐ Consent form/s ☐ Other recruitment documents ☐ Principal researcher/medical supervisor* ☐ Other * *Additions to the research team other than the principal researcher, student supervisor and medical supervisor do not need to be submitted as amendments but a complete list should be available upon request.	
4	**Justification** (give the reasons why the amendment/s are needed) This amendment pertains to adding groups of paprticipants receiving 25mg rapamycin (temsirolimus) to our current protocol. This will enable us to better understand mechanisms of memory restabilisation, as well as elucidate the molecular 'site of action' of ketamine's effects. It will also enable us to effectively interpret any 'paradoxical' effects of ketamine that might be observed. The primary aims of the existing study are to understand the NMDA receptor mediated mechanisms governing destabilisation, restabilisation and updating of maladaptive memories by blocking this receptor with ketamine. However, very recent evidence (Zanos et al, 2016) has demonstrated that ketamine's therapeutic properties in depression may be due to its primary metabolite (6S-hyrdorxynorketamine) and its downstream interactions with the protein synthesis regulator kinase Mammalian Target of Rapamycin Complex 1 (mTORC1; Li et al, 2010), independently of NMDA receptor blockade. The mTORC1 kinase may therefore be proximally responsible for many of the processes we are putatively targeting with ketamine in our study. Following consultation with international mTORC1 experts Segev Barak ad Dorit Ron, it was suggested that ketamine may produce paradoxical memory effects due to downstream activation of mTOR. We have therefore concluded that to fully achieve the aims of our study with ketamine it will be necessary to understand whether any observed ketamine effects are mTORC1-dependent. As ketamine may dose-dependently increase mTORC1 activity via its active metabolite, it is possible that it this may negate any memory reconsolidation-blocking effects of NMDA receptor antagonism via ketamine, causing problematic interpretation of effects. To overcome this issue, we seek to add three groups to our existing protocol with ketamine who receive single-dose rapamycin (temsirolimus-25mg), a blocker of the mTORC1 kinase. This will provide a far more time, resource and cost-effective means of interpreting observed ketamine effects than repeating the whole experiment and re-collecting data using the same ketamine challenge we are currently using. Understanding the interaction between ketamine, 6S hydroxynorketamine and mTORC1 will prove key to the future development of clinical interventions based on the current mechanistic research. We have confirmation from the MHRA Clinical Trials Helpline that our planned usage of rapamycin does not constitute a clinical trial of an investigational medicinal product (see appended email).	
5	**Details of Amendments** (provide full details of each amendment requested, state where the changes have been made and attach all amended and new documentation)	

	1) Add-on groups receiving temsirolimus. The add-on groups will be as follows: i) Memory reactivation + rapamycin: Participants will undergo identical memory reactivation procedures to those currently being employed in the study, but receive a 30-minute infusion of 25mg temsirolimus instead of ketamine. This will allow us to assess whether this produces the same effect as the current reactivation + ketamine group 2) Memory reactivation + ketamine + rapamycin: If ketamine effects are mTORC1 – dependent, these should be prevented by co-administration of 25mg temsirolimus with the currently used (0.35mg/ml) plasma concentration of ketamine currently being used in the study. 3) No memory reactivation + rapamycin: To assess whether any observed effects are attributable to the putative memory reconsolidation mechanisms under investigation, we need to assess whether rapamycin effects are reactivation-dependent. As such, one group will receive the 25mg infusion of temsirolimus without memory reactivation. Given the use of rapamycin, on top of existing exclusion criteria, the following (non-exhaustive) criteria will constitute grounds for exclusion: Current use of rapamycin or analogues (rapalogues), compromised immune function, lung disease, renal function, existing allergies to sirolimus or temsirolimus, high cholesterol/triglyceride count, diabetes, abnormal BMI, recurrent infections or wounds or currently taking any medication that interacts with temsirolimus. Further, participants agreeing to take part must agree not to use any cytochrome P450 inhibitors, not undergo any vaccinations, take antifungals, antibiotics or other immunomodulators during the course of the study. In the instance of unclear medical eligibility for participation, we will seek advice from the medical consultant (Dr. Brigitta Brandner) on the study. ii) Addition of information sheets to include information on rapamcyin/temsirolimus. The addition of the new groups to which participants may be randomised will require us to update the relevant sections of the information sheets. We have updated the information sheets to include information about temsirolimus and added exclusion criteria to our screening to ensure that participants are not contraindicated for temsirolimus infusion. These updated sheets are attached. iii) Extension of study duration: The addition of these groups will require us to test an extra 90 participants (N = 30 per group, as per our existing protocol). As such we will require an extension of 18 months to the current ethical approval (0760/004). Aside from these additions, all procedural aspects of the study (recruitment, consent, tasks and protocols, payment, follow-up and monitoring etc.) will remain exactly the same as those currently being implemented.
6	**Ethical Considerations** (insert details of any ethical issues raised by the proposed amendment/s) A note on temsirolimus: Temsirolimus is a pro-drug that is endogenously converted to its primary active metabolite, sirolimus or rapamycin, which produces the desired mTORC1 blocking effects. Both temsirolimus and sirolimus are FDA and EMEA approved, the former for renal cell carnicoma due to its anti-tumour proliferation properties and favourable tolerability and the latter for lymphangioleiomyomatisis and organ transplant acceptance due to its immunosuppressant properties. Sirolimus has been previously used in related memory research (with PTSD patients) with no reported serious adverse effects (Surís et al, 2013). We have opted to use temirolimus as it has greater specificity and efficacy in blocking mTORC1 (Boni et al, 2009), fewer side-effects and a lower immunosuppressant profile than sirolimus (Bellmunt et al, 2008). Further, since oral sirolimus undergoes extensive first-pass metabolism, it is often unclear that desired plasma concentrations are reached. With the proposed intravenous preparation of temsirolimus and administration over 30 minutes we will have close control over the pharmacokinetics and plasma concentrations of temsirolimus and will be able to terminate infusion rapidly in the very unlikely event of an adverse response. The intravenous preparation will allow us to use the same infusion-pump driven administration protocol we are currently using for administering ketamine, which will greatly simplify the dosing and maintenance of blinding procedures. All infusions, as with our current protocol, will be performed by fully trained anaesthetists, with full medical support, monitoring and emergency resuscitation equipment, in University College London Hospital. Possible side-effects:

	Temosirolimus may produce immunosuppression and may therefore increase the risk of contracting an infection. For this reason, immune-compromised or otherwise unhealthy individuals will be prohibited from participation. Given the excellent tolerability of Sirolimus observed by Surís et al (2013), we do not expect any serious adverse reactions to the generally more tolerable temosirolimus. However, participants will be fully appraised of the potential side effects of the drug and given advice on how to minimise risk. Given that adverse reactions to temsirolimus are observed after months of *continuous* treatment in a population with severe pre-existing compromises to immune function and metabolism (cancer patients) significant adverse responses in healthy volunteers following a single 25mg dose are not anticipated. However, as with our current ketamine administration, full monitoring during and after infusion, along with follow-up assessment and reporting of adverse events will be carried out. Participants will have access to a study medic at all times in order to report any adverse events and for advice should these occur.
7	**Other Information** (provide any other information which you believe should be taken into account during ethical review of the proposed changes) We have sent a copy of our proposed temsirolimus protocol to the MHRA Clinical Trials Helpline, who have confirmed that our planned usage of rapamycin/temsirolimus does not constitute a clinical trial of an investigational medicinal product (see appended).

Declaration (to be signed by the Principal Researcher)

- I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.
- I consider that it would be reasonable for the proposed amendments to be implemented.
- For student projects I confirm that my supervisor has approved my proposed modifications.

Signature: [redacted]

Date: 12/10/2016

FOR OFFICE USE ONLY:

Amendments to the proposed protocol have been ..approved.. by the Research Ethics Committee.

Signature of the REC Chair, Professor John Foreman: [redacted]

Date: 20/10/2016.

Appendix item 7 *NHS approval for study in Chapter 4*

University College London Hospitals NHS
NHS Foundation Trust

Joint Research Office

Office Location:
1st Floor Maple House
149 Tottenham Court Road
London W1T 7DN

Postal Address:
UCL,
Gower Street
London WC1E 6BT

Tel: 020 3447 2177/79833 Fax: 020 3447 9937
Websites: www.uclh.nhs.uk; www.ucl.ac.uk; www.ucl.ac.uk/jro

FINAL R&D APPROVAL – NHS PERMISSION

29/09/2015

Dear Dr Brigitta Brandner,

Project ID: 15/0403 (Please quote in all correspondence)
REC Ref: 0760/004
UKCRN ID:
Title: Examining the role of NMDA receptors in recall of learned information

Thank you for registering the above study with the Joint Research Office (UCLH site). I am pleased to inform you that your study now has local R&D approval (NHS permission) to proceed and recruit participants at University College London Hospitals NHS Foundation Trust, subject to the Sponsor's written 'green light' confirmation.

Please note that all documents received have been reviewed and this approval is granted on the basis of the key documents provided which have obtained a 'notice of favourable opinion' by the UCL Research Ethics Committee (REC):

Document	Date
UCL REC notice of favourable opinion and REC approved documents	12/06/2015

As Principal Investigator you are required to ensure that your study is conducted in accordance with the requirements on the attached sheet. These include the conditions of your NHS permission.

Please note a summary of the study will be obtained from IRAS A6-1 for the **UCLH Research Gateway** which is available to the public at http://www.uclh.nhs.uk/Research/Pages/researchdatabase.aspx.

Do not hesitate to contact a member of the team should you have any queries.

Yours sincerely,

[redacted]

PP: Professor Bryan Williams
UCLH Research Director

Responsibilities of the Researcher

Conditions of NHS permission

Your research has been granted NHS permission by the Joint Research Office on behalf of University College London Hospitals NHS Foundation Trust.

As a condition of the NHS permission you must comply with:

- Applicable Joint Research Office's Standard Operating Procedures available at: http://www.ucl.ac.uk/jro/standingoperatingprocedures
- Department of Health's Research Governance Framework for Health and Social Care
- Research Ethics Committee notice of favourable opinion
- Data Protection Act, Caldicott Principles and Trust Information Governance Policy.
- All other relevant legislation and regulatory approvals including the following *if applicable*
  - Medicines and Healthcare products Regulatory Agency
    - notice of acceptance of a clinical trial of investigational medicinal product (CTIMP)
    - notice of no objection of a clinical investigation for a medical device
  - Human Tissue Act 2004 and the Codes of Practice with special relevance to Code 9 Research
  - Human Tissue (Quality and Safety for Human Application) Regulations 2007

Responsibilities for Research Teams

As Principal Investigator you are required to ensure that:

- The roles and responsibilities of all members of the research team are documented in a delegation log and that all team members are made aware of these.

- All researchers conducting the study have applicable (up-to-date) honorary contracts.

- All researchers are suitably trained, qualified and experienced to carry out duties delegated to them and **if conducting a clinical trial**, have up-to-date Good Clinical Practice (GCP) training (updated every 2 years). Copies of GCP certificates and CVs outlining GCP training are filed in the site file.

Responsibilities for the Principal Investigator in relation to tissue and data in the absence of a study agreement:

- After REC' s 'notice of favourable opinion' for the study has expired, you shall ensure that tissues are disposed of in accordance with the protocol and Human Tissue Act 2004, transferred to a licensed tissue bank or used under a new REC approved research project.

- Ensure that all necessary arrangements are in place for appropriate transfer, storage, handling, retention (archiving) and, if applicable, destruction of study data. The sponsor will act as the custodian of such data.

Reporting on Recruitment

Please ensure that you **notify the Joint Research Office** with:

- Confirmation of **recruiting your first patient** by emailing RandD@uclh.nhs.uk.

- Accrual data on a regular basis. If your study has been adopted onto the NIHR portfolio you will be contacted directly by the NIHR Clinical Research Network Coordinating Centre. For all other studies you are required to provide an update to the Joint Research Office on recruitment **every 6 months**.

Reporting Study Events

Responsibilities of the Researcher

Events and incidents

Please ensure that your study team reports the following **to the Sponsor** as required by the protocol and/or sponsor SOPs:

- For **CTIMPs**
  - All reportable SAEs, suspected unexpected serious adverse events (SUSARs),
  - Protocol violations, serious breaches of protocol and of GCP
  - Urgent safety measures
- For **all other studies**
  - All reportable serious adverse events (SAEs)

Please ensure that your study team reports the following **to the Joint Research Office**:

- For **all research**
  - All **complaints** from NHS patients from UCLH should be reported in the first instance to the UCLH NHS Complaints Manager.
  - All research related incidents occurring at UCLH should be reported through DATIX, the Trust Incident Reporting System (available on InSight).

- For **CTIMPs**
  - Please report Serious Breaches of Protocol and GCP occurring at UCLH through DATIX.
- For **all other studies**
  - Please report unexpected SAEs related to the research protocol, serious breaches of protocol and GCP if applicable through DATIX.

Study progress and changes

Please ensure that your study team reports the following to the Joint Research Office:

- Amendments (including a request to extend the study)
- Monitoring activity information:
  - for non-commercially sponsored clinical trials provide **a summary of corrective and preventive actions from monitoring reports**, as agreed with the sponsor
  - annual progress reports submitted to REC (for UCLH sponsored research)
- Audit activity information:
  - Notification of audits or inspections
  - Audit reports (where possible, and in agreement with the sponsor, to provide a copy of the corrective and preventive actions arising from an audit)
- Notification of end of study or suspension of study
- Publications

Study documentation

Research teams are required to:

- Prepare and maintain a site file to ensure that data and documentation associated with the study are available for audit. The sponsor should provide you with a site file index. If UCLH is the sponsor please refer to the SOP for Preparation of Site File JRO/RM&G/SOP-13 available at: http://www.ucl.ac.uk/jro/standingoperatingprocedures

- Contact the JRO by email RandD@uclh.nhs.uk as soon as the study has been suspended or ended in order to arrange for archiving.

If you require any further information on the above please see the Joint Research Office website http://www.ucl.ac.uk/jro.

Joint Research Office Standard Operating Procedures are available at:
http://www.ucl.ac.uk/jro/standingoperatingprocedures

Appendix item 8. Information sheet for study in Chapter 4

Information Sheet for Heavy Drinkers Involved in Memory and Cognitive Research Studies Using Ketamine in the Clinical Psychopharmacology Unit

You will be given a copy of this information sheet:

Title of the Project: Examining the role of NMDA receptors in recall of learned information

This study has been approved by the UCL Research Ethics Committee (Project ID Number): **0760/004**

Name: Dr. Sunjeev Kamboj & Dr. Ravi Das

Work Address: Clinical Psychopharmacology Unit, UCL, 1-19 Torrington Place, London, WC1E 7HB.

Contact Details: Email: CPUexperiments@gmail.com

Who are we recruiting?

We would like to invite heavy beer drinkers, defined as people who drink twice (or more than twice) the governmental daily recommendation of alcohol at least 4 days out of every 7. The governmental daily guidelines are 2-3 units per day for women and 3-4 units for men (minimum of 30 units/week - female, 40 units/week - male).

Details of Study:

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. This study is being conducted by researchers from the Clinical Psychopharmacology Unit at UCL.

Why are we doing this study?

The way people learn about rewards is thought to be affected by continued alcohol and drug use and important in the development of drug addiction from recreational drug use. Certain brain chemicals are known to be important in the way learned associations are stored, recalled and affect behaviour. One of these that is particularly important is called the NMDA receptor. The functioning of this type of receptor can be affected by long-term drug and alcohol use. We are interested in the role of this receptor in the recall of learned information about alcohol. This receptor is blocked by ketamine. Participants in the study will therefore receive intravenous ketamine or placebo as part of the study. By taking part in this study you will contribute to the scientific knowledge of the brain basis of memory and reward and inform potential future treatments for psychiatric disorders like addiction. If you would like to receive an overview of the study's results once it has been completed, please ask the investigator and this will be arranged.

Who may take part?

We would like to invite healthy males and females, aged 18-65 who drink at least twice the maximum daily allowance of alcohol (3 units per day for females or 4 units for males) at least four days out of every seven *AND* would like to reduce their drinking *AND* who *do not* have a current or past diagnosis of alcoholism *AND* who have no other psychiatric or major physical illnesses *AND* who are not pregnant or breastfeeding and are not likely to become pregnant during the course of the study *AND* who have not taken part in a similar study before. Please note that these are not the only criteria for taking part in the study and you will have to complete a telephone screen to check you meet all our criteria for eligibility. If you have a fear of needles and/or injections, we advise that you do NOT take part in this study.

Do I have to take part?

Your participation in the study is entirely voluntary and you are free to withdraw from the study at any time without giving a reason, even if you have previously given your written consent. If you do agree to take part, you will be asked to sign a consent form and will be given this information sheet to keep.

What will happen to me if I take part?

If you agree to participate in this study you must contact the experimenter by email with contact information and a convenient time to call. You will then receive a call from us and we will ask you a short series of questions to check your eligibility for the study. Please note that we have strict criteria for participation and based on your answers to these questions; you may not be eligible to take part in the study. If you are eligible to take part, you will be asked to come to the Clinical Psychopharmacology Unit (CPU) at UCL in central London and University College hospital at a time convenient for you on 3 occasions. The first two occasions will be around 48 hours apart and the third one week later. You will be required to abstain from drinking alcohol or using any psychoactive drugs (aside from caffeine) for the 12 hours before each testing session. If you take part in the study, we will assess your recent drinking with an alcohol breathalyser test at the beginning of each session. This measures the concentration of alcohol in your blood. If your blood alcohol concentration shows that you have drunk alcohol in the last 12 hours, testing will have to be re-arranged or you will be excluded from the study.

The following is an overview of what will happen on each day of the study:

Day 1: After arriving at the Clinical Psychopharmacology Unit, UCL and completing a breathalyser and consent form, you will go through some questionnaires that measure levels of drinking, mood, attitudes, and levels of different psychological traits and family history of alcoholism. You will also provide some basic health measures, such as BMI, blood pressure and heart rate.

We will then fit you with an EEG (electroencephalography) cap. This measures the electrical activity on your scalp produced by your brain. We will need to measure your head, fit a cap, and then fill the electrodes in the cap with a conductive gel. To get a good signal, we may need to gently rub your scalp to move hair out of the way of the electrodes. All of these procedures will be completely painless, but you will be left with conductive gel on your scalp, which you will be able to wash off in the testing centre. For this reason, we are unable to accept participants with elaborate hairstyles such as dreadlocks, braids or cornrows, or participants with a very small or large head circumference. You should not take part if you are uncomfortable having the cap fitted and gel applied to your scalp. The EEG cap will leave some slight red marks on your forehead where the electrodes press against the skin. These are not painful and will fade away within a few minutes of removing the cap.

When the cap it fitted, you will complete some computer tasks that will involve learning about, rating and measuring reaction times to different stimuli, either pictures or words. These tasks will also involve measuring your eye movements to words and pictures, so it is important you have normal colour vision and no eye-related conditions (e.g. a squint) if you take part, however prescription glasses and contact lenses are fine.

At the end of the session you will be given details about preparing for the ketamine session (Day 2), and particularly, will be asked confirm that you will not take solid foods in the 6 hrs or clear liquids in the 2 hrs prior to ketamine infusion.

Day 2(Day 1 + 24-48 hours): You will come to University College Hospital where you will meet a researcher who will take you to the ward where the testing will take place. You will once again be fitted with the EEG cap and complete some brief questionnaire measures of your current mood. After this, a cannula will be inserted into your arm and a blood sample taken from you. You will then complete an algometer test. This is a device that gradually increases pressure upon your skin to determine your pain threshold. Ketamine is a very potent painkiller, and part of the research is aimed at understanding its pain-killing effects. After this test, you will be asked to briefly rate some pictures and consume a sample of drink you are provided with. This will either be beer or a non-alcoholic drink like orange juice. The infusion of ketamine or placebo will then begin. Whether you receive ketamine (low dose or high dose) or placebo will depend on random allocation to an experimental group and will be 'blind'. That is, you will not be told whether you are going to receive ketamine or placebo infusion beforehand, as you will randomly allocated to one condition. During the infusion, you will repeat the algometer pain-threshold test, but will otherwise be asked to sit quietly until the infusion ends.

After the infusion ends, the cannula and EEG cap will be removed and the experimenters will wait until you feel normal. You will then complete a mood questionnaire and final algometer threshold measure. You will be required to successfully complete some basic physical and mental competency tests before you are allowed to leave the hospital. **Because Ketamine is an anaesthetic drug we have to ask that you are accompanied to the second session by a responsible adult or that someone can collect you once the session is complete**. Please note, you must not drive or operate heavy machinery following *Day 2***, so please do not drive to the hospital**.

pressure, heart, lung, brain, liver or kidney function. If you have any concerns, please discuss this information with your general practitioner before deciding whether or not to take part.

What are the benefits of taking part?

By taking part in this research you will be helping us to gain a clearer understanding of the biological basis of learning and memory about rewards, likes and dislikes and how these stay the same or change over time. We think this information will be important for understanding drug and alcohol use disorders.

How will I be paid?

You will receive payment of £10 per hour for your participation upon completion of the first follow-up. In total, the basic testing should last ~7 hours, so you can expect to earn around £70. You will also be paid £5 for each subsequent follow-up, meaning your total pay will be ~£80-100.

How will my data be stored?

All information which is collected about you during the course of the research will be kept strictly confidential and will be securely stored electronically, using a numbered code so that you cannot be identified. Only researchers directly involved in the study will have access to the data. All data will be stored in accordance with the Data Protection Act 1998. The data will be used only for informing the research question in this study and the results of the research will be disseminated in peer-reviewed scientific journals, but you will in no way be identifiable from such publications. Any biological samples we collect from you will also be anonymised. These samples will be destroyed once they are analysed.

Who should I contact for further information?

To volunteer to take part in the study, please email CPUexperiments@gmail.com with your name, a contact telephone number and appropriate time to call. One of the experimenters will then be in touch to run through a full screening to check you are eligible to take part.

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

It is up to you to decide whether to take part or not; choosing not to take part will not disadvantage you in any way. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

All data will be collected and stored in accordance with the Data Protection Act 1998.

-

Thank you for reading this information sheet and for considering take part in this research.

Note – if you have any further questions regarding this study please do not hesitate to contact any of the researchers above.

Day 3 (Day 2 + 7 days): You will again come to the Clinical Psychopharmacology Unit to repeat the questionnaires from *Day 1*. You will then be fitted with the EEG cap and complete the computer tasks from *Day 1*. After completing these tasks, the cap will be removed and we will take one draw of blood from your arm. This will be a very minimal amount of blood and will be over very quickly. Once this is finished, testing will be complete.

Follow-Up (Day 3 + 14 Days, 3 months, 6 months & 9 months)

After Day 3, you will not be required to come in to UCL again, however, we will need to contact you at occasional intervals to ask you some follow-up questions about your mood and drinking. The first of these follow-up calls will be 14 days after *Day 3*. You will receive basic payment for the study after completion of this follow-up. Subsequent follow-up calls will be completed at 3, 6 and 9 months. You will be paid for each follow-up you complete. The follow-up data is very important to the study, so please only take part if you are willing and able to complete all the follow-up calls.

What are these drugs and are they safe?

Ketamine is an analgesic (pain-killing) and anaesthetic (sedative) drug that has been used for a long time in hospital settings, particularly in young children, as it is much safer than alternative anaesthetics. Ketamine has dissociative and sedative effects, meaning you may feel detached from your body, uncoordinated and sedated. The ketamine we are using in the study will be administered intravenously via a cannula in your arm. You should therefore not take part if you have a fear of needles or would be uncomfortable receiving intravenous ketamine.

Administration of ketamine or placebo will be undertaken by a trained anaesthetist in a clinical area in University College Hospital (UCH), London. We will also collect some blood samples while the intravenous cannula is being prepared. These will be stored anonymously and analysed for levels of various blood chemicals related to ketamine's effects. Single dose ketamine is generally considered very safe and an expert team of anaesthetists will be performing all ketamine infusions.

What are the possible risks of taking part?

Ketamine has some known significant side-effects that you should be aware of when deciding to take part:

In 10+% of cases: *Increases in blood pressure, increases in heart rate, increases in intracranial pressure, tonic-clonic movements (muscular seizures), visual hallucinations, auditory hallucinations, vivid dreams, dissociation*.

In 1-10% cases: *Reductions in heart rate, changes in eye focus and movement (diplopia), reduction in blood pressure, increases in intraocular (eye) pressure, injection-site pain.*

In < 1% cases: *Anaphylaxis, irregular heartbeat (arrhythmia), depressed cough reflex, muscle twitches, increased salivation, increased metabolism, increased muscle tension, spasm of the larynx.*

Due to these possible effects, you must not take part if you have a personal or family history of psychosis or schizophrenia or other psychiatric disorder, or a medical condition affecting blood

This study has been approved by the UCL ethics committee

It is up to you to decide whether or not to take part. If you choose not to participate it will involve no penalty or loss of benefits to which you are otherwise entitled. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

Study Registration Details:

- All data will be collected and stored in accordance with the Data Protection Act 1998. This study has been registered with UCL data Protection. Number **Z6364106/2015/03/12**

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 0760/004

If you have any questions regarding the study please contact the experimenter:

Ravi Das

Clinical Psychopharmacology Unit, UCL, 1-19 Torrington Place, London WC1E 7HB.

Email: CPUexperiments@gmail.com

Telephone: 02076791826

Appendix item 9 *ethical approval study in Chapter 5*

UCL RESEARCH ETHICS COMMITTEE

Amending an Approved Application

Should you wish to make an amendment to an approved study, you will need to submit an 'amendment request' for the consideration of the Chair of the UCL Research Ethics Committee. Applications can only be amended **after** ethical approval has been granted.

You will need to apply for an amendment approval if you wish to:

1. Add a new participant group;
2. Add a new research method;
3. Ask for additional data from your existing participants;
4. Remove a group of participants or a research method from the project, and have not yet commenced that part of the project;
5. Apply for an extension to your current ethical approval.

If you need to apply for an amendment approval, please complete the Amendment Approval Request Form on the next page.

When completing the form, please ensure you do the following:

- Clearly explain what the amendment you wish to make is, and the justification for making the change.
- Insert details of any ethical issues raised by the proposed amendments.
- Include all relevant information regarding the change so that the Chair can make an informed decision, and submit a copy of the sections of your application that have changed with all changes highlighted/underlined for clarity.
- You do not need to submit your original application in full again. However, if the changes you wish to make alters several sections of your application form, you are advised to submit this.

One signed hard copy of the form (and any amended documents), as well as an electronic copy of these same documents must be submitted to the REC Administrator to the address detailed below:

Administrator of the UCL Research Ethics Committee
Graduate School, UCL
North Cloisters
Wilkins Building
Gower Street
London WC1E 6BT

Email: ethics@ucl.ac.uk

Amendment requests are generally considered within 5-7 days of submission.

Amendment Approval Request Form

1	**Project ID Number:** 0760/004	**Name and Address of Principal Investigator:** Dr Sunjeev Kamboj, Research Dept Clinical, Educational and Health Psychology
2	**Project Title:** Examining the role of NMDA receptors in recall of learned information	
3	**Type of Amendment/s (tick as appropriate)** ☒ Research procedure/protocol (including research instruments) ☒ Participant group ☐ Sponsorship/collaborators ☒ Extension to approval needed (extensions are given for one year) ☒ Information Sheet/s ☐ Consent form/s ☐ Other recruitment documents ☐ Principal researcher/medical supervisor* ☐ Other * *Additions to the research team other than the principal researcher, student supervisor and medical supervisor do not need to be submitted as amendments but a complete list should be available upon request.	
4	**Justification** (give the reasons why the amendment/s are needed) This amendment pertains to adding groups of paprticipants receiving 25mg rapamycin (temsirolimus) to our current protocol. This will enable us to better understand mechanisms of memory restabilisation, as well as elucidate the molecular 'site of action' of ketamine's effects. It will also enable us to effectively interpret any 'paradoxical' effects of ketamine that might be observed. The primary aims of the existing study are to understand the NMDA receptor mediated mechanisms governing destabilisation, restabilisation and updating of maladaptive memories by blocking this receptor with ketamine. However, very recent evidence (Zanos et al, 2016) has demonstrated that ketamine's therapeutic properties in depression may be due to its primary metabolite (6S-hyrdorxynorketamine) and its downstream interactions with the protein synthesis regulator kinase Mammalian Target of Rapamycin Complex 1 (mTORC1; Li et al, 2010), independently of NMDA receptor blockade. The mTORC1 kinase may therefore be proximally responsible for many of the processes we are putatively targeting with ketamine in our study. Following consultation with international mTORC1 experts Segev Barak ad Dorit Ron, it was suggested that ketamine may produce paradoxical memory effects due to downstream activation of mTOR. We have therefore concluded that to fully achieve the aims of our study with ketamine it will be necessary to understand whether any observed ketamine effects are mTORC1-dependent. As ketamine may dose-dependently increase mTORC1 activity via its active metabolite, it is possible that it this may negate any memory reconsolidation-blocking effects of NMDA receptor antagonism via ketamine, causing problematic interpretation of effects. To overcome this issue, we seek to add three groups to our existing protocol with ketamine who receive single-dose rapamycin (temsirolimus-25mg), a blocker of the mTORC1 kinase. This will provide a far more time, resource and cost-effective means of interpreting observed ketamine effects than repeating the whole experiment and re-collecting data using the same ketamine challenge we are currently using. Understanding the interaction between ketamine, 6S hydroxynorketamine and mTORC1 will prove key to the future development of clinical interventions based on the current mechanistic research. We have confirmation from the MHRA Clinical Trials Helpline that our planned usage of rapamycin does not constitute a clinical trial of an investigational medicinal product (see appended email).	
5	**Details of Amendments** (provide full details of each amendment requested, state where the changes have been made and attach all amended and new documentation)	

	1) Add-on groups receiving temsirolimus. The add-on groups will be as follows: i) Memory reactivation + rapamycin: Participants will undergo identical memory reactivation procedures to those currently being employed in the study, but receive a 30-minute infusion of 25mg temsirolimus instead of ketamine. This will allow us to assess whether this produces the same effect as the current reactivation + ketamine group 2) Memory reactivation + ketamine + rapamycin: If ketamine effects are mTORC1 – dependent, these should be prevented by co-administration of 25mg temsirolimus with the currently used (0.35mg/ml) plasma concentration of ketamine currently being used in the study. 3) No memory reactivation + rapamycin: To assess whether any observed effects are attributable to the putative memory reconsolidation mechanisms under investigation, we need to assess whether rapamycin effects are reactivation-dependent. As such, one group will receive the 25mg infusion of temsirolimus without memory reactivation. Given the use of rapamycin, on top of existing exclusion criteria, the following (non-exhaustive) criteria will constitute grounds for exclusion: Current use of rapamycin or analogues (rapalogues), compromised immune function, lung disease, renal function, existing allergies to sirolimus or temsirolimus, high cholesterol/triglyceride count, diabetes, abnormal BMI, recurrent infections or wounds or currently taking any medication that interacts with temsirolimus. Further, participants agreeing to take part must agree not to use any cytochrome P450 inhibitors, not undergo any vaccinations, take antifungals, antibiotics or other immunomodulators during the course of the study. In the instance of unclear medical eligibility for participation, we will seek advice from the medical consultant (Dr. Brigitta Brandner) on the study. ii) Addition of information sheets to include information on rapamcyin/temsirolimus. The addition of the new groups to which participants may be randomised will require us to update the relevant sections of the information sheets. We have updated the information sheets to include information about temsirolimus and added exclusion criteria to our screening to ensure that participants are not contraindicated for temsirolimus infusion. These updated sheets are attached. iii) Extension of study duration: The addition of these groups will require us to test an extra 90 participants (N = 30 per group, as per our existing protocol). As such we will require an extension of 18 months to the current ethical approval (0760/004). Aside from these additions, all procedural aspects of the study (recruitment, consent, tasks and protocols, payment, follow-up and monitoring etc.) will remain exactly the same as those currently being implemented.
6	**Ethical Considerations** (insert details of any ethical issues raised by the proposed amendment/s) A note on temsirolimus: Temsirolimus is a pro-drug that is endogenously converted to its primary active metabolite, sirolimus or rapamycin, which produces the desired mTORC1 blocking effects. Both temsirolimus and sirolimus are FDA and EMEA approved, the former for renal cell carnicoma due to its anti-tumour proliferation properties and favourable tolerability and the latter for lymphangioleiomyomatisis and organ transplant acceptance due to its immunosuppressant properties. Sirolimus has been previously used in related memory research (with PTSD patients) with no reported serious adverse effects (Surís et al, 2013). We have opted to use temirolimus as it has greater specificity and efficacy in blocking mTORC1 (Boni et al, 2009), fewer side-effects and a lower immunosuppressant profile than sirolimus (Bellmunt et al, 2008). Further, since oral sirolimus undergoes extensive first-pass metabolism, it is often unclear that desired plasma concentrations are reached. With the proposed intravenous preparation of temsirolimus and administration over 30 minutes we will have close control over the pharmacokinetics and plasma concentrations of temsirolimus and will be able to terminate infusion rapidly in the very unlikely event of an adverse response. The intravenous preparation will allow us to use the same infusion-pump driven administration protocol we are currently using for administering ketamine, which will greatly simplify the dosing and maintenance of blinding procedures. All infusions, as with our current protocol, will be performed by fully trained anaesthetists, with full medical support, monitoring and emergency resuscitation equipment, in University College London Hospital. Possible side-effects:

	Temosirolimus may produce immunosuppression and may therefore increase the risk of contracting an infection. For this reason, immune-compromised or otherwise unhealthy individuals will be prohibited from participation. Given the excellent tolerability of Sirolimus observed by Surís et al (2013), we do not expect any serious adverse reactions to the generally more tolerable temosirolimus. However, participants will be fully appraised of the potential side effects of the drug and given advice on how to minimise risk. Given that adverse reactions to temsirolimus are observed after months of *continuous* treatment in a population with severe pre-existing compromises to immune function and metabolism (cancer patients) significant adverse responses in healthy volunteers following a single 25mg dose are not anticipated. However, as with our current ketamine administration, full monitoring during and after infusion, along with follow-up assessment and reporting of adverse events will be carried out. Participants will have access to a study medic at all times in order to report any adverse events and for advice should these occur.
7	**Other Information** (provide any other information which you believe should be taken into account during ethical review of the proposed changes) We have sent a copy of our proposed temsirolimus protocol to the MHRA Clinical Trials Helpline, who have confirmed that our planned usage of rapamycin/temsirolimus does not constitute a clinical trial of an investigational medicinal product (see appended).

Declaration (to be signed by the Principal Researcher)

- I confirm that the information in this form is accurate to the best of my knowledge and I take full responsibility for it.
- I consider that it would be reasonable for the proposed amendments to be implemented.
- For student projects I confirm that my supervisor has approved my proposed modifications.

Signature: [redacted]

Date: 12/10/2016

FOR OFFICE USE ONLY:

Amendments to the proposed protocol have been …… by the Research Ethics Committee.

Signature of the REC Chair, Professor John Foreman: ……

Date: ……

Appendix item 10 *Information sheet for study in Chapter 5*

Information Sheet for Participants involved in Research Studies on the Causes, Mechanisms and Consequences of Binge Eating in the UCL Clinical Psychopharmacology Unit

You will be given a copy of this information sheet to keep and refer to.

Title of the Project: Probing the role of the mTORC1 metabolic pathway in learning and memory in healthy humans using a single-dose Sirolimus (rapamycin)

This study has been approved by the UCL Research Ethics Committee (Project ID Number): 0760/004

Name: Dr. Sunjeev Kamboj & Dr. Ravi Das

Work Address: Clinical Psychopharmacology Unit, UCL, 1-19 Torrington Place, London, WC1E 7HB.

Contact Details: Email: CPUstudies@gmail.com

Who are we recruiting?

We would like to invite **healthy males and females, aged 18-45, who enjoy eating chocolate, and occasionally overeat chocolate,** to take part in psychological research at University College London that will help us understand binge eating.

Please note, you **MAY NOT** take part if you:

1. Are pregnant or breastfeeding, or are likely to become pregnant during the study
2. Suffer from any major psychiatric or physical health disorder, including hypertension, diabetes, asthma, Hypokalemia, LQTS (long QT syndrome), Torsades de Pointe, Syncope, Congenital deafness, or an autoimmune disease
3. A family history of LQTS
4. Have a current diagnosis of Binge Eating Disorder, Bulimia, Anorexia, or a drug or alcohol use disorder
5. Are currently seeking treatment for any other psychiatric condition
6. Are currently using blood-sugar-control medication (e.g. insulin, or metformin) or statins
7. Are unable to abstain from drugs and alcohol for at least 24 hours prior to each testing session.
8. Are experiencing any physical health issues which may affect immune function, for example chicken pox, the herpes virus or a very recent cold.
9. Frequently suffer from UTIs
10. Have an allergy to Macrolides, i.e. any of the following medications:
    - SiroLIMUS
    - TacroLIMUS
    - PimecroLIMUS

- Oleandomycin
- Solithromycin
- Spiramycin
- Troleandomycin
- Tylosin/tylocine
- Roxithromycin
- Ketolides (Telithromycin, Cethromycin, Solithromycin)
- Fluoroketolides (Solithromycin)
- Antifungal Macrolides (Amphotericine B, Nystatin, Cruentaren)
- Toxic macrolides (Mycolactones)

Please note that these are not the only criteria for taking part in the study and you will have to complete a full online screen to check you meet all our criteria for eligibility.

Details of Study:

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to read the following information carefully and discuss it with others if you wish. Ask us if anything that is not clear or you would like more information. Remember, if you do choose to take part, you have the opportunity to withdraw at any time, without giving reason. Please note that full payment for the study will depend upon completion of the full study. If you do not complete the study, you will be paid according to the time you have spent in UCL for the study. Note we cannot reimburse travel expenses. This study is being conducted by researchers from the Clinical Psychopharmacology Unit at UCL in London.

Why are we doing this study?

Some people occasionally binge on large volumes of food and feel that they lose control over their eating on these occasions. Such bingeing can cause negative thoughts and emotions and have unintended health consequences such as weight gain. It can also lead to more serious eating disorders like Binge Eating Disorder. We wish to understand why some people experience this issue and whether we can develop better ways to help people reduce or manage bingeing. The latest research in psychology and neuroscience suggests that the way people learn about rewards, particularly with regards to food, is an important factor in binge eating. Some people, for instance, find that certain 'trigger foods' tend to cause bingeing. We wish to test this theory by assessing whether trying to change unhelpful responses to foods might affect how often someone will overeat chocolate.

To test this, we will use various computer tasks and questionnaires aimed at understanding your responses to chocolate. You will be asked to view and rate images of certain foods and will be required to taste samples of these foods. You will also be required to complete a task where you respond to images of different foods as quickly as possible.

By taking part in this study you will contribute to the scientific knowledge of how food tastes and preferences are learned and how these might contribute to binge eating. The results of the study may also contribute to improving treatments of eating disorders such as binge eating disorder. If you would

call from us to arrange a date and a time for you to come in. If you do not meet the criteria for the study we will email you. Please note that we have strict criteria for participation and based on your answers to these questions; you may not be eligible to take part in the study. If you *are* eligible, you will be asked to come to the Clinical Psychopharmacology Unit (CPU) at UCL in central London at a time convenient for you on 3 occasions. The first two occasions will be around 48 hours apart and the third 10-14 days later. The first and third session will take roughly 1 hour 30, and the second 2 hours. You will be required to abstain from drinking alcohol or using any psychoactive drugs (aside from caffeine) for at least 24 hours before each testing session and to refrain from eating for 4 hours prior to each session.

What will happen during testing?

The following is an overview of what will happen on each day of the study:

Screening and food diary: Before your first session at UCL, you will need to complete a screening questionnaire to check that you are eligible to take part and that there are no factors that might preclude you from participating. If you are eligible we will get into contact with you to organise a time for you to come into UCL. For the week before testing we will need you to complete a food diary, which will ask you how much chocolate you have consumed on each day.

Day 1 (~1.5hrs): You will need come to the Clinical Psychopharmacology Unit, UCL, 1-19 Torrington Place, London WC1E 7HB. First we will ask you to re-read and countersign the consent form once again, in person. We will take a finger-prick blood sample to assess your blood glucose.

You will then fill out questionnaires that measure your relationship with food, mood, attitudes, psychological traits and family history of eating disorders. You will also provide some basic health measures, such as BMI, blood pressure and heart rate. We will need to take your body weight on each day of the study. You will then complete some computer tasks that will involve rating and measuring reaction times to either pictures or words. These tasks will also involve measuring your eye movements to words and pictures, so it is important you have normal colour vision and no eye-related conditions (e.g. a squint) if you take part. Prescription glasses and contact lenses are fine.

Day 2(Day 1 + 24-48 hours, ~2 hours): You will again come to UCL and take either 10mg of the drug Rapamycin or a placebo. We will not know which of these you have taken. Next you will complete finger-prick blood glucose test and complete some brief questionnaire measures of your current mood. To allow time for the drug to take effect you will be asked to sit quietly (you may read or complete work during this time) for approximately 50 minutes. During this time we will monitor your heart rate and blood pressure. After this, you will be briefly rate some pictures and consume a sample of chocolate. Next, you will complete some basic psychological tests that measure your short-term memory, motor speed and attention. Following these tasks you will again be asked to sit quietly for roughly one hour, during which your heart rate and blood pressure will monitored.

Day 3 (Day 2 + 10-14 days, ~1.5 hrs): You will again come to the Clinical Psychopharmacology Unit to repeat the questionnaires and computer tasks from *Day 1*. After completing these tasks the testing will be complete and you will not be required to attend the testing centre again.

Follow-Up (Day 3 + 1 month)

After Day 3, you will not be required to come in to UCL again, however, you will need to continue to fill out your food diary for one week. We will need to contact you to ask you some follow-up questions about your mood, attitudes towards food and any overeating that has occurred one month after the

What are these drugs and are they safe?

Rapamycin (brand name Rapamune) contains the active substance sirolimus, which is typically used as an immunosuppressant following an organ transplant. Within this study you will be given a single 10mg dose, which will be administered orally. If you are uncomfortable with receiving rapamycin please do not take part. You will be supervised by a qualified medical doctor, who will be available for immediate advice in the unlikely effect of adverse effects.

What are the possible risks of taking part?

Continuous use of Rapamycin has some known side effects that you should be aware of when deciding to take part. Please be aware that the following risks are associated with **continuous use** of Rapamycin, in which accumulation of the medication in the body may produce serious side effects. **You will only be receiving a single dose.**

Side effects of continuous use of Rapamycin:

In 10+% of cases: *Fluid collection around the kidney, swelling of hands and feet, pain, fever, headache, increase blood pressure, stomach pain, diarrhoea, constipation, nausea, low red blood cells, low blood platelets, increased fat in the blood, increased blood sugar, low blood potassium, low blood phosphorus, increased lactate dehydrogenase, increased creatinine in the blood, joint pain, acne, urinary tract infection, pneumonia and other bacterial, viral and fungal infections, reduced white blood cells, diabetes, rash, elevated protein in urine, menstrual disorders, slow healing, rapid heart rate, and collection of fluid.*

In 1-10% cases: *Infections, blood clots, mouth sores, fluid collection, kidney damage, inflammation, nose bleeds, skin cancer, kidney infection, ovarian cysts, allergic reactions, shingles, cytomegalovirus infection.*

In < 1% cases: *Cancer of lymph tissue, bleeding of the lung, protein in the urine, kidney scarring, low blood platelets, tuberculosis, Epstein-Barr virus infection, infectious diarrhoea, liver damage*

Human studies in which a single dose of Rapamycin was administered have recorded either no side effects [1] or dizziness and headache in <10% of participants.[2] No further adverse effects were recorded during testing or in the week following administration.

A medical doctor will be available during and after the testing session. You are required to contact the medical doctor if you experience any of the following symptoms:

- fast heart rate;
- pain when you breathe, feeling short of breath; chest pain, feeling weak or tired;
- coughing up blood or mucus;
- feeling like you might pass out;
- pale skin, easy bruising or bleeding, weakness;
- fever, chills, body aches, flu symptoms;
- night sweats, weight loss;
- swelling in your face, stomach, hands or feet;
- rapid weight gain;
- pain or burning when you urinate; or
- slow healing of a wound

If you experience any of the following side effects, we ask that you contact the researcher who will be able to advise you:

- joint pain;
- nausea, vomiting, diarrhoea, constipation, stomach pain;
- headache; or
- acne or skin rash.

How will I be paid?

You will be paid £60 upon completion of the third testing session. This will be transferred to you via BACs, so please bring the details of the account you wish to be paid into (these are typically found on your bank card). Upon completion of the follow-up questionnaires, you will be paid an additional £10, bringing the total payment to £70. In total, the basic testing should last ~5 hours. If you withdraw before Day 3, you will only be reimbursed for the testing time you have completed.

How will my data be stored?

All information which is collected about you during the course of the research will be kept strictly confidential and will be securely stored electronically, or in locked repositories, with your name replaced by a numbered code so that you cannot be identified. Only researchers directly involved in the study will have access to the data. All data will be stored in accordance with the Data Protection Act 1998. The data will be used only for informing the research question in this study and the results of the research will be published in peer-reviewed scientific journals, but you will in no way be identifiable from such publications. Any biological samples we collect from you will also be anonymised and destroyed once they are analysed.

Note – if you have any further questions regarding this study please do not hesitate to contact the researchers by emailing CPUstudies@gmail.com.

It is up to you to decide whether or not to take part. If you choose not to participate it will involve no penalty or loss of benefits to which you are otherwise entitled. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

Please discuss the information above with others if you wish or ask us if there is anything that is not clear or if you would like more information.

Study Registration Details:

All data will be collected and stored in accordance with the Data Protection Act 1998. This study has been registered with UCL data Protection; **Number** Z6364106/2017/09/28

This study has been approved by the UCL Research Ethics Committee (Project ID 3901/004):

If you have any questions regarding the study please contact the study team:
Clinical Psychopharmacology Unit, UCL, 1-19 Torrington Place, London WC1E 7HB.
Email: CPUstudies@gmail.com

References

1. Surís, A., Suris, A., Smith, J., Powell, C., North, C. S., Surís, A., … North, C. S. (2013). Interfering with the reconsolidation of traumatic memory: Sirolimus as a novel agent for treating veterans with posttraumatic stress disorder. *Annals of Clinical Psychiatry*, *25*(1), 33–40. http://doi.org/10.1016/j.drudis.2011.09.009

2. Shi, J., Jun, W., Zhao, L. Y., Xue, Y. X., Zhang, X. Y., Kosten, T. R., & Lu, L. (2009). Effect of rapamycin

Appendix item 11 *Contact sheet for study in Chapter 5*

UCL

Contact sheet

If you are worried about any symptoms in the following 72 hours please contact our medical doctor at [redacted]

- *fast heart rate*
- *pain when you breathe, feeling short of breath;*
- *chest pain,*
- *feeling weak or tired*
- coughing up blood or mucus
- feeling like you might pass out
- pale skin, easy bruising or bleeding,
- fever, chills, body aches, flu symptoms;
- night sweats
- pain or burning when you urinate
- slow healing of a wound
- joint pain;
- nausea
- vomiting
- diarrhoea
- constipation
- stomach pain;
- headache; or
- Acne or skin rash.

Please attend A&E if you experience any swelling in your face, stomach, hands or feet

Researcher Phone [redacted] | Medical Phone [redacted]

[redacted]