Manipulating Maladaptive Motivational Memories via Reconsolidation

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I, Ravi Das 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

Substance Use Disorders (SUDs), are generally viewed as disorders of maladaptive reward memory and motivation. In SUDs, memories formed during drug use associate environmental stimuli with the rewarding effects of drugs. These stimuli can subsequently trigger craving, highly motivated drug-seeking and relapse, even after years of abstinence. An exciting new approach to combatting these maladaptive memories is via *reconsolidation*, the process by which memories become briefly unstable upon recall in order to strengthen or update before restabilising. In *Chapter 1*, I review reward memory mechanisms in SUDs along with pharmacological and behavioural determinants of memory reconsolidation to identify potential drug targets for interfering with reconsolidation. In *Chapter 2*, I use meta-analysis to assess the effects of two classes of drugs; N-methyl D-aspartate (NMDAR) antagonists and β-Blockers on blocking reconsolidation of reward memory in rats and show that NMDAR antagonism is far more effective. Building on this knowledge, in *Chapter 3*, I show that 10mg of the NMDAR antagonist memantine in combination with the retrieval of smoking cue-drug memory does not affect relapse or craving in a group of quitting smokers. As this null finding may have represented either a failure to destabilise memories or inefficacy of memantine, in *Chapter 4* I use a reward conditioning paradigm in hazardous drinkers to show that NMDAR antagonist Nitrous Oxide can interfere with reconsolidation of cue-alcohol memory, when administered after a reminder of learning that induces a negative prediction error. *Chapter 5* builds on emerging evidence of the necessity of prediction error to destabilise memory, using guided expectancy violation to destabilise naturalistic cue-alcohol memories in hazardous drinkers. Subsequent disgust counterconditioning updated these memories, reducing motivational salience and liking of alcohol stimuli, with associated reduction

in drinking. In *Chapter 6* I discuss the research reported and suggest directions for further study.

Table of Contents

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List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Figures:

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

The work in the following thesis has given rise to the following publications:

Das, RK., & Kamboj, SK. (2012). Maintaining clinical relevance: considerations for the future of research into D-cycloserine and cue exposure therapy for addiction. *Biological Psychiatry*, *72*(11), e29-e30.

Das, RK., Freeman, TP. & Kamboj SK. (2013) The Effects of N-Methyl D-Aspartate and B-Adrenergic Receptor Antagonists on the Reconsolidation of Reward Memory: A Meta-Analysis, *Neuroscience and Biobehavioral Reviews.* 37(3): 240-2155.

Glossary of terms and abbreviations

AMPA - α-amino, 3–hyroxy 5- methyl 4 - isoxazolepropionic acid, a synthetic specific agonist for the AMPA receptor. Mimics glutamate at AMPA receptors, but does not bind to NMDA receptors.

AMPAR - α-amino, 3–hydroxyl 5 – methyl 4-isoxazole propionic acid receptor, a subtype of glutamate receptor involved in fast evoked post-synaptic potentials. It is responsible for much of the rapid excitatory action of glutamate throughout the brain.

Consolidation – the protein synthesis – dependent stabilisation of short-term memory into long term memory following initial learning. Over time this involves a migration from primarily hippocampus-dependent neuronal encoding to distributed cortical encoding of memory traces.

DA – **Dopamine**, a monoamine neurotransmitter found mainly in substantia nigra, tegmental, limbic and striatal neurons. It is heavily implicated in reward, learning and motivation particularly with regard to substance use disorders.

LTP - Long-term potentiation, the lasting increase in evoked post-synaptic potentials following a high-frequency electrical stimulus train from an efferent neuron. This is believed to be the cellular mechanism underlying long-term memory.

LTD – **Long term depression**, the lasting reduction in neuronal firing rates following a low-frequency electrical stimulus train, an important inhibitory neuronal tuning and learning mechanism

mGluR – Metabotropic glutamate receptor, G-protein coupled glutamate receptors involved in synaptic plasticity. Unlike NMDARs and AMPARs, they are not ion channels, and modify cell signalling purely by second-messenger cascades involving cell plasma membranes. MGluRs can act as modulators of the NMDAR, increasing or decreasing its activity.

MMMs – **Maladaptive Motivational Memories**. Memory traces formed through repeated drug use that link environmental stimuli with drug availability and rewarding effects. These memory traces imbue drug-related stimuli with aberrant motivational properties, promoting craving, drug seeking and using when the stimuli are encountered

Model free – Learning that occurs without cognitive representation of the holistic network of states, transition probabilities and possible rewards (i.e. decision trees) that can be achieved in any given state. Such learning requires little cognitive effort, but it less flexible than cognitive, simulation-based learning.

NAcc – **Nucleus Accumbens**, a region nested within the ventral striatum receiving inputs from the amygdala, hippocampus, hypothalamus and frontal cortex. This region is heavily implicated in reward processing and maladaptive learning in SUDs.

NMDA – N-Methyl D-aspartate, a synthetic specific agonist at the NMDA receptor. Mimics the action of glutamate at these receptors, without action at other glutamatergic receptors.

NMDAR - N-Methyl D-aspartate receptor. A subtype of glutamate receptor that requires concurrent binding of glutamate and depolarisation to open its ion channel and allow calcium influx. The NMDAR is critical in synaptic plasticity, learning and memory.

NRT – Nicotine replacement therapy, a form of substitute prescribing for smoking cessation

PE – Prediction Error the mismatch between the outcome of a given stimulus or action stored in an existing memory trace and the outcome that is experienced following that stimulus or action. Acts as the primary driver of reinforcement learning through updating of stored values.

PIT – Pavlovian to instrumental transfer, the process by which Pavlovian reward-associated cues invigorate instrumental responses also associated with reward. This is an example of **conditioned motivation** whereby drug-associated cues motivate drug-seeking and using.

Reconsolidation – A process whereby consolidated memories can become briefly unstable upon retrieval, presumably to update or strengthen, and subsequently restabilise.

Reinforcement learning - The computational description of the creation, storage and execution of memory traces that maximise reward and minimise punishment through updating values concerning the reward associated with different states and actions.

Substitute Prescribing – A treatment approach most commonly used for opiate and nicotine addiction whereby a similar, but less potent compound to the addictive drug or the drug itself via a less harmful and addictive administration route are prescribed to reduce drug-associated harm and manage withdrawal.

SUD – Substance Use Disorder. This term is used to be in keeping with DSM-5 criteria for addictive disorders relating to drugs. The disorder is specific to the drug being abused, for example, one type of SUD is AUD (Alcohol Use Disorder).

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Chapter 1: Introduction

1.1. Addiction: Cost and Treatment

Humans have used recreational psychoactive drugs in various forms and for various sociocultural purposes for millennia. Their use has become deeply ingrained in worldwide cultures in many forms from the culturally normative (coffee, alcohol, nicotine) to the illicit. While there are many reported benefits of drug use, their use is also associated with serious negative outcomes in some individuals, among which addiction is the primary and most prevalent harm. The word 'addiction' comes from the Latin *Addicere*, meaning 'to enslave' and this is an accurate description of the experience of **substance use disorders** (**SUDs**). While the profile of SUDs are relatively unique to specific drugs or drug groups, common across all addictions is compulsive drug seeking and drug using behaviour, characterised by repeated relapse, with an accompanying loss of control over use, despite serious adverse consequences.

Although the legal classification of drugs is not correlated with their holistic potential for harm (Nutt et al. 2010), the rationale for legal control over drugs is largely based in the harm and cost to individuals and society which is associated with addiction. At the individual level and depending on the abused substances, SUDs can be debilitating diseases leading to social recidivism, and can be responsible for health issues caused directly from the drug (such as ketamine-induced ulcerative cystitis) or indirectly (such as HIV/AIDS from sharing needles). There are further societal consequences of addiction, including crime to fund the purchase of drugs in the absence of gainful employment, the breakdown of social networks with the potential for domestic abuse (O'Farrell et al. 1999) and wider socioeconomic costs from treatment and recidivism. While it is difficult to accurately quantify the total social costs stemming from addiction (estimates tend to focus on illegal drugs, or subsets of illegal drugs), the charity Addaction estimates that the 1998 - 2008 cost of problematic drug use in the UK alone

reached £110 billion. £100 billion of this is attributable to the crime and policing costs associated with drug use and £10 billion to primary healthcare costs. Each addict is estimated to cost the taxpayer £44,000 per year that their addiction is not resolved (Addaction, 2008). To contextualise the scale of the economic burden of addiction, if this annual accumulated cost were eradicated, the savings would be sufficient to cover the cost of running the *entire* National Health Service for one year.

There is a striking asymmetry between the cost of addiction and investment into treatment development. In the same period (1998-2008), a total of only £3 billion was spent on addiction treatment in total, a small percentage of which was spent on primary research into how to improve addiction treatments. Yet if 100,000 UK addicts could be 'cured' of their addictions and return to the labour force they could contribute £4.4 billion *per year* in tax revenue. Therefore, development of effective, durable treatments for addiction is an important goal from numerous perspectives. Effective treatments would reduce individual suffering, improving the quality of life for addicts, their families and their communities. From an economical perspective, such treatments would free resources to be deployed elsewhere in the healthcare system, while also enabling former addicts to contribute to the economy.

Unfortunately, current treatments for drug addiction are not particularly effective. Of the 366,217 addicts that entered UK NHS addiction services from 2005-2012, fewer than a third (29%) completed treatment and remained drug-free without returning in 5 years (National Drug Treatment Monitoring System, 2012). It should be noted that these statistics assume that all patients completing treatment and not returning to primary care did not relapse. Thus an unknown percentage of 'successfully' treated individuals is comprised of i) those who relapsed and decided not to return to treatment and ii) those who died. This estimate of treatment success is therefore likely overly

optimistic. As an example, although over half of UK smokers attempt to quit annually, only around 2-3% of 'cold-turkey' quitters are successful (West 2006). Notably, outcomes can be significantly improved by treatment options, but even with Varenicline, the most effective smoking cessation drug available (Fiore 2008), abstinence rates after 9 weeks are still only 21.9% (Jorenby et al. 2006). Given this poor success rate, there is wide scope for improvement in long-term addiction treatment efficacy in the long-term.

One may ask, given the astounding progress over the last half century in treatment of many diseases, why are treatment prognoses for SUDs so poor? Answering this question requires a consideration of the predominant current treatment approach. For some addictive drugs, such as cannabis and ketamine, dedicated treatment programs simply do not exist, obviating a discussion of the major limiting factor in recovery in these populations. For other drugs, such as nicotine and opiates like heroin, the primary treatment approach is **substitute prescribing** usually supplemented by some form of psychosocial support. Nicotine replacement therapy (**NRT**) is currently the most widely-used pharmacological aid to smoking cessation in the UK and methadone and buprenorphine are the most commonly prescribed pharmacological treatments for heroin addiction. These treatments operate on principles of harm reduction, by replacing a primary addictive substance with a less physically harmful analogue (for example by reducing the carcinogenic load of smoking cigarettes). They also reduce secondary harms and risks of drug use, for example by reducing needle sharing among heroin users and ensuring a consistent dose of drug. They are also extremely important for managing the withdrawal syndrome induced by abstinence from drugs.

Withdrawal is caused by homeostatic renormalisation of neurotransmitter, metabolic and endocrine function after continual allostatic changes incurred through drug use. The

recovery of these systems typically results in negative affect and anhedonia and can incur extremely unpleasant physical and psychological symptoms such as gastrointestinal distress, anxiety, hallucinations and seizures. Addicts are strongly motivated to avoid these withdrawal symptoms so negative reinforcement is a major driver for continued drug use or early relapse after abstinence. Thus substitute prescribing can be key to maintaining early abstinence from the abuse drug, but if it is to lead to eventual abstinence, it must follow a progressive titration schedule so that withdrawal symptoms can be managed. However, in the case of methadone, this detoxification is frequently not achieved and addicts often continue to use illicit opiates on top of their prescribed methadone treatment (Lions et al. 2014). As both methadone and nicotine replacement are themselves addictive, some may argue that the approach simply swaps one addiction for another. This 'lesser of two evils' approach is clearly not an optimal strategy for treating SUDs.

Managing physical symptoms of drug withdrawal in drug addiction is undoubtedly important in engendering eventual abstinence. However, physical withdrawal represents only a single, short-term driver in the maintenance of addiction and should be considered a first step in an efficacious program, rather than a satisfactory treatment in its own right. In most addicts, full withdrawal and detoxification upon complete cessation of drug use occurs within seven to 28 days (Glauser et al. 1970; Smolka and Schmidt 1999; Weybrew and Stark 1967), yet addicts remain at high risk for relapse for many years after discontinuation of drug use (O'Brien 1997), highlighting the short-term palliative effect of replacement therapies and the absence of long-term treatment effectiveness.

Rapid and full relapse also demonstrates that persistent - potentially permanent - neuropharmacological adaptations accompany addiction that far outlast the

physiological drivers of relapse (i.e. withdrawal). In recent years, mounting evidence has shown striking similarity between the neural adaptations seen in addiction and those seen in neural systems underlying motivated learning and memory (Kelley 2004). This is compelling evidence that long-term addiction operates primarily through maladaptive engagement of motivated memory processes. Treating these maladaptive memory systems will therefore be key to developing effective long-term treatments for SUDs. In the next section I will outline the research supporting this position and suggest ways in which these aberrant processes might be reversed.

1.2 Drug Addiction as Maladaptive Motivated Memory and Behaviour

Drug addiction is a multivariate problem. Its occurrence is the culmination of one or more of a heritable disposition to addictive behaviours (Kreek et al. 2005), epigenetic alterations caused by prenatal, perinatal or early developmental insults (Kippin et al. 2007), acute and chronic environmental stressors (Gordon 2002), social vulnerability (Broms et al. 2004) and maladaptive physiological and neuropsychopharmacological processes (Belin et al. 2008; Everitt and Robbins 2005). While work is underway to delineate the contributions of these factors to addiction pathology, the required integration of knowledge across disciplines (genetics, developmental biology and psychology, epidemiology and neuroscience) is in its infancy. Further, these risk factors may be additive or interactive and their frequent collinearity currently makes it impossible to define clear cause-effect relationships in the pathogenesis of addiction. As such preventative interventions at the genetic or societal level are not realistic in the short term. Instead, research strategies that aim to understand and remediate the maladaptive neuropsychological processes underlying addiction at the level of ‘the individual’ are both tractable and realistic. This strategy benefits from intervening

proximally to symptomatology of SUDs. That is, these neuropsychological maladaptations are the 'final common pathway' in SUDs and therefore represent a relatively consistent treatment target. This thesis will explore one such strategy: Translating behavioural modelling in laboratory animals to human addicts in an attempt to overwrite maladaptive memories thought to underlie relapse in SUDs (Milton and Everitt 2012).

Like many neuropsychiatric disorders, drug addiction can be thought of as malfunctioning of an adaptive plastic systems due to stresses that exceed the neural capacity for homeostasis and adaptive compensation (Koob and Le Moal 2001; Selye 1936; 1973). In the case of drug addiction, this system is the brain's reward learning and motivational machinery. Although an exhaustive discussion of the neuropsychopharmacology of memory is beyond the scope of this thesis, a brief review of the major neurotransmitter pathways involved in creating **maladaptive motivational memories** (**MMM**s) will contextualise the psychopharmacological issues that arise throughout the experimental work reported later.

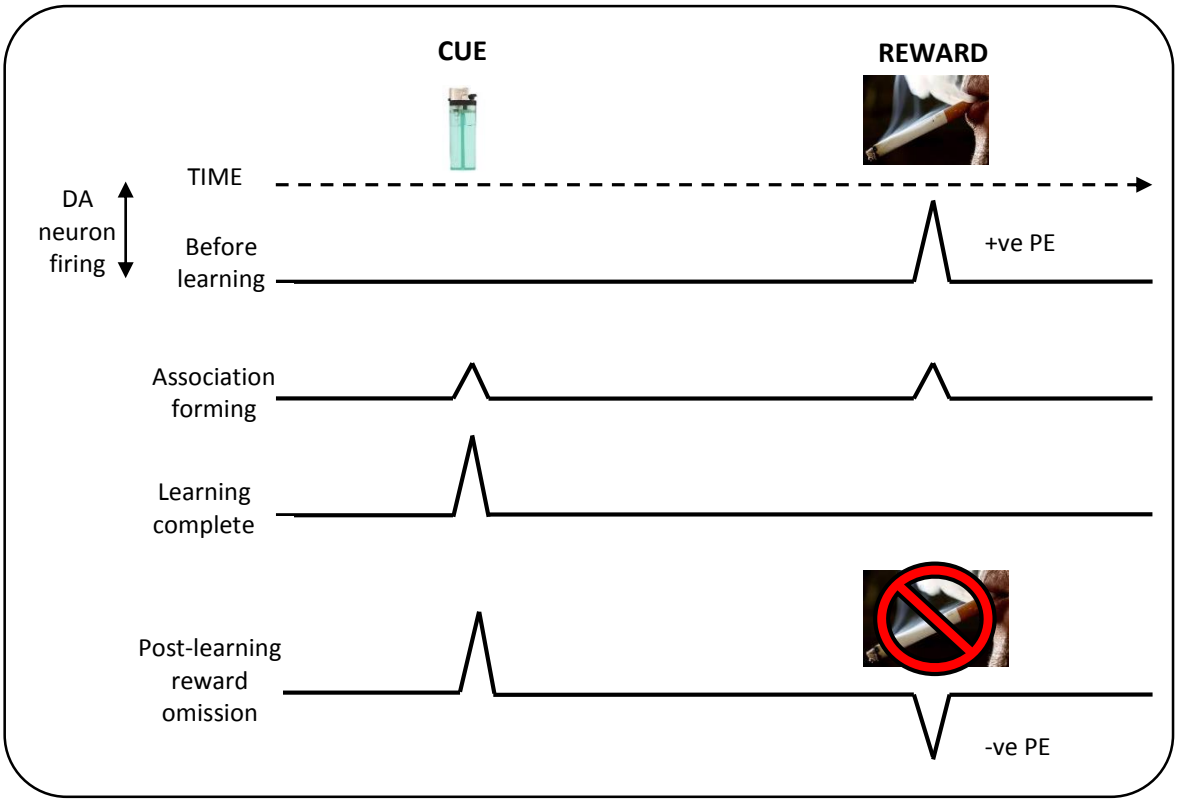
1.3. Drugs produce maladaptive motivational memories through hijacking of neural systems involved in reward learning.

Although addictive drugs vary widely chemically and in their mechanisms of action, they have consistent downstream effects on monoaminergic, glutamatergic, GABAergic or endorphin-system signalling in the brain (Koob 1992b). While different drugs recruit different receptor and signalling pathways to produce their reinforcing effects, abused drugs converge on modulation of **dopamine** (**DA**) signalling in the midbrain, limbic system and cortex as a 'final common pathway.' Those drugs that produce the greatest direct or downstream DA increases in these areas, particularly in neurons terminating in

the **Nucleus Accumbens** (**NAcc)** tend to be the most addictive (Pierce and Kumaresan 2006).

Dopamine is central to **reinforcement learning**, that is, in encoding the rewarding effects of stimuli and representing associations between actions, stimuli and outcomes that control reward-related behaviour (Dayan and Balleine 2002). Via a related mechanism, DA signalling also conveys information about the **salience** or importance and worthiness of attention of environmental stimuli (Berridge and Robinson 1998). Dopamine promotes learning about rewards via phasic bursts of firing of striatal DA neurons. These patterns of responding are shown in *Figure 1.1*. If an unexpected positive outcome occurs, a burst of action potentials is seen in DA neurons when the outcome is presented. If this favourable outcome is predicted by a previous stimulus, through repeated pairing of the stimulus and outcome, phasic DA neuronal bursts begin to occur upon the presentation of the predictive stimulus, rather than the reinforcer. Thus striatal DA neurons encode temporal predictions of rewards (Schultz et al. 1997). If a reward is withheld following its predictor, a phasic suppression of DA neuron firing occurs. Dopamine thus acts as a learning signal, encoding the error between predicted and actual events. Contemporary models of reinforcement learning revolve around this '**prediction error**' (**PE**) signal as the primary driver of learning about rewards and extensive neurobiological evidence now support this proposal (Waelti et al. 2001). This PE signal is central to both Pavlovian and instrumental learning, as it can encode the outcomes of actions, tuning future action selection (Redgrave and Gurney 2006). Clearly not all learning can be reasonably conceptualised as involving reward and other types of learning may be relatively independent of dopamine. These learning modalities are not of immediate relevance to this thesis however, so will not be discussed here.

Figure 1.1. The role of dopamine in reward prediction and learning via phasic prediction error signalling



Unexpected delivery of a reward such as nicotine (used as the example here, but this may be any reward) causes a phasic increase in striatal dopamine burst firing known as a positive prediction error (PE). If a cue consistently predicts the occurrence of a subsequent reward (such as a lighter often predicts the administration of nicotine), the temporally-delayed pairing of these events sees a shift in DA neuron burst firing from the time of reward presentation to the time of cue presentation. As the association between the two is fully formed, the phasic firing becomes completely time-locked to cue occurrence. Once this association is formed, if the reward is omitted, a phasic reduction in striatal DA firing occurs (negative prediction error).

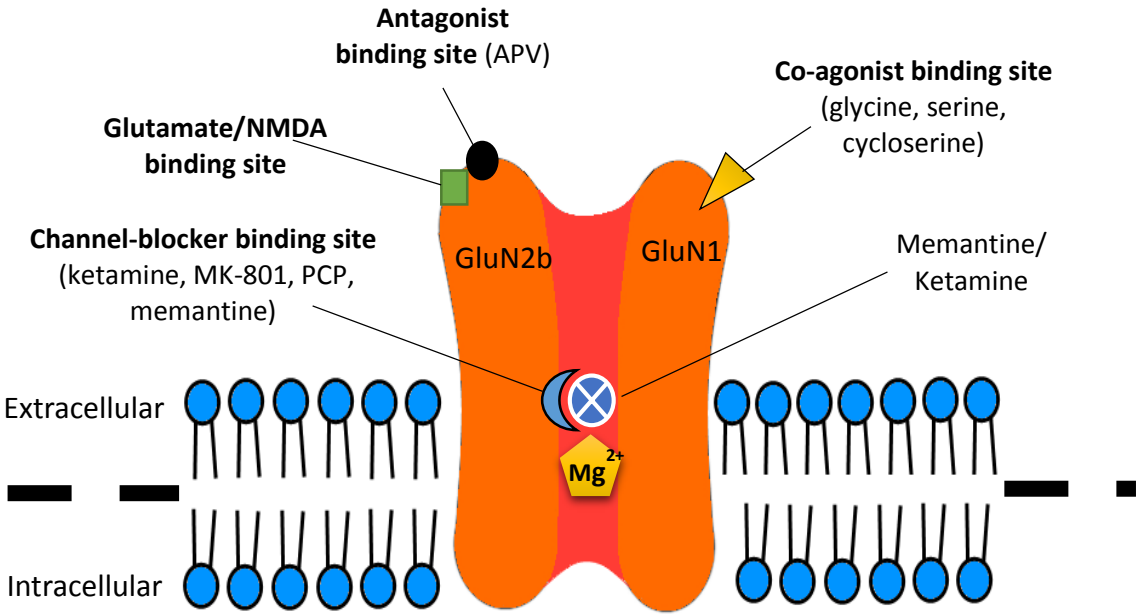
Addictive drugs produce phasic firing of basal-striatal-cortical DA neuronal activity and can create much higher frequency bursts than natural rewards such as food and water (Hernandez and Hoebel 1988). This activity creates a powerful learning signal and reinforcing effect that promotes learning about predictors of drugs and motivates behaviour towards further drug use. Human Positron Emission Tomography (PET) studies show that these spikes in DA-ergic activity correlate with the pleasure and feeling of 'high' derived from drugs of abuse (Volkow et al. 1997; Volkow et al. 1999) and the subjective experience of craving. In addition the salience attached to drugs versus natural rewards can be reduced via pharmacological manipulation of dopaminergic autoreceptors which dampen phasic DA activity (Freeman et al. 2014).

However, dopamine alone is not sufficient to produce lasting changes in neural memory circuits. The excitatory glutamatergic **N-Methyl-D-Aspartate (NMDA**) and **α-amino, 3–methyl 5- hyroxy 4-isoxazolepropionic acid** (**AMPA**) receptors mediate neural signalling necessary to create and stabilise motivational memory traces. Given the complexity and importance of these receptors and their properties in maladaptive memory formation and SUDs, a brief description of their pharmacology is warranted. NMDA receptors (**NMDAR**s) are heterotetrameric ligand-gated ion channels, consisting of a combination of two subunit types (canonically, two GluN1 and two GluN2 subunits[1]). The subunit types themselves are heterogeneous, creating many isoforms of NMDARs throughout the brain. A schematic of the NMDA receptor with ligand binding sites is given in *Figure 1.2*. A unique property of NMDARs, is their requirement for ligand binding at both glutamate and glycine sites and depolarisation of the postsynaptic membrane to remove the resting-state Mg^{2+} block from the ion channel. This requirement for concurrent ligand binding and depolarisation confers

[1] The GluN2 subunit has four different types (GluN2a, b, c and d) which introduce considerable functional diversity to the receptor. These subunit types are variably expressed in the central nervous system.

NMDARs with the properties of a logical 'AND' gate. Moreover, NMDARs are highly permeable to Ca^{2+} a ubiquitous second messenger in neurons, required for the activation of a variety of downstream enzymes which result in long-term synaptic plasticity (see below). As such NMDARs can encode synaptic co-activation, underlying much of the synaptic and dendritic remodelling that comprises Hebbian, experience-dependent neural plasticity. AMPA receptors (**AMPAR**s) have a similar, heterogeneous tetrameric structure to NMDARs and are responsible for the majority of rapid excitatory signalling in the brain and for expressing NMDAR-mediated synaptic plasticity. They are continuously produced and shuttled to the neuronal plasma membrane where they migrate to synapses. Increasing the density of AMPARs on the post-synaptic membrane increases excitability and serves as a key mechanism in creating functional neural networks (Esteban et al. 2003; Song and Huganir 2002).

Figure 1.2. Schematic representation of the NMDA receptor



This schematic shows a front view of a bisected NMDA receptor, with two of the four subunits visible. The existence of the GluN2b subunit here is simply an example and subunit composition may vary widely. Binding sites of endogenous ligands and drugs are shown as coloured shapes. In its resting state, the ion channel is blocked by Mg^{2+}. Removal of this block by depolarisation plus binding at the receptor sites is necessary to allow calcium influx into the cell and subsequent signal transduction mechanisms to be activated. Drugs can antagonise the NMDAR via binding to the extracellular antagonist receptor site, or by binding to the channel receptor site, forming an artificial channel block.

Activating NMDARs triggers a cascade of intracellular events that produce lasting synaptic changes via signal transduction pathways involving intermediate protein kinases and transcription factors, resulting in transcription of DNA into mRNA and mRNA to new proteins. These pathways therefore lead to protein synthesis-dependent synaptic potentiation via modification of NMDAR and AMPAR channels (Ben-Ari et al. 1992; Chen and Huang 1992) and translocation and migration of synaptic and extrasynaptic AMPA/NMDA receptors (Esteban et al. 2003).

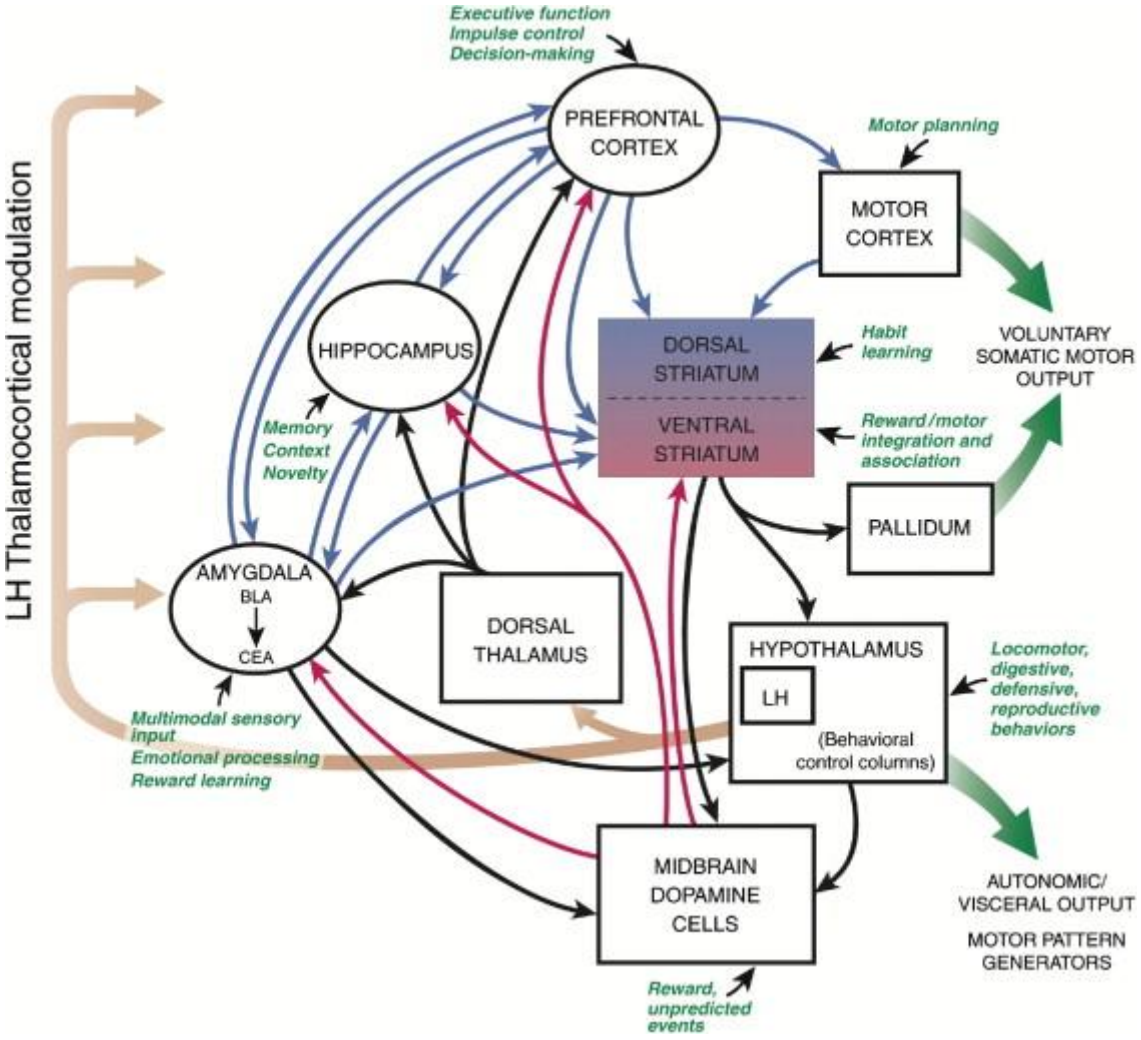
NMDARs and AMPARs are therefore critical in **long-term potentiation** (**LTP**), **long-term depression** (**LTD**) (Muller et al. 1988; Watt et al. 2004) and other forms of long-term plasticity (O'Brien, Kamboj et al, 1998). Antagonism of NMDARs prevents the stimulus-dependent tuning of synaptic networks, and the induction of LTP in the rat hippocampus (Malinow et al. 1988) and impairs learning and memory in rats (Baker and Azorlosa 1996; Butelman 1989) and humans (Krystal et al. 1994; Morgan and Curran 2006). Through these biochemical and neuronal pathways (drawn schematically in *Figure 3*), NMDARs underlie memory **consolidation**, the stabilisation of neuronal modification that converts short-term memory traces into long-term memories (Shimizu et al. 2000) that can persist across an organism's lifetime.

Phasic DA firing is dependent upon NMDAR inputs to ventral striatal DA neurons, as genetic modifications that disrupt the development of these inputs both prevent phasic (but not tonic) firing of DA neurons and the acquisition of conditioned behaviour. Critically, transgenic mice lacking these inputs show greatly reduced learning about the predictive nature of discriminative cues (Zweifel et al. 2009). Recurrent monoaminergic and glutamatergic projections from the NAcc to the amygdala, prefrontal cortex, hypothalamus and hippocampus convert the PE 'learning signal' into neural networks encoding the emotional, contextual and associative components of motivational

memories (Fields et al. 2007). These form integrated mesocorticolimic / nigrocorticostriatal circuits that are recruited by addictive drugs (Wise 2009). These circuits are perfectly suited to encode the sensory inputs of predictive environmental stimuli (primary cortical areas), the outcomes predicted by these stimuli (hippocampus and basolateral amygdala), the valence and relevance of these stimuli (midbrain, striatum) according to physiological state (hypothalamus), the integration of these stimuli and outcomes into a wider allocentric spatial context (hippocampus) and to 'paint' emotional (limbic system), hedonic and motivational (midbrain and striatal) value upon these stimuli in order to control appropriate behaviours (motor cortex) for maximising reward and avoiding punishment (Koob 1992a) (see *figure 1.3* for a schematic of circuits involved in reward learning).

Addictive drugs can directly increase NMDAR-mediated LTP in mesolimbic DA neurons (Bernier et al. 2011; Mansvelder and McGehee 2000) and alter the subunit composition, expression and distribution of AMPARs (Beckerman et al. 2013; Mickiewicz and Napier 2011) thereby directly engaging the neural mechanisms of motivational memory through both glutamatergic and dopaminergic routes. These systems evolved to motivate animals to seek and interact with rewards and it is the hijacking of these systems that imbues drugs with addictive properties. By hyperactivating the neural mechanisms that underpin motivation, salience and reinforcement learning, such drugs produce maladaptive motivational memories that are key to the development of pathological drug use.

Figure 1.3. Neural networks involved in maladaptive motivational memory formation.



This is a schematic circuit diagram and does not represent anatomical location of structures involved, however, although the Nucleus Accumbens is not explicitly drawn in this diagram, it is a structure nested within the ventral striatum. It receives extensive cortical, hippocampal and amygdalar input, interfacing limbic affective processing, motivational processing and motor output to guide motivated behaviour. Training to asymptote performance levels (and continued training beyond this) and habit formation is believed to involve a shift of this interfacing to the dorsal striatum, creating more direct and less flexible input-output (stimulus-response) associations, explaining the resistance of habitual memory to changes in the values of reinforcers. This figure is reproduced from Kelley (2004).

1.4 Maladaptive cue-drug learning in context

Recreational drug-taking occurs in the presence of various environmental stimuli, either distal or proximal to drug consumption. As with natural rewards, drug-induced phasic DA firing and related NMDAR/AMPAR mediated neuronal plasticity drives the formation of mnemonic associations between these stimuli or 'cues' and the availability and reinforcing effects of drugs.

Imagine drinking a glass of red wine. The bottle and glass each have a distinctive shape, pouring the wine from the bottle to the glass produces unmistakable proprioceptive feedback and makes a specific sound. The wine itself has a distinctive colour, aroma and taste. All of these sensory inputs specify with relative certainty that ethanol will soon be entering the central nervous system, leading to a cascade of events ending in dopamine release in the striatum. Simultaneously activated are the cortical, limbic, motor and hippocampal networks encoding sensory inputs, affective status of the events and appropriate actions to be taken, respectively (see *Figure 1.3*). Whenever wine is consumed in this manner, Pavlovian and/or instrumental associative memories are formed between these predictive sensory stimuli and the rewarding effects of wine via the dopaminergic and glutamatergic mechanisms described above. As such, following repeated wine drinking, cues like the aroma of wine and sight of a wine bottle elicit increased striatal dopaminergic activity, which imbues these cues with abnormally increased salience such that they grab attention (Robinson and Berridge 1993; 2001). These learned associations also produce the hallmark conditioned Pavlovian responses of craving and the urge to use drugs (Wong et al. 2006), motivated drug seeking, and instrumental responses, such as buying alcohol or picking up a glass and drinking.

Reward values of stimuli do not simply exist in a unitary, homogenous state within organisms and may further vary across organisms. In normal adaptive motivated

behaviour (including non-dependent recreational drug use), the behavioural consequences of reward memory activation are modulated by both the physical state of the organism and frontal-cortical inhibitory mechanisms. Proprioceptive and homeostatic signals, operating via the hypothalamus, determine the motivational value of a reward in a given internal state of the organism (Berridge 2012).

To illustrate this, let us return to our bottle of wine. Imagine that after a particularly busy and stressful week at work, you manage to submit a grant application just before the 8pm deadline on a Friday evening. Your co-applicant suggests that you go for a drink to celebrate and you decide to share a bottle of wine in the local pub. The combination of having finished the grant, the reduction in stress and the buzz from the bottle of wine is extremely rewarding, so you decide to share another. On the way home you realise you have not eaten any dinner and decide to stop off at the local kebab shop for some food. The next morning you wake up with a pounding headache and find the half-eaten kebab on your kitchen counter. You notice that the smell and sight of it are extremely unpleasant compared to how tasty it had seemed the night before. In the kitchen cupboard is an unopened bottle of wine. The thought of drinking any seems extremely unpleasant and makes you feel nauseous, when the previous night the wine had been highly rewarding. Thus, though food and drugs can be highly rewarding, they do not have a single *a priori* and immutable 'reward value', but rather this exists on a spectrum that is determined by the motivational homeostatic signals.

Research has demonstrated that modulation of these signals can lead to diametric shifts in the motivational status of stimuli, turning highly salty solutions that were previously aversive into sought-after and liked rewards in rats (Robinson and Berridge 2013). In SUDs, drug cues and associated contexts can trigger unjustifiably strong motivation to

obtain and use drugs that is disproportionately large (Berridge 2012), overriding satiety and other inhibitory signals.

The value of short-term rewards, such as drug use are also couched within the value of longer-term goals. Actions (such as drinking) are weighed against the value of their short-term (e.g. intoxication) and longer-term (e.g. hangover) outcomes and rational decisions are made regarding the utility of the action given the current state of the organism (Sutton and Barto 1998). Prefrontal inhibition of mesostriatal dopamine can thus reduce cue-induced drug seeking and prevent drug use, maintaining drug use at a healthy level. However, chronic drug use causes adaptive down-regulation of frontal DA and reduces the expression of prefrontal D_2 receptors, leading to increased drug use in an attempt to recover normal DA levels while reducing the reinforcing effects of natural rewards such as food and sex.

Further, lowered prefrontal D_2 expression is also associated with increased impulsivity (Lee et al. 2009) and reduced capacity for response inhibition (Ghahremani et al. 2012) due to reduced control of the prefrontal cortex over striatal dopamine levels in response to drugs (Volkow et al. 2007) and drug cues (Volkow et al. 2006). In concert with this reduction in regulatory control of drug use, cue-drug memories are continually reinforced, evolving from goal-directed, flexible stimulus-outcome or action-outcome memories to overlearned, inflexible stimulus-response habits (Everitt and Robbins 2005), a process which relies upon NMDAR activity (Wang et al. 2009) and involves a shift of processing from the ventral to dorsal striatum. Development of such habitual memories and associated action patterns are central to the progression from recreational drug use to the compulsive patterns of drug seeking and use that characterise addiction (Belin et al. 2008). The key feature of these stimulus-response habitual memories is that they are independent of any cognitive representation of goal states and therefore

insensitive to modulation by the normal inhibitors of responding, satiety and reward devaluation. Rather they represent a relatively direct input-output circuit whereby stimuli such as drug cues can reliably and automatically produce conditioned responses leading to drug use. As exposure to drug cues is usually inevitable for an addict, habitual stimulus-response memory traces become a primary driver of drug use in addiction (rather than just the reinforcing effects of the drug itself). Once formed, associative cue-drug memories are long-lasting. This is why, after months or years since their last cigarette, under some circumstance whereby sensitised motivational systems are activated by drug cues, an ex-smoker may experience overwhelming cravings for cigarettes and relapse. While allostatic perturbations to normal neurotransmitter levels recover relatively rapidly after long-term cessation of drug use, cue-drug memories persist, underlying the quiescent long-term susceptibility to relapse that characterises addiction and its chronicity.

It will hopefully now be clear that cue-drug memories are central to the pathogenesis and maintenance of SUDs; that sensitised motivational mnemonic systems that increase the control drug cues exert over drug seeking and using explain the limited efficacy of existing pharmacological treatments for substance use disorders. Currently licensed pharmacological treatment options do not primarily address the role of maladaptive motivational memories in relapse. Any changes in these memories likely occur as a secondary consequence of abstinence. Shifting focus to directly targeting the memory mechanisms underpinning relapse may prove critical in developing more effective treatments for substance use disorders. Moreover, as the same basic maladaptive memory mechanisms are thought to be at play in the pathogenesis of addiction to all drugs, despite their widely varying pharmacology and sequelae, this approach may have general utility for a wide range of substance use disorders.

1.5. How to Treat Maladaptive Motivational Memories (MMMs)?

Since Pavlov first delineated the characteristics of associative learning, or conditioning, the most effective and widely used method aimed at reducing conditioned responding has been extinction. Extinction involves repeatedly presenting a conditioned stimulus (for example, a light associated with a shock in a rat or a cigarette packet, which has become associated with smoking) without its reinforcing outcome (i.e. without the shock or without the inhalation of smoke). Such uncoupling of stimulus and outcome can reduce both conditioned Pavlovian (Pavlov 1927) and instrumental responses (Skinner 1938). However, there is now considerable evidence that extinction is not an *un-learnin*g of conditioned associations, but rather the creation of a parallel, inhibitory memory association that competes with the conditioned association for behavioural expression. In the standard rat light-shock paradigm, this means that, following conditioning and extinction two associations exist, one light/shock (the conditioned association) and one light/no shock (extinction association). Input parameters, such as context or the presence of 'scene setting' cues, determine which of these associations is expressed (Bouton and Bolles 1979).

1.6. Out with the old: Extinction to treat MMMs

Perhaps unsurprisingly, extinction has been the focus of experimental medicine that attempts to treat drug addiction through reduction of MMM expression. The homologue of extinction learning in laboratory animals is **cue exposure therapy** (**CET**) in human patients (Bouton et al. 2001). CET for substance use disorders involves repeatedly exposing participants to a drug cue (for example, a pint of beer) while the participant withholds the conditioned response (drinking from the glass) under the supervision of an experimenter or clinician. The aim of CET for SUDs is to extinguish conditioned responding such that drug cues become less likely to activate prepotent conditioned

motivational responses, less able to provoke craving and less likely to lead to drug use. CET has been extensively studied in the treatment of maladaptive motivational memories in substance use disorders (Conklin and Tiffany 2002; Dawe et al. 1993; Drummond et al. 1995; Drummond and Glautier 1994; Loeber et al. 2006; Niaura et al. 1999), but has limited efficacy (Dawe et al. 1993; Drummond et al. 1990; Marissen et al. 2005; Niaura et al. 1999). This is due to a lack of generalisation of in-lab effects to other contexts, (renewal), recovery of drug responding following a single exposure to the drug (reinstatement) and instability of acute changes in outcome measures over time (spontaneous recovery).

These phenomena occur because, as discussed, extinction is not erasure of conditioned memory traces, but increased inhibition of these memories via competing (conditioned stimulus- no drug) traces. In suppressing drug-seeking, extinction will thus only ever be as effective as the continued ability of the inhibitory memory trace to effectively compete with a conditioned drug-taking response. The rapid recovery of cue-induced responding for drugs following CET is one indication of the insufficiency of the inhibition of cue/drug memory traces engendered by CET. These traces therefore invariably recover and precipitate drug-seeking behaviour due to a greater retrieval propensity. When we consider the length of the learning history that established stimuli like lighters as drug cues (tens or hundreds of thousands of learning episodes over many years) compared to the length of typical in-lab or in-clinic cue exposure sessions (tens of trials over minutes or hours), and that repetition of cue-response pairings increases retrieval propensity (Ebbinghaus 1913) the long-term inefficacy of CET is perhaps unsurprising.

One approach to overcome the disparity in learning strength between CET and conditioned cue-drug associations is to pharmacologically prime the neurotransmitter

systems underlying memory during CET to enhance the strength of the memories formed during the treatment. Given the centrality of NMDA receptors to learning and memory, enhancing NMDA-mediated neurotransmission is an attractive candidate for potentiating the strength of extinction learning in laboratory CET paradigms. Drugs which may achieve this goal are the amino acid D-serine (Schell et al. 1995) and the partial agonist antibiotic **D-cycloserine** (**DCS**) (Watson et al. 1990), both of which are co-agonists (with glutamate) at the glycine site of NMDA receptors. These compounds increase channel open probability (see *Figure 1.2*), although the extent to which this occurs is receptor subtype-dependent (Sheinin et al. 2001). Research investigating the effects of D-serine on memory in humans is extremely sparse, although some studies show remediation of cognitive deficits in medicated schizophrenic patients with co-administration of D-serine and antipsychotics (Heresco-Levy et al. 2005; Tsai et al. 1998), suggesting it may have cognitive enhancing effects. In this vein recent research has focused on combining DCS with CET to reduce the context-dependency of extinction learning and improve outcomes in CET for substance use disorders.

While DCS has shown promise in animal models of addiction (Botreau et al. 2006) and human fear learning models (Grillon 2009; Kalisch et al. 2009), it has failed to show utility for CET in human drug-using populations (Hofmann et al. 2011; Kamboj et al. 2012; Kamboj et al. 2011; Price et al. 2013; Yoon et al. 2013) and in some studies has been found to increase cravings following CET (Hofmann, Hüweler, MacKillop, & Kantak, 2011; Price et al., 2013). Some authors have suggested that failures with D-Cycloserine are due to insufficiently powered or sensitive tests in humans to observe subtle effect of DCS on laboratory measures of components of cognitive processes involved in addiction. However, the extinction-enhancing approach may be flawed in more fundamental ways which proponents of this approach have tended to ignore (Myers and Carlezon Jr 2012). Rather than continued unproductive research in this

domain researchers should develop alternative experimental pharmacobehavioural approaches while considering five central criteria in appraising their potential as therapies in addiction (Das and Kamboj 2012). These criteria will be applied in appraising the strategies described in later sections of this thesis.

1.7 Five criteria for appraising pharmacobehavioural treatments for addiction

Treatments that will offer meaningful utility for SUDs must possess the following qualities if they are to be ultimately implemented in the clinic:

1) Robust, reproducible effects on reducing *relapse rates*.
2) Long-lasting efficacy
3) Contextual invariance
4) A feasible mode of clinical implementation
5) Cost- and time-effectiveness.

Considering the nature of associative learning in SUDs and the studies of DCS referred to above, these criteria would suggest that pharmacological enhancement of extinction should be abandoned as a strategy for treating SUDs. No study of DCS/CET has shown long-lasting effects on drug use or relapse rates, the primary outcomes of interest (failing criteria 1,2, and 3). The single small-scale study showing beneficial effects of DCS on CET (Santa Ana et al, 2009) found no such effect at four week follow up (failing criterion 2). In contrast the number and sample sizes of extant clinical studies with DCS/CET showing null or detrimental effects, suggest that if DCS does enhance extinction, the effect is very small and therefore likely to be of negligible practical importance in improving addiction treatment. Moreover, given the ratio of null to positive findings, it is also likely that positive findings with DCS/CET represent Type I errors.

More fundamentally, pharmacologically potentiated CET is likely to fail because extinction does not weaken or change pre-existing MMM traces, but, to a greater or lesser extent, temporarily prevents them from being expressed behaviourally. As previously discussed, the sheer amount of learning that occurs to establish stimuli as drug cues versus the amount of extinction that occurs in-lab may account for null effects of DCS. A 40-a-day smoker will experience 14,600 stimulus-response training episodes per year, which explains the difficulty in retraining these responses. DCS would have to increase memory strength to the extent that the gap is bridged between training histories that differ by orders of magnitude. This likely explains the disparity between the efficacy of DCS for extinction of fear memory in anxiety disorders (Smits et al. 2013) and SUDs and between animal and human models of appetitive memory (Dhonnchadha et al. 2010; Thanos et al. 2011). Prototypical anxiety disorders and lab-based learning paradigms represent extremely abbreviated learning histories compared to naturalistic human MMM formation. One potential solution to this issue is to massively increase the amount of extinction training during a course of CET such that extinction learning begins to approach the habitual levels of responding seen with cue-drug memories. This, however, would require inordinate amounts of treatment time which is unrealistic in terms of retention of patients and insufficiency of healthcare resources, thus failing criteria 4 and 5.

DCS is a partial agonist at the glycine site and a weak potentiator of NMDA receptor activity, especially since normative synaptic glycine levels are likely to be at saturating levels (Forsythe et al. 1988). It may therefore be argued that more efficacious compounds than DCS should be used. While other compounds are currently being tested as memory enhancers (e.g. (Das et al. 2013b), an inescapable fact of human memory is that it is an extremely efficient and highly functional system. It therefore seems unlikely that a single pharmacological compound would produce such a profound

improvement on this evolutionarily fine-tuned system as to fundamentally redress an imbalance created through vastly mismatched training histories. Such an efficacious drug is not currently known to (and may never) exist. These factors reinforce the conclusion that use of cognitive enhancers to potentiate consolidation of extinction in CET is a deeply flawed approach. A more parsimonious approach is to degrade or change MMMs directly.

1.8 In with the new: Reconsolidation interference to overwrite Maladaptive Motivational Memories

The use of extinction in behavioural treatments of MMMs stemmed from the prevailing model of memory **consolidation** and stability over the last century, first proposed by Muller and Pilzecker (McGaugh 2000; Müller and Pilzecker 1900). This model proposes that, following learning, and consolidation of this learning into long-term memory, memories are stored in a stable, inactive state and are therefore resistant to interference. This model implies that there is a single window of opportunity to directly interfere with a maladaptive memory: during its consolidation. This was first evidenced by retroactive interference in word pair learning tasks demonstrating that after learning of cue-response word pairs, subsequent acquisition of a new list of cue-response pairs caused poorer recall of original word pair items at test both immediately and 24 hours later (Müller and Pilzecker 1900). Even non-verbal material (pictures of landscapes) produced this interference effect. However, this effect was only observed if the new items were learned shortly after the original list, before the first memory trace had a chance to stabilise.

Subsequent studies by Duncan and colleagues (Duncan 1949) found that retrograde amnesia for learning was produced by electroconvulsive shock administered soon after learning (Duncan 1949). Research in animals showed that protein synthesis inhibition

did not impair acquisition of learned responses, but did impair their retention (Agranoff et al. 1966; Duncan 1949), suggesting that consolidation is underpinned by a late-phase form of LTP (Matthies et al. 1990) that involves re-scaffolding the synaptic architecture during learning to lay down new memories (Krug et al. 1984). Much research has now characterised late-stage LTP and memory consolidation at the cellular, molecular, systems, and behavioural levels. This shows, for example, that recruitment of immediate-early genes (Jones et al. 2001), changes in gene transcription (McMahon and Jones 1993; Nguyen et al. 1994) and activation of neurotrophic factors (Ying et al. 2002) are involved in the process, which critically relies upon modification of neural activity in the hippocampus (Nadel and Moscovitch 1997; Zola-Morgan et al. 1986)

Various lines of evidence suggested that once consolidated, memories become resistant to interference. For example amnestic drugs administered shortly after learning cause retrograde amnesia but do not impair memory if administered after the time-period in which consolidation occurs (Duncan 1949; McGaugh 1966). Over a longer time-course (weeks to years), a process of systems consolidation occurs, where memory traces become increasingly hippocampus-independent and cortically distributed, such that memories can be recalled by the external activation of neuronal nodes within the memory trace (McGaugh 2000). With more frequent recall and reinforcements, memories continue to become more strongly encoded (Ebbinghaus 1913) until an asymptote is reached. At this point, typical accounts of memory would suggest that there is no means of interfering with the memory trace, as even the archetypical amnesic patient H.M. retained remote memories from before surgery that bilaterally removed his medial temporal lobes (Scoville and Milner 1957).

Traditional consolidation theory would therefore suggest that the modification of overlearned MMMs in SUDs is unachievable as it is virtually impossible to intervene

within the window of opportunity, before these memories are strongly consolidated and well-rehearsed. The last decade, however, has fundamentally challenged standard consolidation theory following the resurgence of interest in memory *reconsolidation*, the process whereby remote (consolidated) memories can become unstable at recall and susceptible to interference. The phenomenon was first observed by D.J. Lewis and colleagues (Lewis and Maher 1965; Misanin et al. 1968) who observed that electroconvulsive therapy following a reminder cue of remotely trained memory produced amnesia for that memory in a similar fashion to post-consolidation electroconvulsive shock. Lewis and colleagues, interested in time-course of consolidation and retrograde amnesia termed the phenomenon 'Cue-dependent amnesia', suggesting that retrieval of a memory renders it temporarily labile, requiring further stabilisation to persist in long-term storage. Subsequently, systemic protein synthesis inhibition following cue-induced memory recall was found to lead to retrograde amnesia for the learned information, in similar time-dependent manner as original consolidation (Judge and Quartermain 1982). After this research, however, interest in 'Cue-dependent amnesia' lost momentum and lay dormant until 1997 when Jean Przybylsawski and Susan Sara found amnesia for an over-trained spatial memory (radial maze) with NMDAR antagonist MK-801 following brief reactivation of this memory (Przybyslawski and Sara 1997). This effect was time-limited for drug infusions up to two hours after reactivation, displaying a gradient similar to that seen in initial memory consolidation. Noting similarities of these amnestic effects with those found in studies of consolidation, the authors named the phenomenon ***re*consolidation**. This 'rebranding' along with an interest in molecular models of memory processes and more sophisticated methods for manipulating the brain's chemistry, sparked a renewed interest in the field. Sara notes, however that the choice of name was unfortunate

because, as will be seen, reconsolidation does not simply represent a recapitulation of consolidation. (Sara and Hars 2006)

The contemporary explosion in reconsolidation research commenced with the publication of a study in *Nature* by Karim Nader and colleagues demonstrating that a Pavlovian conditioned tone-shock fear memory could be 'erased' by infusion of protein synthesis inhibitor, anisomycin, into the rat basolateral amygdala immediately following a brief reminder of the memory (a single presentation of the tone in the conditioning context) (Nader et al. 2000). This demonstrated that memory retrieval can spark a period of memory lability (memory *destabilisation*) and a subsequent requirement for *restabilisation* of the trace, which requires protein synthesis in the same brain areas necessary for initial consolidation. Although 'reconsolidation' literally refers to the ongoing requirement for restabilisation of memories following retrieval-induced destabilisation, the term is now generally used to refer to the entire phenomenon of memory destabilisation and restabilisation following retrieval. The potential of interfering with reconsolidation processes should be self-evident in that it offers the first viable means to weaken or change maladaptive memories, be they motivational as in addiction, or fear-based as in PTSD and anxiety disorders.

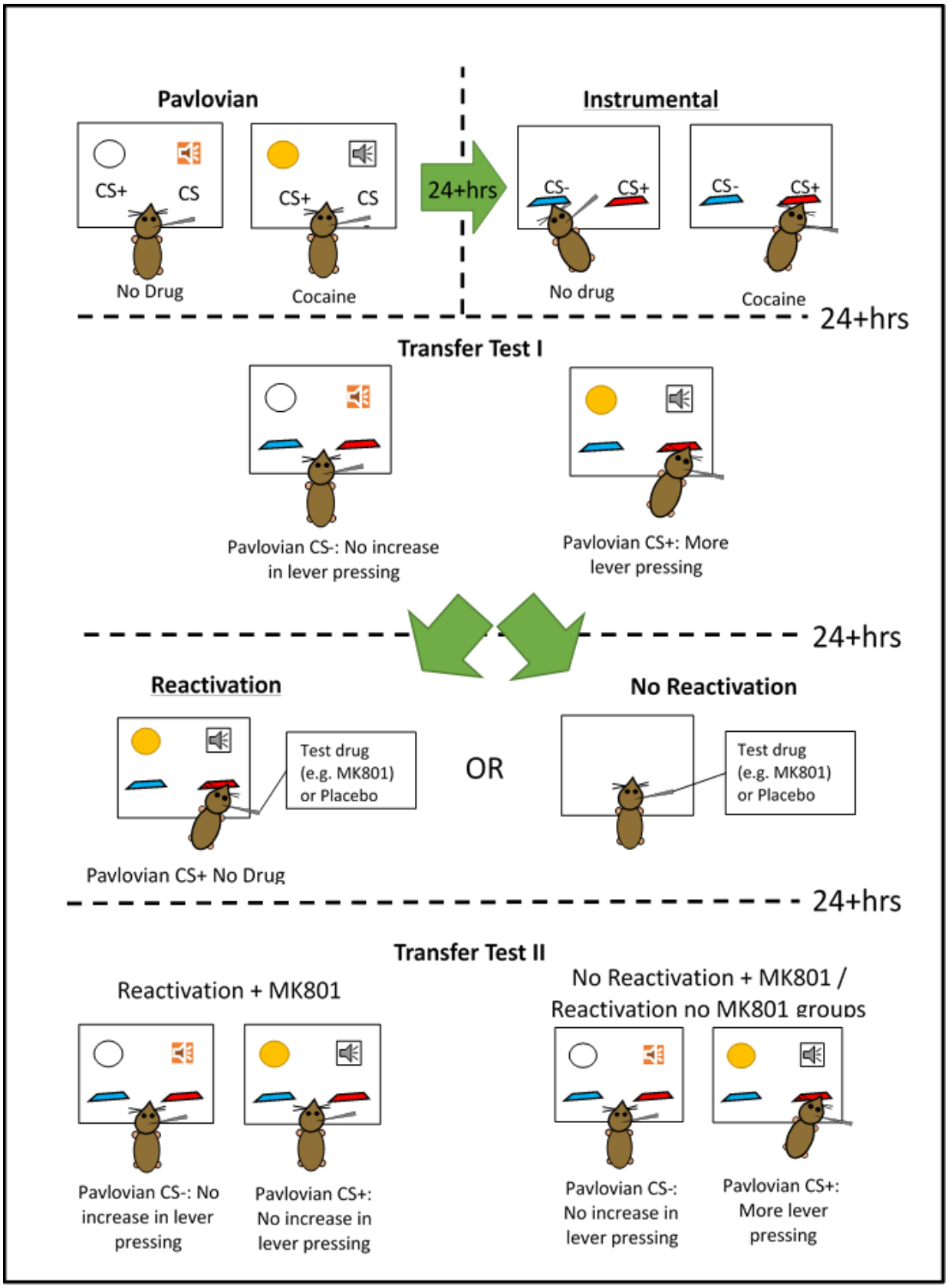
Since publication of Nader et al's study, hundreds of further papers examining the phenomenon of reconsolidation have been published, using various learning paradigms and post-retrieval interventions to understand its behavioural and molecular determinates. It is now generally accepted that reconsolidation exists as a computationally efficient and evolutionarily essential means for updating consolidated memory traces to maintain their relevance by incorporating pertinent new information (Lee 2009). Although animal reconsolidation studies are numerous and rapidly expanding, there is thankfully a high review to research ratio, with up-to-date reviews

being steadily published (Alberini and LeDoux 2013; Besnard et al. 2012; Dudai 2006; Dudai and Eisenberg 2004; Finnie and Nader 2012; Lee 2009; Milton and Everitt 2012; Soeter and Kindt 2011; Sorg 2012; Torregrossa and Taylor 2013; Tronson and Taylor 2007). I believe repeating such a review process will create redundancy, so a full synthesis will not be repeated here (the interested reader is encouraged to read any of the excellent referenced reviews). Instead, I will focus upon issues pertinent to the efficient translation of preclinical reconsolidation research into novel treatment modalities for SUDs.

1.9. Pharmacological mechanisms of memory reconsolidation

Findings of consolidation research have largely driven the cellular and molecular interrogation of the reconsolidation process and therefore much of the preclinical research into memory reconsolidation has probed the process with drugs that are known to interfere with initial consolidation. The aim of this research is to understand the molecular pathways underlying reconsolidation, their relationship to behaviour and to identify potential drug targets that may be of therapeutic benefit in the clinic. *Figure 1.4* shows and describes a prototypical rat appetitive memory reconsolidation paradigm known as the **Pavlovian to Instrumental Transfer (PIT)** task. This task is used as an exemplar because it includes both Pavlovian and instrumental training and represents a means by which Pavlovian learning can modulate operant responding. Although the exact stimuli and reinforcement schedules differ across paradigms, the general procedure for interrogating a molecular pathway in reconsolidation is similar across paradigms.

Figure 1.4: The Pavlovian to Instrumental Transfer (PIT) paradigm in reconsolidation research



All preclinical reconsolidation studies consist of three key phases, typically separated by at least 24 hours: Acquisition, Test I, Reactivation/Intervention and Follow-up Test. Above is a schematic illustration of these phases in the PIT paradigm. In this paradigm, instrumental and Pavlovian associations are trained during separate acquisition phases. In the Pavlovian phase, one stimulus (in this example a yellow light CS+) preceded delivery of cocaine via an indwelling cannula located in the ventricles. Both the reinforcer and method of delivery may vary in reconsolidation research. The reinforcer may be morphine, nicotine, ethanol or dietary such as sucrose solution or chow. The drugs may be injected intracerebroventricularly, systemically, or in the case of food or sucrose, accessible via a tube or chow magazine. A control Pavlovian CS- (in this case a tone) never signals delivery of cocaine. The rat will associate the light with the drug reward and in some cases, begin to approach and interact with the light CS+. Subsequently, in the instrumental phase, two levers are available in the operant conditioning chamber. Pressing ten times on the CS+ lever (this reinforcement schedule may vary or be progressed to increasingly infrequent rewards) will cause an infusion of cocaine. Pressing on the other (CS-) lever will do nothing. The rat learns to repeatedly press the CS+ lever to receive an administration of cocaine.

Once both of these are established, during the first transfer phase, both Pavlovian and instrumental stimuli are presented together. Presentation of the Pavlovian CS+ (light) can invigorate instrumental responding (increasing lever pressing rate) and reinstate lever pressing even following instrumental extinction. This is the 'transfer' effect of Pavlovian to instrumental learning. Presenting the Pavlovian CS- (tone) will not enhance lever pressing. Subsequently, during the reactivation/intervention phase, the rat is randomised to receive either memory reactivation + test drug (in this case NMDAR antagonist MK801), reactivation with placebo (in this case saline) or no reactivation + test drug. The MK801 is infused intracranially via the same cannulae that administered the cocaine and can be administered either prior to or after reactivation.

The reactivation session consists of a brief (several minutes) reminder of the Pavlovian memory trace. The CS+ light is presented, but the cocaine is not delivered. It is thus similar to a single 'extinction' type trial. This procedure aims to reactivate and destabilise the memory trace, without causing extinction. Infusion of the test drug begins either immediately before or immediately after the brief reactivation session. Since abolition of the Pavlovian light→ cocaine memory trace will necessarily abolish the PIT effect (there is nothing to transfer), the subsequent follow-up transfer test should find no evidence of PIT if reconsolidation was successfully blocked by the reactivation + drug procedure. The PIT effect should still be evident in the groups that received the drug without reactivation and reactivation without the drug. These controls are necessary to determine that the abolition of responding was both reactivation-dependent and drug-dependent.

Any subsequent tests can determine the longevity of any reconsolidation blockade effects and susceptibility to spontaneous recovery. Reinstatement can also be assessed by giving the animals a priming dose of cocaine and re-introducing them to the test chamber. A lack of reinstatement and spontaneous recovery are often taken as evidence of memory erasure during reconsolidation.

The PIT paradigm described in *Figure 1.4* is one of the more complex conditioning designs used to investigate drug seeking and abolition of reward memory by reconsolidation. However, it is the most direct measure of **conditioned motivation,** the modulation of motivated responding for drugs by a Pavlovian association. This type of conditioned responding is thought to be important in SUDs, where drug stimuli invigorate drug seeking, often via initiating craving.

Simpler Pavlovian discriminative stimulus and instrumental paradigms are often conducted with a training phase consisting of either the Pavlovian *or* instrumental training described above. In these more basic paradigms, the test/reactivation phase, then aims to reactivate (or does not aim to reactivate) this single memory trace with or without the test drug: subsequent follow-up test phases assess memory abolition by reconsolidation blockade. For simple instrumental conditioning, lever pressing rates are the primary outcome. Purely Pavlovian responding is harder to measure, as the reinforcer does not require action from the animal. However, one frequently observed phenomenon (and primary outcome) in purely Pavlovian paradigms is **conditioned approach**, whereby the animal will move towards and make consummatory actions towards the location of reward delivery (e.g. the chow magazine) when a predictive CS+ is presented. The human analogue of this behaviour, however is poorly defined.

One other important question to ask of drug-paired Pavlovian CS+s is whether they themselves gain reinforcing properties. Given the discussed role of dopamine in assigning reward value and salience to stimuli and the temporal shift of phasic DA firing from reward to cue during learning, it should be expected that Pavlovian drug cues themselves become motivational targets via **conditioned reinforcement,** indeed this is central to theories of drug addiction (Berridge and Robinson 1998). Conditioned reinforcement may be illustrated by the consumption of decaffeinated coffee. The

rewarding effects of caffeine imbue the olfactory predictor of coffee's flavour with conditioned reinforcing properties, such that some people may enjoy drinking coffee even without the caffeine. Further, smokers may report experiencing more reward from smoking a denicotinised cigarette than from intravenous nicotine itself (Rose et al. 2000). Conditioned reinforcement is assessed in the **acquisition of a new response** paradigm. In this paradigm, an instrumental memory is trained whereby a correct lever press illuminates a CS+ light and delivers a reward. Subsequently, the light alone is used as the reinforcer to condition a new action (such as a nose-poke into a chow magazine). The speed with which the animal acquires the new response and persistence with which it makes this response to illuminate the light is a measure of the conditioned reinforcing effects of the stimulus. New response acquisition should be abolished if the original memory trace is reactivated and reconsolidation blocked.

Research using paradigms such as this has shown that protein synthesis inhibitors, NMDAR antagonists and the β-Blocker Propranolol interfere with drug memory reconsolidation and disrupt the expression of conditioned approach, motivation (Lee and Everitt 2008b) and reinforcement (Milton et al. 2008a). These all represent ways in which Pavlovian memory traces interact with and modulate actions to increase drug seeking and using to provoke relapse. That these processes can be disrupted during reconsolidation is promising for the use of this approach to treat SUDs.

Pharmacological studies such as these have shown that serial cascades of molecular processes underlie memory reconsolidation and that many of these processes overlap with those involved in initial consolidation. There are, however, doubly dissociable pathways that are unique to consolidation and reconsolidation, indicating that reconsolidation is not simply a 'second round' of consolidation (Lee et al. 2004; Lee and Hynds 2013a). As with consolidation, reconsolidation is reliant upon gene

transcription and protein synthesis to instantiate and stabilise changes in synapses and dendrites. The first step in the process - destabilisation of memories following retrieval - requires degradation of synaptic proteins via ubiquitination, a biochemical process that tags protein molecules, allowing them to be recognised and targeted by proteasomes (Lee et al. 2008). As such, proteasome inhibitors – drugs that interfere with degradation of ubiquitin tagged proteins - prevent retrieval-induced memory destabilisation. After this degradation of proteins underlying potentiated synaptic communication, de novo protein synthesis is subsequently required to re-build the synapse with new protein-based elements such as neurotransmitter receptors, anchoring proteins and general scaffolding molecules. This synaptic remodelling is the physical and biochemical basis of memory-updating.

This is why post-retrieval protein synthesis inhibition is the prototypical pharmacological means for interfering with memory restabilisation (Nader et al. 2000). Protein synthesis inhibitors such as anisomycin and cycloheximide interfere with translation of mRNA to proteins in the ribosome. As such, they directly interfere with the protein 'building blocks' of memory restabilisation, but are also highly toxic, as they indiscriminately prevent ongoing protein synthesis in all organs and tissues. For this reason, they cannot be used in humans and do not represent a drug class with translational potential. Further, the lack of specificity of protein synthesis inhibitors does not help to advance our understanding of specific mechanisms linking transcription, translation and myriad other biochemical events involved in restabilising reactivated memories, other than demonstrating the requirement for new proteins.

More targeted manipulations of signal transduction pathways have revealed the requirement for various transcription factors in reconsolidation. Among these, cyclic adenosine monophosphate (cAMP) response element binding protein (CREB), Nuclear

Factor Kappa-B (NF-κB)(Yang et al. 2011b) and zinc-finger 268 (zif268, also known as Egr1) (Lee et al. 2006) are heavily implicated in reconsolidation of reward memory (Miller and Marshall 2005).The action of Zif268 and BDNF on consolidation and reconsolidation in the hippocampus show a double dissociation, with BDNF necessary, and Zif268 unnecessary for consolidation, and vice versa for reconsolidation.

Membrane receptor activation targets transcription factors via intermediate enzymes known as protein kinases. During reconsolidation these kinases phosphorylate transcription factors and allow divergent and common routes to gene transcription from upstream activation of membrane-bound receptors (see *Figure 1.5*). Two particular kinases; Extracellular signal-Regulated Kinase (ERK) and Protein Kinases A (PKA) and C (PKC) have been shown to be necessary for reconsolidation, with PKA implicated more in fear memory consolidation (potentially due to its activation via β-AR signalling and the key role of amygdalar β-AR in fear conditioning (Tronson et al. 2006) and ERK critical in drug memory reconsolidation (Valjent et al. 2006). Together, these biochemical studies provide compelling evidence that reconsolidation, at least at the molecular level, is not simply lingering consolidation, but a separate process.

More recent assessment of the involvement of receptor-kinase-transcription factor pathways has further delineated divergent hippocampal cellular mechanisms underlying consolidation and reconsolidation, suggesting that the picture may be more complex. Lee and colleagues (2013b) have shown that despite common requirement of NMDAR activation, the processes diverge in the intracellular kinases and transcription factors recruited. Consolidation recruits an NMDA-ERK1-BDNF signalling pathway, while reconsolidation relies upon an NMDA- Inhibitor of Kappa Kinase alpha (IKKα)-NF-κβ – Zif268 pathway. This differentiation may be due to the differential dendritic neurotrophic requirements for reconsolidation versus initial consolidation. The latter

involves ‘building’ a whole new memory trace, while the former, if updating is relatively subtle, involves finessing or modifying these existing traces, rather than ‘starting from scratch’.

It should be clear that the signal transduction pathways involved in memory reconsolidation are complex and far from fully elucidated. There are undoubtedly many more as yet unidentified co-factors, limiting steps and signal transduction mechanisms that will affect the extent to which memories successfully reconsolidate. Further, the requirement of certain intracellular events may vary interactively across neural regions and across types of memory, due to the variable input requirements (e.g. spatial, associative, and emotional) of different forms of memory and the differential behavioural outputs required from these memories. Despite these unknowns, there is obviously a great deal of potential for development of drug targets with increasing levels of specificity to modulate the molecular cascades referred to above and in *Figure 1.5*. For the time being, it is also highly promising that neuropharmacological research consistently identifies the activation of glutamatergic and noradrenergic membrane receptors – the most upstream events in the cascades described above - as necessary for reconsolidation. Since safe, tolerable and pharmacologically potent drugs with high specificity for these receptors already exist for use in humans they deserve serious consideration as drugs for weakening MMMs in humans.

As with other forms of synaptic plasticity, activation and trafficking of NMDARs and AMPARs receptors (Clem and Huganir 2010) is critical in reconsolidation. NMDAR manipulations have been consistently shown to affect reconsolidation, but the involvement of these receptors is complex. NMDAR activity is required for restabilisation of appetitive memory traces as evidenced by significant weakening of memory by infusions of NMDAR antagonist MK-801 following memory retrieval

(Brown et al. 2008; Milton et al. 2008a; Milton et al. 2012; Przybyslawski and Sara 1997), yet the effect is highly time-sensitive, with potentially opposite effects depending upon drug timing.

Activation of NMDARs in the basolateral amygdala is necessary for the *de*stabilisation of memory traces as antagonism of these receptors prior to retrieval prevents subsequent memory interference by post-retrieval anisomycin (Mamou et al. 2006). Therefore, depending on timing of drug administration, NMDAR antagonism may either interfere with memory reconsolidation, or prevent it from occurring in the first place. Recent research has shown that the temporal changes in the requirement for NMDARs activation during reconsolidation is receptor-subunit-specific, the GluN2b subunit being critical in the destabilisation, but not restabilisation of memories and GluN2a containing NMDARs critical in the restabilisation, but not destabilisation of memories (Milton et al. 2013).

AMPARs are not involved in destabilisation, as pre-retrieval antagonism does not prevent post-retrieval amnesia following anisomycin (Mamou et al. 2006), but the phosphorylation of the AMPAR[2] GluA1 subunit and removal of AMPA receptors from synaptic membrane is a necessary component of post-retrieval memory erasure (Clem and Huganir 2010). AMPA receptor translocation, protein kinase activation, immediate early gene expression and protein synthesis are all downstream consequences of NMDAR activation, so pharmacologically blocking NMDA receptors may be an effective means of preventing the subsequent molecular cascades necessary for restabilising memory traces.

A key problem with the use of NMDAR antagonists for weakening MMMs is their tendency to be poorly tolerated and have numerous and hazardous side effects. For

[2] Like NMDARs, AMPARs are heterotetrameric and comprise four subunits (GluA1,2,3 and 4) which assemble in various configurations.

example while the prototype NMDAR antagonists [5R,10S]-[+]-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801) and phencyclidine (PCP) produce sustained blockade of NMDARs they have powerful psychotomimetic effects and can produce organic damage to neural tissue (Olney's lesions; (Olney et al. 1989).

1.10. β-AR and NMDAR antagonism and human reconsolidation

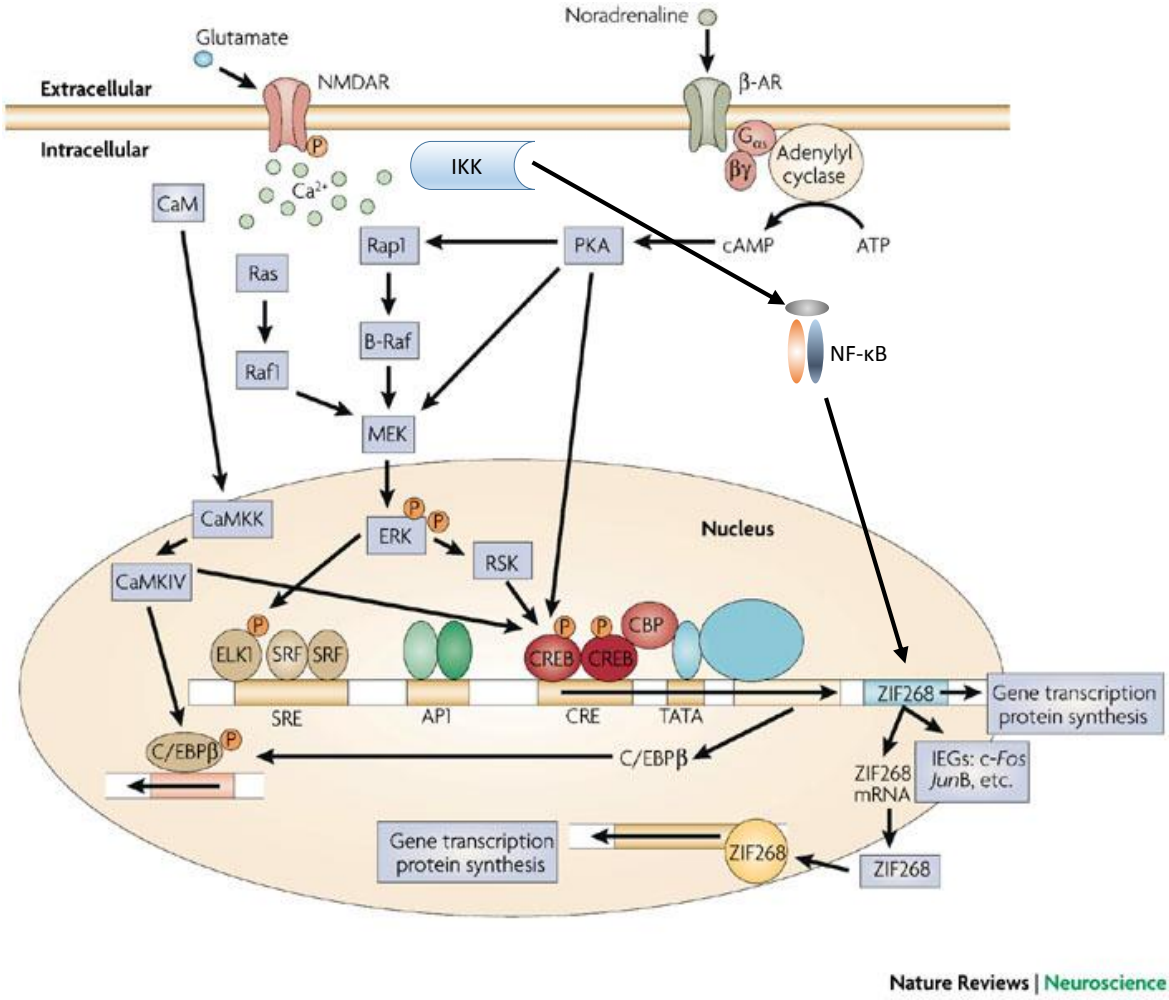
Within the pharmacopoeia of NMDAR antagonists, the substances that can be used *relatively* safely in humans are limited to **ketamine**, used frequently in paediatric anaesthesia and **memantine**, used to treat cognitive decline in Alzheimer's. Like PCP, ketamine produces powerful dissociative and psychotomimetic effects, has relatively high abuse potential (Morgan et al. 2004) but is not seriously neurotoxic in the context of medically supervised and isolated dosing. Importantly however, it has very high affinity for the NMDAR and impairs the reconsolidation of morphine CPP in rats (Zhai et al. 2008). Conversely, memantine at therapeutic doses does not produce overt subjective effects, but has lower affinity at the NMDAR than ketamine (Rammes et al. 2008). Memantine has been found to block cocaine and morphine CPP reconsolidation (Alaghband and Marshall 2013; Popik et al. 2006) in rats, although it may paradoxically have opposite effects in the day old chick (Samartgis et al. 2012). Given that memantine is well tolerated, it is an extremely promising compound for blocking reconsolidation of MMMs in humans, although it has never been tested for this purpose. Testing of ketamine for use in MMM reconsolidation blockade should therefore be dependent on null effects from memantine, and efficacy and safety that outweighs its need for inpatient treatment through intravenous administration.

Independently of the NMDAR, reconsolidation of appetitive memory in rats is also reliant upon on β-adrenergic (**β-AR**) signalling. Few systemically administered β-blockers cross the blood-brain barrier although **propranolol**, which is widely used for

hypertension, does enter the central nervous system (CNS) and, in combination with memory reactivation, reduces the strength of morphine conditioned place preference (Robinson and Franklin 2010), cocaine cue-drug and cue-sucrose memory (Milton et al. 2008a; Milton et al. 2008b) and instrumental sucrose memory (Diergaarde et al. 2006) in rats. Propranolol has also consistently been shown to block fear memory reconsolidation, with the potential for clinically-relevant benefits in humans (Kindt et al. 2009; Sevenster et al. 2013; Soeter and Kindt 2011). However, research into human appetitive memory reconsolidation blockade with Propranolol is lacking. β-ARs affect long-term plasticity through downstream signal transduction and protein synthesis in pathways that are different to, but sometimes convergent with, those initiated by NMDAR activation, as discussed previously (these are shown in *Figure 1.5*).

In summary, both β-AR and NMDAR antagonists have potential therapeutic uses in blocking MMM reconsolidation in humans, but few studies in animals have directly compared their efficacy. Those that have found that both β-AR and NMDAR antagonism prevented the reconsolidation of Pavlovian conditioned reinforcement in an acquisition of a new response paradigm, but only NMDAR blockade prevented reconsolidation of Pavlovian conditioned approach (Milton et al. 2012) and PIT (Lee and Everitt 2008b). This suggests that NMDAR antagonism may have a more general and consistent effect in blocking the reconsolidation of memory processes important in relapse (outlined in *Figure 1.4* and accompanying text), although this conjecture requires clarification from objective statistical treatment of the issue. Further, although many narrative reviews have highlighted the potential of interfering with memory reconsolidation via NMDAR and β-AR blockade, the approaches have not been trialled in humans and the lack of direct comparisons between the two make it unclear which class of drug shows most promise for weakening human MMMs and should be prioritised for testing in human models of SUDs.

Figure 1.5. Molecular pathways in memory reconsolidation.



NMDAR and β-ARs are the key upstream mediators of memory reconsolidation and operate through both separate and convergent signal transduction pathways, recruiting protein kinases, cAMP response element binding protein (CREB) and ultimately immediate early genes, gene transcription and de novo protein synthesis. In the case of NMDAR activation, influx of Ca^{2+} sparks the activation of the MEK – ERK pathway through recruitment of small enzymes of the family Ras, Raf and Rap. This leads to gene transcription via CREB and zif268. Binding of noradrenaline to β-ARs activates Protein kinase A (PKA) through cyclic AMP (cAMP) and can either directly, or indirectly through ERK and ribosomal protein S6 kinase (RSK), activate transcription via CREB and zif268. This figure has been modified to include the recently identified Ca^{2+} - IKKα – NF-κB – zif268 pathway (Lee and Hynds 2013a) that dissociates consolidation from reconsolidation. Figure adapted from Kelley (2004).

1.11 Methodological issues in reconsolidation

Other than the sensitivity of reconsolidation to different pharmacological manipulations, a key issue in the field is the fact that reconsolidation does not occur under all circumstances when a memory is recalled. Null results in a reconsolidation study could lead to three possible conclusions:

1) The manipulation used to interfere with reconsolidation was ineffective
2) The reminder procedure employed failed to destabilise the memory in the first place, yielding a false negative for the manipulation
3) The memory process being studied does not undergo reconsolidation.

While early studies tended to default to conclusion three to explain observed null findings (Cammarota et al. 2004; Hernandez and Kelley 2004), subsequent positive results in experiments examining the same types of memory (Diergaarde et al. 2006; Tronel et al. 2005) suggest that conclusion one or two are more likely to explain null results. Where the same reconsolidation-blocking drug has been found *not* to affect reconsolidation in one study (Hernandez and Kelley 2004) but does in another (Exton-McGuinness et al. 2014) and as there is no intuitive evolutionary reason why some forms of memory should undergo reconsolidation and others should not, conclusion two is often the most parsimonious explanation for null results. As any intervention that interferes with reconsolidation of destabilised MMMs is predicated upon successful destabilisation of the memory in the first place, the methodological parameters determining memory destabilisation, commonly known as '**boundary conditions**' have become the focus of reconsolidation research.

This research has shown that, in line with previous findings on amnesia gradients and the occurrence of systems consolidation, older (Eisenberg and Dudai 2004; Frankland et al. 2006; Milekic and Alberini 2002), stronger (Eisenberg et al. 2003; Robinson and Franklin 2010; Robinson et al. 2011) memories are more resistant to destabilisation upon recall. In this context, 'older' memories refer to greater chronological age of memories (i.e. a greater amount of time since the memory was initially consolidated) and memory 'strength' refers to the number of training trials reinforcing the memory. One central function of reconsolidation is to strengthen memories when they are recalled and found to have positive predictive benefit, so 'stronger' memories will have potentially undergone successful reconsolidation many times. This is particularly pertinent for the translation of reconsolidation-based research to human drug users. Typically, conditioning in animal paradigms takes place over tens of trials within a few days, whereas in human MMM formation, conditioning takes place over tens of thousands of trials over the course of years. As such they may not amenable to reconsolidation interference in the same way as animal MMMs.

However, research has demonstrated that reconsolidation of old, hippocampus-independent memories does occur (Debiec et al. 2002) suggesting that appropriate manipulation of retrieval parameters allows older memories to destabilise, but these parameters may be somewhat different to those for younger, more weakly trained memories (Besnard et al. 2012). Robinson et al (2011) demonstrate that introducing a thirty day delay between training and memory reactivation (i.e. allowing more cortical consolidation) destabilises strongly trained memories upon retrieval, while a retrieval session closer to training does not. Thus allowing time for memory traces to consolidate independently of hippocampal involvement may be key to the disruption of older memories.

A further complication in interpreting null findings is that the length of the retrieval trial used may determine a switch between the mutually exclusive processes of extinction and reconsolidation (Pérez-Cuesta and Maldonado 2009). Long retrievals, or retrievals with multiple presentations of conditioned stimuli, tend to cause memory extinction, rather than destabilisation. As only one of these processes can occur and there is a discrete switch in the molecular machinery responsible for the two processes (involving the exclusive recruitment of BDNF (Kirtley and Thomas 2010), shorter retrieval trials are desirable when attempting to engage reconsolidation. On the other hand, retrieval trials cannot be *too* short because neither reconsolidation nor extinction will be engaged (Suzuki et al. 2004). Determining the optimum length of retrieval for memory destabilisation is thus a fine balancing act and as yet, there are no hard rules for determining optimal retrieval length based on learning history. Indeed, even if such rules for optimal retrieval lengths were found in animal models of addiction, their utility in humans would be questionable, given that learning history in human MMMs is difficult to determine. Reconsolidation research is therefore currently limited to inferring memory destabilisation at recall from the effects of post-retrieval manipulations on memory and somewhat arbitrarily determining the length of recall required to destabilise memories.

Recently, an alternative requirement for memory destabilisation has been identified that may resolve the seemingly variable capacity of different memories to destabilise. Since one of the main functions of reconsolidation is to update memories, it follows that novel information regarding the memory should be available at recall. Indeed empirical research (Pedreira et al. 2004) and computational modelling (Osan et al. 2011) has shown this to be the case, with the correct level of mismatch between expectation and

actual reinforcement (value) driving memory updating via reconsolidation. Too great a mismatch, generated by massed presentation of CSs without their reinforcer (i.e. extinction) creates an entirely new memory trace. Thus the switch between reconsolidation and extinction may be determined by a mismatch comparator mechanism that assesses whether to update an existing trace or create a new one.

The 'mismatch' referred to here can be formalised in terms of the Prediction Error (PE) previously discussed. As the putative learning signal in reinforcement learning, PE is necessary for updating memories and therefore for initiating reconsolidation (Sevenster et al. 2012; 2013). This could take the form of unexpected omission or reduction in value of a reinforcer (negative PE), unexpected presentation or increase in value of a reward (positive PE) (Liu et al. 2014) or temporal mismatch between expected and actual presentation of a reinforcer (Díaz-Mataix et al. 2013). Memory formation is not linear, but follows an approximately logarithmic or power curve, with early experience creating a large prediction error and correspondingly large changes in the memory, with early experience creating a large prediction error and correspondingly large changes in the memory. However, as memories age and more experience relevant to the trace is incorporated, the potential for large PE in the expected outcome decreases. In overlearned, habitual memory, such as some MMMs, there is likely to be no (or extremely little) PE in simple naturalistic recall as reinforcement follows such a repetitive, predictable pattern. This may explain both the resistance of strongly-trained memories to retrieval-induced destabilisation and the naturalistic persistence of MMMs.

To date very few experimental studies have looked at truly strong or remote memories, on the scale that is seen in humans MMMs and virtually no experimental examination of the approach in humans exists. It is proposed here that the disparity lies in 1) the

sometimes inconsistent results with reconsolidation-based interventions in animal reward learning studies (due to the subtleties of the boundary conditions described) 2) the limited choice from the pharmacopeia of potential reconsolidation-blockers in humans and 3) the exponentially greater complexity of determining what constitutes 'reactivation' and prediction error in human memory where the training history is typically outside of experimental control.

Many excellent narrative reviews of preclinical reconsolidation research exist, taking different positions of the scope and limits of the phenomenon while attempting to reconcile both positive and null findings (Dudai 2006; Dudai and Eisenberg 2004; Finnie and Nader 2012; Milton and Everitt 2012; Torregrossa and Taylor 2013; Tronson and Taylor 2007). However, a common methodological limitation in rodent research is that small and variable numbers of animals are used, creating situations of potentially low and variable power. Behavioural observations based purely on the binary logic of null hypothesis significance testing (NHST) can occlude very similar effect sizes that may be equally important, but due to differences in group Ns and power, fall on different sides of the 0.05 'statistical cliff'. The conclusions of narrative reviews are biased by this issue and are likely to be the source of much of the inconsistency and disagreement between researchers in the field and the lack of headway made in translational research. A more objective assessment of effect sizes with regards to identified boundary conditions would thus be highly beneficial in progressing this field.

1.12 Aims of the current thesis

The work presented in this thesis represents an attempt to make the first steps in addressing these identified shortcomings, by translating the preclinical work on

reconsolidation of drug memories to clinical or clinically relevant populations of human drug users. The work presented herein is agnostic with regards to reconsolidation interference as a methodology, but aims to assess whether it genuinely represents an improvement upon current approaches. In all the studies described herein, one eye is on the ultimate aim of improving long-term treatment outcomes for drug addicts, if only by ruling out potential dead-ends in research. While development of actual treatment programs is far beyond the scope of this modest work, I hope to lay the initial groundwork for future research into methods for treating MMMs in human addicts.

1.12.1 Research Questions

With this in mind, there are several overarching questions that this thesis aims to answer, at least in part.

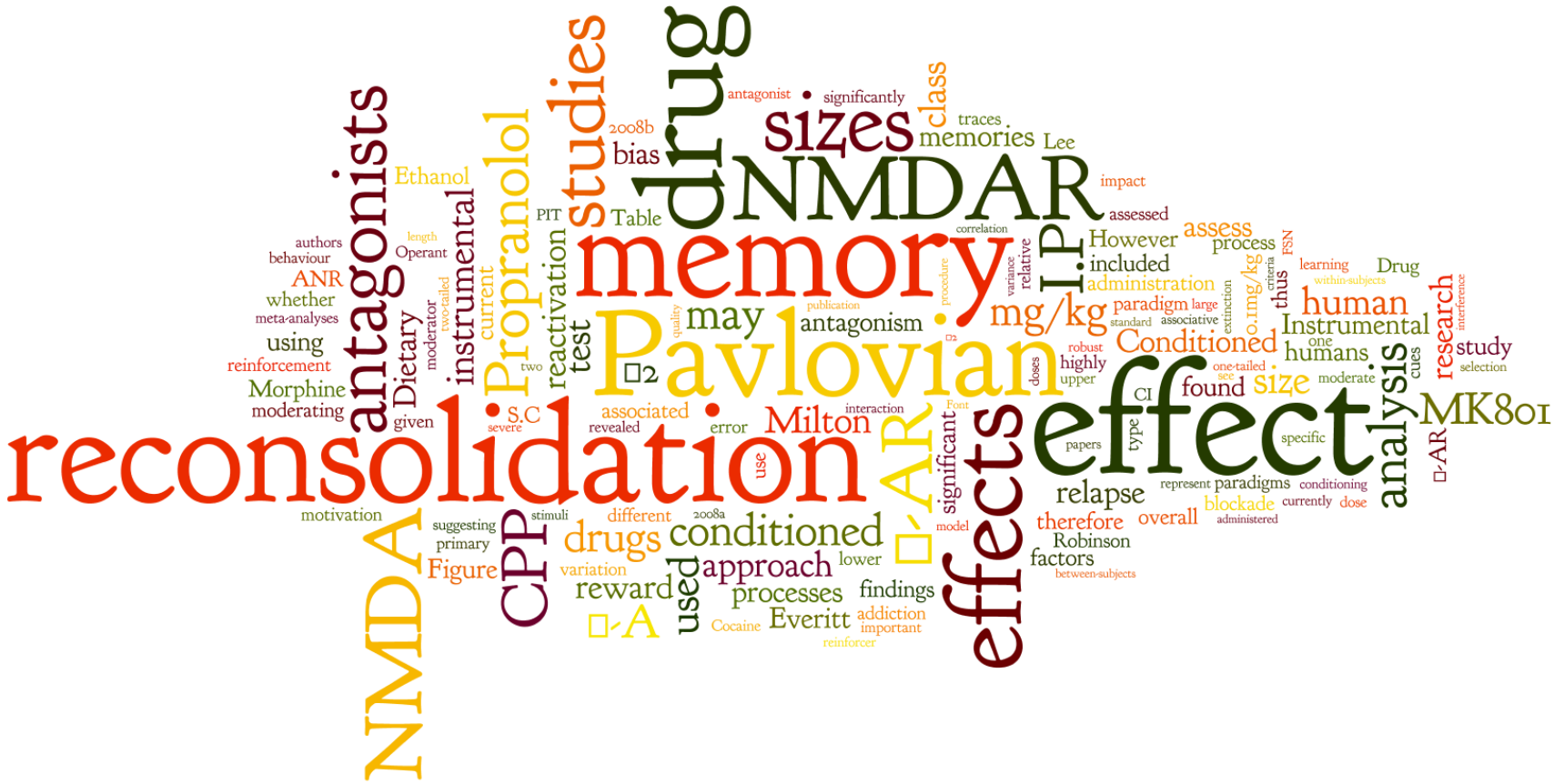
1) Of the established reconsolidation-blocking drugs, which are more likely to be effective at blocking MMM reconsolidation in humans? Are these effects related to identified boundary conditions and what are the sources of heterogeneity in findings?
2) Can potential reconsolidation-blocking drugs reduce MMM strength in clinically relevant human samples?
3) Are human MMMs resistant to destabilisation upon retrieval and if, so, what procedures are necessary to destabilise them?
4) Are there effective drug-free approaches to targeting MMMs?

1.12.2. Methodological approach to addressing these questions

The first of these questions can be effectively addressed by systematic statistical evaluation of appropriate studies. Adding to the extant narrative reviews of research is

unlikely to represent an important advance in the field. The first empirical study of this thesis will therefore be a meta-analysis of preclinical studies using the two most translationally promising drug classes for blocking reconsolidation (NMDAR and β-AR antagonists) examining the moderating impact of identified 'boundary conditions'. Building on the findings of this analysis and to address question two, I will adopt an 'experimental medicine' approach to assess the feasibility and efficacy of pharmacological reconsolidation-interference in treating human MMMs. *Chapter 3* examines the effects of cue-drug memory retrieval with the NMDAR antagonist memantine in a population of quitting smokers. The third study attempts to extend our NMDA-ergic pharmacopoeia for reconsolidation blockade by examining the effects of Nitrous Oxide on the reconsolidation of associative alcohol memory in hazardous drinkers. Due to evidence from Chapter 2 that MMMs may be destabilisation-resistant, Chapter 5 then takes a slightly different tack, trialling a novel memory destabilisation procedure, extending recent research using a pharmacobehavioural approach to modify cue-drinking memories in heavy drinkers. Finally in Chapter 6 I discuss what I believe we can learn from the work contained in this thesis and what I evaluate to be the logical progression of reconsolidation research in human drug users going forward.

Chapter 2: The Effects of N-Methyl D-Aspartate and B-Adrenergic Receptor Antagonists on the Reconsolidation of Reward Memory: A Meta-Analysis



2.1. Introduction

2.1.1. Memory reconsolidation in addiction treatment

Substance Use Disorders are chronic relapsing disorders that can be viewed as a disease of persistent maladaptive memory (Hyman, 2005, 2006; Everitt & Robbins, 2005; Milton & Everitt, 2012; Robinson & Berridge, 1993, 2005; Berridge, 2011) whereby adaptive associative memory processes that are used to direct action towards rewards are usurped by drugs and their associated stimuli or 'cues'. Cue-drug associations are long-lasting and direct behaviour towards drug-seeking and using long after withdrawal. As such, any effective long term anti-relapse treatment for addiction must address the control these memories exert over behaviour.

Recent years have witnessed a proliferation of research interest in reward memory reconsolidation as a target model for weakening aberrant memory processes while memories are in a briefly labile state that putatively allows new information to enter the trace (Forcato et al, 2010; Lee, 2009). Protein synthesis inhibitors (Nader et al, 2000), inhibitors of transcription factors such as Zif268 (antisense oligodeoxynucleotides; Lee et al, 2004) and certain pharmacological challenges (Bernardi et al, 2006, 2009; Fricks-Gleason & Marshall, 2008; Lee & Everitt, 2008a, 2008b, Milton et al 2008a, 2008b, 2011) given after the reactivation of a conditioned reward memory can produce a profound deficit in expression of that memory at test. If similar effects can be reproduced for human maladaptive drug memories, long-term, context independent relapse attenuation may be achievable (Milton et al, 2012), or the efficacy of cognitive behavioural interventions may be improved by relative weakening of cue-drug associations.

To date, virtually all work examining reward memory reconsolidation has been conducted on laboratory animals. Using a variety of drug and genetic challenges, the neural pathways and processes involved in memory reconsolidation are beginning to be elucidated. While these assays have begun to map the molecular pathways in memory reconsolidation, the only drug interventions currently with real translatable utility for human investigation are those using classes of drugs that can be administered systemically and are either currently used, or have suitably safe analogues, in humans.

Of drugs fitting this description, by far the most extensively studied are the N-Methyl D Aspartate (NMDA) and β-Adrenergic (β-A) system antagonists. NMDA receptor (NMDAR) and β-Adrenoreceptor (β-AR) antagonism has been shown to interfere with the reconsolidation of conditioned reward memory (Przybyslawski et al, 1997; Bernardi et al, 2006, 2009), leading to decrements in memory expression. As antagonists at both of these receptors are available for use in humans, pharmacological NMDAR and β-AR blockade is thus a promising approach for interfering with drug memory reconsolidation, although human studies using these drugs are currently lacking and there is a clear need for translational work.

Further, preclinical studies have not universally found that NMDAR or β-AR antagonism interferes with reward memory reconsolidation, indicating the involvement of boundary conditions determining the susceptibility of memories to post-retrieval NMDAR and β-AR antagonism. To effectively translate this treatment approach to human drug users it is critical to understand the limiting effects of these conditions. As several excellent, recent narrative overviews of this literature exist (Diergaarde et al, 2006; Milton & Everitt, 2010; Milton & Everitt, 2012; Sorg, 2012, Torregrossa & Taylor, 2012; Tronson & Taylor, 2007), they will not be reviewed in depth here.

Rather, the purpose of the current chapter is to take an objective, statistical approach to assessing the relative magnitude of effects of β-AR and NMDAR antagonists on reward memory reconsolidation and examine the heterogeneity of effects herein based on putative moderating factors identified by previous research.

2.1.2. Memory reconsolidation in addiction treatment

Variability in findings has generally been addressed on the basis of theoretical, pharmacokinetic and methodological factors. Methodological factors are length of memory reactivation session (differentiating between reconsolidation and extinction; Suzuki et al, 2004), primary reinforcer used (drug or dietary rewards) and conditioning paradigm. Pharmacokinetic factors include drug dose, administration route and timing of administration relative to memory reactivation. Primary research is currently under way assessing how these factors affect the destabilisation and restabilisation of drug memories and constrain the effects of pharmacological assays on reconsolidation.

As discussed in *Chapter 1*, multiple memory processes thought to contribute to relapse are studied in reconsolidation paradigms. Conditioned reinforcement (CR), conditioned motivation (CM) and conditioned approach (CA) (Milton & Everitt, 2010, Milton et al, 2012) may be differentially susceptible to interference during episodes of reconsolidation. To clarify, these are processes by which drug-associated cues come to control drug seeking and using behaviour. They interact with situational factors controlling the *expression* of memory such as renewal, reinstatement and spontaneous recovery in order to maintain drug seeking and using.

Conditioned motivation as measured by the Pavlovian-to-instrumental transfer (PIT) test, increases motivated drug seeking in the presence of a drug-paired CS or context, due to the signalling of drug availability. Conditioned motivation is thus able to support increased drug use through environmental exposure to certain stimuli. Conditioned motivational properties of cues trigger a negative internal state akin to craving via peripheral stress hormones and amygdalar noradrenaline that promotes drug seeking, or that motivational cues trigger habitual responding, the interruption of which is experienced as craving (Tiffany, 1990). **Conditioned approach** assessed with approach and autoshaping or maze procedures precipitates relapse by steering individuals towards the spatial locations previously paired with drugs, increasing proximity to both drug conditioned stimuli and drugs themselves. Conditioned approach appears to be dependent on spatial localisation of drugs and related stimuli as it is hard to induce conditioned approach when drugs are infused directly into an animal, for instance (Tomie et al, 2006). Its human analogue is measured through oculomotor and approach biases to drug cues in laboratory tests. These approach biases are both a function of the incentive value of drug cues (Robinson and Berridge 2001) and, through continued drug use, of stimulus-response habits (Tiffany, 1990; Mogg et al. 2005).

Conditioned reinforcement can support responding for drug cues themselves despite drug reinforcement (DiCiano & Everitt, 2004) or reinforcer devaluation (Parkinson et al, 2005), and thus maintain drug-seeking for distal rewards. The conditioned reinforcing properties of drug-related stimuli can therefore play an important part in binding together the chains of instrumental responses necessary for drug attainment and use in human addicts.

The conditioned place preference (CPP) paradigm, where rats are trained to associate a contextual chamber with drug, deserves special consideration. There is some debate as to what memory process is tapped in CPP, with the potential involvement of several processes. I argue that the responding seen in CPP reflects conditioned reinforcement. The lack of spatial localisation of drug administration precludes conditioned approach (Tomie et al, 2006; CPP paradigms almost always use experimenter-injected drugs *before* confinement to a specific chamber). The association of a single chamber during learning to drug effects, rather than motivational drug-seeking states meaning conditioned motivation is unlikely. Thus the bias towards the drug-paired chamber at test is most likely to reflect reinforcing properties of the chamber's contextual cues. Another possibility is that CPP could be supported by instrumental learning. This is to say, the extra time spent in the drug-paired chamber at test could be reflective of an instrumental drug-seeking response, much as in a maze paradigm. However, the passive administration of drug and subsequent lack of contingency upon behaviour make the acquisition of an instrumental association, or even superstitious conditioning, unlikely. For the current analysis, we therefore categorise CPP paradigms as measuring conditioned reinforcement.

It is unclear whether reconsolidation of these three processes is equally susceptible to interference from β-AR and NMDAR antagonists. Different kinds of memory trace have been shown to be amenable or resistant to reconsolidation (Brown et al, 2008; Cammarota et al, 2004) and β-ARs and NMDARs may interactively or differentially be involved in the reconsolidation of different memory processes, particularly with regard to the role of arousal and stress in those processes (Roozendaal et al, 2009). Furthermore, all three processes may not contribute to relapse in human addicts equally. Treatment outcome may therefore be determined both by the relative expression and

dominance of variable memory processes and the amenability of these processes to intervention. Assessing the moderating impact of these processes on drug effects will be important for the identification of human targets for MMM reconsolidation.

2.1.3. Pavlovian and instrumental memories

A particularly prevalent proposed moderator of reconsolidation effects is the Pavlovian or instrumental nature of memory traces. While the former 'class' of memories have reliably been shown to undergo reconsolidation in several instances (Brown et al, 2008; Frick-Gleason & Marshall, 2008, Zhou et al, 2012; Wu et al, 2012), it has been suggested that evidence is weaker for the reconsolidation of instrumental memories (Hernandez & Kelley, 2004, but see Diergaarde et al, 2006 and Wouda et al, 2010). However, both types of memory putatively arise as a result of experience-dependent, NMDAR-mediated synaptic plasticity (Riedel et al, 2003; Yin et al, 2005) and should theoretically be susceptible to NMDAergic reconsolidation blockade under the right circumstances. In practice, *both* types of trace and their interaction are critical in understanding human addiction. This analysis will therefore seek to confirm whether reconsolidation effects truly are weaker for instrumental memories, based on previously conducted research.

2.1.4. Rationale for meta-analysis

Although putative moderators of memory susceptibility to reconsolidation interference have been identified, due to the problems of null hypothesis significance testing with relatively small Ns used in animal research and paucity of replication studies, the

importance and reliability of these moderators is currently unknown. The current study therefore used meta-analysis (Glass, 1977, Hedges & Olkin, 1980; Hedges & Vevea, 1998; Hunter & Schmidt, 2000) to assess the overall magnitude of interference with reward memory reconsolidation by β-AR and NMDAR antagonists, compare the overall effects of these two classes of drug and assess the impact of the outlined methodological, pharmacokinetic and mnemonic variables on these effects using meta-regression. The main aim of this analysis is to provide a quantitative synthesis of preclinical research that may be used for hypothesis generation in translational studies, particularly those geared towards relapse-prevention in substance dependence disorders and addiction.

2.2. Methods

2.2.1. Search Strategy

ISI web of knowledge, PubMed, PsychInfo and SIGLE online databases were searched with the following term "*Reconsolidation* OR re-consolidation* OR post-retrieval* OR post-reactivation* OR post-recall*"* on 15/11/2011. The search was restricted only to papers written in English, as paper translation was not feasible within the scope of this review. No other restrictions were placed on the search criteria.

The reference lists of included papers and recent review papers in the field were hand-searched along with back issues and conference abstracts to 1996 of all journals in which included studies were published and other prominent journals in the field. This yielded no new studies. Authors of all included studies and other authors known to be actively researching in the field were contacted for unpublished or pre-publication information. This yielded one unpublished dataset (Milton et al, unpublished). Updating of the search on 20/03/2012 retrieved three studies that had been published since the original search (Wu et al, 2012, Zhou et al, 2012 Font & Cunningham, 2012) that were included in the review.

2.2.2. Study selection

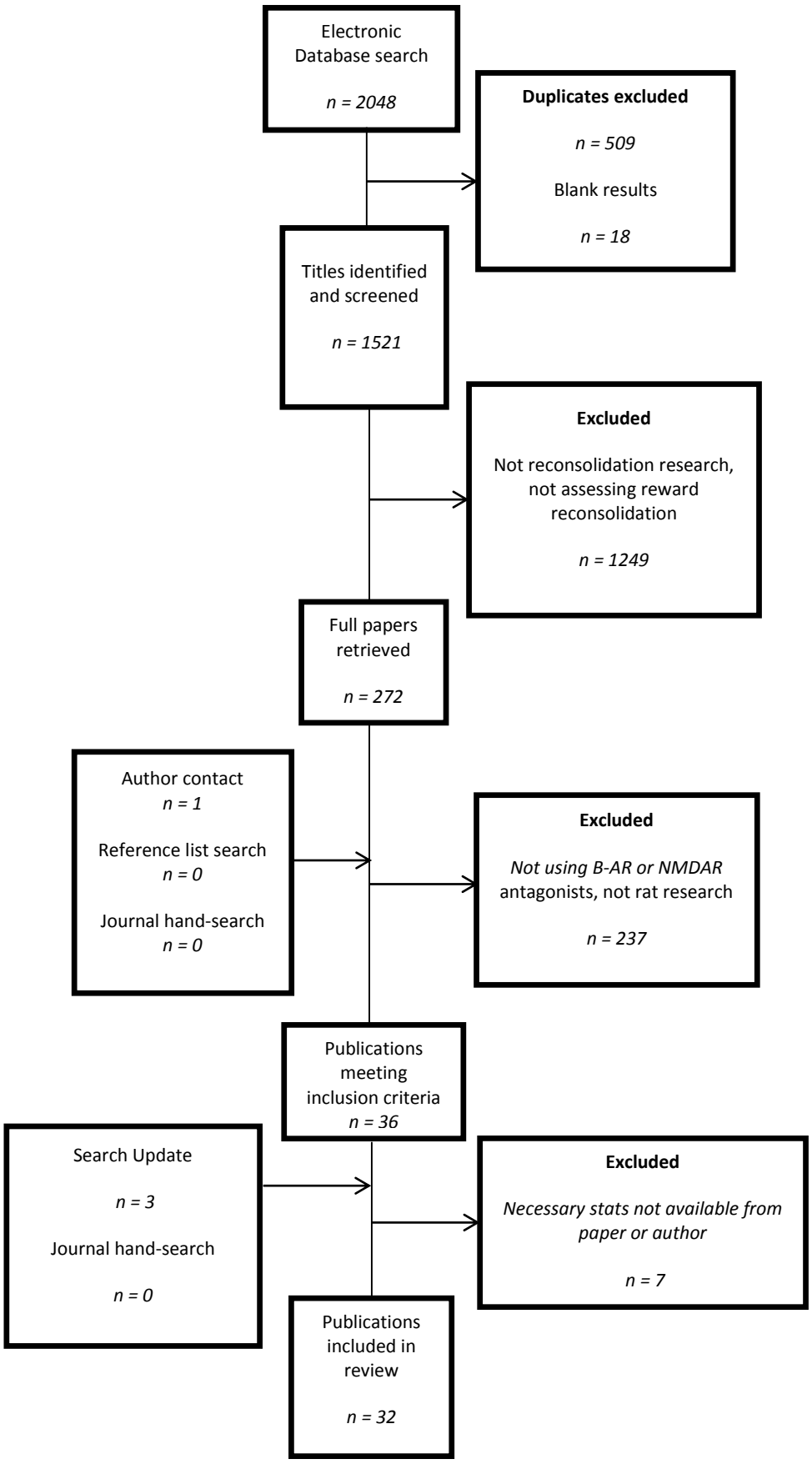
Studies were included if they examined the effects of a β -AR or NMDAR antagonist on the reconsolidation of a reward memory in laboratory animals. A PRISMA schematic of the iterative study exclusion procedure is given in *Figure 2.1*. Excluded studies were independently cross-checked by myself and two colleagues for ineligibility. There are likely boundary conditions that differentiate whether reconsolidation or extinction processes are preferentially activated such as length of the reactivation procedure. Very short reactivations (< 1 minute) do not appear to trigger memory reconsolidation, but

much longer procedures (>30min) may preferentially activate extinction (Suzuki et al, 2004). Furthermore, stimulus offset in the absence of reinforcement may be critical in determining whether extinction or reconsolidation occurs, independent of length of the reactivation procedure (Pedreira & Maldonado, 2003). Given that there are no definite cut-off criteria for determining whether extinction or reconsolidation occurs, studies in the current search were not excluded on the basis of reactivation length if the studies claimed to examine reconsolidation effects. Also, given that β -Adrenergic and NMDAergic antagonists tend to interfere with both extinction and reconsolidation and that this interference should manifest in opposite behavioural effects, if extinction were being targeted by drug challenges, an increase in responding at test should be seen.

2.2.3 Study Quality Coding

All identifying information relating to authors, institution from which studies originated and significance of results was removed from included papers. These blinded studies were then independently assessed by myself and two colleagues using a 23-item quality rating instrument created for this review (see Appendix I). The form was piloted by all authors and discrepancies and difficulties in applying the coding form were resolved by author discussion prior to coding of the entire study sample. Note that, quality ratings were determined based on published descriptions included in the methods and materials sections of papers. Thus incomplete reporting would impact negatively on study quality ratings.

Figure 2.1: PRISMA flow chart of study inclusion screening process



2.2.4 Data Extraction

Information about study paradigm, species of animal studied, drug dose, drug timing and route of administration were extracted. Effect sizes were computed for simple between-subjects contrasts at primary post-reactivation test between reactivated, drug treated and reactivated, saline treated groups. This contrast was chosen as it best represents the effect of drug on reconsolidation. Some papers (Bernardi et al, 2006, 2009; Itzhak et al, 2008; Otis & Mueller, 2011, Zhai et al, 2011) did not report between-subjects effects at test, but rather gave statistics for single post-test, within group contrasts (i.e. time spent on drug paired floor vs. time spent on saline paired floor at test in the CPP paradigm). This caused two problems with data extraction. Firstly, in this paradigm, a successful blockade of reconsolidation is evidenced by a non-significant differential conditioned response at test when assessed within-subjects, whereas a between-subjects contrast of the same effect should yield a significant reduction in responding in the test group. The within-subjects single time-point contrast effect size is thus not representative of the effect of drug on reconsolidation.

Secondly, due to the inherently correlated nature of within-subjects measurements and lack of reporting of this correlation, there are systematic differences in variance when comparing within- and between-subjects designs. As such, effect sizes for within-subjects contrasts tend to be inflated relative to their between-subjects equivalents (Dunlap et al, 1996). There is therefore no meaningful way to combine effect sizes from within-subjects and between-subjects test statistics, unless the correlation of within-subjects measures is known (Morris and DeShon, 2002). As only one paper in the current sample reported the relevant descriptive statistics (Milton et al, 2008a), primary authors were contacted for the necessary statistics to calculate effect sizes for between-subjects comparisons or adjustments of within-subjects (pre-test /post-test)

contrasts. If this information was not available from papers or authors, the study was excluded from the analysis. 11 effect sizes were excluded for this reason (*Figure 2.1*).

2.3. Results

2.3.1. Included Data

The literature search yielded 32 independent papers. For all relevant independent samples contrasts between test drug/reactivated and saline/reactivated groups were included. Pearson's product moment correlation coefficient, *r*, was calculated from descriptive or test statistics for each of these contrasts. Pearson's *r* was chosen due to ease of interpretation and several advantages over Cohen's *d* (Rosenthal, 1991; Field, 2001). In four studies (Font & Cunningham, 2012; Lee & Everitt, 2008b; Popik et al, 2006; Milton et al, 2011), a common placebo group was used to calculate an effect size in two drug groups. In total, 30 effect sizes for NMDAR antagonists and 26 for β - Adrenergic antagonists were extracted. These are listed by drug used, memory class and paradigm in *Table 2.1*.

Table 2.1: Effect sizes from all studies with extracted information on moderating variables, arranged by drug class.

Study Authors	Year	Drug Class	Paradigm	Memory Class	React Length	UCS	Test Drug	Drug Timing	Dose	Admin Route	Effect direction	N	r
Brown et al	2008	NMDA	CPP	Pavlovian	15	Cocaine	MK801	before	0.2mg/kg	I.P	+	20	0.55
Kelley et al	2007	NMDA	CPP	Pavlovian	20	Cocaine	MK801	before	0.3mg/kg	I.V	+	18	0.89
Lee & Everitt	2008a	NMDA	Autoshaping	Pavlovian	24	Dietary	MK801	before	0.1mg/kg	I.P	+	16	0.82
Lee & Everitt	2008a	NMDA	PIT	Pavlovian	24	Dietary	MK801	before	0.1mg/kg	I.P	+	14	0.88
Lee & Everitt	2008b	NMDA	ANR	Pavlovian	30	Dietary	MK801	after	0.1mg/kg	I.P	+	14	0.2
Lee & Everitt	2008b	NMDA	ANR	Pavlovian	30	Dietary	MK801	before	0.1mg/kg	I.P	+	14	0.43
Lee & Everitt	2008b	NMDA	ANR	Pavlovian	10	Dietary	MK801	after	0.1mg/kg	I.P	+	18	0.43
Lee & Everitt	2008b	NMDA	ANR	Pavlovian	10	Dietary	MK801	before	0.1mg/kg	I.P	+	18	0.62
Lee & Everitt	2008c	NMDA	Operant	Instrumental	15	Dietary	MK801	after	0.1mg/kg	I.P	+	16	0.1
Lee & Everitt	2008c	NMDA	Operant	Instrumental	15	Dietary	MK801	before	0.1mg/kg	I.P	+	16	0.34
Milton et al	2012	NMDA	PIT	Pavlovian	30	Ethanol	MK801	before	0.1mg/kg	I.P	+	13	0.35
Milton et al	2008b	NMDA	ANR	Pavlovian	15	Cocaine	MK801	before	0.1mg.kg	I.P	+	18	0.50
Milton et al	2008b	NMDA	ANR	Pavlovian	15	Cocaine	APV	after	0.5µg/side	BLA	+	8	0.57
Milton et al	2008b	NMDA	ANR	Pavlovian	24	Cocaine	APV	before	0.5µg/side	BLA	+	12	0.63
Milton et al	2012	NMDA	Autoshaping	Pavlovian	45	Ethanol	MK801	before	0.1mg/kg	I.P	+	13	0.80
Popik et al	2006	NMDA	CPP	Pavlovian	45	Morphine	Memantine	before	3.5mg/kg	I.P	+	19	0.30
Popik et al	2006	NMDA	CPP	Pavlovian	45	Morphine	Memantine	after	7.5mg/kg	I.P	+	23	0.59
Popik et al	2006	NMDA	CPP	Pavlovian	45	Morphine	Memantine	before	7.5mg/kg	I.P	+	17	0.65
Przybyslawksi & Sara	1997	NMDA	Maze	Instrumental	5	Dietary	MK801	after	0.05mg/kg	I.P	+	15	0.61
Przybyslawksi & Sara	1997	NMDA	Maze	Instrumental	5	Dietary	APV	after	0.05mg/kg	I.P	+	15	0.81
Przybyslawksi & Sara	1997	NMDA	Maze	Instrumental	5	Dietary	MK801	after	0.05mg/kg	I.P	+	14	0.86
Sadler et al	2007	NMDA	CPP	Pavlovian	30	Amphet	MK801	after	0.1mk/kg	I.P	+	22	0.38
Storozheva et al	2011	NMDA	Pavlovian	Pavlovian	3	Dietary	MK801	before	50µg/Kg	I.P	+	16	0.89
Torras-Garcia et al	2005	NMDA	Pavlovian	Pavlovian	1.5	Dietary	APV	after	2.5µg/µL	I.V	+	26	0.55
von der Goltz et al	2009	NMDA	Operant	Instrumental	5	Ethanol	MK801	after	0.1mg/kg	I.P	+	18	0.37
Wouda et al	2010	NMDA	Operant	Instrumental	20	Ethanol	MK801	after	0.1mg/kg	I.P	+	15	0.48
Wu et al	2012	NMDA	CPP	Pavlovian	45	Morphine	APV	after	5µg/side	NAcc	+	20	0.22
Wu et al	2012	NMDA	CPP	Pavlovian	45	Morphine	APV	before	5µg/side	NAcc	+	20	0.77
Zhou et al	2011	NMDA	CPP	Pavlovian	15	Cocaine	7-CTKA	before	5µg/µl	VTA	+	20	0.67
Zhou et al	2011	NMDA	CPP	Pavlovian	15	Cocaine	7-CTKA	after	5µg/µl	VTA	+	17	0.78

Diergaarde et al	*2006*	*β -A*	*Operant*	*Instrumental*	*10*	*Dietary*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*+*	*14*	*0.23*
Diergaarde et al	*2006*	*β -A*	*Operant*	*Instrumental*	*20*	*Dietary*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*+*	*16*	*0.64*
Font & Cunningham	*2012*	*β -A*	*CPP*	*Pavlovian*	*15*	*Ethanol*	*Propranolol*	*after*	*10 mg/kg*	*I.P*	*+*	*47*	*0.08*
Font & Cunningham	*2012*	*β -A*	*CPP*	*Pavlovian*	*15*	*Ethanol*	*Propranolol*	*after*	*30 mg/kg*	*I.P*	*+*	*48*	*0.11*
Font & Cunningham	*2012*	*β -A*	*CPP*	*Pavlovian*	*15*	*Ethanol*	*Propranolol*	*after*	*10 mg/kg*	*I.P*	*+*	*47*	*0.13*
Font & Cunningham	*2012*	*β -A*	*CPP*	*Pavlovian*	*15*	*Ethanol*	*Propranolol*	*after*	*10 mg/kg*	*I.P*	*+*	*48*	*0.14*
Font & Cunningham	*2012*	*β -A*	*CPP*	*Pavlovian*	*15*	*Ethanol*	*Propranolol*	*after*	*30 mg/kg*	*I.P*	*+*	*46*	*0.15*
Fricks-Gleason & Marshall	*2008*	*β -A*	*CPP*	*Pavlovian*	*15*	*Cocaine*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*+*	*24*	*0.46*
Lee & Everitt	*2008a*	*β -A*	*PIT*	*Pavlovian*	*24*	*Dietary*	*Propranolol*	*before*	*10 mg/kg*	*I.P*	*+*	*16*	*0.32*
Milton	*Unpub.*	*β -A*	*ANR*	*Pavlovian*	*15*	*Ethanol*	*Propranolol*	*after*	*10 mg/kg*	*I.P*	*+*	*33*	*0.40*
Milton et al	*2012*	*β -A*	*Autoshaping*	*Pavlovian*	*45*	*Ethanol*	*Propranolol*	*before*	*10 mg/kg*	*I.P*	*+*	*13*	*-0.31*
Milton et al	*2012*	*β -A*	*PIT*	*Pavlovian*	*24*	*Ethanol*	*Propranolol*	*before*	*10 mg/kg*	*I.P*	*+*	*13*	*0.27*
Milton et al	*2008a*	*β -A*	*ANR*	*Pavlovian*	*10*	*Cocaine*	*Propranolol*	*after*	*10 mg/kg*	*I.P*	*+*	*14*	*0.35*
Milton et al	*2008a*	*β -A*	*ANR*	*Pavlovian*	*10*	*Dietary*	*Propranolol*	*after*	*10 mg/kg*	*I.P*	*+*	*18*	*0.47*
Przybyslawksi et al	*1999*	*β -A*	*Maze*	*Instrumental*	*5*	*Dietary*	*Propranolol*	*after*	*10 mg/kg*	*I.P*	*+*	*14*	*0.31*
Robinson & Franklin	*2010*	*β -A*	*CPP*	*Pavlovian*	*30*	*Morphine*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*+*	*24*	*0.37*
Robinson & Franklin	*2007*	*β -A*	*CPP*	*Pavlovian*	*10*	*Morphine*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*+*	*19*	*0.37*
Robinson et al	*2011a*	*β -A*	*CPP*	*Pavlovian*	*30*	*Morphine*	*Propranolol*	*after*	*40 mg/kg*	*S.C*	*-*	*18*	*-0.82*
Robinson et al	*2011a*	*β -A*	*CPP*	*Pavlovian*	*30*	*Morphine*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*-*	*18*	*-0.75*
Robinson et al	*2011a*	*β -A*	*CPP*	*Pavlovian*	*30*	*Morphine*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*-*	*18*	*-0.67*
Robinson et al	*2011a*	*β -A*	*CPP*	*Pavlovian*	*30*	*Morphine*	*Propranolol*	*after*	*40 mg/kg*	*S.C*	*-*	*18*	*-0.59*
Robinson et al	*2011b*	*β -A*	*CPP*	*Pavlovian*	*30*	*Morphine*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*+*	*26*	*0.10*
Robinson et al	*2011b*	*β -A*	*CPP*	*Pavlovian*	*30*	*Morphine*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*-*	*28*	*0.21*
Robinson et al	*2011b*	*β -A*	*CPP*	*Pavlovian*	*30*	*Morphine*	*Propranolol*	*after*	*10 mg/kg*	*S.C*	*+*	*21*	*0.24*
Roullet & Sara	*1998*	*β -A*	*Maze*	*Instrumental*	*5*	*Dietary*	*Timolol*	*after*	*2.5µl/side*	*I.C.V*	*+*	*16*	*0.26*
Wouda et al	*2010*	*β -A*	*Operant*	*Instrumental*	*20*	*Ethanol*	*Propranolol*	*after*	*10 mg/kg*	*I.P*	*+*	*22*	*0.52*

Paradigm Key: ***CPP*** *= Conditioned Place Preference;* ***Operant*** *= Self-administration paradigm,* ***ANR*** *= Acquisition of a New Response;* ***PIT*** *= Pavlovian-Instrumental Transfer;* ***Pavlovian*** *= Classical Conditioning.* ***Administration route key:*** ***I.P*** *= Intraperitoneal;* ***I.V*** *= Intravenous;* ***S.C*** *= Subcutaneous;* ***I.C.V*** *= Intracerebroventricular;* ***BLA*** *= Basolateral amygdala;* ***VTA*** *= Ventral tegmental area;* ***NAcc*** *= Nucleus Accumbens.* ***Drugs Key:*** ***MK801=*** 5--methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine, ***APV*** = amino-5-phosphonovaleric acid, ***7-CTKA*** = 7 Chlorokynuernic acid.

2.3.2. Data synthesis and Statistical Approach

Basic analysis was performed using hand-written formulae in Microsoft Excel and moderator analyses were performed with custom-written syntax by Field and Gillett (2010) in IBM SPSS Statistics v.19. As an initial test of the homogeneity of variance of the effect sizes within the analysis revealed a highly significant degree of heterogeneity (see *overall analysis* below) and as there is rarely reason to assume that fixed effects models are appropriate when attempting to make inferences about real-world population effects (Field, 2001;2003; Hunter & Schmidt, 1990, 2000), a random-effects analysis model (Hedges & Vevea, 1998) was used to assess the effect sizes. All data thus represent Hedges and Vevea's (1998) method applied on Fisher r-z transformed correlation coefficients after back-converting to Pearson's *r* (product moment correlation coefficient). Effect size values were corrected for the positive bias inherent in Fisher's r-z transformation using the equation $r - [r(1 - r^2)]/2(n - 3)$ prior to applying the transformation.

For analysis of moderator effects, a mixed-model was used whereby moderator variables are treated as fixed, but effect sizes random (Overton, 1998). Chi-square tests of moderator effects were used to assess categorical moderators and t-tests used to assess continuous variables entered into a meta-regression model.

Due to the small sample size in the current analysis, it was not possible to examine the effects of all moderators simultaneously, as the resulting power to detect moderating effects would be extremely low, leading to a large type II error rate. As such, moderators were examined in conceptual clusters in order to best address the questions set out in the introduction of this chapter.

To assess sensitivity of findings to publication bias, the procedure developed by Vevea and Woods (2005), implemented in R (R development core team, 2008) by Field and Gillett (2010) was used. This procedure uses four a priori weight functions to assess the impact of four different levels of possible publication bias on estimates of population effect size. These four weight functions represent moderate one-tailed, severe one-tailed, moderate two-tailed and severe two-tailed publication bias.

2.3.4. Overall Analysis

A basic overall analysis was first performed on all effect sizes regardless of moderating variables. This assessed the effect size of the effects of any β-Adrenergic or NMDAergic antagonist on the reconsolidation of reward memory trained in any of the included paradigms. A stem and leaf plot of these effect sizes is given *Figure 2.2*, showing a modal class of 0.3 with a slight positive skew towards large effect sizes. The highly negative cluster of values at the top of the distribution are the effect sizes from Robinson et al (2011) where Propranolol significantly increased CPP scores versus placebo at test. As these effect sizes came from a single study and were more than 3 standard deviations from the mean effect size, they were excluded from all analyses as outliers, leaving 52 effect sizes (30 NMDA, 22 β -A) remaining in the analysis. The overall analysis yielded a population effect size of 0.47 (95% CI = $0.386_{(lower)}$ to $0.546_{(upper)}$) with a highly significant associated z-score ($z = 9.728$, $p < 0.001$), suggesting a moderate-large overall effect of drug intervention on reconsolidation blockade, by Cohen's (1977) criteria. A chi square test of Cochran's Q statistic was highly significant [$\chi^2(51) = 121.312$, $p < 0.001$], indicating heterogeneity in effect sizes and confirming that a random effects conceptualisation of the data was appropriate. Between-study variance, computed as Hedges and Vevea's τ^2 was 0.0787, suggesting a large degree of between-study variance. To assess robustness against the file-drawer problem, a fail-

safe N (FSN) was calculated using Rosenthal's (1991) procedure. This revealed that 4089 'file drawer' studies would be required to make the calculated effect size non-significant. A FSN of greater than 5 times the number of studies in the analysis plus 10 is considered robust against the file-drawer effect. As such, this effect can be considered robust against the file-drawer problem.

Figure 2.2. Stem and leaf plot of effect sizes obtained from all studies:

Stem	*Leaf*
-1	
-.9	
-.8	2
-.7	5
-.6	7
-.5	9
-.4	
-.3	1
-.2	
-.1	
.0	8
.1	0, 0, 1, 3, 4, 5
.2	0, 1, 2, 3, 4, 6, 7
.3	0, 1, 2, 4, 5, 5, 7, 7, 7, 8
.4	0, 3, 3, 6, 7, 8
.5	0, 2, 5, 5, 7, 9
.6	1, 2, 3, 4, 5, 7
.7	7, 8
.8	0, 1, 3, 6, 9, 9
.9	0
1	

2.3.5. Separate Analyses by Drug Class

NMDAR

Stem and leaf plots of effect sizes for NMDA and β -AR studies is are given in *Figure 2.3*. For NMDAR effects, a population effect size of 0.613 (95% CI = $0.522_{(lower)}$ to $0.69_{(upper)}$) with a highly significant associated z-score ($z = 10.38$, $p < 0.001$) was found, suggesting a large overall effect of NMDAR antagonism in reconsolidation blockade, by Cohen's (1988) criteria. Cochran's Q was highly significant [$\chi^2(29) = 55.919$, *p*

=0.02], indicating heterogeneity in effect sizes. Between-study variance, computed as Hedges and Vevea's τ^2 was 0.0673. Rosenthal's (1991) Fail-safe N (FSN) calculation revealed that 2274 'file drawer' studies would be required to make the calculated effect size non-significant. The effect is thus robust against the file-drawer effect.

Figure 2.3. Stem and leaf plot of effect sizes obtained from NMDA studies (k = 30) and β-adrenergic studies (k = 22):

Stem	***NMDAR Leaf***	***β-AR Leaf***
-.3		1
-.2		
-.1		
.0		8
.1	0	0, 1, 3, 4, 5
.2	0, 2	1, 3, 4, 6, 7
.3	0, 4, 5, 7, 8	1, 2, 5, 7, 7
.4	3, 3, 8	0, 6, 7
.5	0, 5, 5, 7, 9	2
.6	1, 3, 4, 7	
.7	7, 8,	4
.8	0, 1, 2, 6, 8, 9, 9	

β-AR

Inspection of the stem-and-leaf plot given in *Figure 2.3* suggests lower overall effects of β-AR antagonists. The population effect size estimate for these studies was 0.24 (95% CI = $0.156_{(lower)}$ to $0.321_{(upper)}$) with a highly significant associated z-score (z = 5.485, $p < 0.001$), suggesting a small-medium overall effect of β-AR antagonism in reconsolidation blockade by Cohen's (1977) criteria. Heterogeneity in these effect sizes was not found [$\chi^2(21) = 17.073$, $p = 0.707$] and the τ^2 statistic was cut off at 0, meaning a fixed-effects conceptualisation of these effects may be appropriate. However, as the power of this test is low, and this analysis examined heterogeneity in the absence of data stratification, this was not used to preclude the inclusion of β-AR effect sizes in

subsequent moderator analysis. A FSN calculation revealed that 246 'file drawer' studies would be required to make the calculated effect size non-significant. The effect estimate can therefore be considered robust against the file-drawer problem.

2.3.6. Moderator Analysis

Drug Effects

The effect of drug class was highly significant [$\chi^2(1) = 29.5$, $p < 0.001$], indicating that effect sizes associated with NMDAR blockade were significantly higher than those associated with β-AR blockade. Thus NMDAR antagonism more robustly interferes with reward memory reconsolidation than does β-AR antagonism.

Moderating effects of specific drug compound within drug class were assessed by entering this predictor into the model. For NMDAR antagonists, there was no effect of specific compound (p =.806), suggesting NMDAR antagonism *per se*, regardless of compound used, interferes with reconsolidation. All β-AR studies used Propranolol, with the exception of one (Fricks-Gleason & Marshall, 2008), which used Timolol, thus moderator analysis of specific compound was not appropriate.

2.3.7. Pharmacokinetic Factors

An important question for the implementation of pharmacotherapy in the clinic is whether drug effects vary according to dose and time of administration relative to the reactivation session. Moderator analysis of pharmacokinetic factors for NMDAR and β-AR drugs was performed for each drug class separately.

Drug Timing

It is possible that drugs given prior to memory reactivation might have state-dependent effects on retrieval reconsolidation. To assess the impact of this on effect size, studies were split into those that administered drugs before and after reactivation. This dummy variable was entered into a moderator analysis along with drug class (NMDAR or β-AR). Drug class significantly moderated effect sizes [$\chi^2(1) = 17.253$, $p < 0.001$], but drug timing did not [$\chi^2(1) = 1.991$, $p < 0.001$], suggesting that administration of drug before reactivation does not significantly influence effect sizes compared to after reactivation. The interaction between drug class and timing did not predict variation effect sizes above drug class ($p >$. 05)

Dose

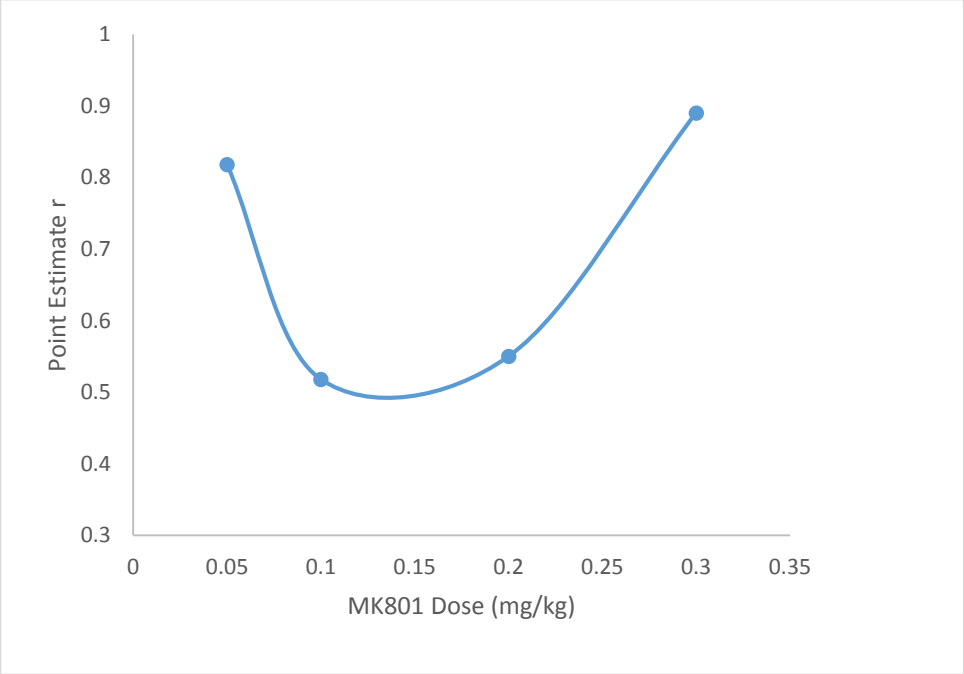
Since dosages of NMDAR and β -AR drugs across different administration routes cannot be converted to a common metric, moderator analysis of these factors was performed for each drug class separately. 19 studies gave doses (0.05, 0.1, 0.2 or 0.3mg/kg) of MK801 systemically). For these studies, dose significantly moderated effect size [$\chi^2(3) = 27.805$, $p < 0.001$]. An approximate U-shaped dose-response curve between MK-801 dose and effect size was found (*figure 2.4*), with the highest r_s for 0.05 and 0.3 mg/kg doses, but lower r_s for 0.1mg/kg and 0.2mg/kg doses. Note, however that only one study used the 0.2 and 0.3mg/kg doses each, so this relationship should be interpreted with caution. For β-AR studies, with the exception of one contrast using Timolol, Propranolol was the test drug and always administered systemically. No effect of Propranolol dose on effect sizes was found ($p = .464$), however only two doses

(10mg/kg and 30mg/kg) were used across all studies, with only two studies using 30mg/kg.

Table 2.2. Separate meta-analyses for different doses of MK801

Dose MK801 (mg/kg)	***k***	τ^2	***Q(df)***	95% CI for r			**z**	***p***	***FSN***
				lower	**mean**	**upper**			
0.05	*3*	*.736*	*3.673(2)*	*.605*	*.818*	*.921*	*5.023*	*<0.001*	*50*
0.1	*14*	*.0508*	*21.612(13)*	*.368*	*.518*	*.642*	*5.989*	*<0.001*	*296*
0.2	*1*	*0*	*N/A*	*.55*	*.55*	*.55*	*N/A*	*N/A*	*0*
0.3	*1*	*0*	*N/A*	*.89*	*.89*	*.89*	*N/A*	*N/A*	*0*

Figure 2.4: U-shaped dose-response curve of reconsolidation blockade by MK-801



Administration route

As NMDARs in different neural loci may be involved in dissociable reconsolidation processes (e.g. labilisation vs., restabilisation; Ben Mamou et al, 2006), effects of systemic administration of NMDAR antagonists were compared to intracerebral administration as a whole and specific site of injection was assessed with moderator analysis. There was no difference between systemic and intracerebral administration (p = .445) or any moderating effect of neural injection site (p = .804). Note, however, as the latter test only assessed eight studies, sensitivity to variations in effect size was extremely low. β-AR studies only administered drugs systemically, so moderator analysis was not possible.

2.3.8. Mnemonic Factors

Pavlovian/Instrumental

Table 2.3 shows the results of separate meta-analyses of NMDAR and β -AR antagonist effects in learning paradigms that putatively require Pavlovian only (CPP, classical conditioning, autoshaping, PIT, ANR) vs. instrumental (operant, maze) memory traces. NMDAR antagonists caused the greatest disruption of reconsolidation of both types of memory. β -AR antagonism was associated with a larger effect on instrumental memory than Pavlovian memory. Memory type (Pavlovian vs. instrumental) and a dummy code for the interaction between memory type and drug class (NMDA vs. β -A) separately entered as predictors in a meta-regression found that memory type alone was not a significant predictor of effect size variation [$\chi^2(1) = .141$, $p = .707$], but the interaction was [$\chi^2(3) = 32.836$, $p < 0.001$] . Post-hoc analyses revealed greater effects of NMDAR than β -AR antagonists on reconsolidation of Pavlovian memory traces [$\chi^2(1) = 33.525$,

$p < 0.001$] but not instrumental memories [$\chi^2(1) = .753$, $p = .386$]. Within drug class, memory type did not moderate the size of NMDAR [$\chi^2(1) = .452$, $p = .5$] or β-AR antagonist [$\chi^2(1) = 3.059$, $p = .08$] effects.

Table 2.3. Separate meta-analyses of instrumental and Pavlovian memory paradigms for NMDAR and β -AR antagonists

Drug Class	Memory Type	*k*	τ^2	*Q(df)*	95% CI for r			z	*p*	*FSN*
					lower	mean	upper			
NMDA	*Pavlovian*	*23*	*.062*	*22.264(22)*	*.529*	*.628*	*.714*	*9.638*	*<0.001*	*1460*
NMDA	*Instrumental*	*7*	*.102*	*6.127(6)*	*.305*	*.559*	*.739*	*3.912*	*<0.001*	*83*
β -AR	*Pavlovian*	*17*	*0*	*11.066(16)*	*.118*	*.210*	*.298*	*4.437*	*<0.001*	*115*
β -AR	*Instrumental*	*5*	*0*	*2.675(4)*	*.210*	*.424*	*.599*	*3.705*	*<0.001*	*19*

Relapse Process

Table 2.4 shows the paradigms included in the current analysis along with the putative association and memory type being tapped. Separate meta-analyses for these effects are shown in *Table 2.5.*

Table 2.4: Overview of conditioning tasks by process measured PIT = Pavlovian to Instrumental Transfer, ANR = Acquisition of a New Response, CPP = Conditioned Place Preference

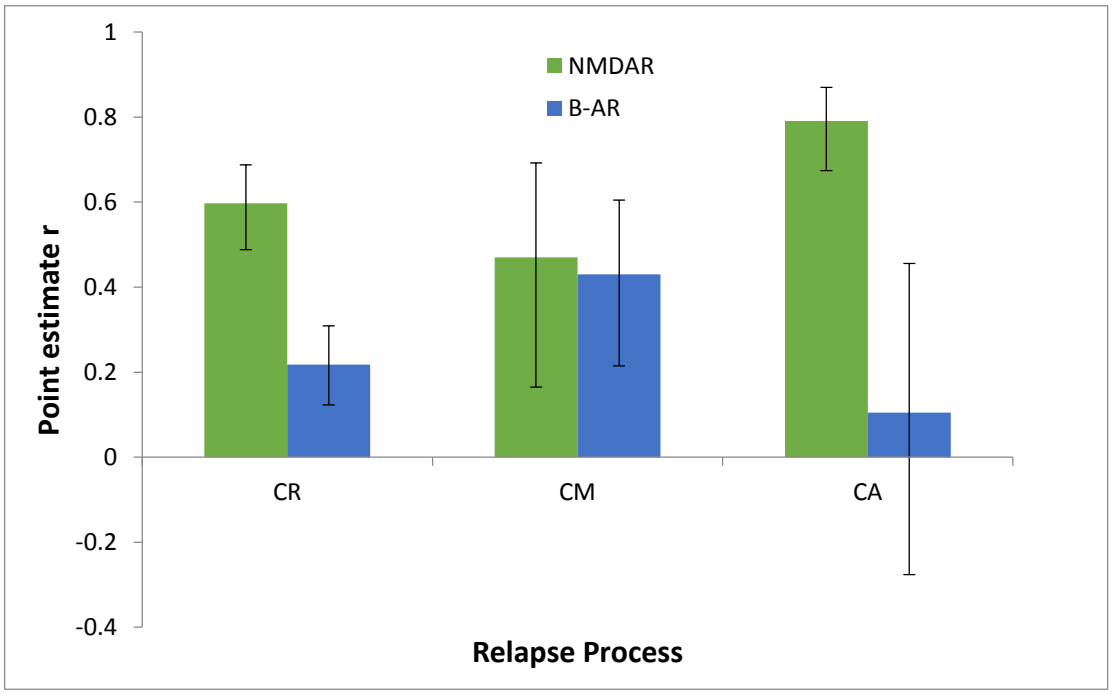
Association Type	***Tasks***	***Memory Class***
Conditioned Approach	*Autoshaping* *Maze*	*Pavlovian* *Instrumental*
Conditioned Motivation	*PIT* *Operant*	*Pavlovian* *Instrumental*
Conditioned Reinforcement	*ANR* *CPP* *Classical*	*Pavlovian* *Pavlovian* *Pavlovian*

Relapse process alone did not significantly explain variance in effect sizes (p =.312) but the interaction between drug class and relapse process significantly *did* moderate effect sizes [$\chi^2(5) = 47.466$, $p = <0.001$]. To assess this interaction, post-hoc tests were used to compare variations in effect sizes across relapse process within-drug-class and between drugs within relapse process. Alpha was set at 0.01 to control for the Type I error rate. No difference was observed between conditioned reinforcement (CR) and conditioned approach (CA) (p =.028) or conditioned motivation (CM) and CR (p = .29) for NMDAR antagonists, but effects were larger for CA than CM [$\chi^2(1)$ =6.951, p=.008]. No differences were found in any relapse process contrasts for β -AR antagonists (all $p > 0.08$). NMDAR antagonists interfered with CR [$\chi^2(1)$ =26.266, p <0.001] and CA [$\chi^2(1)$ =18.763, p <0.001] reconsolidation , but not CM reconsolidation [$\chi^2(1)$ =.063, p =.802] significantly more than β -AR antagonists (see *figure 2.5*)

Table 2.5: Separate meta-analyses for putative relapse process by drug class.

Drug Class	*k*	τ^2	Q(df)	95% CI for r			z	*p*	FSN
				lower	mean	upper			
NMDA									
Conditioned Reinforcement	19	.0489	31.067(18)	.488	.597	.688	8.726	<0.001	903
Conditioned Motivation	*6*	*.1016*	*11.2(5)*	*.165*	*.470*	*.692*	*2.915*	*0.004*	*36*
Conditioned Approach	*5*	*0*	*2.395(4)*	*.674*	*.791*	*.87*	*8.817*	*<0.001*	*119*
β -A									
Conditioned Reinforcement	14	0	7.982(13)	.123	.218	.309	4.429	<0.001	1.04
Conditioned Motivation	5	0	2.558(4)	.215	.430	.605	3.733	<0.001	19
Conditioned Approach	3	.029	2.653(2)	-.276	.105	.456	.53	.596	-0

*Figure 2.5: Interaction between relapse process and drug class showing meta-analysis point estimates ± 95& CI.**CR** = Conditioned Reinforcement, **CM** = Conditioned Motivation, **CA** = Conditioned Approach*



2.3.9. Methodological Factors

Primary reinforcer

Table 2.6 shows separate meta-analyses carried out on effect sizes split by the primary reinforcer (UCS) used in the study. Only one study (Sadler et al, 2007) used amphetamine as a primary reinforcer, so analysis was not possible for amphetamine. UCS significantly predicted effect size variation [$\chi^2(4) = 16.524$, $p = .002$]. To assess where the significant moderating impact of primary reinforcer lay, Bonferroni-corrected post-hoc comparisons were used to compare effect sizes for different reinforcers. This revealed no significant difference between ethanol and morphine ($p = .146$), but larger effects for dietary reinforcers [$\chi^2(1) = 10.055$, $p = .002$] and cocaine [$\chi^2(1) = 15.037$, $p < 0.001$] than ethanol. The difference was not significant between morphine and dietary reinforcers ($p = .106$) or cocaine ($p = .022$) or between dietary reinforcers and cocaine ($p = .545$).

Table 2.6: Separate meta-analyses for primary reinforcers

				95% CI for r					
UCS	***k***	**τ^2**	***Q(df)***	**lower**	**mean**	**upper**	**z**	***p***	***FSN***
ethanol	*13*	*.0203*	*13.608(12)*	*.068*	*.214*	*.359*	*3.606*	*<0.001*	*90*
morphine	*10*	*.0285*	*9.06(9)*	*.236*	*.396*	*.534*	*4.606*	*<0.001*	*112*
dietary	*12*	*.0634*	*11.321(11)*	*.330*	*.505*	*.646*	*5.112*	*<0.001*	*198*
cocaine	*9*	*.0334*	*7.768(8)*	*.488*	*.635*	*.746*	*6.816*	*<0.001*	*214*

Conditioning Paradigm:

Table 2.7 shows the results of separate meta-analyses carried out for each conditioning paradigm. Drug class moderation of effect sizes was assessed for each paradigm. Significant moderating effects were found for CPP, maze and autoshaping paradigms, with NMDAR antagonist effects higher than β -AR for each (χ^2 values and probabilities given in *table 2.7*). Note that the number of samples using the maze, PIT, autoshaping and operant procedures was very low, so results should be interpreted with caution. There was no effect of conditioning paradigm overall (p =.747).

Table 2.7: Separate meta-analyses of conditioning paradigms

				95% CI for r							
Paradigm	***k***	**τ^2**	***Q(df)***	**lower**	**mean**	**upper**	**z**	***p***	***FSN***	***Drug mode ration*** χ^2 (df)	***p***
CPP	*23*	*.095*	*70.95 (22)*	*.312*	*.444*	*.559*	*6.069*	*<0.001*	*890*	*26.52 (1)*	*<0.001*
OPERANT	*7*	*0*	*3.87(6)*	*.227*	*.406*	*.559*	*4.221*	*<0.001*	*37*	*.947 (1)*	*.331*
MAZE	*5*	*.132*	*10.2(4)*	*.320*	*.629*	*.817*	*3.557*	*<0.001*	*54*	*7.53 (1)*	*.006*
PIT	*4*	*.190*	*9.24(3)*	*.067*	*.528*	*.803*	*2.214*	*0.027*	*18*	*1.366 (1)*	*.243*
AUTO-SHAPING	*3*	*.585*	*14.75(2)*	*-.273*	*.572*	*.919*	*1.369*	*.171*	*13*	*13.71 (1)*	*<0.001*
ANR	*10*	*0*	*2.74(9)*	*.315*	*.457*	*.579*	*5.778*	*<0.001*	*111*	*.242 (1)*	*.623*

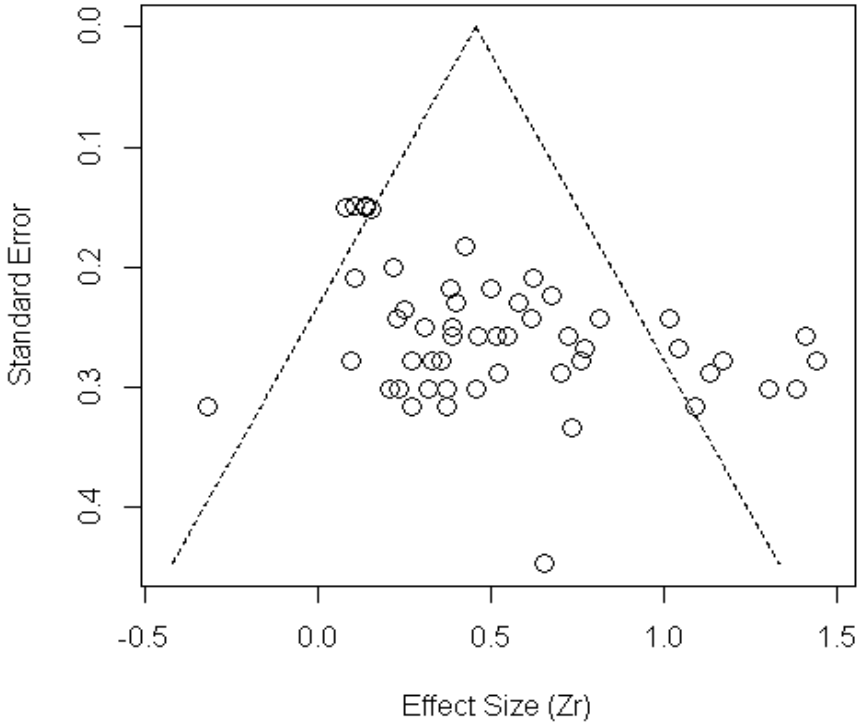
Reactivation Length:

Length of reactivation session entered as a continuous predictor in a random-effects meta-regression did not predict variation in effect sizes [$t(49) = -.647$, $p = .521$].

2.3.10. Sensitivity Analysis

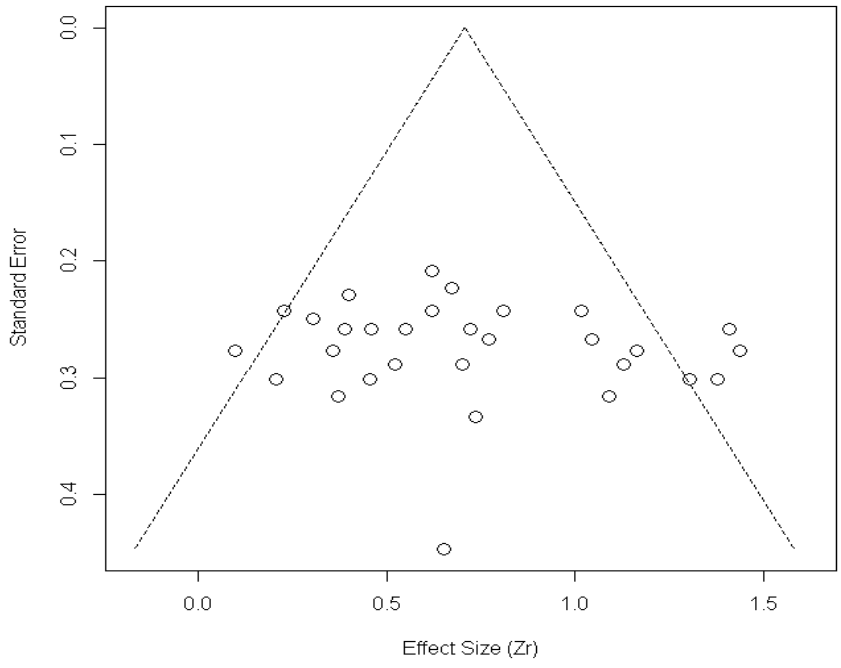
To assess potential publication bias, funnel plots were first created and assessed for asymmetry. As larger study samples tend to provide better estimates of true effect sizes, an inverted funnel shape should be observed when effect sizes are plotted against standard error. Publication bias manifests in under-representation in the negative tail of the plot. Overall, the included studies were relatively homogeneous in standard error, although more studies were included with high effect sizes (see *Figure 2.6*). Application of a priori weights for moderate one-tailed and two-tailed selection bias did not appreciably alter the point estimate (0.42 and 0.44 respectively). If severe one-tailed selection bias were assumed, the point estimate would be reduced to 0.34 (0.39 for severe two-tailed selection bias; see (*Figure 2.6*). As severe selection bias represents an extreme scenario, and does not nullify the findings of the present analysis, the current effects appear relatively robust and publication bias is unlikely to represent a threat to the validity of the findings. Discussion of these effects will therefore concern the unadjusted effect estimates.

Figure 2.6: Funnel Plot of Fisher Z-r transformed effect sizes against standard error. Lines represent 95% confidence intervals



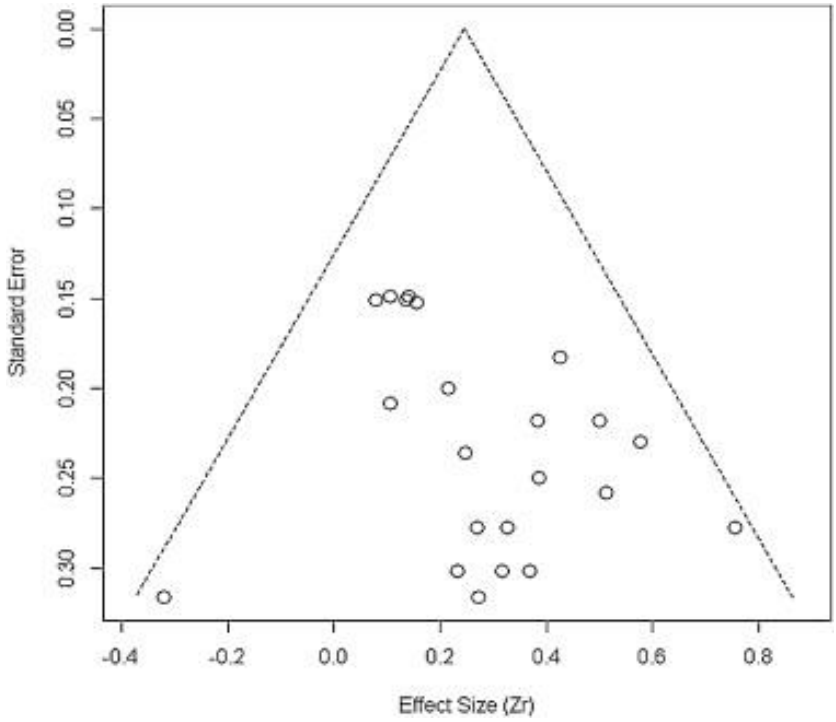
For NMDAR antagonist studies, effect size distribution looked largely symmetrical, with little variation between studies in standard error (see *Figure 2.7*). No a priori selection bias weighting had an appreciable impact on this estimate (moderate one-tailed = 0.59, severe one-tailed = 0.56, moderate two-tailed = 0.59 and severe two-tailed 0.56).

Figure 7: Funnel Plot of Fisher Z-r transformed effect sizes against standard error for NMDAR antagonist studies. Lines represent 95% confidence intervals



Inspection of the funnel plot for β-AR studies appears to show bias for large positive effect sizes (the left tail of the distribution is under-represented in *Figure 2.8.*). However, analysis revealed no significant impact of a priori selection bias weight functions on the point estimate. The point-estimate for this model was 0.24 and neither moderate (one-tailed = 0.208, two-tailed = 0.218) or severe (one-tailed = 0.168, two-tailed = 0.19) selection bias would invalidate the findings.

Figure 2.8: Funnel Plot of Fisher Z-r transformed effect sizes against standard error for β-AR antagonist studies. Lines represent 95% confidence intervals



2.3.11. Study Quality Ratings

Inter-rater reliability of the three judges' (myself and two colleagues) study quality scores was assessed by calculating the intraclass correlation coefficient using a two-way mixed-model of consistency. This found an acceptable degree of inter-rater reliability, with an intraclass correlation coefficient of .787 (95% CI = $.617_{(lower)}$ to $.889_{(upper)}$). Averages of judges' ratings were therefore entered into a meta-regression model to assess whether rated study quality predicted variation in effect sizes. Study quality was not related to effect size variation [β-coefficient = .0088 (95% CI = $-.008_{(lower)}$ to .024

$_{(upper)}$); t (49) = 1.027, p = .309], indicating that reported effects are not due to variations in methodological quality.

2.4. Discussion

2.4.1. Overview of findings

The current meta-analysis examined the effects of β-AR and NMDAR antagonists on the reconsolidation of reward memory. Overall, antagonism at either receptor caused a significant decrement in memory expression at test. However, large differences were found between NMDAR and β-AR antagonists, with NMDAR antagonists more robustly interfering with reconsolidation. Effects of relapse process (conditioned motivation, reinforcement or approach), primary reinforcer and dose were found on variation in effect sizes. An interaction between drug class and memory trace type (Pavlovian or instrumental) was also found, but no effect of memory type itself.

β-AR antagonists were associated with a significant, but small (by Cohen's criteria) overall effect (r = 0.24). Furthermore, there was relatively little variation in these effect sizes, suggesting that moderating influences of experimental variables on the effects of these drugs are minimal. Conversely, NMDAR antagonists showed fairly large and reliable effects on reward memory reconsolidation (r = 0.613), with considerable heterogeneity in the effect sizes, highlighting the importance of moderating factors. A direct test of the relative effects of NMDAR and β-AR antagonists confirmed that overall, NMDAR antagonists had a far larger effect on reconsolidation than β-AR antagonists. This is in line with the fundamental role of post-synaptic NMDAR activity in mediating synaptic plasticity and associative learning (Cotman et al, 1988; Malenka & Nicoll, 2002). The extent of the difference between NMDAR and β-AR antagonists was unexpected however, as both are thought to play important roles in associative memory formation and consolidation.

2.4.2. Impact of moderators

Pharmacokinetic, mnemonic and methodological factors may all affect the impact of drugs on reconsolidation and these factors were assessed with meta-regression. Drug administration route and timing relative to reactivation did not systematically modulate effect sizes for either drug class. It is possible, however, that the lack of moderating effect of administration route was due to a lack of studies injecting drugs at specific neural loci (only two effect sizes each for drugs injected into the basolateral amygdala, nucleus accumbens and ventral tegmental area were included, and only one where drug was given intracerebroventricularly). Previous research has suggested that separate neural loci might be important for distinct stages of reconsolidation at different times (Théberge et al., 2010). In the basolateral amygdala, for example, NMDARs (particularly GluN2bs) are important in destabilisation of memory traces (Ben Mamou et al., 2006), such that NMDAR antagonism prior to reactivation may *prevent* reconsolidation from occurring. However, intracranial drug administration at specific loci is unfeasible in humans, so the current observation of robust effects for systemically administered drugs is encouraging for the translation of the reconsolidation blockade approach to humans. Also encouraging is that timing of drug relative to reactivation did not moderate effect sizes. This demonstrates that the observed drug effects on reconsolidation are not attributable solely to state-dependent effects at recall and are in contrast to previous findings suggesting NMDAR antagonism only prior to reactivation can disrupt drug memories (Milton et al., 2008b).

A tentative U-shaped dose-response curve was found for MK801 effects, but no moderating effect of dose was seen for Propranolol. Note, however, that only two doses of Propranolol were used in the included literature (10mg/kg and 30mg/kg) and only

two samples used the latter dose. This should therefore not be taken as firm evidence against dose-response effects of Propranolol on reconsolidation and highlights the need for dose-finding studies with both β-AR and NMDAR antagonists in human reconsolidation. For NMDAR antagonists, dosing will also depend upon the specific pharmacodynamics of NMDAR antagonists to be used in humans, as MK801 is contraindicated for human use. However, the analysis revealed that the specific NMDA antagonist used (i.e. Memantine, MK801, D-APV, 7-CTKA) was not associated with variation in effect sizes. That is, inhibition of NMDAR activity through a diverse range of pharmacological mechanisms - channel blocking (MK801, Memantine), competitive antagonism of the glutamate binding site (D-APV) or the glycine site (CTKA) - is sufficient to disrupt reconsolidation. This is encouraging for translational research, as it suggests that NMDAR antagonists that *are* suitable for human use, such as Memantine, ketamine and Nitrous Oxide may effectively block reconsolidation.

Destabilisation does not occur under all circumstances following memory retrieval (see Finnie & Nader, 2012 for a review), suggesting certain parameters may modulate the viability of reconsolidation disruption as a treatment strategy. It has been suggested that one such parameter is memory trace type (Hernandez & Kelley, 2004; Milton et al 2012), with instrumental memories thought to be less susceptible to reconsolidation interference than Pavlovian memories. The current analysis found that memory type did not affect the size of reconsolidation effects overall. However an interaction between memory type and drug class was found, with NMDAR antagonists disrupting Pavlovian memory traces only to a greater extent than β-AR antagonists.

This challenges the notion that instrumental memory traces are less susceptible to reconsolidation interference *per se* (NMDAR and β-AR antagonism were both still associated with fairly large effects on instrumental traces; r = .559 and .424,

respectively), and suggests that β-AR antagonists such as Propranolol may be as effective as disrupting instrumental memory traces as NMDAR antagonists, but may be far less effective for targeting Pavlovian memories. Targeting the NMDA receptor should therefore still be the first choice for interfering with memory reconsolidation. Instrumental memory reconsolidation should thus not be ruled out as a target for pharmacotherapy, but research should focus on delineating the roles of the different neurotransmitter systems in this process. It remains to be seen whether interfering with the reconsolidation of Pavlovian cue-drug memories alone will be sufficient for long-term relapse reduction in human addicts, or whether their instrumental counterparts also need to be targeted to optimise relapse prevention.

Drug effects varied according to the putative 'relapse process' targeted by conditioning paradigms. Conditioned approach was more readily disrupted than conditioned motivation by NMDAR antagonists, and NMDAR antagonists caused greater disruption in conditioned reinforcement and conditioned approach (but *not* conditioned motivation) than β-AR antagonists. This dissociation is consistent with findings of separate amygdalar neuronal assemblies encoding conditioned motivation and reinforcement (Tye & Janak, 2007) and the involvement of amygdalar noradrenergic neurons in regulating autonomic arousal, stress and affective content associated with memory (reviewed by Roozendaal et al, 2009). As conditioned motivation reflects a state of cue-induced arousal, involving affective states, hyperlocomotor activation and possibly conditioned withdrawal, it is likely to involve both neurotransmitter systems regulating learned associations (i.e. glutamatergic projections from the BLA to the PFC and NAcc) and the arousing, affective correlates of those associations, via projections from the central nucleus of the amygdala, hypothalamus and brainstem. β-A receptors may therefore be an important target for reducing conditioned motivation in particular. Indeed, human studies of fear memory reconsolidation using Propranolol have shown

that the treatment attenuates the autonomic and affective components of fear memory, but not the knowledge of associations themselves (Brunet et al, 2008; Kindt et al, 2009). Conditioned approach and reinforcement, on the other hand, may rely more upon midbrain dopaminergic signalling modulated by glutamatergic afferents, to control behavioural outcomes (Kalivas & Volkow, 2005). There is currently limited research with compounds acting on the dopaminergic system to assess the role of dopamine in reward reconsolidation, which is surprising, given the extensive literature on its role in addiction, reward learning and prediction error. We therefore encourage research using dopaminergic drugs to examine the contribution of dopamine to reconsolidation.

The primary reinforcer used to train behaviour significantly moderated the effect of reconsolidation blockade on memory expression at test. This was due to greater effects on animals conditioned to dietary rewards (sucrose, food pellets) and cocaine than ethanol. It is unclear why this should have been the case, given the putative centrality of midbrain dopamine release and associative memory to addictive processes (Hyman, 2005; Kelley, 2004). However, dissociable neuronal ensembles have been identified that encode different motivationally relevant stimuli (Carelli & Wondolowski, 2003) and dopaminergically-encoded reward prediction error learning signals are thought to be key to memory destabilisation (Schultz et al, 1997; Pessiglione et al, 2006; Osan et al, 2011) by signalling expectancy mismatch. This raises the intriguing possibility that compounds whose self-administration is more directly associated with increased DA transmission (food and cocaine; Hernandez & Hoebel, 1988; Ritz et al, 1987) may form memory traces that are more amenable to destabilisation through more robust negative prediction error upon their omission. Ethanol primarily acts to increase DA via GABAergic mechanisms and directly antagonises NMDARs at high doses (Mukherjee et al, 2008), potentially causing adaptations that interfere with NMDAergic memory destabilisation. Thus memories related to those drugs that rely more on non-

dopaminergic neurotransmitter systems for their reinforcing effects may be less susceptible to reconsolidation blockade by NMDAR and β-AR antagonists. This is highly speculative, however, and remains to be tested in humans. However, this does highlight the fact that the differences, as well as the commonalities between addictions to different substances should be considered when designing interventions (Badiani et al, 2011). It will be important to assess reconsolidation-blocking interventions in human alcohol users to assess whether ethanol reinforced memories are genuinely resistant to reconsolidation. Given the legality of alcohol and its subsequent frequent use across varying contexts, alcohol MMMs perhaps represent some of the hardest memory traces to destabilise. Thus if therapeutic effects can be shown in this group, this will be promising for the application of the approach to other addictions.

Interestingly, neither learning paradigm nor length of reactivation procedure explained variation in effect sizes. Various parameters putatively determine whether reconsolidation or extinction is activated by cues (Pedreira & Maldonado, 2003; Suzuki et al, 2004), but the current analysis revealed that effects of NMDAR and β-AR antagonists are relatively invariant across length of reactivation procedures. Again, this is encouraging for translational work, as a concern with the use of reconsolidation interference for treating addiction is the sensitivity of the procedures to unavoidable variance in clinical application. The current findings suggest that other factors, such as mismatch between expected and actual outcomes, new information about stimuli and stimulus offset may thus be more important for triggering reconsolidation than absolute length of memory reactivation (Pedreira, 2004; Osan et al, 2011).

Publication bias is a constant threat to the validity of research based on published data. However, accounting for potential publication bias did not dramatically affect the estimates of effect size in the current analysis. As such, there is reasonable certainty that

the observed pattern of results is genuine and not an artefact of the propensity of journals to publish positive findings.

2.4.3. Limitations and recommendations for future research

While the current analysis aimed to inform the early translation of preclinical work in reward memory reconsolidation, I acknowledge some limitations associated with such a task. As previously mentioned, while preclinical studies can provide a good model of the basic learning processes involved in addiction, they are unable to represent the full complexity and inherent variability of human drug addiction. If, for example, we accept the maladaptive memory model of relapse, in reality there is virtually never a case where *both* Pavlovian and instrumental memory traces are not involved in addictive behaviour. In humans, illicit drugs are never passively administered in the absence of action nor consumed in the absence of rich sensory stimuli. The Pavlovian learning that imbues cues with conditioned motivational, reinforcing or attractive properties feeds into habitual actions, or chains of actions required in order to obtain and consume a drug. In humans, relapse behaviour is likely to be determined by more complex, higher-order associative networks of Pavlovian and instrumental memories over longer periods of time than in rats. In rat paradigms, drugs are often administered contingent upon, or relatively soon after, the presentation of a simple CS or execution of a simple instrumental response. In humans, there can be extended periods of time and a multitude of necessary intermediate responses between cue presentation and drug seeking and reward. It is unclear, for instance, how well the behaviour of a heroin addict who must first steal money, perhaps travel considerable distance to meet a dealer and procure the drug, then prepare the drug for use, is modelled by a simple approach or lever press response. Disruption of Pavlovian or instrumental 'links' in extended

associative memory chains may thus reduce the ability of drug cues to precipitate relapse, but some of the links may be more easily targeted or lead to greater improvements in outcome. This is a general issue for the translation of preclinical research to humans, as the associative networks maintaining addictive behaviour in humans are almost certainly more complex, variable and temporally extended than those created by experimental manipulation in rats. There is thus much human research to be done to assess the relative impact on relapse rates of degrading different links in these associative networks with reconsolidation-based pharmacotherapy. The clinical viability of such an approach is still currently unknown.

Because preclinical studies are highly controlled and thus sensitive to experimental manipulation, we can expect some attenuation of effects in humans. This may be problematic for β-AR antagonists in the present instance, as the observed effects in animals were modest. This would fall foul of Criterion 1 of the appraisal criteria for assessing drug efficacy outlined in *Chapter 1*. Accounting for attenuation, β-AR antagonism may not be the optimal way for interfering with maladaptive reward memory in humans. This is not to say that we discourage research with β-AR antagonists in humans, but direct comparison with NMDAR antagonists in humans will be critical in determining the extent to which these results translate to clinical intervention.

There is currently a lack of human research into the effects of NMDAR and β-AR antagonists on appetitive memory reconsolidation. However, early findings in human fear and anxiety studies, which typically precede equivalent addiction studies, are encouraging (Kindt, 2009; Soeter & Kindt, 2010; Schwabe et al, 2012). However, the neural, behavioural and psychological divergence between appetitive and aversive memory mean it is no simple task to translate findings from one domain to the other.

More primary research into the role of β-ARs and NMDARs in the reconsolidation in human appetitive memory is therefore encouraged. One human study examining Propranolol for blocking the reconsolidation of MMMs in cocaine addicts has been conducted since the publication of this meta-analysis (Saladin et al. 2013). The findings of improvements following Propranolol with memory reactivation were in the modest range predicted by this meta-analysis and were not enduring (thus not meeting *Criterion 2* outlined in *Chapter 1*).

In summary, the present analysis is highly encouraging for translational use of NMDAR antagonists to interfere with reward memory reconsolidation. Weaker support was found for the use of β-AR antagonists, but further human research will be needed to assess whether effects are robust enough to be of utility in the clinic. Pharmacokinetic, mnemonic and methodological factors can modulate the effects of these drugs on reward memory reconsolidation, but these effects must be replicated in humans, where basic proof-of-principle work is required. Translating this research approach to human drug users, it would be prudent to focus on modulation of the NMDAR as a key drug target.

Chapter 3: Translating Basic Reconsolidation Research to Human Addiction: An Investigation of Memantine with Cue-Drug Memory Retrieval as an Adjunct to Voluntary Smoking Cessation.

3.1. Introduction

Antagonising NMDARs or β-ARs during reconsolidation (Lee and Everitt 2008a; Milton et al. 2008a) should putatively inhibit reward memory restabilisation, weakening reactivated memory traces. However, meta-analytic evidence (see *Chapter 2*) demonstrates that of these two drug types, NMDAR antagonists display much more robust effects in blocking restabilisation (Das et al. 2013a).

Blocking NMDARs may therefore be an effective pharmacological strategy for weakening MMMs, yet it is unclear how well this approach will translate to human drug-using populations. This uncertainty is driven primarily by the sensitivity of pharmacological memory blockade to boundary conditions at recall, the paucity of tolerable NMDAergic antagonists available for human use and the simple lack of research using the approach in humans.

Regarding the first of these issues, as reconsolidation is a memory updating and maintenance process (Lee 2009), unexpected new information regarding the retrieved memory is necessary to destabilise the trace at recall (Lee 2009; Pedreira et al. 2004). The necessity of this this mismatch between predicted and occurring events (Sevenster et al. 2013), known as a prediction error (Schultz et al. 1997) is consistent with computational models of reinforcement learning, in that it acts as a learning signal to spark memory updating via reconsolidation. Incorporating uncertainty into reward availability during MMM retrieval procedures may thus be key to their destabilisation and subsequent weakening.

Further, the length of the memory retrieval period and number of unreinforced presentations of cues are also critical in destabilising memories, as they determine the switch between the mutually exclusive (Merlo et al. 2014) processes of reconsolidation and extinction (Osan et al. 2011; Pérez-Cuesta and Maldonado 2009; Suzuki et al.

2004). Longer reactivations with more unreinforced cue presentations bias in favour of extinction, which is undesirable due to the fragility of extinction-based therapeutic effects. Very short reminders fail to engage either process, however (Suzuki et al. 2004), so reactivation procedures should be brief, but not too brief. Exactly how long is optimal in human drug users is still unknown, however, and no clear moderating effect of retrieval length was observed taking the 'bird's eye view' during meta-analysis in *Chapter 2*.

The limited NMDAergic antagonist pharmacopoeia of reconsolidation-blocking agents available for human use is due to the tendency of high-affinity channel blocking NMDAR antagonists like ketamine and phencyclidine to cause dissociation, hallucinations and psychosis-like symptoms (Muetzelfeldt et al. 2008) and potentially lesions in neural tissue (Olney et al. 1989).

Memantine, a novel, channel blocking non-competitive NMDAR antagonist does not exhibit the side effects of other NMDAR antagonists at low doses (Parsons et al. 1999), is very well tolerated in humans and is already prescribed for memory loss in humans with Alzheimer's disease. Memantine has been shown to interfere with MMM restabilisation in preclinical studies (Alaghband and Marshall 2013; Popik et al. 2006). It is therefore an attractive candidate for translational attempts to pharmacologically weaken MMMs during reconsolidation.

However, findings with memantine in reconsolidation are inconsistent, as it has been shown to *enhance* reconsolidation in day-old chicks (Samartgis et al. 2012). It also promotes neurogenesis, which may lead to enhancement *or* weakening of memory depending on the nature of hippocampal memory encoding (Akers et al. 2014). Further, it has not yet been tested in the context of blocking human MMMs. Given the aforementioned differences between human MMMs and lab-learning paradigms, the

current study sought to test whether memantine could interfere with the reconsolidation of cue-smoking memories in quitting tobacco smokers, a prototypical addicted population. If so, it should ameliorate cognitive measures of MMM strength and ultimately reduce relapse.

3.1.1.Specifying an appropriate memantine dose

Due to the lack of human research looking at blocking MMM restabilisation and the novel pharmacodynamics of memantine at the NMDA receptor (Rammes et al. 2008; Xia et al. 2010), there is very little information upon which to base a memantine dose for the current study. Meta-analysis suggested a non-linear dose-response effect of MK-801 on reward memory reconsolidation blockade, with low doses exhibiting greater efficacy than moderate doses. Although memantine has lower NMDAR affinity than MK-801 (Rammes et al. 2008), low doses of the drug have been found to induce memory impairments in rats, with higher doses generating an intolerable side effect-profile (Creeley et al. 2006). Further, in populations of cloned human receptors, memantine in high concentrations antagonises both NMDARs and nicotinic acetylcholine receptors (Maskell et al. 2003). This lack of specificity impedes the attribution of any observed effects to glutamatergic systems.

Human research in smokers has utilised up to 40mg memantine. At this dose, memantine produced significant dizziness, light-headedness, detachments from reality and temporal distortion and prevented the 'buzz' smokers experienced following a cigarette (Jackson et al. 2008). The current study aimed to minimise this side-effect profile, maximise NMDAR specificity of memantine and avoid the potential dip in efficacy of moderate-dose NMDA antagonism. As such, a relatively low dose of 10mg memantine was selected. This is generally the maximum single oral dose prescribed to Alzheimers patients (www.namenda.com).

3.2 Methods

3.2.1. Participants & Design

Power analysis based on a meta-analytic effect size of $r = 0.67$ determined a total N of 12 was required for 0.8 power at $\alpha = 0.05$. However, to account for attenuation in NMDAR antagonist effects across species, a more conservative r of 0.35 was adopted for all studies using NMDAR antagonist studies, yielding a required N of 57 for 0.8 power at $\alpha = 0.05$. Expecting minimal attrition rates, fifty nine smokers were therefore recruited via online advertisements and the University College London postgraduate subject pool. Inclusion criteria were scoring >4 on the Fagerstrom Test of Nicotine Dependence (FTND) (Heatherton et al. 1991), smoking > 10 cigarettes per day, seriously wanting to stop smoking as indexed by endorsing item 1 or 2 on the Motivation to Stop Smoking Scale (Kotz et al. 2013). Exclusion criteria were ages <18 or >65, current / history of mental health or neurological conditions, concurrent addiction to any other substance, use of any illicit drug more than once per week, use of an NMDAR antagonist (e.g. ketamine) more than once per month, pregnancy or breastfeeding, compromised renal or hepatic function. Of the participants randomised to a group, four did not attend the second study session and were lost to all further follow-up. We utilised an intention-to-treat approach such that all participants randomised to groups contributed data to the statistical analyses.

A randomised, double-blind, placebo-controlled design was used to assess the effects of 10mg memantine in blocking reconsolidation. To assess drug and reactivation dependency of effects, participants were therefore randomly assigned to one of three groups: Reactivation of smoking MMMs with memantine (REACT + MEM, N = 19), Reactivation of smoking MMMs with Placebo (REACT+PLA, N =20), or memantine without reactivation of smoking MMMs (NO REACT + MEM, N = 20). 10mg

Memantine Hydrocloride in pill form (Namenda) was obtained from UCH pharmacy and formulated in opaque gelatine capsules with lactose powder filler for those groups receiving memantine. Placebo capsules were identical red gelatine capsules filled with lactose powder only.

3.2.2. Apparatus & Tasks:

Smoking memory reactivation stimuli consisted of in-vivo smoking cues (a pack of Marlboro cigarettes, lighter and ashtray) and six thirty second video clips depicting smoking paraphernalia (cigarettes, lighters and ashtrays) and people smoking in various locations. The non-reactivation cues consisted of six similar thirty second clips that did not depict smoking or smoking-related cues and numbered cards and a pencil. The videos were kindly provided by Joel Erblich (Tong et al. 2007).

A visual probe task was used to assess attentional bias to smoking cues on Day 8 of the study. The task consisted of smoking pictures paired with composition-matched neutral images (n = 20) or control neutral-neutral (n = 20) pairs. The picture pairs were developed and kindly provided by Karin Mogg and are described fully in Mogg et al. (2003). Pairs of pictures appeared for 500ms or 2000ms and were replaced by a probe either contralateral or ipsilateral to the target (smoking-related). Trial presentation was counterbalanced for stimulus onset asynchrony (500/2000ms), target side (left or right) and probe congruence with target (congruent/incongruent). The shoulder buttons on a Microsoft Sidewinder gamepad were used to respond to the side upon which the probe appeared.

A saccade/antisaccade task was used to assess baseline oculomotor response inhibition. In both phases, red target circles appeared either on the near (x,y screen location

412,384) or far (212,384) left or near (612, 384) of far (812, 384) right of the screen, twelve times in each location in a random order. Correct saccades or antisaccades ended the trial, otherwise it timed out after 3000ms. The saccade phase required participants to saccade to red target circles from fixation in the centre of the screen. Participants were instructed to 'Simply look from the central fixation to the red circle' during this phase. The antisaccade phase required participants to make a saccade to the point on the screen in the exact mirror-image location of the red target circle without first looking at the target. Participants were told that they had to 'Look at the opposite point on the screen to where the red circle appears, while inhibiting their natural tendency to look at the circle first'. The saccade phase was always completed prior to the antisaccade phase to familiarise participants to the oculomotor response requirements.

An adaptation of the Effort Expenditure and Reward Responsivity Task (EEfRT) by Treadway and colleagues was used to assess motivation for non-drug reward at baseline and on Day 8. The reader is referred to Treadway et al (2009) for a full description of the task. Briefly, on each of 48 trials the participant was required to choose between an 'easy task' (tapping the space bar 30 times in 7 seconds) and hard task (tapping it 100 times in 21 seconds). All tapping had to be performed with the little finger of the non-dominant hand. On each trial, a fixed reward of 50p was available for successful completion of the easy task and a variable reward between 70p and £2.00 (in 10 pence increments) for the hard task. Participants had 5 seconds to choose one of the tasks to complete in each trial or one was chosen randomly. An associated 'win probability' was displayed as the choice was being made that showed probability of winning on that trial *if* the task was completed. This was either low (12%), medium (50%) or high (88%). The monetary value of the sum of two randomly selected trials on which the participant won was added to their payment.

Level of nicotine dependence at baseline and at each follow-up was assessed with the Fagerstrom Test of Nicotine Dependence (FTND) (Heatherton et al. 1991). Participants completed an online 'smoking diary' every evening for a minimum of one week prior to Day 1 and continuously from then up to three weeks following Day 8. The diary was implemented in UCL Opinio (http://opinio.ucl.ac.uk) and recorded how many cigarettes participants smoked on a daily basis. At baseline, cigarette craving was assessed using the 10-item Questionnaire on Smoking Urges (QSU) (Tiffany and Drobes 1991). Withdrawal-related symptoms were assessed with the Mood and Physical Symptoms Scale (MPSS) (West and Hajek 2004). State anxiety was assessed with the Spielberger State-Trait Anxiety Index (STAI, (Spielberger et al. 1970), depressive symptomatology with the Beck Depression Inventory (BDI)(Beck et al. 1988), levels of anhedonia with the Temporal Experience of Pleasure Scale (TEPS) (Gard et al. 2006) and levels of social support with the Perceived Social Support scale, friend (PSS-FR) and family (PSS-FA) (Procidano and Heller 1983) versions. Single-item VAS scales given pre and post-video assessed cue-induced craving from the videos used in the memory reactivation procedure. These 100m scales required participants to mark down the strength of their urge to smoke and were anchored 'No urge at all' and 'Strongest Urge Ever'. Visual analogue scales were used to assess drug-related changes in mood from baseline to peak effects. These were the mood rating scale (MRS) and bodily symptoms scale (BSS) (Bond and Lader 1974).

For tasks involving eye tracking (dot probe and saccade/antisaccade), eye movements were recorded with a desktop-mounted Eyelink 1000 eye tracker (SR Research, Ontario, Canada) using a stabilised head configuration where participants' heads were stabilised in a chin rest 70cm from the 1024x768 monitor used to display all computer tasks. Prior to, during and after participants watched the reactivation or control videos, Electrocardiogram (ECG) and skin conductance was recorded using and Equivital EQo2

Lifemonitor belt and sensor with auxiliary skin conductance electrodes (Hidalgo, Cambridge, UK) attached to the medial phalanges of the participants' left hand with AMBU white sensors. Blood pressure was measured with an Omron 708-BT electronic blood pressure cuff (Omron, Japan).

3.2.3. Procedure

Following screening, the first study session (Day 1) was arranged with the participant such that it would fall on their target 'quit day'. This was at least 7 days from screening and participants began filling out the daily online smoking diary immediately for the week preceding Day 1. Participants were asked to make any necessary arrangements to make their quit as successful as possible, including informing friends and family, getting rid of cigarettes and smoking paraphernalia prior to Day 1 and purchasing nicotine gum if they intended to use it. Participants were required to refrain from smoking for 1 hour prior to the beginning of Day 1, to fast for at least 3 hours prior and to avoid the use of alcohol or any illicit drug in the 24 hours preceding sessions.

Day 1

After providing written informed consent, participants were given the capsule containing either 10mg memantine or placebo and took it immediately with water. Breath carbon monoxide was then measured with a Micro+ carbon monoxide meter (Bedfont Scientific, Kent, United Kindgom). Participants then completed the baseline MRS, BSS, QSU, MPSS, STAI, BDI, TEPS, PSS-FR and PSS-FA. Following this, they completed the saccade/antisaccade and EEfRT tasks. At the end of the EEfRT task, participants were required to sit in a waiting room until 3 hours had elapsed since they

took the pill. This was based on oral memantine reaching peak plasma concentrations at 3 – 7 hours post-administration (Product Monograph, Lundbeck; (Periclou et al. 2006)). We aimed to conduct the memory reactivation at 3.5 hours to coincide with plasma levels peaking during the reconsolidation window. After the break, participants re-completed the MRS and BSS and then were fitted with the electrode belt for ECG and skin conductance electrodes.

Reactivation Procedure

Participants were given a box and told not to open it until instructed to do so via the computer screen. Two boxes were prepared for the smoking memory reactivation groups (MEM + REACT, PLAC + REACT), which contained a lighter, an ashtray and a full, open box of Marlboro cigarettes with one cigarette protruding. The items were anchored in the boxes in the same configuration. In the no-reactivation group, the box contained stacks of cards with numbers written on their surfaces. The experimenter was blind to the contents of the boxes and the correct box/videos were selected via an alphabetic code attached to each participant number. Participants were informed that that they would shortly watch a video and open the box and that the box contained the materials for a task *they may need to complete after watching the videos*. This instruction was included to induce uncertainty in reward availability, with the aim of destabilising cue-drug MMMs. Prior to starting the videos, a five minute heart rate baseline, blood pressure and single-item cigarette craving was recorded.

When the experimenter started the videos, instructions appeared on screen asking participants to watch carefully and try to imagine being in the depicted scenes as much as possible, imagining the sights smells, sensations and sounds as if they were really there. They were then instructed to open the box in front of them and take note of its contents. They were reminded that, following the video, they may have to complete the

task in the box. The videos then played and after their conclusion, more instructions appeared informing participants that they would not complete the task, to close the box and to alert the experimenter. Another blood pressure and single item VAS craving measure was then recorded and a 5 minute period of heart rate post-video collected.

Following this, to ensure 'cognitive offset' of the scenes depicted in the videos, high load working-memory tasks were completed by the participants. These were Verbal (M) and Category (fruit) fluency, digit span forwards and backwards and the Wisconsin Test of Adult Reading (WTAR). This concluded Day 1 testing.

Day 8

Participants returned to the study centre and a carbon monoxide reading was taken. They then re-completed all questionnaire measures from Day 1 along with the EEfRT task. Following this, the video procedure was again performed along with heart rate, blood pressure and craving measures. Finally, the visual probe task was completed and this concluded testing. Participants were reminded that they must continue to fill out the smoking diary for three more weeks. To incentivise this, payment was split between the end of Day 8 and the end of the three week diary period, with full payment only being made if completed diaries were received.

Follow-up measures were completed by telephone at three month intervals for up to one year. Due to time constraints on the study, continued follow-ups were terminated following relapse to baseline levels of smoking. If participants became uncontactable for 3 month follow-up, their scores on the FTND and cigarettes smoked per day were returned to baseline level.

3.2.4. Data Preparation

Heart rate data from the EQo2 were extracted in plain text format for the pre, peri- and post-video epochs. Artefacts were removed by calculating mean R-R interval for the recording period and excluding any R-R intervals that fell more than 3 standard deviations outside of this mean. Inspection of waveforms before and after this confirmed that this successfully removed all visually identified artefacts. From trimmed data, single heart rate (HR) and heart rate variability (HRV) measures were taken as the mean and standard deviation of R-R (SDRR) intervals across the record, respectively.

Eyetracking data were extracted using SR Research's dedicated Dataviewer program. For the visual probe, dwell time (summated duration of all fixations occurring on each picture during the trial period), initial fixation times and durations on each image were extracted. Data were not included for any trials that did not have a dwell time of at least 100ms on each image. Fixations on images occurring <100ms after image onset were excluded, as these reflect anticipatory fixation upon image locations, rather than stimulus-base orienting (Mogg et al. 2005). For the probe reaction time data, trials where reaction times were >3 SDs from mean RT were excluded. For the saccade/antisaccade task, saccade latency and prosaccade errors in the antisaccade phase were extracted. The latter were calculated as any saccade ending in the area occupied by the target circle.

We used an intention-to-treat analysis, so scores from all participants randomised into the study were used to assess intervention effects on primary outcomes. Four participants did not attend their Day 8 sessions (one participant from MEM no REACT and 3 from MEM + REACT). For these participants, variables about which we had information from baseline (smoking and mood variables, EEfRT performance and peri-

video psychophysiological data) were returned to their baseline values to give a conservative estimate of treatment effects.

For the visual probe data, scores were imputed using the estimation maximisation method as Little's test indicated that data were missing completely at random (χ^2 (69) = 77.094, p = 0.236). Variables used to predict scores were baseline FTND, baseline and Day 8 cigarettes per day, behavioural inhibition scores, Day 8 craving (QSU and pre-post video craving VASs) and existing visual probe data. In the neutral-neutral pairs, one image was arbitrarily designated as the 'target' image to aid comparison with the smoking-control image pairs. All data were analysed in IBM SPSS Version 21 for Windows. Data were analysed blind and Group identity codes were only unblinded after analysis was completed.

3.3. Results

3.3.1. Baseline Smoking and Subjective measures:

Descriptive statistics for baseline measures of smoking dependence and questionnaire-based assessments of factors important in successful smoking abstinence are given in *Table 3.1*. All statistics are presented as mean± standard deviation. One-way ANOVAs were used to assess whether the groups differed at baseline on any of these measures, and a more conservative alpha of 0.01 was adopted due to the large number of comparisons. Welch's ANOVA was used to compare time since last cigarette and MPSS mood due to heterogeneity of variance between groups in these variables. The groups did not differ on any of the variables at baseline, although there was a trend for higher craving as measured by the QSU in MEM no REACT compared to PLAC+REACT.

Table 3.1: Descriptive statistic and associated significance of tests of group means for smoking and mood variables at baseline. All tests were one-way ANOVA except where marked with a subscript W, indicating that Welch's ANOVA was used. = significant at 0.05. Values are means ± SD*

	MEM No REACT *(N = 20)*	PLAC+REACT *(N = 20)*	MEM+REACT *(N =19)*	*Significance of Difference*
Age	*27.45±6.91*	*28.35±7.04*	*29.32±9.9*	*P = 0.769*
Years in Education	*15.33±1.98*	*16.45±3.02*	*15.47±2.9*	*P = 0.304*
Pre Quit FTND	*5.4 ± 1.05*	*5.6 ± 1.05*	*5.0 ± 0.75*	*P = 0.150*
Pre Quit Cigarettes Per Day	*14.2±4.27*	*14.45±3.33*	*14.53±3.2*	*P = 0.958*
Years smoking	*11.68±5.41*	*10.75±6.59*	*11.24±7.36*	*P =0.904*
Pre Quit CO (ppm)	*7.84±5.7*	*9.8±4.4*	*11.95±6.5*	*P =0.081*
Number previous quits	*2.11±1.2*	*2.45±1.7*	*2.53±2.25*	*P =0.736*
Previous longest quit (days)	*188.58 ± 358.3*	*121.85±249.67*	*169.16±493.95*	*P =0.852*
Last cigarette (mins)	*833.7±184.51*	*248.45±294.94*	*204.16±227.08*	*$P=0.416_w$*
QSU Baseline	*37.75±14.93*	*25.45±10.23*	*32±12.88*	*P=0.014**
MPSS Mood	*0.96±0.69*	*0.63±0.35*	*0.72±0.48*	*$P=0.185_w$*
MPSS Urge Frequency	*2.15 ±1.31*	*1.75±1.07*	*1.95±1.13*	*P =0.563*
MPSS Urge Strength	*2.40±1.31*	*1.90±1.21*	*1.63±0.68*	*P =0.098*
BIS Total	*69.65±11.40*	*61.6±12.51*	*69.63±11.28*	*P =0.053*
STAI	*36.95±11.83*	*32.6±7.87*	*33.05±6.91*	*P =0.266*
BDI	*2.1±2.51*	*2.1±1.83*	*2.26±1.48*	*P =0.958*
TEPS Anticipatory	*4.56±0.79*	*4.7±0.58*	*4.59±0.77*	*P =0.815*
TEPS Consummatory	*4.59±0.84*	*4.74±0.8*	*4.72±0.79*	*P =0.818*
BAS DRIVE	*8.9±1.74*	*9.05±2.21*	*8.42±2.29*	*P =0.623*
BAS FUN	*7.2±2.21*	*7.6±2.01*	*6.53±2.34*	*P =0.311*
BAS REWARD	*8±1.75*	*7.45±1.93*	*6.89±1.79*	*P =0.177*
BIS	*12.9±3.6*	*12.3±2.56*	*13.26±2.98*	*P =0.615*
PSS-FR	*14.85±4.92*	*15.3±3.05*	*14.84±3.88*	*P =0.919*
PSS-FA	*11.75±6.23*	*12.6±5.92*	*10.53±6.34*	*P =0.576*

3.3.2. Changes in Smoking behaviour:

A mixed 2 (baseline, test) x 3 (Group) ANOVA found a significant reduction in breath carbon monoxide [Time main effect $F(1,56) = 141.822$, $p < 0.001$, $\eta_p^2 = 0.717$] and number of cigarettes smoked [Time main effect $F(1,56) = 10.586$, $p = 0.002$, $\eta_p^2 = 0.159$] in all groups from Day 1 to Day 8. However, no main effects of Group or Group x Time interactions were observed. Reductions in momentary craving measured by the QSU, were seen between Days 1 and 8 in all groups [Time main effect $F(1,56) = 19.333$, $p < 0.001$, $\eta_p^2 = 0.257$]. A main effect of Group was also observed [$F(2,56) = 5.788$, $p < 0.001$, $\eta_p^2 = 0.171$], driven by consistently higher general craving in MEM NO REACT than PLAC+REACT [$t(38) = 3.39$, $p = 0.004$, $r = 0.48$]. QSU at test was positively correlated with Day 8 smoking levels ($r(59) = 0.367$, $p = 0.004$) and predicted 3 month FTND score ($r(59) = 0.5$, $p < 0.001$) and time to relapse ($r(59) = -0.367$, $p = 0.004$), however there were no group differences in FTND [$F(2, 56) = 0.569$, $p = 0.569$, $\eta^2 = 0.02$] or cigarettes smoked per day [$F(2, 56) = 0.355$, $p = 0.703$, $\eta^2 = 0.01$] at 3 months.

Assessment of the MPSS found no change in mood [Time main effect $F(1, 56) = 2.239$, $p = 0.14$, ns], but significant decreases in all groups in urge to smoke frequency [Time main effect $F(1,56) = 6.393$, $p = 0.014$, $\eta_p^2 = 0.102$] and strength [Time main effect $F(1,56) = 4.778$, $p = 0.033$, $\eta_p^2 = 0.079$]. Again, no group differences or Group x Time interactions were observed.

Table 3.2: Smoking outcomes across the experimental groups.
Values are counts or mean ± SD

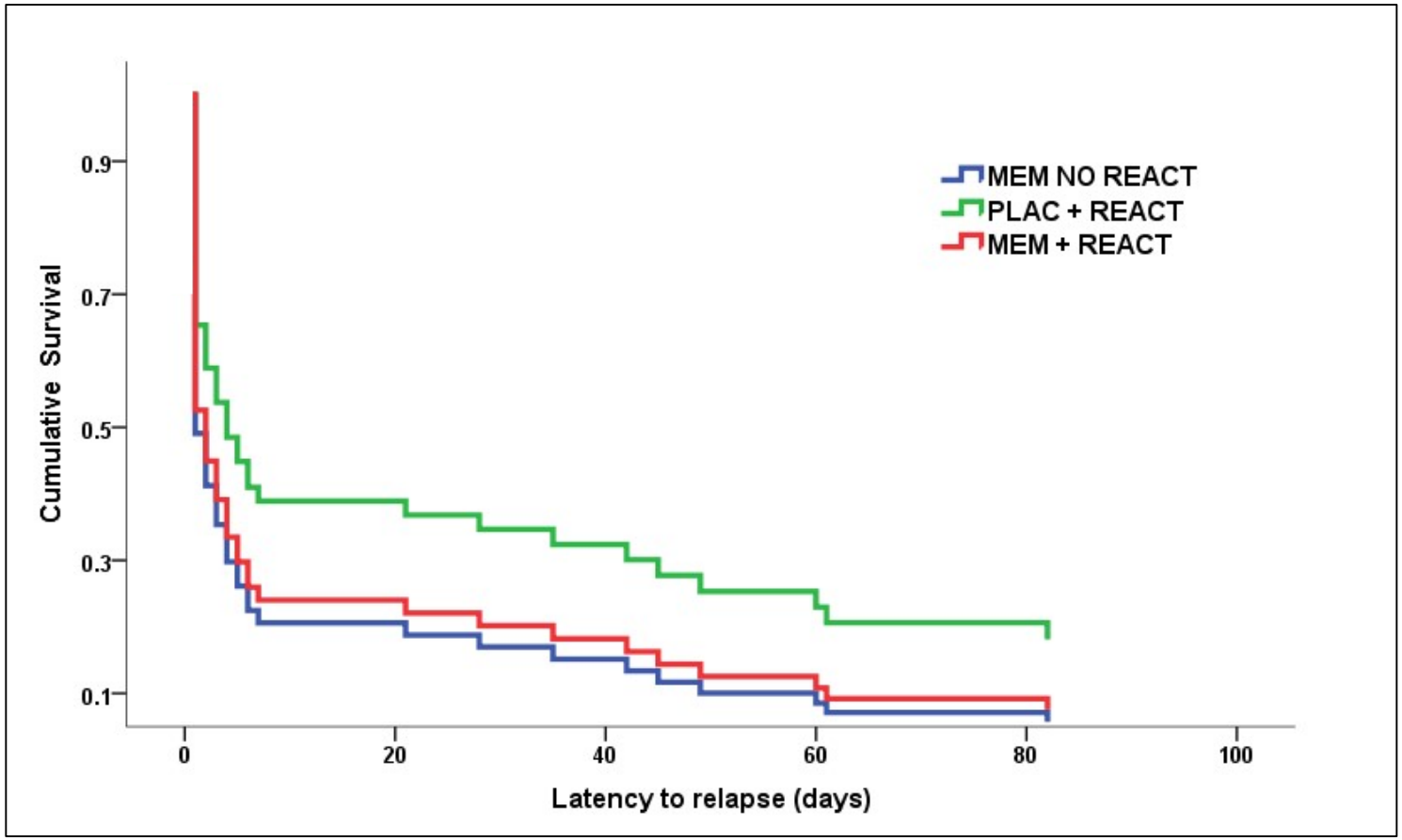
Group	MEM No REACT (N = 20)	PLAC+REACT (N = 20)	MEM+REACT (N = 19)
Day 8 N abstinent/still smoking	7/13	11/9	6/13
Day 8 CO (ppm)	6.45±6.38	6.8±6.12	8.21±4.65
Day 8 N smoking less/smoking as much	15/5	18/2	12/7
Pre Quit Cigarettes Per Day	14.2±4.27	14.45±3.33	14.53±3.2
Day 1-Day 8 cigs per day	4.13 ±4.48	3.2±3.93	6.66±6.3
Post Day 8 cigs per day	3.84±4.97	3.58±4.78	5.89±5
3 month cigs per day	7.91±6.45	8.55±7.0	10.44±5.45

3.3.3. Survival Analysis:

Cox regression analysis was used to assess binary coded relapse latency (relapsed at time N/ abstinent at time N) across the three groups. Participants' relapse latencies were censored if they were not smoking at the final follow-up time point available for that participant. A survival plot for each group is shown in *Figure 3.1*. Although the plot shows better absolute survival in the PLAC+REACT group, group was not significantly predictive of relapse latency [χ^2 (2) = 4.453, p = .109]. Curves are only plotted to 85 days as there was no change in relapse status up to 365 days in any participants who were abstinent at this time point.

Figure 3.1: Survival curves for relapse latency by experimental group.

Group did not significantly predict relapse latency. Records are truncated at 80 days due to no change from this point to 365 days.



3.3.4. Reactivity to smoking cues

A 2 (Day 1, Day 8) x 2 (pre-video, post-video) x 3(Group) mixed ANOVA found a decrease in craving in all Groups from Day 1 to Day 8 [Time main effect F(1,56) = 22.114, $p < 0.001$, $\eta_p^2 = 0.283$] and a trend for an increase in craving pre to post-video on both study days [pre-post main effect F(1,56) = 3.017, $p = 0.088$, $\eta_p^2 = 0.051$]. No effects of Group or interactions were found. Systolic and Diastolic blood pressure were assessed using mixed ANOVAs with factors identical to that for craving. No effects of the video, Group or study day were observed for systolic blood pressure (all *F*s < 2.3, *p*s > 0.1). No effects of video or study day were found for Diastolic blood pressure (all Fs < 1, *p*s > 0.45), but a main effect of Group was observed, with lower diastolic blood pressure in MEM no REACT than PLAC+ REACT and MEM + REACT [Group main effect F(2,56) = 3.728, $p = 0.03$, $\eta_p^2 = 0.117$].

Table 3.3. Descriptive statistics for measures of smoking cue reactivity. Data represent means ± SD

	MEM No REACT (N = 20)			PLAC+REACT (N = 20)			MEM+REACT (N = 19)		
	Pre	**Peri**	**Post**	**Pre**	**Peri**	**Post**	**Pre**	**Peri**	**Post**
SC Day 1	*3.16 ± 2.91*	*3.58 ± 3.62*	*4.5 ± 4.05*	*3.84± 2.16*	*4.70 ± 2.22*	*5.54 ± 2.68*	*4.47 ± 2.83*	*5.13 ± 3.18*	*6.05± 3.61*
SC Day 8	*3.57 ± 2.6*	*4.04 ± 3.116*	*4.85 ± 3.64*	*3.57 ± 1.86*	*3.6 ± 2.06*	*4.23 ± 2.53*	*4.27 ± 2.64*	*4.39 ± 2.53*	*5.42± 2.87*
HRV Day 1	*10.14 ± 6.57*	*6.86 ± 4.8*	*8.34 ± 4.34*	*6.87 ± 3.81*	*5.58 ± 4.05*	*7.46 ± 4.34*	*7.05 ± 3.75*	*6.08 ± 4.13*	*7.69± 4.35*
HRV Day 8	*9.53± 5.42*	*7.86 ± 5.39*	*10.64 ± 5.66*	*7.92 ± 4.18*	*7.25 ± 7.37*	*7.79 ± 5.57*	*8.17± 4.70*	*5.69 ± 3.70*	*6.71± 3.97*
Craving Day 1	*48.35 ± 14.87*	-	46.55 ± 22.75	*41.73± 27.93*	-	*49 ± 27.18*	*48.5 ± 25.77*	-	*53.7± 31.78*
Craving Day 8	*34.75 ± 25.95*	-	35.19 ± 24.64	*22.55± 17.47*	-	*25.8 ± 22.75*	*29.42± 26.75*	-	*39.37± 34.12*
Systole Day 1	*106.9 ± 11.9*	-	105.1 ± 9.35	*110.25± 15.21*	-	*108 ± 14.70*	*108.84± 13.12*	-	*109.9± 14.87*
Diastole Day 1	*65.25 ± 6.912*	-	66.4 ± 5.932	*71.3± 12.13*	-	*71.7 ± 10.87*	*70.736± 9.825*	-	*71.95± 10*
Systole Day 8	*103.2 ± 10.13*	-	101.55 ± 8.438	*109.35 ± 12.59*	-	*107.5 ± 13.52*	*109.47± 15.77*	-	*110.4± 15.42*
Diastole Day 8	*63.9 ± 8.12*	-	65.3 ± 4.47	*71.85 ± 10.25*	-	*70.45 ± 9.47*	*71.11 ± 11.13*	-	*71.37± 9.91*

A 3 (Time: pre-video, peri-video, post-video) x 2 (Day: day 1, Day 8) x 3 (Group) ANOVA on HRV data found a quadratic main effect of Time [$F(2, 112) = 11.925$, $p < 0.001$, $\eta_p^2 = 0.176$], with an overall reduction of HRV peri-video [pre vs. peri $t(58) = 4.262$, $p < 0.001$, $r = 0.49$; peri vs. post $t(58) = 3.938$, $p = 0.001$, $r = 0.46$]. A trend-level Time x Day x Group interaction was also found [$F(4,112) = 2.354$, $p = 0067$, $\eta_p^2 = 0.078$]. This interaction showed that the reduction of HRV from pre-to-peri video was significant only in the MEM NO REACT group [$t(58) = 2.917$, $p = 0.015$, $r = 0.36$].

Skin conductance data also showed a Time main effect [F(2,112) = 47.211, $p < 0.001$, $\eta_p^2 = 0.457$], with mean conductance increasing in a linear fashion from pre-to-peri [$t(58) = 4.01$, $p = 0.001$, r = 0.47] and peri-to-post video [$t(58) = 7.197$, $p < 0.001$, $r = 0.69$]. This was qualified by a Day x Time interaction [F(2,112) = 3.688, $p = 0.029$, $\eta_p^2 = 0.062$], with skin conductance rising across all time points but not pre-video to peri-video on Day 8 [$t(58) = 0.91$, $p = 0.429$, $r = 0.12$].

3.3.5. Visual Probe

Dwell times were assessed independently for 500ms trials and 2000ms trials, as dwell time the 2000ms trials may represent intentional inhibition of gaze to target images and is therefore of separate interest. 2 (Type: smoking pairs, neutral pairs) x 2 (Target: target image, control image) x 3 (Group) mixed ANOVAs were used to assess all eye tracking data.

Analysis of 500ms trials found main effects of Type [F(1,56) = 5.729, $p = 0.02$, $\eta_p^2 = 0.093$], Target [F(1,56) = 5.295, $p = 0.025$, $\eta_p^2 = 0.086$] and importantly a Type x Target [F(1,56) = 7.428, $p = 0.009$, $\eta_p^2 = 0.117$] and Type x Target x Group interaction [F(2,56) = 3.31, $p = 0.043$, $\eta_p^2 = 0.106$]. The Target x Type interaction confirmed the salience of the smoking images, as there was greater dwell time on the smoking target image in the smoking-control image pairs [$t(58) = 3.183$, $p = 0.002$, $r = 0.39$] but not in the neutral-neutral image pairs [$t(58) = 0.29$, n.s]. The Type x Target x Group interaction indicated greater attentional bias in MEM NO REACT than PLAC + REACT and MEM+REACT, evidenced by greater dwell time on the smoking target vs. control image in this group [t(58) = 3.846, $p < 0.001$, r = 0.45], but not MEM+REACT and PLAC+REACT [ts < 1, n.s.]

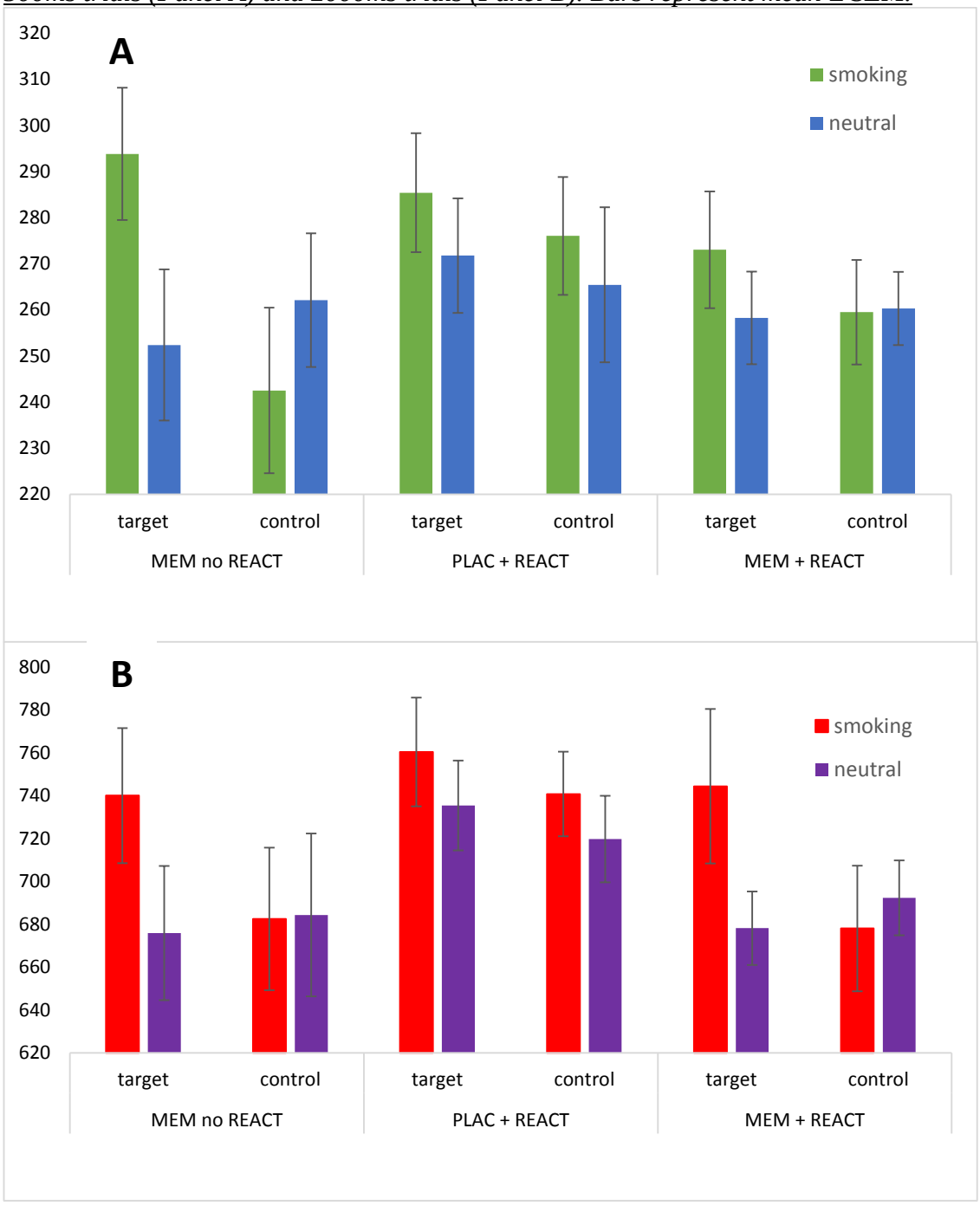
Analysis of the 2000ms dwell times found a main effect of Type [F(1,56) = 22.706, $p < 0.01$, $\eta_p^2 = 0.288$] and a borderline Type x Target interaction [F(1,56) = 3.891, $p =$

0.053, η_p^2= 0.065], indicating greater overall attention to image pairs containing a smoking image and, within these pairs, borderline longer looking at the smoking target image [t(58) = 2.01, p = 0.05, r = 0.25].

Analysis of first fixation times found a main effect of Target F(1,56) = 10.004, p = 0.003, η_p^2= 0.152] subsumed under a Target x Type interaction F(1,56) = 6.617, p = 0.013, η_p^2= 0.106]. The interaction was driven by more rapid fixations on smoking target images than control images [t(58) = 5.376, p < 0.001, r = 0.58], with no difference in initial fixation times in neutral-neutral pairs[t < 0.5, n.s.].

A trend for a Target x Type x Group interaction was found for first fixation durations F(2,56) = 2.908, p = 0.063, η_p^2 = 0.094]. In accordance with the 500ms dwell time data, this was driven by longer initial fixation on smoking images relative to control images in MEM NO REACT [t(58) = 2.982, p = 0.004, r = 0.36].

Figure 3.2: Dwell times during visual probe task on smoking and neutral images in 500ms trials (Panel A) and 2000ms trials (Panel B). Bars represent mean ± SEM.



3.3.6. EEfRT Task

Probability of choosing the hard task was calculated as per Treadway et al. (2009) and GLM analysis performed with a mixed ANOVA with a within-subjects factor of probability (12%, 50%, 88%), Day (Day 1, Day 8) and a between-subjects factor of

Group with probability of choosing hard as the dependent variable. In line with Treadway et al. (2009) a main effect of Probability was found [$F(1,56) = 105.98$, $p < 0.001$, $\eta_p^2 = 0.654$], with a significant linear increase in proportion of hard task choices as probability of win increased [12% vs 50% $t(58) = 7.966$, $p < 0.001$, $r = 0.72$; 50% vs 88% $t(58) = 8.344$, $p < 0.001$, $r = 0.74$]. No effects of Group [$F(2, 56) = 1.303$, $p = 0.28$, $\eta_p^2 = 0.044$] , Day [$F(1, 56) = 0.583$, $p = 0.448$, $\eta_p^2 = 0.001$] or interactions [all Fs < 1.7, ps > 0.15] were observed. We also found no correlation between performance on the EEfRT task measures of smoking abstinence or anhedonia as assessed by the TEPS and BDI [all rs < 0.2, ps > 0.1].

3.3.7. Drug guess

A chi square of group x participant's guess on drug (don't know, drug, placebo) found a significant effect of group [$\chi^2(4) = 11.74$, $p = 0.019$], with fewer participants in the MEM NO REACT group guessing that they received the drug than the other two groups. Ns per group were as follows : Guessing Drug MEM + REACT = 9, PLAC + REACT = 9, MEM NO REACT = 4; Guessing Placebo, MEM + REACT = 6, PLAC + REACT = 4, MEM n REACT = 7, Guessing Don't know MEM + REACT = 4, PLAC + REACT = 7, MEM NO REACT = 12.

3.4. Discussion

Employing a translational medicine paradigm, the current study assessed the potential of memantine as an adjunct to voluntarily quitting in cigarette smokers via inhibiting the reconsolidation of maladaptive cue-smoking memories. Ten milligrams of memantine in combination with smoking cue memory retrieval did not significantly impact smoking levels, latency to relapse, craving, cue salience or reactivity to smoking-related stimuli, indicating that memantine did not block the reconsolidation of retrieved cue-smoking MMMs.

Memantine, in the absence of reactivation, was associated with greater attentional bias to smoking cues at test. However, this group experienced higher craving prior to and after capsule treatment and lower belief in receiving the active drug. Correlations between craving and outcome measures suggest this may be responsible for observed group differences, that these differences are not reconsolidation-dependent and that the experimental manipulation did not affect smoking outcomes.

All groups reduced their smoking over the course of the study as measured by smoking diary and confirmed by CO levels. The high rates of short-latency relapse observed here are typical of smoking cessation and may have masked intervention effects by reducing power to assess long-term group differences. The physiological allostatic drivers of early relapse may be unaffected by memantine, as it fails to improve partial abstinence in reducing smokers (Thuerauf et al. 2007). The effects of successful MMM reconsolidation blockade also likely appear later in abstinence, when following homeostatic restoration, sensitised mnemonic reward systems play a more significant role in relapse.

Reconsolidation-blocking treatments may be best employed as relapse-preventing, rather than abstinence-promoting interventions (Milton and Everitt 2012), or may need

to be employed in combination with withdrawal management strategies such as nicotine replacement therapy. A repeat of the current study in smokers who have already achieved abstinence for a minimum period (one month, for instance) may therefore be warranted.

NMDAR antagonists have potential for reducing the potency of MMMs. Our failure to translate preclinical findings to human addicts is important for the future development of this field. Previously, attempts to translate preclinical memory-based SUD pharmacotherapies have persevered despite poor chances of achieving clinically relevant outcomes (Das and Kamboj 2012; Kamboj et al. 2012; Kamboj et al. 2011) and lack of a cohesive methodological framework, incurring substantial financial and research costs. This research highlights several priority areas of experimental refinement in response to the observed null results, which, whilst being mindful of clinical relevance, should take precedence in the advancement of this field.

As reconsolidation of drug memories is a 'silent' process, only inferred via interference during the reconsolidation window, an epistemological problem exists for null findings which may be attributable to a drug's inefficacy in interfering with restabilisation, or a lack of memory destabilisation during retrieval. In order to disentangle these, retrieval procedures that consistently destabilise MMMs and alternative compounds that effectively and consistently block restabilisation are required.

In animals, robust blockade of restabilisation of MMMs is achieved using compounds that interfere directly or upstream of neuronal protein synthesis or transcription. This action makes these compounds highly toxic and unsuitable for human use. To date no drug has shown reliable blockade of MMM reconsolidation in humans (Saladin et al. 2013). For safety and tolerability, memantine is an attractive NMDAR antagonist for use in the context of interfering with human MMM reconsolidation. However the

current findings do not support this application. Although the dose used here was low, memantine (Creeley et al. 2006) shares with other NMDAR antagonists (Das et al. 2013a) a complex, non-linear dose-response relationship in mnemonic function implying that optimal dosing is not simply a case of 'more-is-better.'

Memantine also has unique kinetic properties at the NMDAR (Black et al. 1996; Blanpied et al. 1997) which may be undesirable in the context of blocking memory restabilisation. In particular, it may not produce the sustained level of NMDAR blockade necessary for sufficient disruption in synaptic plasticity during the temporally limited reconsolidation window due to its relatively low affinity, rapid off-rate receptor kinetics (Rammes et al. 2008) and preference for extrasynaptic rather than synaptic NMDARs (Xia et al. 2010).

In contrast, MK-801 (Dizoclipine), the prototypical antagonist for reconsolidation blockade – is paradigmatic with regard to its selectivity, affinity, voltage-dependence and essential irreversibility of blockade during memory destabilisation. The dissociative and psychotomimetic effects are products of the same kinetic profile at NMDARs that cause robust interference with restabilisation, so these effects may be necessary when blocking MMM reconsolidation via NMDARs. While neurotoxicity precludes the use of MK 801 in humans, ketamine may be a realistic alternative. It is approved for human use despite its side effects and already shows some promise for the treatment of SUDs (Krupitsky and Grinenko 1997).

Oral memantine's slow peak plasma latency means it must be administered *prior* to memory retrieval in order to peak post-retrieval. As activation of GluN2b subunit-containing NMDARs is required for memory destabilisation at recall, prior antagonism can *reduce* the ability of memories to destabilise (Mamou et al. 2006) and it is possible that this occurred in the current study. Further, NMDAR blockade can engender

aberrant prediction error, potentially interfering with successful destabilisation or producing paradoxical effects on memory retention (Corlett et al. 2013). Dosing after retrieval would potentially allow restabilisation of memory before sufficient NMDAR blockade was achieved, reducing the efficacy of the intervention (Milton et al. 2008a; Wu et al. 2012). Ideally, NMDARs should be rapidly antagonised, with high receptor saturation, following memory destabilisation. This may preclude the use of oral preparations of NMDAergic drugs for this purpose and in humans and may require intravenous or intranasal dosing post-reactivation. If these formulations prove ineffective in reducing MMM strength, NMDAR antagonism may need to be abandoned as a pharmacological target in favour of alternative receptor pathways implicated in memory restabilisation (Blundell et al. 2008; Carrera et al. 2012; de Oliveira Alvares et al. 2008; Makkar et al. 2010). Identifying tolerated pharmacological means for consistently blocking MMM reconsolidation in humans will be key in moving this field forward.

An alternative explanation for these null findings is that memanatine *does* interfere with memory restabilisation, but that smoking MMMs were not effectively destabilised by the reactivation procedure used here. This procedure was designed in an attempt to improve the potential for memory destabilisation by presenting prototypical smoking cues and engendering uncertainty about reinforcement by telling participants they ‘may or may not be required to complete the task in the box’ (i.e. smoke) following the cue videos, while withholding reward. This is equivalent to the prototypical reminder without reinforcement in animal reconsolidation studies.

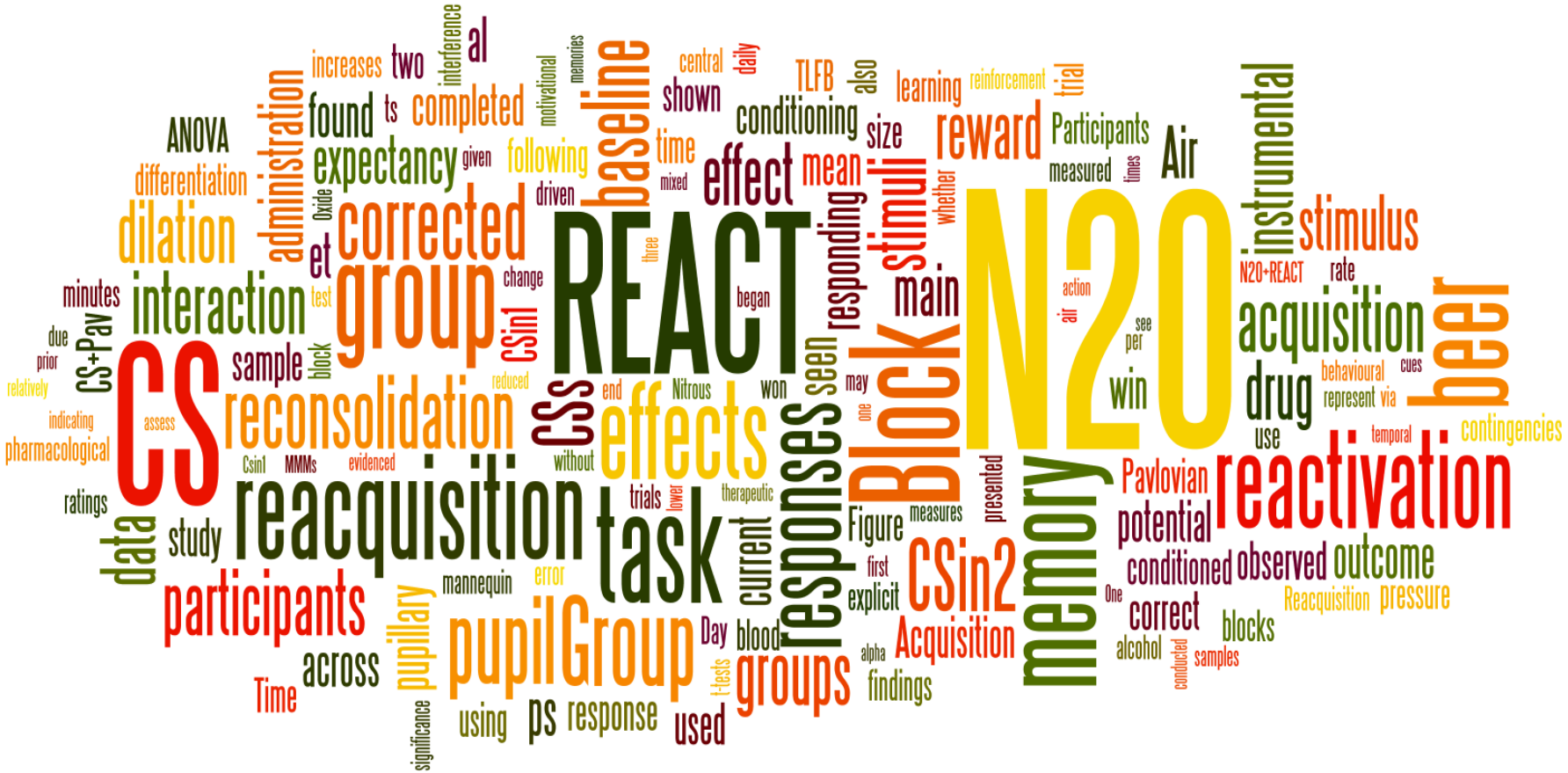
However, given their age and strength (Gräff et al., 2013; Robinson and Franklin, 2010), it is possible that the reminder structure did not destabilise smoking MMMs. It remains unknown whether the same reminder parameters that destabilise the relatively

recently-acquired, univariate memory traces studied in lab reconsolidation paradigms also successfully destabilise years-old human MMMs. This research suggests that they may not. This failure of MMMs to destabilise may be due to insufficient generation of prediction error at retrieval (Sevenster et al. 2013). As learning reaches asymptotic levels (as is the case with MMMs in smoking), trace flexibility decreases along with prediction error magnitude (Schultz et al. 1997). Knowing what constitutes a prediction error during retrieval of MMMs, where the learning history is unknown, represents a major challenge for this field. A fruitful approach may be to explicitly maximise the occurrence of prediction error through verbal instructions to participants, however this must be assessed experimentally.

Alternatively, it is possible that reconsolidation simply does not occur at any meaningful level for memories as strongly encoded as cue-smoking MMMs in daily smokers. Many researchers have identified the potential of reconsolidation interference for treating SUDs, however there is a notable paucity of human research directly assessing whether the laboratory findings are applicable to clinical populations. This research shows we next need to re-assess whether destabilisation of extremely robustly trained MMMs is possible and, if so, what retrieval procedures can reliably produce these effects.

In summary, this study found no evidence for 10mg memantine blocking the reconsolidation of cue-smoking memories on any measure of cue reactivity, craving, salience or relapse in quitting smokers. So while memantine in combination with memory reactivation does not appear to be a clinically useful strategy for smoking cessation given the current findings, methodological and epistemological issues must be addressed in reconsolidation research to allow the accurate assessment of the clinical potential of post-destabilisation interventions for SUDs.

Chapter 4: An Investigation of the Potential Use of Nitrous Oxide for Blocking Reconsolidation of Alcohol Memories in Heavy Drinkers



4.1 Introduction

Despite the theoretical clinical promise of pharmacologically weakening maladaptive motivational memories during their reconsolidation, my own (see *Chapter 3*) and others' findings with available 'reconsolidation blockers' in substance-using populations have so far produced disappointing outcomes. This inefficacy may be attributed to insufficient interference with the cellular signalling mechanisms responsible for restabilising memories. My meta-analysis suggests that limited effectiveness is particularly evident with β-adrenergic receptor (β-AR) antagonists.

Given the null findings with memantine in smokers (*Chapter 3)* and the potentially limited efficacy of β-blockers, there is a serious need for drug discovery for human MMM reconsolidation blockade. As a potent NMDAR antagonist, ketamine is one potential option, but its pharmacokinetics and psychoactive profile currently dictate medically supervised, in-patient administration. Less invasive alternatives are therefore needed but, as already discussed such NMDAergic drugs are lacking. One potential therapeutic agent in this context is Nitrous Oxide (N_2O). N_2O, also known as 'laughing gas' has been used as an obstetric and dental analgesic and anaesthetic as well as a recreational dissociative drug for over two hundred years (Goerig and Schulte am Esch 2001).

Humphry Davy was a famous proponent of the drug (Davy 1800) which is still widely used today owing to its ease of administration, rapid onset/offset kinetics and excellent safety profile. Despite its recreational and medical popularity, the mechanism of action of N_2O remained elusive until relatively recently when it was found to act upon GABAergic and NMDAergic neurotransmission. In vitro, N_2O reduces NMDAR transmission by 31% (Yamakura and Harris 2000). In rats, N_2O has now been shown to

operate primarily through NMDA antagonism (Jevtović-Todorović et al. 1998; Jevtovic-Todorovic et al. 2001).

Likely due to its NMDAergic action, N_2O also acts as a dose-dependent amnestic with mild memory impairment at 30% N_2O in air (Dunlosky et al. 1998) and an inability of humans to encode new information at concentrations of 40% N_2O in air (Robson et al. 1960). This pharmacological profile, along with its ease and safety of administration, make N_2O an attractive compound for interfering with memory reconsolidation. However its ability to do so has never been tested. Prior to trialling a drug of unknown efficacy, off-label, for reconsolidation blockade in a clinical population, proof-of-principle research into its efficacy is both prudent and important.

Owing to the null results of *Chapter 3* the current study sought to test whether N_2O could block the reconsolidation of human associative reward memory. To do so, a more sensitive experimental approach was used than in *Chapter 3*, using in-lab reward conditioning to assess the effects of N_2O in combination with retrieval of conditioned memories. This approach has several important advantages when testing a compound as a reconsolidation blocker for the first time: 1) Control over conditioning histories, allowing a guarantee of prediction error at retrieval; 2) Ability to incorporate experimental biomarkers of efficacy (e.g. pupil dilation to in-lab conditioned stimuli) that would not be possible in a more clinical study; 3) Excellent cost- and time-effectiveness when considering the uncertainty of translation following my results in *Chapter 3;* 4) Greater certainty in null results, as the memory traces being targeted are not naturalistic and heterogeneous.

To maintain the clinical relevance of the experiment, an alcohol-reinforced Pavlovian and instrumental conditioning task was therefore utilised with a sample of hazardous beer drinkers who could win beer reward through effective performance in the task. The

experiment followed a typical experimental memory reconsolidation paradigm (*Figure 1.4* and accompanying text), with acquisition of reward contingencies during session one, reactivation or no reactivation with drug or placebo during session two and test of memory retention during session 3 taking the form of a reacquisition task. The inclusion of Pavlovian and instrumental elements in the conditioning task were to allow direct comparison of drug effects on the two types of memory, something that has been lacking in previous research. A simple, 100% reinforcement schedule was employed between conditioned stimuli and their respective outcomes. While this is not necessarily representative of real-world reinforcement contingencies, it allows a prediction error to be generated at retrieval from a single unreinforced presentation of a CS.

The task was designed such that pupil dilation could be reliably measured during task performance, as pupil size is a reliable measure of outcome certainty (Preuschoff et al. 2011), reward prediction and dopaminergic function (O'Doherty et al. 2003) and autonomic arousal. It is therefore the ideal measure for assessing the strength of reward memory associations. It was hypothesised that N_2O administration after reactivation of learned information would reduce pupil dilation to conditioned stimuli at test and, more tentatively, impair correct responding to these stimuli.

4.2 Methods

4.2.1. Participants and Design

The power calculation was as per *Chapter 3*. Fifty-nine hazardous beer drinkers were recruited from University College London using internal study advertisement networks and convenience sampling from the local community. Participants were randomly allocated to one of three groups: those who would receive N_2O after reactivation of the conditioning task (N_2O + REACT, N = 20), those who would receive it without reactivation of the task memory (N_2O no REACT, N = 22) and those would receive normal air after reactivation of both tasks (Air + REACT Group, N = 17). Treatment administration was single-blind (participants, but not experimenter were blinded). Differing Ns per group were due to drop-outs from initial randomisation and experimenter error in re-randomising replacement participants.

Inclusion criteria were: current hazardous drinking defined as a score of 8 or more on the Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al. 1993); consumption of more than twice the daily governmental allowance of alcohol (i.e.> 3 units for females, > 4 units for males) on at least four days per week; fluent English and normal or corrected-to-normal colour vision.

Exclusion criteria were past or current diagnosis of drug or alcohol use disorders as determined by endorsement of three or more items coded as '3' on the Structured Clinical Interview (SCID) of the DSM IV (First, Spitzer, Gibbon, & Williams, 2002); current mental health issues requiring treatment; any current major physical health issue; current pregnancy or breastfeeding, regular (>4 times per month) recreational use of N_2O or other NMDA antagonists, vitamin B12 deficiency (owing to N_2O effects on B12 metabolism) and pneumothorax. Participants were reimbursed at the rate of £7.50

per hour. All procedures were approved by the University College London research ethics committee.

4.2.2. Apparatus and Tasks

Subjective Assessments:

The digit span forward and backward assessed baseline working memory capacity (Baddeley 1992). Alcohol use assessments were the timeline follow-back (TLFB; Sobell and Sobell 1992), stages of change readiness and treatment eagerness scale (SOCRATES) (Miller and Tonigan 1996).The behavioural inhibition/behavioural activation scale (BIS/BAS) was used to assess trait reward responsiveness (Carver and White 1994).

The acute subjective effects of nitrous oxide were measured with the Clinician Administered Dissociate States Scale (CADSS) (Bremner et al. 1998) and Bodily Symptoms Scale (BSS)(Bond and Lader 1974).

Beer Conditioning Task:

The task consisted of three phases: Acquisition, Reactivation and Reacquisition, performed on sessions 1, 2 and 3 of the study respectively. A schematic of the acquisition phase is given in *Figure 4.1*.

Acquisition

During acquisition, participants were informed that they were able to win beer during the task by learning the association between shapes appearing on-screen, responses and outcomes and that they would consume this beer at the end of the task. Four black and white simple shapes (a triangle, star, plus sign and circle) were used as the conditioned stimuli (CSs) in the task. All stimuli were presented centrally on a 21 inch VGA colour

monitor with a static red (RGB = 200, 0, 0) background for 4000ms per trial. The stimuli were luminance-matched to prevent non-specific effects on pupillary responses. One stimulus was designated as a Pavlovian CS+ (*CS+Pav*), such that it was rewarded with 10ml beer without participants making any response. Two stimuli were designated as instrumental CS+s. For both of these a mannequin figure would appear above or below the stimulus in a counterbalanced fashion and participants could move the mannequin towards or away from the central stimulus using designated 'up' and 'down' keys. For *CSin1*, participants were rewarded with 10ml beer if they moved he mannequin *towards* the central stimulus. For *CSin2*, participants were rewarded 10ml beer if they moved the mannequin *away* from the central stimulus. The final stimulus was designated the CS- and was never rewarded. If participants made the correct response, or were shown the *CS+Pav*, they would see an outcome screen informing them they had won 10ml beer. If they made the incorrect response or were shown the CS-, they saw an outcome screen informing them they had won nothing. Outcome screens were displayed for 3000ms. Following the outcome screen, a running total of the amount of beer won so far was displayed for 2000ms. All CS/outcome contingencies were on a 100% reinforcement schedule.

A schematic of typical Pavlovian and Instrumental trials is given in *Figure 4.1*. As eye movements affect pupil dilation, participants were asked to maintain their gaze upon a central fixation spot throughout the task. Each trial began with experimenter-verified drift correction to ensure central fixation. The CS then appeared, followed by outcome and running total. So that participants could practice moving the mannequin without looking away from the central fixation, a baseline practice block began the task, where participants moved the mannequin first toward, then away from all stimuli. Prior to beginning acquisition, participants rated how much they thought each shape was

associated with winning beer from 0-‘definitely will not win beer’ to 10 ‘definitely will win beer’.

Acquisition was split into four blocks, each block consisting of four presentations of each stimulus, pseuorandomised with the stipulation that no more than two consecutive presentations of each stimulus could occur. At the end of the first and second halves of acquisition, outcome expectancies were re-rated on the same scale used at baseline.

Reactivation

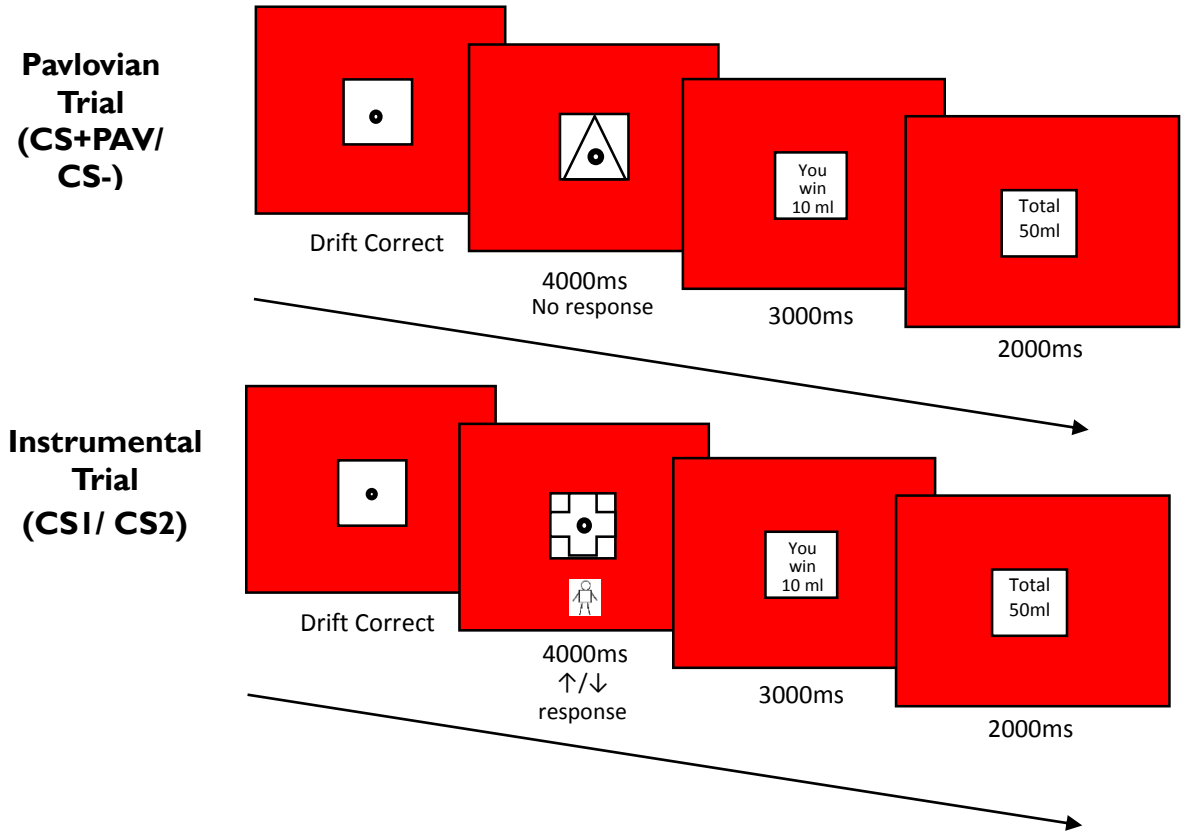
At the beginning of the reactivation task, participants were told they would resume the task from day one in which they could win beer and that they would again consume the won beer at the end of the task. They were explicitly told that the contingencies between the stimuli and outcomes were exactly the same as previously. They then re-rated all stimuli for explicit outcome contingency from 0-‘definitely will not win beer’ to 10 ‘definitely will win beer’. Following this the task proper began. Each stimulus conditioned on Day 1 was only presented *once* in a randomised order. For *CSin1* and *CSin2* the mannequin appeared and could be moved, however following 4000ms of stimulus presentation, the stimuli disappeared and no feedback was presented. Due to the 100% reinforcement schedule during acquisition, this lack of feedback and reinforcement forced a negative prediction error, which is necessary for memory destabilisation. During reactivation, all stimuli were presented on a laptop with a 15 inch screen with identical stimuli and background luminance to those used in the Acquisition phase.

Reacquisition

This task was identical to the first half of *Acquisition*, with the following exceptions. First, there was no practice block at the start of the task; the task began with the explicit

outcome contingency rating for each stimulus. Second, in addition to the trial randomisation stipulation of no more than two consecutive presentations of a given stimulus, each stimulus was presented once in each four trials so that reacquisition rates were comparable across stimuli. Each stimulus was presented eight times and the task finished with a final explicit contingency rating.

Figure 4.1*: Example of a Pavlovian and an instrumental trial in the beer conditioning task. Although 'win' trials are shown here, the feedback could also show 'you win nothing' if the incorrect instrumental response is made or CS- is presented.*



Physiological Measures:

During acquisition and reacquisition of the beer conditioning task, pupil dilation was measured at 1000Hz with an Eyelink 1000 desktop-mounted infrared eye tracker (SR Research, Ontario, Canada). Blinks were detected using the manufacturer's algorithms on default settings. Where pupil data was unavailable due to blinks, the fifty samples prior to and after the blink were discarded and linear interpolation used to fill in the

missing sample data. Pupillometry data were exported then smoothed, epoched and down-sampled to 100Hz using custom-written scripts in Matlab (Mathworks, Boston, MA).

Systolic/diastolic blood pressure was measured via an Omron 708BT electronic blood pressure cuff (Omron, Tokyo, Japan). Blood alcohol content measurements were collected with a Lion 500 portable Alcometer (Lion Instruments, UK). Heart rate was measured using a pulse oximeter (HeartMath UK, London, UK) attached to the participant's earlobe.

4.3.3. Procedure

The experimental sessions occurred in two study centres. Sessions one and three were conducted in an experimental psychology unit in an academic centre. Session two was conducted in a medical setting where N_2O could be safely administered.

Session 1

Upon arrival at the study centre, all participants were breathalysed and if Blood Alcohol Concentration was below 0.001 ng/dl, completed written informed consent. One participant was excluded for having a BAC over the cut-off. Participants then completed an immediate prose recall and digit span. Following this, the TLFB for the previous two weeks, SOCRATES and BIS/BAS, were completed prior to the acquisition phase of the beer conditioning task. After acquisition, the experimenter measured the millilitres of beer won in the task into a chilled pint glass. Participants then had ten minutes to drink as much of the beer as they wanted. The beer was chilled Stella Artois. This completed Day 1 testing.

Session 2 (Day 1 + 48 hours)

Participants attended the medical centre where their BAC was again confirmed via breathalyser prior to testing. The correctly sized face mask for N_2O administration was then determined to minimise time between reactivation and administration of N_2O or air placebo. The pulse oximeter was then attached to record baseline heart rate and participants completed the BSS and CADSS baseline measures followed by a baseline blood pressure reading. Participants then completed the 48 hour delayed prose recall. Participants in the N_2O + REACT and N_2O no REACT groups then completed a short filler N-back task so that the number and timing of tasks and cognitive load was consistent across groups during the session. Immediately after this, the N_2O + REACT group completed the beer conditioning reactivation and N_2O no REACT group completed a no-reactivation control task (a word-pair learning task). The Air + REACT group completed both the control task and beer conditioning reactivation tasks in a counterbalanced order.

Drugs

Drug or air (placebo) administration began as quickly after the reactivation tasks as possible. Drug was a gaseous solution of 45% N_2O and 55% Oxygen. This was filled into 100 litre administration bags from regulated canisters of N_2O and O_2 (British Oxygen Supplies, UK) prior to participant arrival to avoid potential hazards of pressurised gas inhalation. A one-way pressure valve attached to a hose was connected to the face mask so that exhaled air could not re-enter the administration bag and dilute its contents. In both drug and placebo conditions, the hose was attached to the valve sealing the full administration bag. In the drug groups, the valve was then opened so that the participant began breathing the contents of the bag, whereas in the placebo group, the valve was left closed so that participants continued to breathe normal air through the hose. The bag was placed out of sight so that the participants could not see

the bag volume decreasing or remaining the same. In all conditions, participants were equilibrated for five minutes to attain a standard blood concentration of 45% N_2O. The CADSS, BSS and blood pressure reading were completed 10 minutes after onset of drug administration. Following equilibration, participants were maintained on N_2O or air for a further 15 minutes (20 minutes total administration time) after which time, the mask was removed, participants had five minutes re-equilibration time and a final blood pressure reading was taken.

Session 3 (Day 1 + 96 hours)

Participants returned to the original study centre and after providing a BAC reading, completed the TLFB, beer winning task reacquisition phase and free consumption of the beer they had won. Following this, participants were fully debriefed and reimbursed according to the hourly rate. This concluded the testing sessions.

4.3.4. Statistical approach:

With the exception of sample-by-sample pupillometry data which were analysed with custom routines written in Microsoft Excel and Matlab, all data were analysed in IBM Statistical Package for the Social Sciences v. 21 for Windows. Data were analysed using the General Linear Model with between and within-subjects factors as appropriate. Where the assumptions of homogeneity of variance and normality of error distribution were violated in t-tests and one-way ANOVA, unequal variances t-tests and Welch's ANOVA were used with bootstrapped parameter estimates, respectively. Where sphericity was violated in repeated measures, the Huynh-Feldt correction was used. Uncorrected degrees of freedom are reported in this case for ease of interpretation, but *p*-values represent post-correction significance levels. For multiple comparisons

following omnibus tests, the Bonferroni correction was applied to maintain alpha at 0.05. For baseline self-report and demographic variables, alpha was set at 0.01 to reduce Type I error and avoid unacceptably low power. To examine differences in the temporal dynamics of pupil responses across the different CSs, paired samples t-tests were conducted on a sample-by-sample basis. These data were sampled at 100 Hz, resulting in 400 samples in the 4 seconds of CS presentation. A false discovery rate (FDR) correction (Benjamini and Hochberg 1995) was applied to the sorted significance levels of these analyses to control Type I error rate at 0.05.

To remove the effects of non-specific, slow-changing pupil size variation across trials during conditioning and reacquisition, pupil dilation for each trial is expressed as a proportion change from the pupil size during the first sample of each trial (i.e. pupil size at trial onset) such that, in every trial for sample 1 to 400, pupil dilation n = (pupil size at sample n – pupil size at sample 1)/pupil size at sample 1. The mean of this proportional increase in each trial was used as the outcome measure of pupillary responses to assess learning effects on pupil dilation (O'Doherty et al. 2003).

4.3 Results

4.3.1. Baseline self-report and demographic

Descriptive statistics for baseline alcohol use measures, BIS/BAS and working memory are given in *Table 4.1*. One way ANOVAs conducted on these data found no significant group differences in any of these measures at the lower alpha = 0.01. Therefore the groups did not differ on any trait or alcohol use measures relevant to the study.

***Table 4.1**: Descriptive statistics for baseline variables. Where within-group variance was heterogeneous, Welch's ANOVA was used and this is denoted by F_W. Due to the large number of ANOVAs conducted, a more conservative alpha of 0.01 was adopted. Values are mean ± SD*

	N_2O no REACT (n = 20)	N_2O + REACT (n = 22)	Air + REACT (n = 17)	ANOVA (df = 2,56)
Age	23.27±3.57	24.2±2.85	23.35±4.82	F = 0.372, p = 0.690
Yeas Education	16.61±2.29	17.6±2.08	16.76±1.71	F = 1.327, p = 0.273
BMI	22.29±2.89	24.28±2.84	22.18±2.03	F = 3.884, p = 0.026
AUDIT	12.27±3.84	11.9±2.9	13.53±3.81	F = 1.050, p = 0.356
SCID	1.41±1.09	0.75±0.96	1±1	F = 2.209, p = 0.119
Last alcohol drink (hours ago)	29.86±14.93	27.5±14.72	32.68±14.40	F = 0.568, p = 0.569
Drinking days per month	14.64±4.95	12.36±6.45	16.03±4.52	F = 1.946, p = 0.153
Drinks per session	6.28±2.79	7.13±2.54	8.03±2.94	F = 1.689, p = 0.195
TLFB daily beer baseline	1.65±0.62	1.61±1.18	1.90±0.92	F = 0.512, p = 0.601
TLFB daily wine baseline	0.39±0.734	0.25±0.29	0.45±0.49	F = 0.886, p = 0.417
TLFB daily spirits baseline	0.49±0.54	0.54±0.64	1.08±0.88	F = 1.65, p = 0.201
TLFB daily beer post	1.61±0.61	1.53±1.32	1.87±1.00	F = 0.556, p = 0.576
TLFB daily wine post	0.28±0.48	0.30±0.48	0.47±0.60	F = 0.684, p = 0.508
TLFB daily spirits post	0.42±0.43	0.45±0.48	0.75±0.67	F = 2.143, p = 0.126
SOCRATES ambivalence	9.68±3.10	7.75±2.97	9.29±1.99	F = 1.241, p = 0..297
SOCRATES recognition	12.59±2.98	10.2±2.66	12.29±2.97	F = 0.452, p = 0.639
SOCRATES taking steps	18.13±6.10	13.4±4.04	17.64±5.23	F = 1.33, p = 0.273
BAS drive	10.63±1.98	11.1±2.71	9.52±3.14	F = 1.732, p = 0.186
BAS fun	13.09±2.09	13.3±1.83	12.52±3.60	F = 0.441, p = 0.645
BAS reward	16.77±1.90	17.15±1.69	15.82±4.82	F_w = 0.675, p= 0.516
BIS	20±3.20	20.9±3.52	18.58±7.02	F_w = 0.864, p =0.431
Digit Span forward	6.72±1.80	5.85±2.18	5.23±1.64	F = 3.053, p = 0.055
Digit Span Backward	5.90±1.30	5.9±1.29	5.52±1.17	F = 0.531, p = 0.590

4.3.2. Beer Conditioning Task

For acquisition and reacquisition data, in order to assess changes across time while constraining degrees of freedom in statistical tests, trials were split into four equal 'blocks' for each CS for both acquisition and reacquisition.

Contingency awareness

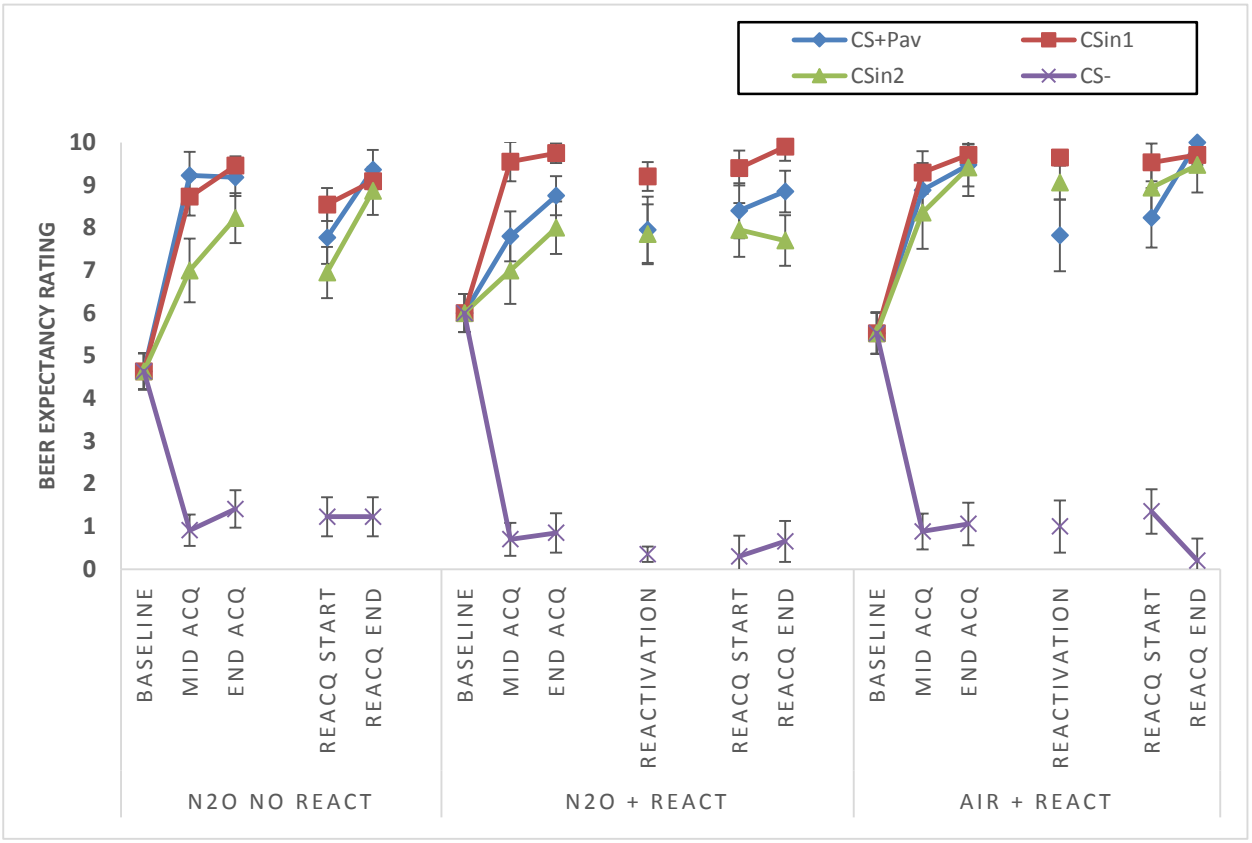
Outcome expectancy ratings across Acquisition, Reactivation and Reacquisition are shown in *Figure 4.2*. A 4 (CS type) x 3 (time: Baseline, Middle & End ratings) x 3 (Group) mixed ANOVA indicated successful explicit acquisition of outcome contingencies, evidenced by main effects of CS [$F(3, 168) = 153.107$, $p < 0.001$, $\eta_p^2 = 0.732$] and Time [$F(2, 112) = 14.927$, $p < 0.001$, $\eta_p^2 = 0.210$], subsumed under a CS x Time interaction [$F(6, 336) = 53.528$, $p < 0.001$, $\eta_p^2 = 0.489$]. This interaction represented significant *increases* in beer reinforcement expectancy from baseline to the later time points for *CS+Pav, CSin1* and *CSin2* [all *t values* (58) > 3.833, *p value*s < 0.0015, *r*s > 0.44, corrected], with no change from mid-acquisition onwards for *CS+Pav* or *CSin1* [all *ts* (58) < 2, *p*s > 0.44, corrected], but a borderline-significant increase from mid-to end acquisition for *CSin2* [$t(58) = 2.466$, $p = 0.05$, $r = 0.308$, corrected] a significant *decrease* in reinforcement expectancy for the CS- from baseline to the subsequent time points [all *ts* (58)> 11.67, *p*s < 0.001, *r*s > 0.83 corrected], again with no change from mid-acquisition until the end phase [all *ts* (58) < 2, *p*s > 0.95, corrected]. No main effects or interaction with Group were found.

For reacquisition, retention of contingencies was evidenced by a main effect of CS [$F(3,168) = 340.452$, $p < 0.001$, $\eta_p^2 = 0.859$], with lower reward expectancy for CS- than all other CSs [*ts*(58) > 20.77, *r*s > 0.93] and greater expectancy for *CSin1* than *CSin2* [$t(58) = 3.51$, $r = 0.42$]. Further reacquisition of contingencies was shown by a main

effect of Time [F(1,56) = 6.046, p = 0.017, η_p^2 = 0.097] and CS x Time interaction [F(3,168) = 3.545, p = 0.025, η_p^2= 0.06]. The interaction was driven by increases in expectancy ratings across time for *CS+Pav* [t(58) = 2.858, p = 0.006, r = 0.351, corrected] and *CSin2* [t(58) = 2.467, p = 0.017, r = 0.308, corrected] only. No main effect of Group [F(2,56) = 1.34, p = 0.27, η_p^2 = 0.046] or interactions with Group (all ps > 0.1) were observed.

Exploratory post-hoc analysis of reacquisition by Group found significant increases in expectancy ratings for all CSs except CS- in **N_2O no REACT** [all *ts* (21) > 2.01, ps < 0.047, rs > 0.4, corrected], with no increases in expectancy of any stimuli in the **N_2O + REACT** group [all *ts*(19)<1.8, ps> 0.08, corrected] and an increase only in *CS+Pav* expectancy in the **Air + REACT** Group [t(16) = 2.147, p = 0.036, r = 0.47, corrected]. CS rating data from reactivation showed a main effect of CS [F(3, 105) = 115.438, p< 0.001, η_p^2 = 0.767], driven by higher expectancy ratings for all CSs than the CS- (all ts (38) > 11.35, ps < 0.001, rs > 0.87, corrected) and for *CSin1* than *Csin2* [t(37) = 2.836, p = 0.045, r =0.41, corrected].

Figure 4.2. Changes in explicit outcome expectancy ratings through Acquisition, Reactivation and Reacquisition. Note there are no data for the ***N_2O no REACT*** *group for Reactivation, as they did not have the task reactivated. Bars represent mean ±SEM*

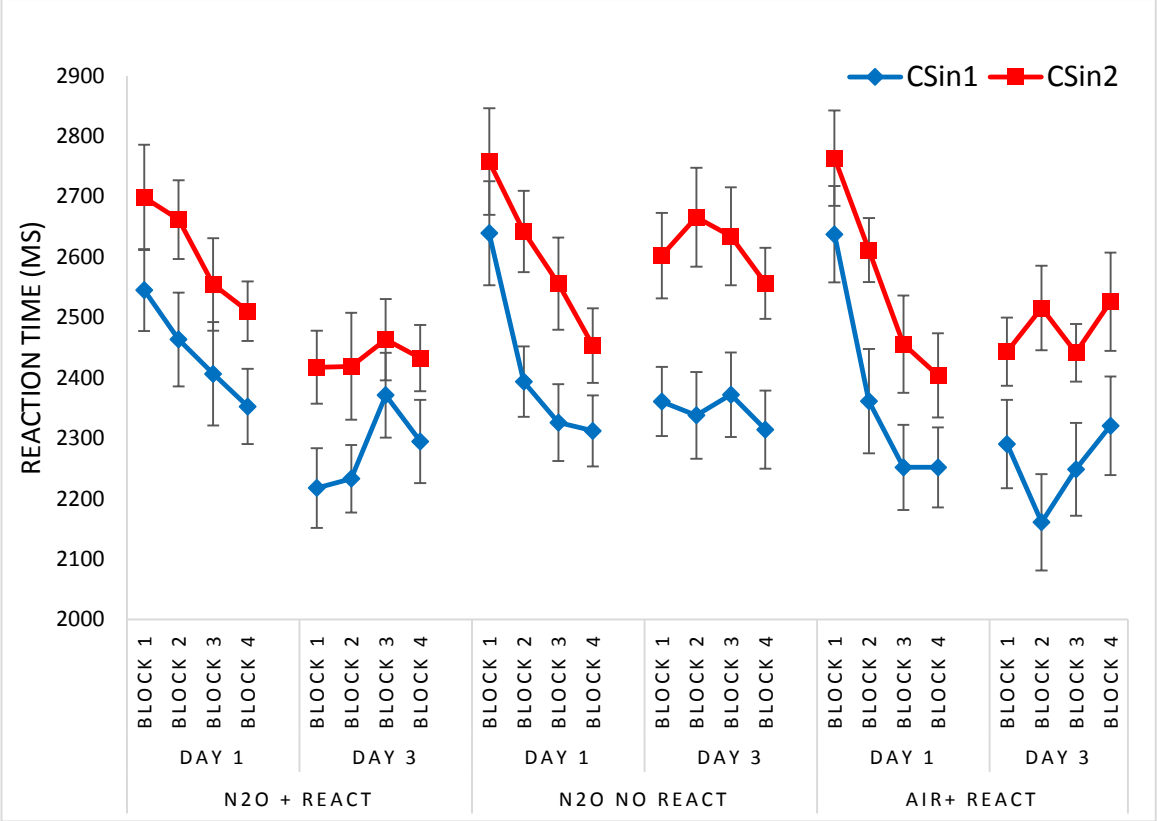


Reaction times for Instrumental stimuli

Reaction times to *CSin1* and *CSin2* during Acquisition were analysed with a 2(CS) x 4(Block) x 3 (Group) ANOVA. Conditioning was evidenced by a main effect of Block $F(3, 168) = 35.151$, $p < 0.001$, $\eta_p^2 = 0.386$], with reaction times to both CSs reducing significantly across blocks 1, 2 and 3 (all *p*s < 0.001) but not between blocks 3 and 4 [$t(58) = 1.78$, $p = 0.482$], indicating ceiling-level instrumental responding by the end of Block 3. A main effect of CS was also observed [$F(1, 56) = 41.438$, $p < 0.001$, $\eta_p^2 = 0.425$] , indicating significantly faster RTs for *CSin1* (approach-to-win) than *CSin2* (avoid-to-win). No group effects or interactions were observed (see *Figure 4.3.* for reaction time data). RTs to CSs during reacquisition were assessed via an identical

ANOVA to Acquisition. This showed a main effect of CS only [F(1, 56) = 83.016, p< 0.001, η^2_p = 0.597], with faster RTs to *Csin1* than *CSin2*. Again, no effects of Group or interactions were found (all ps > 0.12).

Figure 4.3: Reaction times to instrumental stimuli across acquisition and reacquisition. Bars represent mean ± SEM.



Accuracy

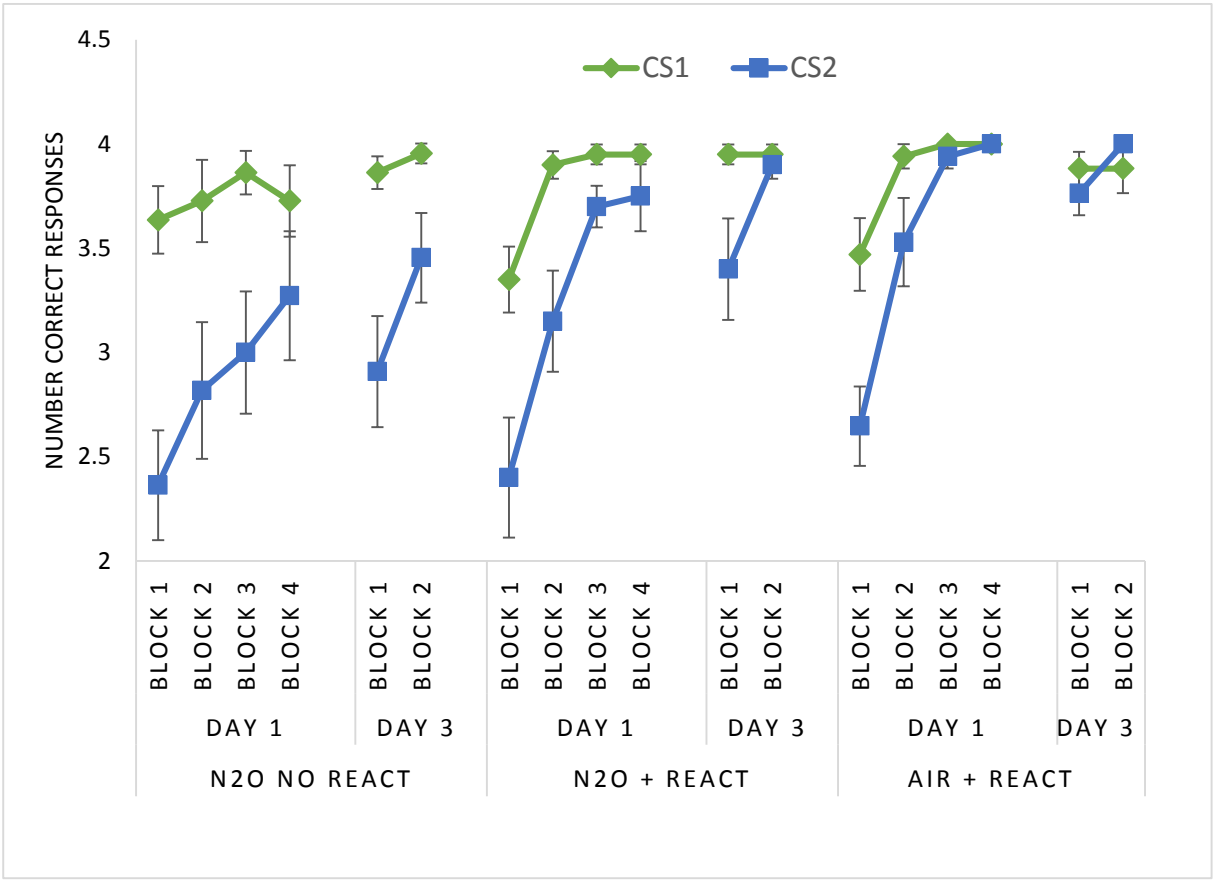
Accuracy data are represented in Figure 4.4. Acquisition of correct responses was assessed with a 2 (*CSin1*, *CSin2*) x 4 (Block) x 3 (Group) mixed ANOVA. Main effects of CS [F(1, 56) = 16.581, p< 0.001, η_p^2=0.228], and Block [F(3, 168) = 45.803, p< 0.001, η_p^2=0.45] were found, subsumed under a Block x CS interaction [F(3, 168) = 10.253, p< 0.001, η_p^2=0.155]. This was driven by more correct responses to *Csin1* (approach to win) than *CSin2* (avoid to win) in the first two blocks (both p< 0.001), but no difference in correct responding in the final two blocks of acquisition (ps >0.58),

consistent with pre-potent Pavlovian approach-to-win biases for potential reward stimuli retarding learning rate (Guitart-Masip et al. 2012).

Note that there are only two 'blocks' of accuracy data for *Day* 3, as accuracy is the number of instrumental trials correctly responded to and there are twice as many trials overall on *Day 1* as *Day 3*. Splitting accuracy data into four blocks (each representing only two trials) resulted in single incorrect responses exerting too much influence over means and artificially decreasing the apparent accuracy on *Day 3.*These were therefore split into two blocks of four trials, to allow comparison across days.

Analysis of Reacquisition accuracy data again found main effects of CS [$F(1,56) = 14.165$, $p< 0.001$, $\eta_p^2=0.202$] and Block $F(1, 56) = 11.541$, $p =0.001$, $\eta_p^2=0.171$] plus CS x Group [$F(2, 56) = 3.425$, $p = 0.039$, $\eta_p^2=0.109$], and CS x Block interactions [$F(1, 56) = 10.941$, $p = 0.002$, $\eta_p^2=0.163$]. The CS x Group interaction was driven by lower accuracy in responding to *CSin2* compared to *Csin1* in **N_2O + REACT** [$t(19) = 3.013$, $p = 0.004$, corrected] and **N_2O no REACT** groups [$t(21) = 3.757$, $p < 0.001$, corrected], but not the **Air + REACT** group where correct responding was equally high for both ($p > 0.99$), reflecting a practice and memory strengthening effect of the retrieval session (Karpicke and Roediger 2008; Karpicke and Roediger III 2007). The CS x Block interaction indicated an increase in response accuracy across blocks for *Csin2* only [$t(58)= 3.619$, $p = 0.001$, $r = 0.429$].

Figure 4.4. Accuracy of instrumental responding across acquisition and reacquisition. Date represent means ± SEM.



4.3.3. Pupillometry Data

Acquisition

4(CS) x 5 (Baseline, Block 1 - 4) x 3 (Group) mixed ANOVA found differential conditioning of pupillary responses to CSs evidenced by main effects of CS [$F(3, 168) = 92.604$, $p < 0.001$, $\eta^2_p = 0.623$], Block [$F(4, 224) = 10.774$, $p < 0.001$, $\eta_p^2 = 0.161$] and a CS x Block interaction [$F(12, 672) = 7.833$, $p < 0.001$, $\eta^2_p = 0.123$]. The interaction represented statistically equivalent pupillary responses to all CSs at baseline [all *p*s > 0.09, corrected], but greater pupil dilation for instrumental CSs than *CS+Pav* and *CS-* from *Block 1* onwards [all *t*s (58) > 8.5, ps < 0.001, corrected], with no differences between *CS+Pav* and *CS-* (all ps> 0.9) or *CSin1* and *CSin2* (all *ps* > 0.33). No main effects or interactions with Group were found, indicating equivalent conditioning of pupillary responses across groups.

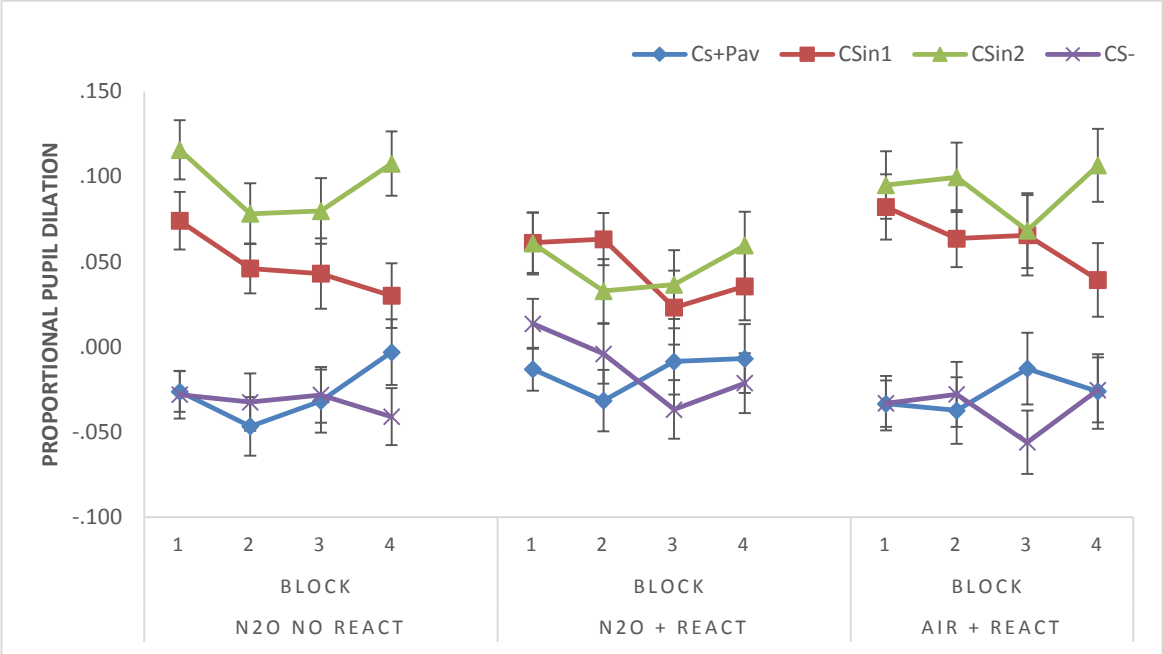
Participant-averaged temporal dynamics of pupil dilation in each block of trials during acquisition is shown in *Figure 4.4 panel A*. To further investigate temporally-dependent differentiation of responses to the *CS+Pav* and *CS-* and between *Csin1* and *CSin2*, two-tailed t-tests were employed on a sample-by sample basis using Benjamini and Hochberg's (1995) False Discovery Rate (FDR) correction with an alpha of 0.05. This analysis showed differentiation of waveforms for *CS+Pav* and *CS-* in *Block 2* of acquisition, with greater pupillary contraction to *CS+Pav* relative to *CS-* (*p* FDR < 0.05 from sample 182 to 400, i.e. 1.82 to 4s). This likely indicates the parasympathetic nervous effect of reward expectation without the requirement to respond.

Reacquisition

A 4(CS) x 4(Block 1 to 4) x 3 (Group) mixed ANOVA found main effects of CS [$F(3, 168) = 58.51$, $p < 0.001$, $\eta^2_p = 0.476$], Block [$F(3, 168) = 4.79$, $p = 0.003$, $\eta_p^2 = 0.32$]

and CS x Group interaction [F(6, 168) = 2.267, p= 0.039, η^2_p = 0.075]. The CS x Group interaction was investigated by testing the simple effect of Group for each CS. Consistent with the behavioural data, this was driven by significantly lower pupil dilation to *CSin2* in the **N_2O + REACT** group than **N_2O no REACT** [t(41) = 3, p = 0.014, r = 0.42, corrected] and **Air + REACT**[t(37) = 2.647, p = 0.036, r = 0.4, corrected] groups, with no difference between the latter two [t(35) = 0.176, p> 0.95, corrected] see *Figure 4.5*.

Figure 4.5: Pupillary responses to CSs during reacquisition across experimental groups. Data points represent mean ± SEM.



The temporal dynamics of pupillary responses during acquisition and reacquisition are shown in *Figure 4.4 A and 4B* respectively. Inspection of the latter qualifies the point-estimate analysis of block data and the behavioural effects observed. Lower magnitude and less differentiated pupillary responses to CSs can be observed in the **N_2O + REACT** group, driven mainly by reduced pupil dilation to the instrumental CSs. The

reduced differentiation can be seen to be relatively consistent across blocks in this group, mirroring impaired reacquisition observed in the accuracy data. The high level of differentiation between CSs evident in Block 1 in the Air + REACT group again is in line with the accuracy data and suggests reacquisition of behavioural responses was not observed in this group due to ceiling-level retention of contingencies during *Block 1*. Descriptive statistics of FDR corrected sample-wise t-tests between CSin1 and CS- and CSin2 and CS- are shown in *Table 4.2*, where it can be see that peak significance of curve differentiation and number of significantly different samples is generally lowest for N_2O + REACT, but especially so for *Csin2* vs *CS-*.

Table 4.2. Descriptive statistics of sample-wise paired t-tests on CSin1 and CSin2 vs CS- across groups.

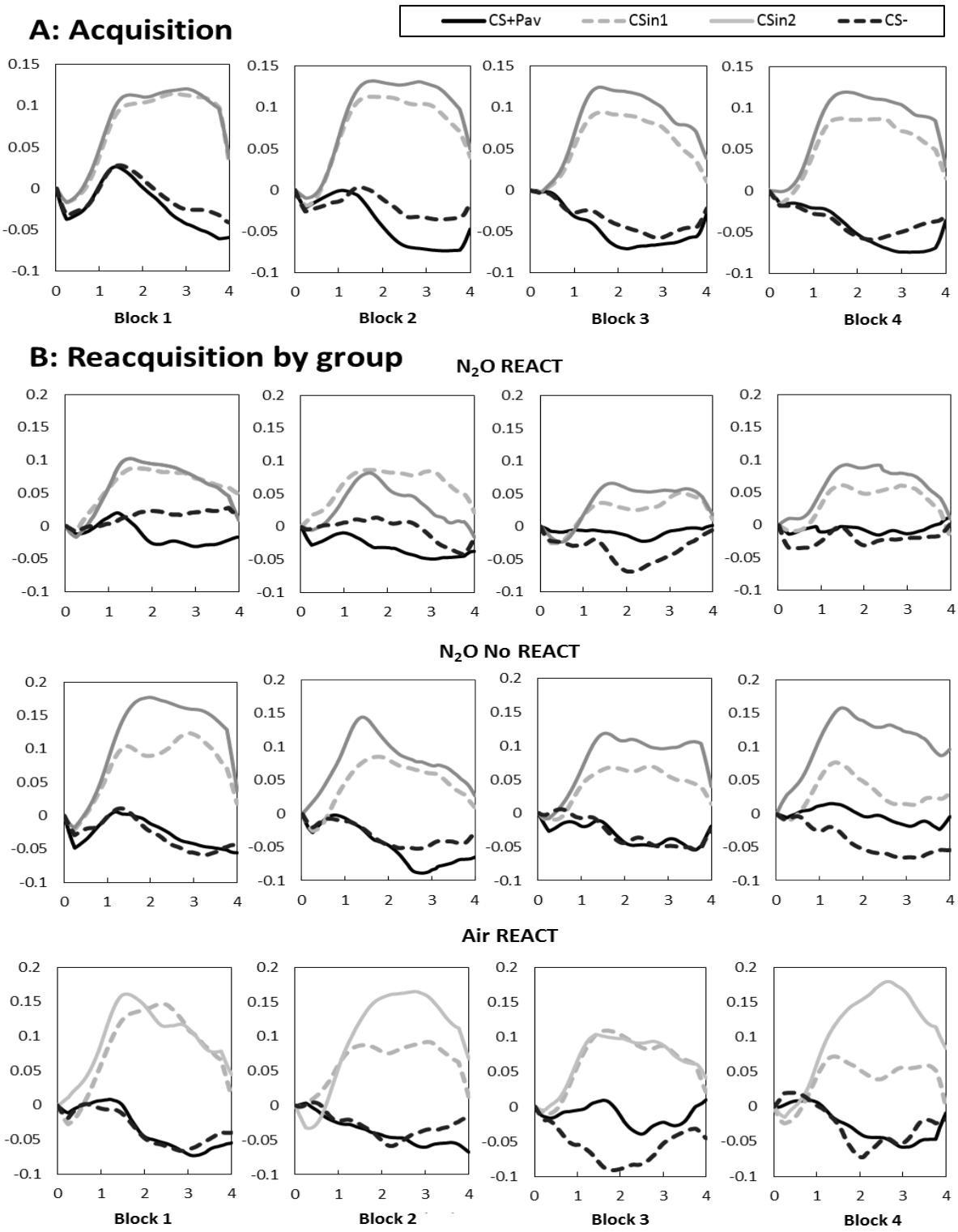
		CSin1 vs. CS-		**CSin2 vs. CS-**	
		N sig samples	peak significance	N sig samples	peak significance
N_2O + REACT	Block 1	89	0.00310132	47	0.00192076
	Block 2	320	0.00267599	0	0.02762411
	Block 3	0	0.02562344	102	0.00084196
	Block 4	263	0.00100590	318	0.00123484
N_2O no REACT	Block 1	319	0.00014829	334	0.00002452
	Block 2	271	0.00165630	301	0.00003757
	Block 3	0	0.01051599	308	0.00081022
	Block 4	0	0.00929262	356	0.00003123
Air + REACT	Block 1	300	0.00003668	357	0.00002250
	Block 2	300	0.00553294	277	0.00103307
	Block 3	306	0.00035630	241	0.00010061
	Block 4	0	0.01694430	289	0.00020743

4.3.4. Acute Effects of Nitrous Oxide

The analysis of BSS measures of subjective drug effects focused on items measuring anxiety, memory impairment, euphoria, drowsiness, difficulty concentrating and confusion as these were most pertinent to the known effects of N_2O. In all cases, these items were assessed with 3 (Group) x 2 (baseline, gas treatment) mixed ANOVAs. Significant decreases in anxiety were seen in all groups after 15 minutes of inhalation [$F(2, 56) = 27.119$, $p < 0.001$, $\eta_p^2 = 0.326$], with no change in self-rated memory impairment or drowsiness. Main effects of Time [$F(2, 56) = 5.373$, $p = 0.024$, $\eta_p^2 = 0.088$] and Group [$F(2, 56) = 4.048$, $p = 0.023$, $\eta_p^2 = 0.126$] were found for concentration, with difficulty concentrating increasing across time overall and lower overall concentration in **N_2O+REACT** than **Air + REACT** [$t(35) = 2.656$, $p = 0.031$, $r = 0.41$]. Although confusion increased only in the groups receiving N_2O [**N_2O + REACT** baseline = 27.45, on-drug = 35; **N_2O no REACT** baseline =20.77, on drug = 36.27, **Air + REACT** baseline = 13.41, on-air = 12.02], the Group x Time interaction did not reach significance. A Time x Group interaction was observed for confusion [$F(2,56) = 4.586$, $p = 0.014$, $\eta_p^2 = 0.141$], with increases in confusion in the **N_2O+REACT** [$t(19) = 5$, $p < 0.001$, $r = 0.75$] and **N_2O no REACT** [$t(21) = 2.389$, $p = 0.02$, $r = 0.46$] groups only. A Time x Group interaction was also found for euphoria [$F(2,56) = 3.76$, $p = 0.029$, $\eta_p^2 = 0.118$] with euphoric increases in the **N_2O+REACT** [$t(19) = 4.324$, $p < 0.001$, $r = 0.7$] and **N_2O no REACT** [$t(21) = 2.697$, $p = 0.009$, $r = 0.5$] groups only. Dissociation also increased significantly in the **N_2O+REACT** [$t(19) = 5.734$, $p < 0.001$, $r = 0.8$] and **N_2O no REACT** [$t(21) = 4.34$, $p < 0.001$, $r = 0.69$] groups, but not the **Air + REACT** group [Group x Time interaction: $F(2,56) = 9.078$, $p < 0.001$, $\eta_p^2 = 0.245$].

Effects of N_2O were seen on systolic blood pressure from baseline to on-drug [Group x Time interaction: $F(2, 56) = 7.114$, $p = 0.002$, $\eta_p^2 = 0.22$], with a reduction in blood pressure from baseline to on-drug seen only in the Air + REACT group [$t(16) = 3.998$, $p = 0.005$, $r = 0.71$] indicating N_2O counteracted the hypotensive effect of sitting still and anxiolysis from habituation to wearing the breathing apparatus. No effects were seen on diastolic blood pressure.

Figure 4.5. Within-trial temporal dynamics of pupillary dilation. ***A: Acquisition****, pupil dilation responses during the four acquisition blocks, subject-averaged across groups as groups did not differ in acquisition. Differentiation of* CSin2 *and* CSin2 *and* CS+Pav *and* CS- *can be seen from Block 1 onward, with earlier temporal shift of curve differentiation as the blocks progress.* ***B: Reacquisition*** *Pupil data split by Group. Reduced differentiation and lack of reacquisition of pupillary responses among stimuli can be seen in the* ***N_2O + REACT*** *group. Y axes represent proportional pupillary dilation, X axes represent time (in seconds) since trial onset.*



4.4. Discussion

The current study sought to test whether the NMDAR antagonist gas Nitrous Oxide would interfere with the reconsolidation of Pavlovian and instrumental reward memories trained in the lab in hazardous drinkers. The main finding was that 45% N_2O in O_2 following reactivation of conditioned alcohol reward memory produced lower differentiation of conditioned pupillary responses to CSs during a test reacquisition task compared to groups who received N_2O without reactivation or reactivation without N_2O. This was in line with behavioural data, showing fewer correct instrumental responses in the N_2O+REACT group during reacquisition relative to the Air + REACT group. No evidence of reacquisition in the Air REACT group (as evidenced by no increases in responding through the reacquisition task), was found, due to ceiling-level retention of conditioned responding from the beginning of reacquisition. This is likely due to the practice effect of Day 3 reactivation in this group, consistent with the well-known effect of successful retrieval (Ebbinghaus 1913) and reconsolidation (Inda et al. 2011; Lee 2008) on memory strengthening. Exploratory analysis of group effects on explicit expectancy ratings during reacquisition corroborated this finding, showing no increase across the reacquisition in the N_2O+ REACT group. Together, these findings indicate that N_2O in combination with reactivation of a conditioned reward memory negated any reactivation-dependent memory enhancement, reduced discriminative responding to discrete reward cues and prevented reacquisition of these responses. This pattern of results is best explained by interference of memory restabilisation by N_2O, possibly through its action at the NMDA receptor, although this mechanism will require verification by in vivo pharmacological studies.

Although the effect of reconsolidation interference on reacquisition seen here is tentative, it is reminiscent of Monfils and colleagues' (2009) findings using a retrieval-

extinction procedure to persistently attenuate fear memory and retard reacquisition of conditioned responses compared to a conditioning-naïve group. Soeter and Kindt (2011) have also shown preventative effects of pharmacological reconsolidation interference on generalising reacquired conditioned responding, leading them to suggest that reacquisition following reconsolidation is somehow qualitatively a different process to initial learning. The pharmacological and behavioural nature of this qualitative difference is currently highly speculative and will require further investigation. From a clinical standpoint, a pharmacological intervention that can both reduce the strength of cue-based responding for drug rewards and have a latent inhibiting effect on the reacquisition of these responses is highly desirable, as it would provide longer-term inoculation against the re-formation of MMMs that could re-ignite compulsive drug use in vulnerable individuals. This is encouraging for the potential of N_2O as a therapeutic intervention, however this effect will require examination and replication in a larger sample.

However, it should be clear that the effects of Nitrous Oxide observed hardly constitute 'memory erasure', as has been observed in other pharmacological reconsolidation interventions with animals (Nader et al. 2000). The explicit knowledge of outcome contingencies was relatively unaffected by post-retrieval N_2O, despite less accurate responding to instrumental stimuli in the N_2O + REACT group than the Air + REACT group. A similar decrease in performance at the beginning of acquisition was seen in the N_2O no REACT group whose lack of a memory reactivation session should have caused between-session decay of memory trace strength, thus N_2O may be said to prevent the memory strengthening effect of successful reconsolidation. In this capacity it could still have important therapeutic benefits and due to its excellent safety profile and minimal side effects at the doses used here, could be administered during repeated therapy sessions. However, N_2O does carry abuse potential and can lead to neuropathy in

repeated high doses (Nevins 1980), so further research will be required to investigate optimum dosing schedules that maximise therapeutic benefit while minimising the potential for harm.

The observed effects of N_2O and memory reactivation on pupil dilation to CSs, an autonomic measure of learning and reward responsiveness, are analogous to findings in fear conditioning with the β-Blocker propranolol. Propranolol following fear memory reactivation leaves the expectancy-related aspects of the memory intact, but reduces the autonomic fear component, indexed by the potentiated startle response (Kindt et al. 2009). Indeed, despite reports of episodic memory loss following ketamine (Muetzelfeldt et al. 2008) and N_2O (Parbrook 1967), pharmacological 'erasure' of explicit episodic or semantic memory during reconsolidation has yet to be demonstrated experimentally in humans. This in itself may be an unachievable and undesirable aim, as, ethical concerns of this possibility aside, the aim of targeting MMMs in addiction is to remove their relapse inducing potential. This does not necessitate explicitly 'forgetting' that a cue is associated with a drug, rather involves removing the aberrant motivational properties of MMMs that lead to drug seeking. The current findings suggest N_2O may therefore have utility for this purpose, but its fulfilment of the clinical utility criteria outlined in *Chapter 1* still needs to be established, particularly with regard to levels of drinking and abstinence in populations of heavy drinkers wishing to cut down or stop their alcohol use.

Pupil dilation is a measure of autonomic arousal (Bradley et al. 2008) but also expectancy of reward (O'Doherty et al. 2003) and certainty of outcome, with greater dilation indicating greater certainty of reward (Preuschoff et al. 2011). These moderators of pupil size are obviously inter-related and likely represent part of a larger motivational preparatory response in response to reward cues. Thus the reduction in

pupillary responses to instrumental CSs observed here is suggestive of a reduction in potential maladaptive motivational processes that underlie relapse in addiction. The question of the mechanism of action of this effect, however, cannot be answered by the current study which, along with the limited sample size, represents its main limitation.

It cannot be determined, for instance, whether the N_2O after reactivation increased uncertainty of appropriate cue-response relationships via direct degradation of cue-outcome contingencies (which would explain the reduction in pupil dilation and accuracy relative to the placebo group; Preuschoff et al., 2011) or whether it reduced the subsequent capability of those cues to recruit motivational circuitry (and respond in order to win beer) via downstream dopaminergic mechanisms. N_2O may thus have reduced conditioned motivation (and therefore pupillary responses) through degrading upstream associative components of memory traces, but this may have been mediated by a subsequent reduction in dopaminergic reward mechanisms. In future research, motivational manipulations, such as fluid deprivation or priming doses of beer may be employed to differentiate these mechanisms. Another avenue of investigation could be to vary the effort costs associated with different CSs and assess whether N_2O effects are dependent upon these costs (and therefore motivation).

It is interesting to note that, in the current experiment, N_2O after reactivation did not appear to affect responding to Pavlovian cues. Historically, reconsolidation is more readily seen for Pavlovian memory traces, although recent evidence has confirmed its occurrence even for well-learned instrumental memories (Exton-McGuinness et al. 2014). Given the 100% reinforcement schedule in the current experiment, which was employed so that prediction error could be guaranteed during reactivation, the learning of cue-outcome relationships reached ceiling level very rapidly and it is perhaps unsurprising that these responses were relatively unaffected, given their ease of

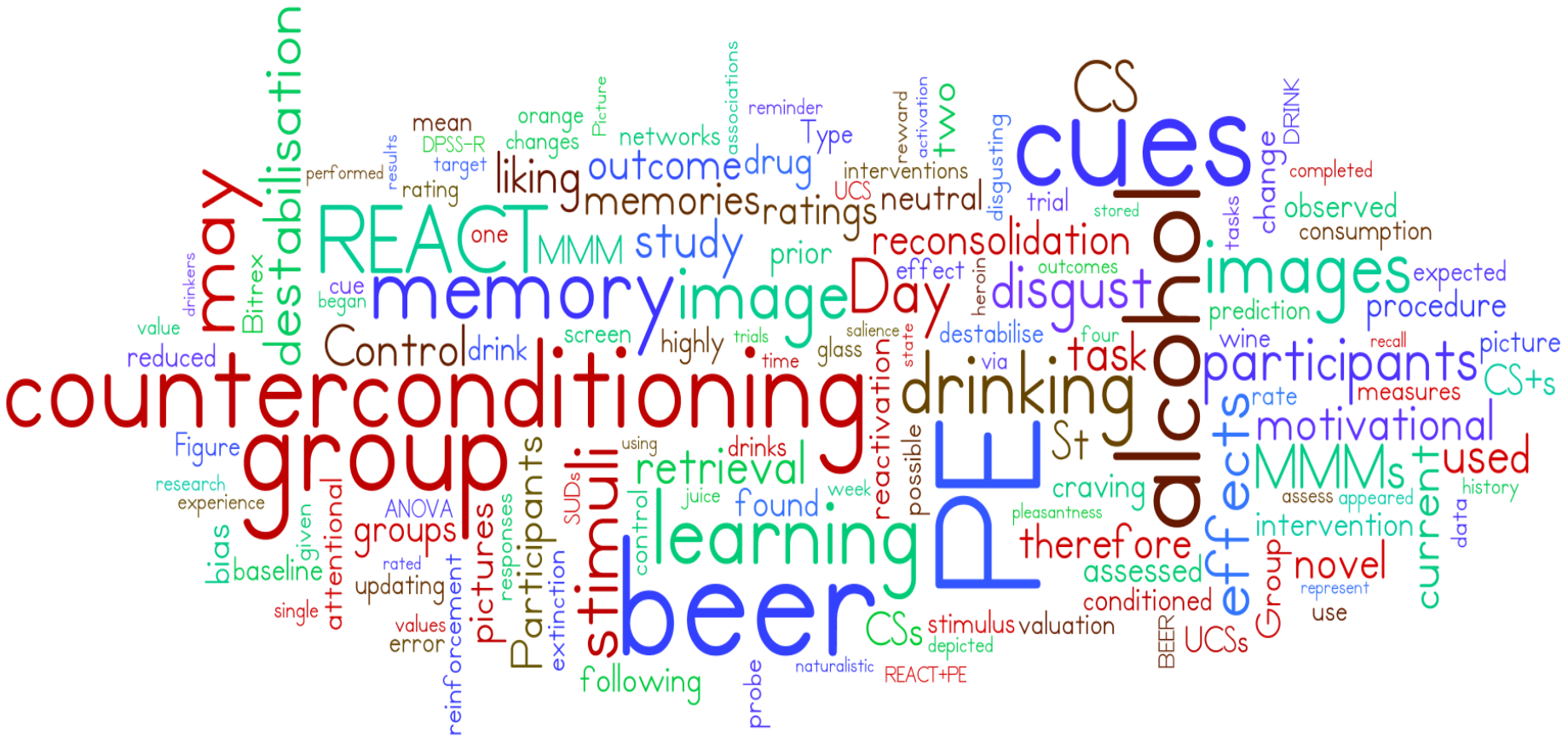
recollection. Indeed, the main effects of reconsolidation interference by N_2O were seen for the avoid-to-win instrumental stimulus. This is objectively the hardest stimulus for which to learn the correct response, as it requires model-based cognitive flexibility to make an unusual response, rather than relying upon the frequently encountered, model-free approach-to-win response, causing a Pavlovian action learning bias (Guitart-Masip et al. 2014). That this more difficult-to-acquire response was most affected by the N_2O suggests that over-learning may be a constraint in memory interference by N_2O following reactivation. This must be tested to fully assess its potential in substance use disorders, where overlearned, habitual responding is the norm. Indeed, instrumental and Pavlovian cues should be employed more frequently in the same learning paradigms to allow their direct comparison in terms of reconsolidation interference effects, as naturalistic drug use always involves an instrumental component.

The current findings further support the importance of prediction error in destabilising memories. The reactivation procedure explicitly included a negative prediction error for each CS and the effects of N_2O suggest this was sufficient to destabilise a lab-trained reward memory. It remains to be seen whether this would hold for more robustly learned associations and the logical progression of this work would be to replicate and extend the findings to naturalistic MMMs. A stronger effect of N_2O might be seen with a longer dosing period. The reconsolidation window lasts up to several hours (Przybyslawski and Sara 1997) and N_2O was only administered for 20 minutes following reactivation, with rapid offset of central effects following cessation. Oral drugs have much longer centrally-active half-lives but are slow to peak, meaning they must be administered prior to memory reactivation to interfere with reconsolidation. The current findings raise the intriguing possibility of using N_2O's rapid-onset to block NMDARs immediately after reactivation and simultaneously using a slower-peaking

oral preparation of an NMDA antagonist to maintain receptor occupancy throughout the reconsolidation window.

In summary, this is the first study to identify Nitrous Oxide as a reconsolidation-blocking drug with potential therapeutic benefits in humans. Its safety, accessibility and ease of administration make it an attractive option for this purpose versus other compounds of the same class. Although the current study had a limited sample size and the findings were relatively modest, given the risk-to-benefit potential of using N_2O to block the reconsolidation of MMMs, further work examining the drug for this purpose is clearly warranted.

Chapter 5: Reversing the motivational status of alcohol cues via counterconditioning during reconsolidation



5.1 Introduction

Despite the promise of preclinical research pharmacologically neutralising the impact of MMMs during reconsolidation, early translational attempts to do the same in humans have had little to no impact on relapse rates (see *Chapter 4* and (Saladin et al. 2013). As discussed in previous chapters, these null effects may be attributable to insufficient blockade of memory restabilisation by the test drug or failure of the reminder procedure to destabilise relevant memories (Piñeyro et al. 2014).

Recent research has demonstrated that prediction error (PE) is a key mediator of memory destabilisation at recall (Pedreira et al. 2004; Sevenster et al. 2012; 2013). Failures to destabilise memory traces may therefore be attributable to insufficient generation of PE during reminder procedures. Building on this hypothesis, in *Chapter 4* I reported evidence for memory weakening by NMDA antagonist gas Nitrous Oxide when administered after a reminder procedure that engendered negative prediction error. This is supportive both of the necessity of PE for destabilising human cue-alcohol memories and the importance of NMDAR activation for memory restabilisation. However, it does not follow that such a retrieval procedure would be sufficient in all circumstances.

Although the N_2O effect was demonstrated in a clinically relevant population (hazardous beer drinkers), with a clinically and ecologically relevant reward outcome (beer consumption), the memories targeted were of novel stimuli, conditioned in a single context (the lab), which is dissimilar to a naturalistic drinking environment. The number of pairings of cues with beer reward was therefore far fewer than those occurring naturally in a real-world context. Further, in the lab, where learning history was controlled, it was possible to guarantee PE at recall as the reinforcement schedule

during training was 100%, so the reminder structure necessitated only single unreinforced presentations of CSs to generate a negative prediction error. In naturalistic human drug use, where learning history is unknown but likely involves thousands or hundreds of thousands of learning trials in multiple contexts, such a guarantee is not possible. Such extensive training and aging of memories involves a shift to distributed cortical encoding of memories (Morris 2006). This may confer the resistance to destabilisation observed in older, more robust memories (Gräff et al., 2013 ; Robinson and Franklin 2010; Wang et al. 2009).

Furthermore, temporal difference models of associative learning predict that the magnitude of changes in stored values of stimuli or states decrease as learning continues. Consider the following prototypical equations describing reinforcement learning, based on the Bellman equation (Bellman and Dreyfus 1962):

$$\mathbf{V\,(S\mathit{t})} = \mathbf{E[R\mathit{t} \mid S\mathit{t}]} + \mathbf{E[V(S\mathit{t} + 1) \mid S\mathit{t}]}$$

Where V (S*t*) is the value of the state S*t*, E[R*t* | S*t*] is the expected reward R, in state St and E[V(S*t* + 1) | S*t*] is the expected reward R in the next state S*t* + 1, given the current state (Sutton and Barto 1998). Clearly the two sides of these equations only balance when the value V is correct, that is if the expected rewards (or lack of rewards) in these states occur. If incorrect, there is a discrepancy between the expected and actual outcomes such that:

$$\boldsymbol{\delta t} = \mathbf{R\mathit{t}} + \mathbf{V\mathit{t}\,(S\mathit{t} + 1)} - \mathbf{V\,(S\mathit{t})}$$

Where δt is the PE between predicted and actual reward. This error is summed onto the stored value V, updating expected V such that:

$$\mathbf{V_{t+1}\,(S\mathit{t})} \leftarrow \mathbf{Vt\,(St)} + \boldsymbol{\alpha\,\delta t}$$

That is, the updated value upon the next encounter with state will equal the old value plus constant coefficient of the prediction error. This constant represents the learning rate, with a smaller constant equating a slower learning rate. Variations on this function form the basis of many forms of associative learning theory. Note that this type of learning is '**model free**' in that it requires no overall cognitive representation of states, their available rewards and how they are connected, but simply sums prediction errors on to stored values upon every encounter with a state. As experience accrues, the stored values thus home in on an accurate representation of the average reward available given different states or stimuli. Magnitude of iterative 'updating' thus decreases as learning continues (Dayan and Balleine 2002; O'Doherty et al. 2003; Rescorla and Wagner 1972; Schultz et al. 1997; Sutton and Barto 1981; Sutton and Barto 1998). That is to say, the PE signal (the putative primary driver of associative learning) magnitude decreases as learning continues and predictions of outcomes become more accurate.

Because this kind of learning is thus based purely on trial-and-error experience it can operate at a relatively simplistic, automatic level. However, this also makes model-free Pavlovian learning somewhat inflexible compared to goal-directed, model-based cognitive representations of values in certain states. If the learning rate constant α is low, changes in stored values can be very slow. Within this formalisation of learning, reconsolidation should be a primary process by which state or action values are updated (Lee 2009). However, this represents a problem with reconsolidation of MMMs in SUDs (Torregrossa and Taylor 2013), where inflexible, hyper-valuation of drugs and their predictors promotes consistent drug-seeking and using (Huys et al. 2014). Unless interventions are staged to deliberately generate large prediction errors, in naturalistic experience, large PEs are unlikely to occur in memories as well-trained as MMMs. In this case, the resistance to destabilisation of strongly trained MMMs may be due to the

difficulty in engendering a PE large enough to challenge the outcome predicted by the memory trace and spark updating.

It is currently unknown whether such over-trained naturalistic MMMs such as those in Alcohol Use Disorder and smoking can be reliably destabilised at all in humans. A key *preliminary* goal for this field is therefore developing memory reactivation procedures that reliably destabilise over-learned MMMs in the absence of knowledge of learning history. If this achievable, maximising PE during MMM retrieval should, theoretically, maximise the probability of destabilisation. If such a procedure is successful, it will be possible to begin to assess the efficacy of post-reactivation interventions in reducing clinically relevant markers of disordered substance use.

The only study to date showing lasting benefits of reconsolidation interference in a human drug-using population utilised a behavioural, rather than pharmacological intervention following memory destabilisation (Xue et al. 2012). This study built on the retrieval-extinction procedure developed by Monfils and colleagues (2009) and Schiller and colleagues (2009) and involved extinction training following a brief reminder of a conditioned memory. Extinction following memory destabilisation in the 'reconsolidation window' putatively updates and overwrites conditioned cue-outcome associations with cue-no outcome associations while the memories are unstable, causing a permanent change in memory expression.

Adopting this procedure in detoxified inpatient heroin addicts, Xue and colleagues (2012) showed that a single video presentation of heroin-associated cues ten minutes prior to repeated exposure of these cues in the absence of heroin use (extinction) reduced cue-induced craving for heroin up to 6 months later compared to standard extinction. A purely behavioural approach to updating MMMs is appealing, as it can be more targeted than systemic drug administration and avoids the side-effects of NMDAR

antagonists. However results with this retrieval-extinction method have been inconsistent in both fear learning (Chan et al. 2010; Soeter and Kindt 2010) and cue-drug learning paradigms (Millan et al. 2013) and it is unknown whether equivalent effects to Xue et al (2012) would be observed in non-abstinent users in an outpatient setting or extend to different drugs of abuse.

As with pharmacological interventions, null results may be due to failure to destabilise memory, but may also be attributable to the insufficiency of extinction as a corrective learning modality. Extinction following retrieval primarily targets associative components of memories (Costanzi et al. 2011), with any changes in conditioned reinforcing or motivating effects of drug cues occurring secondary to a reduction in their predictive utility.

Pairing drug cues instead with an aversive consummatory outcome may more directly and powerfully reverse conditioned reinforcement and motivational sensitisation to drug cues by devaluing cues and engendering disgust, a salient (Berridge 2009), universal (Olatunji and Sawchuk 2005) and robust (Olatunji et al. 2007b) anti-consumption response. If such a **counterconditioning** procedure is performed during reconsolidation, it may be possible to reverse the valuation (conditioned reinforcement) and attractive value (conditioned approach) of drug-related cues, manifesting in reduced liking and attentional capture by these cues respectively. It is possible that this will also reduce drinking by decreasing the positive modulating effect these cues have on alcohol consumption (conditioned motivation).

The development of reconsolidation-based therapies for SUDs therefore requires a procedure for generating sufficient PE during recall of cue-drug memories that 1) does not require knowledge of learning history 2) can destabilise networks of well-learned cue-drug associations and 3) is clinically practicable (*Chapter 1 criterion 4*) and enables

changes that persist across changes in context (*Chapter 1 criterion 3*) . Further, corrective learning following memory destabilisation should ideally have broad spectrum effects on the motivational salience of alcohol cues.

The current study therefore aimed to generate a large PE during recall of well-learned cue-alcohol memories in a sample of hazardous beer drinkers. We hypothesised that explicitly instructing participants that they would drink beer after viewing prototypical beer cues, but then withholding the alcohol at the moment of expected reinforcement would generate maximal PE at recall and destabilise alcohol MMM networks. Following this, it was hypothesised that counterconditioning of reactivated cues with disgusting outcomes would update cue-drinking MMM networks, replacing motivational alcohol associations with disgust/avoidance associations. If successful, this procedure should reduce the valuation, motivational salience and positive outcome expectancies of alcohol stimuli and increasing cue-induced disgust in response to these stimuli.

5.2. Methods

5.2.1. Participants and Design:

The study by Xue and colleagues (2012) does not contain sufficient information to calculate an effect size for the retrieval + extinction effect on heroin craving. However, highly significant effects were found at 180 days with Ns of 16 per group. Expecting attenuation of effects owing to the 'Winner's curse' phenomenon (Young et al. 2008), we therefore conservatively retained the moderate effect size estimate of $f = 0.35$ used previously to calculate required N. Fifty-nine hazardous, non-dependent beer drinkers were recruited from University College London internal study advertisement networks and from via convenience sampling from the locale. Participants were randomly assigned to one of three groups that differed only in the nature of the MMM 'reactivation session' 10 minutes prior to counterconditioning on Day 1. The Control group (n = 19) received no reactivation of cue-alcohol memories; the REACT + PE group (N = 20) received a 'reminder' of cue-drinking memories with an explicitly guided PE prior to counterconditioning. Finally, the REACT-no PE group (N = 20) received a reminder of cue-drinking memory with no PE.

Inclusion criteria were current hazardous drinking defined as a score >8 on the Alcohol Use Disorders Identification Test (AUDIT)(Saunders et al. 1993) but <3 items coded as 3 on the Structured Clinical Interview for DSM (SCID; First, Spitzer, Gibbon, & Williams, 2002); consumption of > 3 units for females, > 4 units for males on at least three days per week; fluent English and normal or corrected-to-normal colour vision.

Exclusion criteria were age <18 >65, past or current diagnosis of drug or alcohol use disorders, any currently medicated mental health issues, any current major physical health issue; current pregnancy or breastfeeding. Participants were reimbursed at the

rate of £7.50 per hour. All procedures were approved by the University College London Research Ethics Committee.

5.2.2. Apparatus and Tasks

Questionnaire Measures:

Drinking in the week prior to the study and after the intervention was assessed with the Timeline Follow Back (Sobell and Sobell 1992). Disgust sensitivity at baseline and following re-exposure to cues was measured with the Disgust Propensity and Sensitivity Scale –Revised (DPSS-R; (Olatunji et al. 2007a) and drinking concern/readiness to change was assessed with the Stages Of Change Readiness And Treatment Eagerness Scale (SOCRATES; Miller and Tonigan, 1996). SOCRATES yields three subscales of 'Recognition', 'Ambivalence' and 'Taking Steps', representing stages of behaviour change. Momentary craving for alcohol was assessed with the Alcohol Craving Questionnaire (ACQ-NOW) (Singleton et al. 1994) and expectancies of drinking-related outcomes were assessed with the Negative Alcohol Expectancy Questionnaire (NAEQ) (McMahon and Jones 1993). The NAEQ yields subscales of negative consequences on the 'Same Day' as drinking, 'Next Day' and 'Continued' consequences of alcohol use. Reward Responsivity was assessed with the Behavioural Inhibition/Behavioural Activation Scale (BIS/BAS) which yields three 'activation' subscales of 'Drive', 'Fun' and 'Reward' and one 'Inhibition' subscale.

CSs

Four prototypical beer images were selected to act as MMM reactivation cues and subsequently as CS+s in the counterconditioning task. These depicted beer taps on a bar, a poured pint of beer, an ice bucket filled with beer bottles and a can of beer being poured into a pint glass, in order to represent the major modes and stages of beer consumption. Multiple, prototypical beer CSs were used to maximise activation of

MMM networks and generalisation of the association between beer-related stimuli and disgusting outcomes as the use of single discrete stimuli in reconsolidation paradigms can lead to effects that are highly specific to that stimulus, rather than generalising to novel stimuli within the class of the reactivated stimulus (Pearce 1987).

Two novel beer images, used on *Day 8* in the liking (picture rating) and attentional bias task depicted pints of beer on a table and a pint of beer being consumed along with two novel wine images depicting a glass of wine next to a wine bottle and a glass of wine being consumed.

Two CS-s were used in retrieval and counterconditioning to control for non-associative effects of the procedures. These depicted a cup of coffee and a can of cola with a glass. Soft drink images were used as CS-s to rule out generic decrease in liking of consumable stimuli due to anti-consummatory effects of exposure to the disgust UCSs.

Control 'no-reactivation' cues presented during the retrieval stage in the Control group depicted whole oranges, an orange being squeezed, a glass of orange juice and a woman consuming orange juice. The beer and orange juice cues were equated as much as possible to minimise any effects that were not specific to the reactivation manipulation. All stimuli were presented via 1024x768 pixel flat screen 21 inch monitor.

UCSs

Four pictorial disgusting images were used as UCSs. Three were taken from the International Affective Picture System database (Lang et al. 2005). These depicted a toilet covered in fecal matter (image 9301), a man vomiting (image 9325) and a badly wounded human hand (image 9405). An additional image was sourced from the internet depicted a septic wound on a human foot that was infested with maggots. Highly bitter drinks were made by mixing 80μL 2.5% Denatonium Benzoate (Bitrex solution;

Macfarlan Smith Ltd, City, UK) in 120ml water. This solution was divided into eight 15ml drinks. Bitrex was kindly provided free of charge by Macfarlan Smith Ltd.

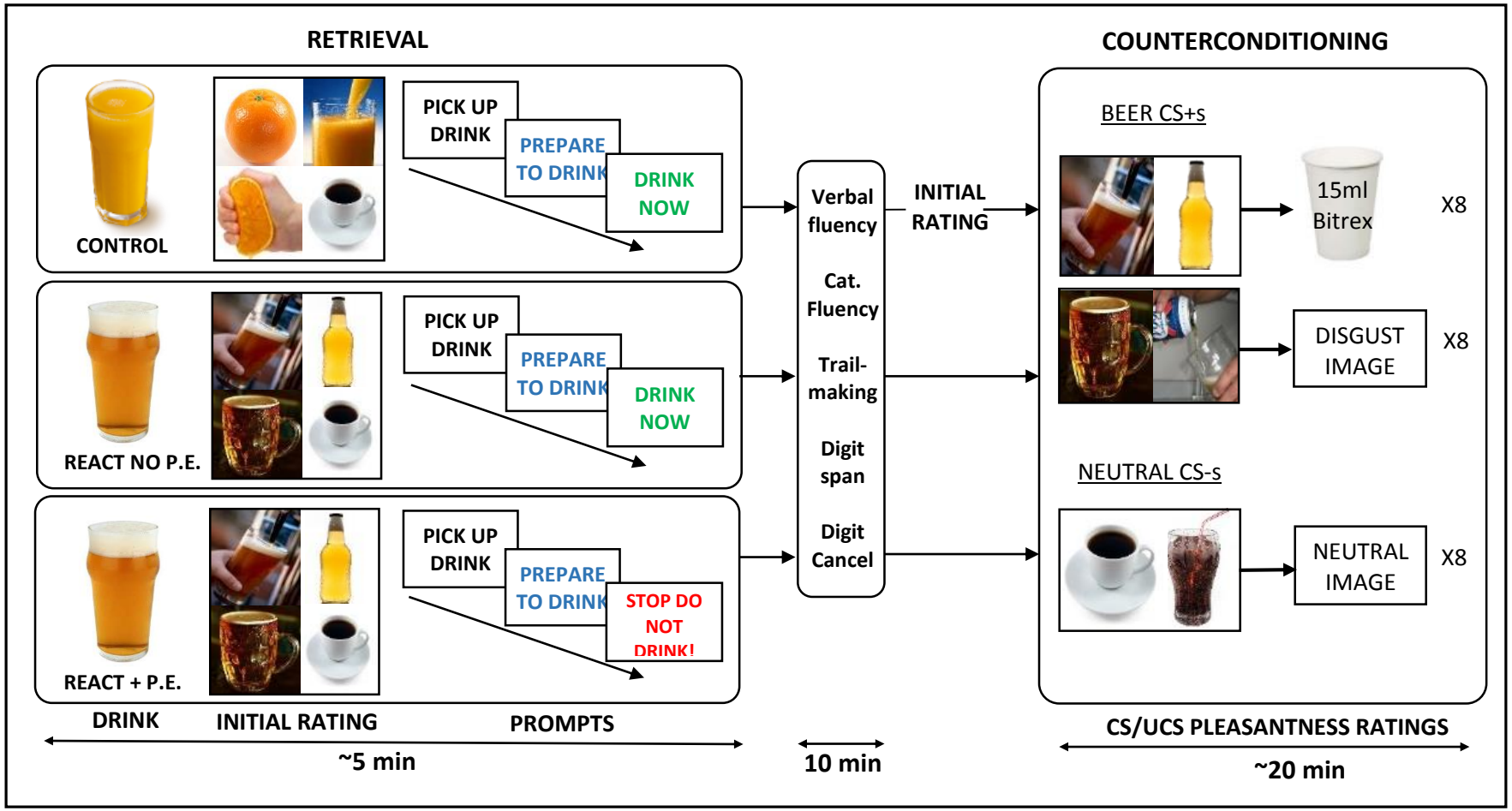
Memory retrieval:

A schematic representation of the retrieval-counterconditioning tasks is given in *Figure 5.1*. In the control condition, a 150ml glass of chilled orange juice was placed in front of the participants as the computer task began. In both the REACT+PE and REACT No PE conditions, an identical glass of 150ml chilled non-alcoholic beer was placed in front of the participants. Non-alcoholic beer was used to avoid any confounding effects of priming doses of alcohol. Participants were not aware that the beer was non-alcoholic.

On screen-instructions informed participants that the experiment examined how viewing images affected their perception of the taste of drinks and that they would rate a series of images, then consume the drink in front of them. They were told that they were to consume the entire amount according to on-screen prompts, which were a sequence of screens displaying 'PICK UP DRINK' 'PREPARE TO DRINK' and 'DRINK NOW', each screen displayed for 2000ms. An example of these screens was given and participants were told only to drink when 'DRINK NOW' appeared on screen. Participants' understanding of the instructions was confirmed before they began rating the images. The Control group then rated the four orange juice images and two CS- images (coffee and cola); the REACT+PE and REACT no PE groups rated the four beer CSs and two CS-. Order of CS presentation was randomised. All ratings were made on the scale 0 (extremely unpleasant) to 10 (extremely pleasant) via labelled keys on the keyboard. Following the final rating, the drinking prompts began. In the Control and REACT no P.E. groups, these screens proceeded as per the instructions and they consumed the beer or orange juice, as expected. In the REACT no PE group, this recapitulated a standard drinking episode, and therefore no new information was

available to destabilise memory. In the REACT + PE group, the first two prompt screens were as expected, but the final screen unexpectedly displayed the words 'STOP! DO NOT DRINK', followed by 'Put the drink down and alert the experimenter'. In all groups, the glass was then removed and the distractor tasks began.

Figure 5.1 Schematic of Day 1 retrieval/counterconditioning procedure. Four beer or orange juice cues plus two control soft-drink cues were baseline-rated during 'retrieval' of cue valuation memory. Beer images were subsequently paired with Bitrex or disgusting pictures. The control group baseline rating of beer images was at the start of counterconditioning, prior to any pairing with UCSs, to provide a baseline in this group and equate the number of cue exposures in each group.



Distractor Tasks:

Participants completed verbal and category fluency tests, digit span forwards and backwards (Wechsler 2008), Trail making version A (numeric) and B (alphanumeric) (Reitan 1958) and digit cancellation tasks. These distractor tasks were chosen due to their high attentional and working memory demands. As offset of reactivated stimuli is critical for the switch between memory reconsolidation and extinction, high working memory tasks prevented maintenance of rated stimuli in working memory during the 10 minute period between retrieval and counterconditioning. Performance in the distractor tasks was not of primary interest to the current study and is therefore not reported here.

Counterconditioning:

Counterconditioning began immediately after completion of the distractor tasks. On-screen instructions told participants that they would now continue rating pictures and consuming samples of drinks, some of which may be extremely bitter, but were completely harmless. As before, participants were instructed to consume the entire drink placed in front of them whenever the words 'DRINK NOW' appeared on the screen.

An example of typical 'drink' trial and example 'picture' trial are shown in *Figure 5.2*. During the task, a CS image appeared in a box on the left side of the screen and then an 'outcome' image would appear on the right (UCSs). The outcome image was either a disgusting picture from the IAPS picture (visual UCS) or the words 'DRINK NOW' (gustatory US). Participants were required to make two pleasantness ratings per trial; the first when the CS image appeared to rate its pleasantness and the second after offset of the UCS outcome (after viewing the UCS image or consuming the drink) to rate the pleasantness of the outcome. All ratings were on the scale 0 (Extremely Unpleasant) – 10 (Extremely Pleasant). Each CS was presented four times during the counterconditioning. Two of the beer CS+s were paired with the disgusting picture

outcomes (each CS was paired once with each of the UCS images) and two of the beer CSs were followed by 15ml 0.067% aqueous Bitrex solution on a 100% reinforcement schedule. The soft-drink images were paired with neutral images from the IAPS.

A single, pseudorandomised trial order was used for all participants. Trials were randomised with the stipulation that the same UCSs could not occur in consecutive trials and that no more than two trials of the same CS could occur consecutively. Participants in the Control condition rated the four beer CSs at the beginning of the counterconditioning session. The purpose of this was firstly to provide a baseline measure of liking for these stimuli and secondly to ensure that identical numbers of CS presentations were given in each group so that effects could not be attributed to differential amounts of CS exposure, a problem with previous retrieval-extinction paradigms (Millan et al. 2013)

Visual probe

On day eight a visual probe task was conducted to index attentional bias. Ten pairs of composition-matched image pairs were used as stimuli in this task, with each pair including a 'target' and 'non-target' image. The 'targets' consisted of the four beer CS+s used in the counterconditioning task, two neutral CS-s used in counterconditioning, two novel beer images and two novel wine images, all of which were paired with composition-matched 'non-target' images that did not depict alcohol or soft drinks. All image pairs were rendered as high definition .jpegs of 300 x 300 pixels. On-screen, the left image was centred at screen coordinates 256,384 and right image at 768, 384.

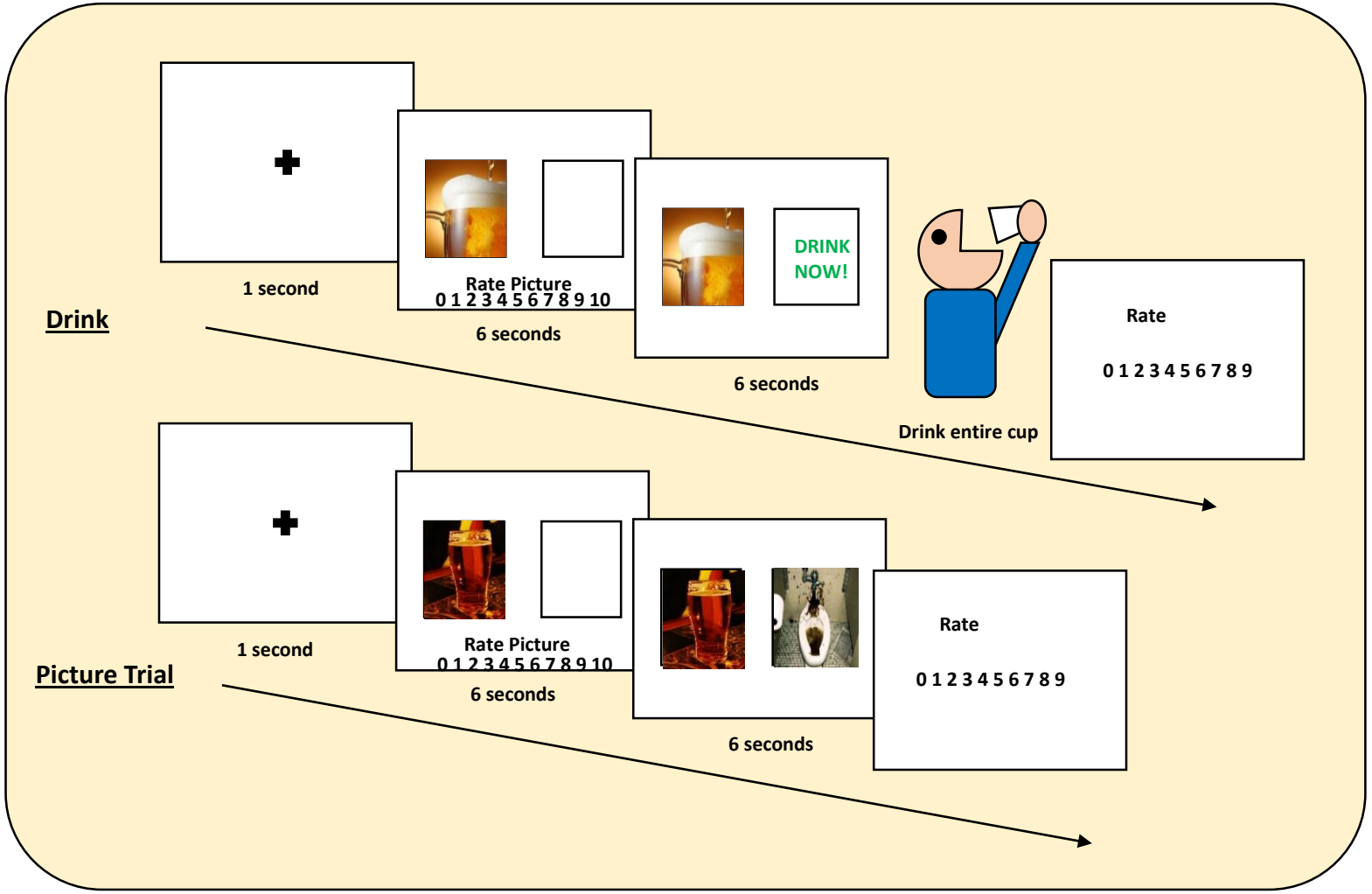
Trials began with a drift correction, where central fixation was verified by the experimenter. Image pairs then appeared for 2000ms, after which the images disappeared and a triangular probe appeared in the location where one of the images had

been. The triangle either pointed upwards or downwards and participants had to respond as to the orientation of the probe as quickly and accurately as possible using 'up' and 'down' arrow keys on the keyboard. These behavioural measures were simply to ensure continued engagement in the task as in practice they were superseded by eye-tracking which directly measures attentional allocation to stimuli. Participants were instructed to try and take in both pictures while they were displayed on- screen.

Each image pair was presented 8 times in a random order, balanced for laterality of target image (left or right), laterality of probe location (ipsilateral to target image, contralateral to target image) and probe orientation (pointing up or down). Eye movements during the task were tracked with an Eyelink 1000 desktop mounted eye tracker (SR Research, Ontario, Canada) with a sampling rate of 1KHz. Participants' heads were stabilised 60 cm away from the computer screen throughout.

Blinks were removed using the manufacturer's algorithms on default settings. For each image pair, mean attentional bias scores were calculated by subtracting total time fixating on matched control image from total time spent fixating on target image –, so positive scores indicate a bias towards target images and negative scores a bias away from them. Fixations occurring <100ms after image pair onset were excluded from dwell time calculation, as they represent pre-emptive looking to stimulus locations (Mogg et al. 2003).

Figure 5.2. Schematic of a typical 'drink' trial and 'picture' trial during counterconditioning. There were 8 gustatory CS-UCS (drinking) trials and 8 pictorial CS-UCS trials in total. On each trial, a pleasantness rating of both the CS and 'outcome' was made.



5.2.3. Procedure

Baseline measures:

Twenty four hours prior to attending the study centre, participants completed and returned the SOCRATES, NAEQ and TLFB measures. TLFB was assessed for the week prior to the study and a daily average beer consumption was computed. These measures were completed prior to Day 1 to minimise the amount of alcohol memory retrieval immediately prior to the manipulation on *Day 1*.

Day 1:

Participants were not informed of the exact nature of the study in order to maintain the necessary surprise element for generating prediction error. Instead they were told that the experimenters were interested in taste perception and learning processes in heavy drinkers. As part of this, participants were told they would be required to rate pictures and consume samples of different drinks, some of which might be very bitter. Participants were randomly allocated to one of the three groups. After providing written informed consent, participants completed the DPSS-R and immediately began the relevant retrieval phase of the retrieval-counterconditioning procedure. After this, all participants completed the distractor tasks, which lasted 10 minutes in total, before completing the counterconditioning task. The eight Bitrex-containing drinks used in the counterconditioning were stored in an opaque box, so that participants were unaware how many more drinks they were required to consume. New drinks were placed in front of participants immediately after consumption of the previous drink. At the end of the task, participants received two squares of milk chocolate to get rid of the taste of Bitrex.

Day 8:

One week later (*Day 8*), participants re-rated the pleasantness of all CSs from *Day 1*, along with novel beer and wine images. Attentional bias, an index of motivational salience, towards these images was then assessed using the visual probe task. Participants then completed the ACQ-NOW and DPSS-R to assess alcohol craving and disgust sensitivity following re-exposure to alcohol cues. Amount of beer consumed over the week since intervention was then recorded with the TLFB. Participants were then fully debriefed as to the nature and aims of the experiment and paid. This completed the testing.

5.2.4. Statistical Analysis

All data analysis was performed using IBM SPSS version 21 for Windows. All data were checked for normality, homogeneity of variance and sphericity (for repeated-measures with K>2 comparisons) inspection of histograms and z-scored skewness/kurtosis, Levene's test and Mauchly's test, respectively. Any outliers more than 3 standard deviations away from the sample mean for that variable were replaced with a score falling 3 standard deviations from the mean. Non-normal data were transformed where skewed. If this did not normalise the distribution, non-parametric equivalents of tests were used as appropriate. Descriptive statistics represent untransformed data, unless stated otherwise, in order to aid interpretation of results. Where homogeneity of variance was violated in one-way ANOVA, Welch's F test is reported. Where sphericity was violated, the Huynh-Feldt correction was applied to the degrees of freedom and significance levels. Uncorrected degrees of freedom are reported here, with corrected *p* values. For single time-point measurements, one-way ANOVA was used to assess group differences and for repeated measurements, mixed ANOVA with a between-subjects factor of Group was used. Significant k >2 main

effects and interactions in omnibus ANOVAs were investigated with independent or paired-samples t-tests on marginal means, where appropriate. Although all follow-up comparisons on omnibus tests were planned a priori, *p* values for these tests are Bonferroni- corrected to control Type I error.

5.3 Results

5.3.1. Baseline drinking and questionnaire data

Descriptive statistics for baseline measures of drinking behaviour, attitudes to alcohol, disgust sensitivity, readiness to change and behavioural inhibition/activation are given in *Table 5.1*. Groups did not differ on any of these measures prior to the retrieval/counterconditioning intervention (all *p*s > 0.07).

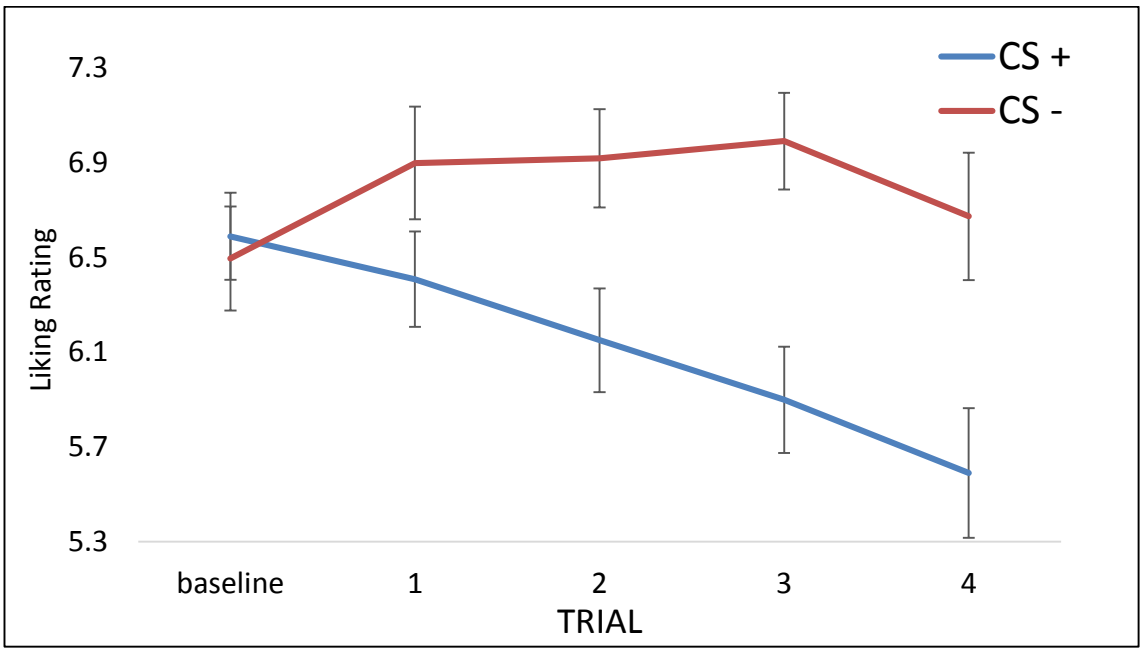
Table 5.1: *Baseline demographic variables, drinking level and questionnaire measures. Values are mean ± SD*

		Control (N = 19)	**REACT + PE (N = 20)**	**REACT No PE (N=20)**
AUDIT		*14.58±4.72*	*15.8±4.05*	*15.1±4.75*
NAEQ	SAME DAY	*40.74±7.87*	*41.75±9.73*	*42.350±7.59*
	NEXT DAY	*36.21±9.37*	*36.1±11.43*	*36.65±8.94*
	CONTINUED	*29.47±13.04*	*29±8.52*	*31.1±6.42*
SOCRATES	RECOGNITION	*11.32±3.16*	*11.2±3.81*	*13.2±3.38*
	AMBIVALENCE	*8.16±3.56*	*8.25±3.02*	*10.3±3.21*
	TAKING STEPS	*17.63±7.15*	*14.9±5.7*	*17.25±5.9*
DPSS-R	SENSITIVITY	*12.68±3.61*	*14.8±6.69*	*12.6±3.03*
	PROPENSITY	*18.16±2.19*	*16.9±3.32*	*16.7±2.64*
	TOTAL	*30.84±4.86*	*29.9±5.44*	*29.3±4.49*
AGE		*23.16±7.49*	*21.5±1.73*	*23.15±7.44*
DRINKING	DAILY PINTS BEER	*1.36±1.15*	*1.31±0.75*	*1.86±1.27*
	DAILY SINGLE SPIRITS	*0.74±.0.7*	*1.26±0.86*	*1.68±1.79*
	DAILY GLASSES WINE	*0.5±0.67*	*0.65±0.71*	*0.23±0.34*
BIS/BAS	BAS DRIVE	*11.68±1.95*	*11.95±1.54*	*10.8±1.82*
	BAS FUN	*13.68±2.26*	*13.25±1.25*	*13.1±1.97*
	BAS REWARD	*17.63±1.67*	*17.45±1.93*	*16.95±1.54*
	BIS	*20.58±3.01*	*21.15±3.31*	*19.75±3.67*

5.3.2. Counterconditioning

Counterconditioning of stimulus valuation was assessed via a 2 (CS Type: beer picture CS+s, neutral picture CS-s) x 5 (Trial: Baseline, trial 1 – 4) x 3 (Group: Control, REACT + PE, REACT no PE) mixed ANOVA. CS pleasantness ratings during the retrieval phase were the baseline ratings in the REACT+PE and REACT No PE groups and ratings at the beginning of the counterconditioning were the baseline in the Control group (see *Figs 5.1* and *5.2*). Main effects of CS Type [$F(1,56) = 7.842$, $p = 0.007$, $\eta_p^2 = .123$] and Trial [$F(4, 168) = 3.026$, $p = 0.041$, $\eta_p^2 = .051$] and critically, a CS Type x Trial interaction $F(4, 224) = 9.902$, $p < 0.001$, $\eta_p^2 = .15$] were found. Planned follow-up pairwise comparisons of the interaction found no significant difference between liking of beer and neutral beverage CSs at baseline $t(58) = 0.29$, $p > 0.5$, $r = 0.04$], but greater liking of neutral CSs from trial 2 of conditioning $t(58) = 3.38$, $p = .001$, $r = 0.41$], subsequently [Trial 3 $t(58) = 3.93$, $p < .001$, $r = 0.46$; Trial 4 $t(58) = 3.52$, $p = .001$, $r = 0.42$] (see *Figure. 3*).

Figure 5.3. Differential evaluative conditioning of CS+s and CS-s across the counterconditioning task. Data are mean ± SEM



UCS ratings

Ratings of UCSs (IAPS disgust pictures, Bitrex or neutral pictures) were assessed with 3 (UCS) x 3 (Group) x 4 (Trial) mixed ANOVA. A large effect of UCS [$F(2, 112) = 156.65$, $p = < 0.001$, $\eta_p^2 = .737$] was observed, indicating unconditioned aversion to the Bitrex [$t(58) = 13.6$, $p < 0.001$, $r = 0.87$] and picture UCSs [$t(58) = 15.79$, $p < 0.001$, $r = 0.9$] relative to the neutral pictures. Descriptive statistics are given in *Table 5.2*.

Table 5.2. Ratings of aversion to UCSs (0 = most disgusting thing ever to 10 = most pleasant thing ever). Values represent mean ± standard deviation.

	Control	**REACT no PE**	**REACT + PE**
Bitrex	*1.59±1.3*	*2.11±1.6*	*1.29±1.45*
Disgust Pictures	*1.89±1.44*	*2.22±1.18*	*2.26±1.61*
Neutral Pictures	*5.23±1.77*	*5.36±1.45*	*5.45±1.73*

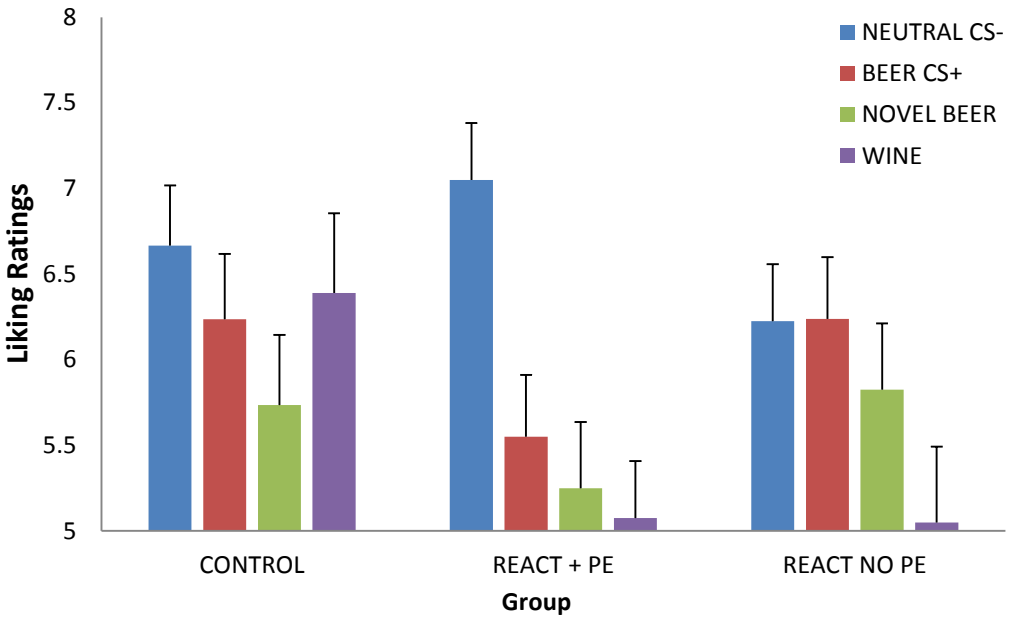
Contingency Awareness:

Chi Square analysis comparing awareness of contingencies between CSs and UCSs across groups found no differential frequency of contingency awareness across groups $\chi^2(2) = 2.636$, $p = 0.268$. Ns of contingency awareness were as follows: Control group, 10 aware, 9 unaware, REACT+PE group 12 aware, 8 unaware, REACT no PE group 7 aware, 12 unaware.

5.3.3. CS Rating Task

A 3 (Group) x 4 (Picture Type; Beer CS+s, Neutral Cs-s, novel beer, wine) mixed ANOVA assessed ratings of CSs from Day 1, along with ratings of novel beer pictures and novel wine pictures. Novel stimuli were included to assess generalisation of liking effects to unconditioned alcohol stimuli. A Picture Type main effect [$F(3, 168) = 8.44$, $p < 0.001$, $\eta_p^2 = .131$] and Group x Picture Type interaction was observed [$F(6, 168) = 2.622$, $p = 0.028$, $\eta_p^2 = 0.086$]. Planned, Bonferroni-corrected comparisons of alcohol CS+s and novel alcohol pictures to neutral CS- ratings were performed in each group. These comparisons showed lower liking for previously rated beer CS+s [t(19) =3.27, p = 0.011, r = 0.6], as well as new beer [t(19) = 3.91, $p = 0.001$, $r = 0.67$], and wine stimuli [t(19) = 3.72, p = 0.003, r = 0.65] in the REACT + PE group (see *Figure. 5.4*). Spontaneous recovery of stimulus valuation was evidenced by differences in liking between stimuli in the REACT no PE and Control group.

Figure 5.4. Reduction in liking of alcohol cues at day eight via counterconditioning after memory destabilisation. *Bars represent mean ± SEM*

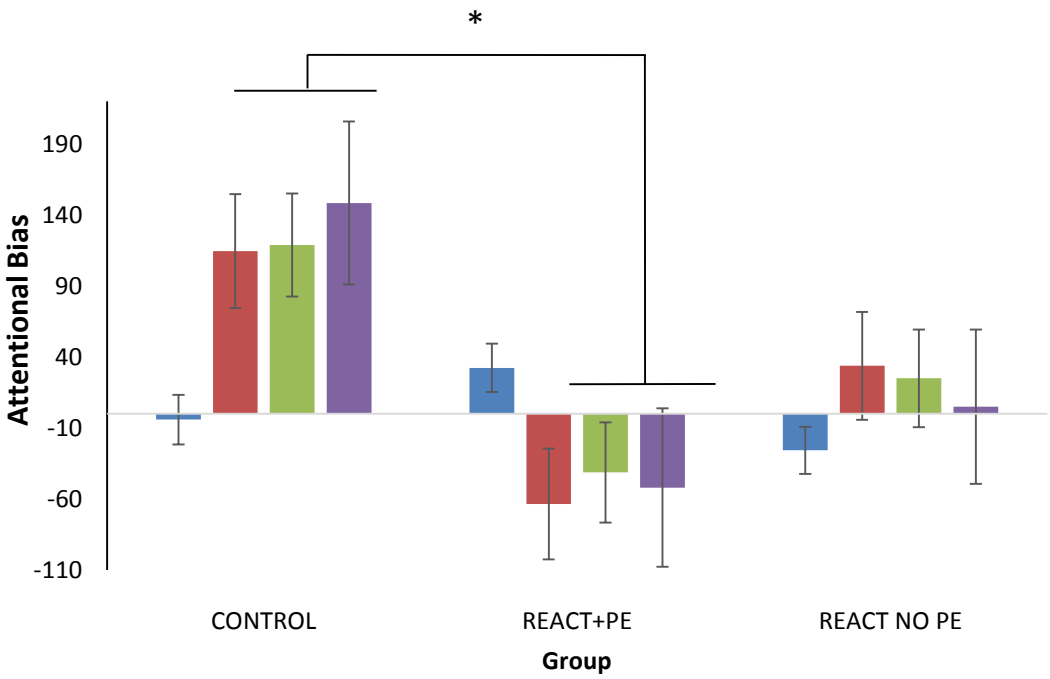


5.3.4. Attentional bias to CSs

Two participants' eye tracking data were discarded (one from the control group and one from the REACT + PE group) due to insufficient fixations on any images during the task. A 3 (Group) x 4 (Picture Type; Beer CS+s, novel beer, wine, neutral) mixed ANOVA was performed on attentional bias scores in the visual probe task. These were calculated as target image dwell time minus matched control image dwell time. A Group main effect [$F(2, 54) = 4.768$, $p = 0.012$, $\eta_p^2 = 0.15$] and Group x Picture Type interaction were found [$F(6, 162) = 3.293$, $p = 0.013$, $\eta_p^2 = 0.109$], providing an oculomotor index of aversion to all alcohol pictures, [Beer CS+s $t(35) = 3.19$, $p = 0.007$, $r = 0.47$; NEW BEER $t(35) = 3.16$, $p = 0.008$, $r = 0.47$ WINE $t(35) = 2.5$, $p =$

0.046, $r = 0.39$] but not neutral CS- pictures [$t(35) = 1.5$, $p = 0.422$, $r = 0.25$] in the REACT + PE group, relative to the control group (see Figure 5.5).

Figure 5.5: Attentional bias (in msec) to alcohol cues reduced by counterconditioning following retrieval + PE. Bars represent mean ± SEM.



To assess the convergence of the liking ratings and attentional bias data and craving, responses for each stimulus type were correlated across the measures. Highly significant correlations were found between Day 8 cue valuation and attentional bias to alcohol stimuli (but not neutral stimuli). These were also highly correlated with craving for alcohol. These correlations are given in *Table 5.3*.

Table 5.3 Correlations of liking ratings with attentional bias and alcohol craving. **Significant at p < 0.05, ** Significant at p < 0.01 *** Significant at p < 0.001.*

		ATTENTIONAL BIAS				
		BEER CS+s	**NOVEL BEER**	**WINE**	**NEUT CS-**	**ACQ**
LIKING RATINGS	**BEER CS+s**	*.606****	*.593****	*.572****	*-.391***	*.542****
	NOVEL BEER	*.464****	*.523****	*.497***	*-.295**	*.515****
	WINE	*.261*	*.32**	*.329**	*-.007*	*.244*
	NEUT CS-	*.053*	*.027*	*.193*	*.123*	*-.056*
	ACQ	*.462***	*.496***	*.483***	*-.344**	-

5.3.5. Cue-induced disgust and craving

There were no baseline differences in the DPSS-R, but the REACT+PE group rated themselves as more sensitive to disgust following the picture rating on Day 8 than at baseline [$t(19) = 2.81$, $p = 0.007$, $r = 0.54$], indicating stronger recall of the aversive reinforcement used during the counterconditioning task [Day x DPSS-R subscale x Group interaction $F(2,54) = 5.189$, $p = 0.009$, $\eta_p^2 = .161$].

As the retrieval-counterconditioning was designed to update outcome expectation in response to beer cues, we conducted planned analysis on the expectancy subscale of the ACQ-NOW. This showed an effect of GROUP [$F(2, 57) = 3.69$, $p = 0.031$, $\eta^2 = .13$], driven by more negative expectancy of alcohol-related outcomes in the REACT +PE group than the Control Group [$t(36) = 2.81$, $p = 0.008$, $r = 0.42$]. Group differences on the other subscales were not observed.

5.3.6. Changes in Drinking

All groups reduced their self-reported drinking pre-to-post intervention [$F(1, 56) = 5.167$, $p = 0.027$, $\eta_p^2 = 0.089$]. However, no main effect of Group [$F(2, 53) = 1.924$, $p = 0.156$, $\eta_p^2 = 0.068$] or interaction [$F(2, 53) = 0.098$, $p = 0.907$, $\eta_p^2 = 0.004$] was observed. The overall reduction in drinking reduction could be due either to the intervention or to non-specific effects such as the Hawthorne effect, regression to the mean or increased drinking awareness. To assess the change in beer drinking attributable to the intervention, overall liking rating of beer cues (mean of *Day 8* ratings for old beer CSs and novel cues) were correlated with changes in beer drinking from pre to post-intervention.

The relationship between liking ratings and drinking was moderated by group, with change in drinking being highly related to stimulus liking in the REACT + PE group ($r(20) = .623$, $p = 0.003$) and not the Control [$r(19) = 0.078$, $p = 0.75$] or NO PE group $r(20) = -0.296$, $p = 0.205$). Z tests on Fisher transformed correlation coefficients found that the difference in these associations was highly significant between the REACT no PE and REACT + PE groups [$z = 3.02$, $p = 0.003$] and borderline significant between REACT + PE and Control [$z = 1.87$, $p = 0.06$].

5.4. Discussion

The current study found that using guided expectancy violation to maximise prediction error during retrieval sufficiently destabilised strongly-trained, multivariate MMM networks to allow updating through subsequent counterconditioning, which produced broad-spectrum effects on cue value, motivational salience and alcohol craving. These findings demonstrate that robust alcohol MMMs do undergo reconsolidation and corroborate the proposed necessity of PE in this process, as evidence of destabilisation was not observed when cue-drinking MMMs were reactivated without PE. Importantly, destabilisation occurred in the absence of advanced knowledge of learning history, suggesting this may be a clinically practicable means for producing MMM destabilisation and potentially overcoming a major obstacle in the development of reconsolidation-based interventions for SUDs, where the training history and idiosyncratic nature of cues is inherently unknown.

Surprisingly, the reconsolidation-dependent reduction in cue motivational salience as indexed by oculomotor approach and cue valuation (Huys et al. 2011a) generalised to novel alcohol cues. Reconsolidation-interference effects to date have generally been shown to be specific to discrete reactivated cues, with second-order associations unaffected (Debiec et al. 2002). This level of pattern-separation could make the reactivation and updating of discrete drug cues insufficient to have an appreciable impact on drug seeking and using. Here, reactivation and counterconditioning was performed on four prototypical beer cues, thus the simplest rule to learn to predict cue outcome was 'beer cue → disgusting outcome'. If the reactivation + PE potentiated the retention of this rule learning by incorporating it into existing memory networks, this would explain the generalisation of effects to novel cues in the REACT + PE group at test.

This finding is also consistent with research showing that responding to multiple outcome-predictive cues can be reduced by blocking reconsolidation following reactivation of one of these cues but only if the cues are highly interconnected (Yang et al. 2011a), as was the case in the current task. That is, activation of memory networks by pattern completion from subsets of inputs (Rudy and O'Reilly 1999) may engender widespread destabilisation of pathological memory through activation of a more holistic neural representation of cue-outcome contingencies. The high degree of inter-connectedness, possible hippocampal independence (the hippocampus has very high-resolution pattern separation capabilities) and susceptibility to activation by individual nodes of MMMs in humans may therefore work in favour of reconsolidation-based interventions for SUDs.

Some evidence for reduced motivational salience of alcohol cues was observed in the REACT no PE group, although this was not significantly different from the Control group. This raises the interesting possibility that the reactivation procedure used in this group partially destabilised cue-drinking MMM networks, raising the question of whether destabilisation is binary or may occur on a continuum. If the latter, it is possible that the level of destabilisation is determined by the size of prediction error at retrieval. Testing this hypothesis will require parametric variation of PE during retrieval and concomitant assessment of reconsolidation effects. Maximising PE may therefore be the optimal approach to MMM destabilisation.

We found disgust counterconditioning with pictures and Bitrex solution to be an effective way of targeting motivational and evaluative components of alcohol cues (Huys et al. 2011a). This may be a useful alternative or adjunctive treatment modality to extinction post-destabilisation. Xue and colleagues (2012) found retrieval-extinction to effectively reduce craving in abstinent heroin addicts, suggesting a purely associative

intervention in MMMs sufficiently targeted craving in this group. It is unclear whether the same effects would be observed in our sample. The differential legal status and methods of consumption of alcohol and heroin mean heroin cues tend to be few (needle, spoon, drug and tourniquet), trained in fewer environments and over fewer episodes compared to drinking cues. The reinforcement schedule between exposure to alcohol cues and drinking is also likely to be more variable than that between heroin cues and heroin use.

Although currently speculative, it seems reasonable that corrective retraining of MMMs would benefit from being highly salient and aversive, providing a more emotional and memorable learning experience (Cahill et al. 1995). Engaging emotional responses is a key aspect of successful therapy and may work in concert with reconsolidation to provoke lasting change (Lane et al. 2014). If one is aiming to redress aberrantly high motivational influences of Pavlovian drug cues, it may therefore be best to 'fight fire with fire', pairing these cues with a highly de-motivating outcome. In extinction learning, the 'corrective' outcome is simply lack of reinforcement. In the laboratory context (an unusual setting in which to drink) this is unlikely to be a highly salient, or memorable reinforcer if it does not engage affective mechanisms. In the abstinent heroin-addicted group of Xue et al (2012), the omission of heroin after exposure to heroin-related cues produced powerful craving and therefore created a highly salient learning experience. However, pure reward omission may not sufficiently affect motivational and affective components of alcohol and other MMMs to produce therapeutic change.

Given the effects observed here, disgust counterconditioning may provide a reliable 'probe' for appetitive memory destabilisation. As mentioned previously, attributing null findings to failure of post-retrieval intervention or failure to destabilise memories in

MMM reconsolidation research is problematic. If disgust counterconditioning is a robust corrective learning modality, it may go some way towards addressing this issue, as it will allow attribution of null results to failure to destabilise MMMs.

The oral consumption modality of alcohol may make alcohol MMMs particularly amenable to disgust-based counterconditioning. As disgust is a potent and universal anti-consumption mechanism that can be reliably elicited by bitter tastes and various images (Olatunji and Sawchuk 2005), further investigation into its use as an intervention to reduce the motivational impact of drug cues is encouraged. The benefits of disgust counterconditioning over extinction for reducing drinking must be assessed in an experimental paradigm directly comparing the two. Recent evidence, examining pathological disgust in anxiety disorders, found that counterconditioning, but not extinction, was effective in reducing disgust responses, supporting counterconditioning as a more potent learning modality (Engelhard et al. 2014).

As motivational sensitisation to drug cues is thought to be a key process in the pathogenesis of SUDs (Berridge and Robinson 1998; Robinson and Berridge 1993; 2001) and relapse in abstinent addicts, effectively reducing this via memory updating is a promising approach to both prevent and treat SUDs. While the results described here represent an important advance in this approach, further research is needed. The current study found no overall difference in drinking change among groups. Aditionally, all groups considerably reduced their beer drinking over the course of the study, suggesting that counterconditioning itself may be an effective strategy for reducing drinking. However, only in the REACT + PE group was stimulus valuation related to greater drinking change, implying that a memory updating mechanism may have been responsible for, or at least contributed to drinking change in this group. Changes in self-reported alcohol consumption as a result of assessment reactivity or the Hawthorne

effect are widely recognised in the alcohol literature and can be observed even after simply administering a single screening (McCambridge and Day 2008). It may therefore be that observing genuine treatment effects of REACT+PE dependent counterconditioning on drinking behaviour will rely on a sufficiently long follow-up or 'dose' of counterconditioning to supersede non-specific effects over time.

Alternatively, naturalistic experience of conditioned disgust responses when drinking in the time between *Day 1* and *Day 8* may have contributed to reduced motivational status of alcohol stimuli on Day 8. If memory destabilisation prior to counterconditioning *updated* existing memory traces, it would be expected that disgust conditioned responses were retrieved more when beer cues were encountered naturalistically, causing further experience-dependent changes in appraisal of those stimuli. This would explain both the correlation between reduced drinking and reduced cue valuation in the REACT + PE group and the increased self-reported disgust sensitivity in this group. Such an effect is in keeping with recent research employing Propranolol to disrupt the reconsolidation of fear responses to spiders in spider phobics. Although behaviourally, participants receiving Propranolol with reactivation displayed no fear in spider handling at initial test, there was a lag before self-reported spider phobia decreased to non-phobic levels (Merel Kindt, personal communication). This suggests that naturalistic experience with the 'updated' status of a stimulus (i.e. the phobics encountering spiders without fear or the drinkers encountering beer cues and feeling disgust) contributes to the long-term efficacy of reconsolidation interventions.

Limitations

A limitation of the current study was that these naturalistic interim responses were not measured. It would have been highly informative, for instance, to assess 'intrusive' disgust responses in the groups after *Day 1* and collect self-report liking and wanting of

beer with momentary assessment. This would allow appraisal of the specific mechanism of the changes observed in the study following destabilisation and counterconditioning. A six week follow-up was attempted in the current study, however, despite the researchers' best efforts, too few participants were contactable for analysis of follow-up data to be meaningfully performed. It therefore cannot be determined whether the current intervention fulfilled *Criterion 2* of the appraisal criteria laid out in *Chapter 1* (long lasting efficacy). Further, although the participants in the current study were hazardous drinkers (and therefore an at-risk group for further alcohol use disorder), they were by no means a clinical group. It therefore remains to be tested whether the current results will extend to patients with AUDs.

A criticism of the design of the current study may be the single time point post-intervention assessment of attentional bias, meaning pre-existing group differences cannot be definitively ruled out. Such a pre-existing difference is highly unlikely, however, given the randomisation to groups and equivalent levels of drinking, AUDIT scores and initial ratings of CSs across groups. Using a three day design, it would have been possible to assess attentional bias to CSs prior to the intervention and test days reported here. However, this would necessarily include pre-exposure to CSs, potentially having a latent inhibiting effect on counterconditioning and would render the 'novel' CSs used on *Day 8* familiar. Nonetheless, such a design may be used in the future to rule out non-intervention-dependent effects.

Lastly, although the counterconditioning used here was effective, it may not have been optimal. Much future research will be required to determine the optimum parameters of retrieval and counterconditioning (i.e. number of cues, number of trials, reinforcement schedules) for producing greatest therapeutic benefit.

In summary, counterconditioning after maximising PE during retrieval of alcohol MMMs appeared to cause destabilisation and updating of these memories in the current study, leading to a generalised reduction in the motivational status of alcohol cues which was associated with decreased drinking at least one week later. This preliminary finding is promising for the use of a reconsolidation based interventions in SUDs, as it suggests robust MMMs can be destabilised via relatively simple procedures without knowledge of learning history. The extent to which this is clinically applicable and the optimal parameters for post-destabilisation interventions remain to be assessed.

Chapter 6: General Discussion

6.1. Introduction

The work presented in this thesis comprises research conducted over three years spanning from autumn 2011 to 2014. When the work began, the exponential rise in publication rate of studies on the phenomenon of memory reconsolidation was in full swing. Some authors had suggested that this approach could be the 'golden bullet' for the pernicious problem of maladaptive memory processes that underlie relapse in addiction (Milton and Everitt 2012). However, at the time, virtually all publications on the phenomenon of appetitive memory reconsolidation were based on studies in rats. The treatment of the issue in humans was confined to narrative review articles, of which there were many.

While the approach was not without its critics who identified limitations in extant studies and urged caution in empirical design and interpretation (Dudai and Eisenberg 2004; Eisenberg and Dudai 2004; Tronel et al. 2005), it seemed when I started out in 2011 that, on the basis of translational success in fear learning, a wave of human studies on appetitive memory reconsolidation was about to break, bringing with it answers to many of the questions concerning its translational potential for substance use disorders.

Three years later the wave has, disappointingly, still not broken. The empirical work in this thesis began with an exhaustive literature search of animal and human appetitive memory reconsolidation studies. The preclinical focus of the meta-analysis presented in *Chapter 2* was driven by necessity, as at the time no equivalent human research existed. Since then (to the 12th October 2014), two studies have been conducted examining reconsolidation interference as a therapeutic intervention in human drug users. The first of these (Xue et al. 2012) used a relatively novel (at the time) retrieval-extinction procedure, rather than building upon the wealth of preclinical pharmacological evidence

and found a lasting effect of craving reduction, albeit in an already abstinent, inpatient heroin-addicted sample, meaning objective assessment of abstinence and relapse rates was impossible. The second (Saladin et al. 2013) did use a pharmacological intervention, building upon rat research showing efficacy of Propranolol for reconsolidation blockade, but failed to show any improvement following the intervention in cocaine addicts. It is perhaps telling that, in a recent review of the area (the publication rate of which has remained high relative to primary research) there are section headers entitled '*Reconsolidation of Human Fear Memory*' and *'Reconsolidation of Human Episodic Memory*' but sadly, no '*Reconsolidation of Human Appetitive Memory*' (Schwabe et al. 2014).

Thus the research presented in this thesis, to my knowledge, represents some of the first and only translational work examining reconsolidation of human drug memories with a view to improving treatments. Given this paucity of research in the area over the last few years, there is not a great deal of context in which to discuss the studies presented here, although I believe they have generated answers to some important questions, created perhaps more questions than they have answered and provided some directions for future research. This discussion will attempt to synthesise these findings by first recapping the major results, considering what we may learn from this research and what this means for the future of SUD treatments and finally speculating where the work may lead next.

6.2. Overview of findings

This thesis aimed to take the first steps necessary to translate promising preclinical findings with appetitive memory reconsolidation blockade to human drug using populations. To this end, *Chapter 2* reported the results of a meta-analysis of preclinical studies on NMDAR and β-AR antagonists with the aim of establishing which class of drugs (if either) was most effective at blocking appetitive memory reconsolidation and statistically addressing some of the methodological issues and controversies thrown up by this research. It was found that both β-AR and NMDAR antagonists robustly interfered with the restabilisation of appetitive memories, but that NMDAR antagonism did so to a much greater extent. The analysis also challenged the generally held view that instrumental memories were resistant to reconsolidation (Hernandez and Kelley 2004; Tronson and Taylor 2007), as robust reconsolidation-interference effects were seen for both Pavlovian and instrumental memory tasks. The clinical utility of this distinction in SUDs will be revisited later. Other issues, such as the timing of drug administration, also appeared to matter less than has been suggested in the interpretation of individual studies relying on the binary logic of null hypothesis significance testing (Milton et al. 2008a; Wu et al. 2012). However dose of the NMDAR antagonist MK-801 showed an unexpected non-linear dose-response curve in reconsolidation blockade, indicating that low doses may be more effective than moderate doses.

These results formed the basis of the study reported in *Chapter 3*, which examined the effect of 10mg of the NMDAR antagonist memantine alongside retrieval of smoking MMMs on relapse latency and measures of MMM strength in quitting smokers. This study found no therapeutic benefit of memantine and memory reactivation on relapse rates or any measure of addiction severity, suggesting either that memantine was

ineffective at blocking smoking MMM restabilisation or that the retrieval procedure did not successfully destabilise memories.

To help disentangle these two alternative explanations for null results, *Chapter 4* took a step back, using an in-lab conditioning paradigm with an ecologically valid reinforcer (beer) in a clinically relevant sample (hazardous drinkers). Building upon recent evidence demonstrating the necessity of prediction error for memory destabilisation and the known amnestic properties of the NMDAR antagonist of Nitrous Oxide, this study found that twenty minutes of inhaling 45% N_2O following a brief reminder of conditioning reduced conditioned responding at initial test compared to a group that received air, as evidenced by pupil dilation dynamics and response accuracy. These findings showed promise firstly for the use of methods that induce prediction error for destabilising drug memories and secondly in identifying N_2O as a potential promising therapeutic agent. However they did not assess whether these results would be achieved for naturalistically trained MMMs.

To address these questions, the study reported in *Chapter 5* extended the logic of using prediction error to destabilise memories by setting up an explicit expectancy of beer consumption, but violating this expectancy last-minute. Using an exploratory form of corrective learning, disgust-based counterconditioning, following this retrieval procedure, it was found that liking and motivational salience of alcohol cues could be reduced for at least one week. Correlated decreases in drinking (but not different overall to control groups) were observed, along with increases in self-rated disgust sensitivity at test, following re-exposure to counterconditioned cues.

6.2. Lessons from reconsolidation research

The null findings in the first experimental study in this thesis highlighted what has become the central issue in this field of research; the problem of memory destabilisation. We do not currently possess the tools to independently determine whether reconsolidation is occurring at a given time and as such it remains an essentially 'silent' process. An inference that reconsolidation has occurred is currently made on the basis of the effects of post-retrieval manipulations (with the appropriate no-reactivation and no-manipulation controls precluding confounding explanations) and there is currently no algorithm for designing retrieval sessions to maximise the probability of destabilisation.

Going beyond MMMs, there is evidence that the parameters of retrieval necessary to destabilise memories may vary depending upon the type and strength of memory being destabilised. In word-pair list learning for example, subtle reminders, such as brief re-exposure to the training context or a single 'cue' word, successfully destabilise word pair memories, allowing interference by new word list learning (Forcato et al. 2007). However, destabilisation does not occur if participants correctly retrieve the response word associated with the cue word during retrieval (Forcato et al. 2009; Forcato et al. 2010). Note that these findings are not inconsistent with the necessity of PE for memory destabilisation as brief exposure to the learning-associated context or a single cue, without the opportunity to respond (as per Forcato and colleagues; 2009, 2010) is equivalent to a negative prediction error generated by omission of outcome. In this case, because the 'outcome' (i.e. the response word) is internally generated and can be retrieved relatively quickly, more subtle reminders are required to prevent successful retrieval and preclude destabilisation.

In support of this, due to the retention-enhancing practice effect of successful retrieval (Karpicke and Roediger 2008; Karpicke and Roediger, 2007), full retrieval (without PE) can counteract the interference effect of new list learning on originally learned word pairs where more subtle reminders, such as exposure to the learning environment, display a retrieval-induced interference effect (Potts and Shanks 2012). Such subtle reminders are certainly not sufficient to destabilise MMMs, but this discrepancy in destabilisation requirements between relatively brief verbal learning and extensive appetitive conditioning highlights the fact that retrieval procedures must be scaled to memory type and learning history (Frankland et al. 2006; Suzuki et al. 2004) to achieve memory destabilisation. If retrieval is incorrectly tailored to memory type, it cannot be said with certainty that the nominal 'no reactivation' group in an experiment did not destabilise a target memory or that the nominal 'reactivation' group did.

The procedures developed in the current work (*Chapter 5*) may therefore be effective for destabilising well-learned motivational memories, but could be entirely inappropriate for destabilising verbal or procedural memory. Further, they may be sufficient to destabilise cue-drinking MMMs, but still ineffective in an addiction like smoking, where training history is even greater. Related to this, there is a further problem of engaging reconsolidation vs. extinction, as both rely on prediction error-driven plasticity, with reconsolidation requiring hitting a 'sweet spot' between too little prediction error at retrieval to destabilise memory and too much (or more accurately, too *many*), which causes new learning in the form of extinction (Osan et al. 2011)

Development of tools that can reliably measure memory destabilisation will be paramount to the progression of this field. Only then can the epistemic issue of null effect attribution be solved and the development of effective post-retrieval manipulations begin in earnest. Has the current research illuminated mechanisms by

which this might be achieved? Certainly, it has highlighted how a relatively simple 'reactivation' procedure for a rat pressing a lever in a cage becomes exponentially more nuanced and complex when applied to human drug users with all their idiosyncrasies and heterogeneity. However, it has also provided strong support for the importance of prediction error in destabilising MMMs. In *Chapter 5*, explicitly guided prediction error was found to be sufficient for destabilisation of robust, multivariate MMMs in hazardous drinkers. It remains to be determined whether PE is universally sufficient for memory destabilisation. The previously discussed word-pair learning experiments and accounts of reconsolidation as an updating mechanism (Lee 2009) suggest that this may be so. If this is the case, the search for procedures that destabilise MMMs will parallel the search for means of producing and measuring robust PE upon the retrieval. This is perhaps a more tractable goal, as biobehavioural metrics for measuring PE are beginning to be elucidated. Of these, electroencephalographic (EEG) measures hold particular promise. Certain event-related potentials (ERPs) such as error-related negativity (Holroyd et al. 2003), medial frontal negativity and P2a signals (Potts et al. 2006) have been identified that track errors in reward processing, although reliable assessment of these signals requires averaging over hundreds of trials whereas destabilisation of MMMs is really a 'one-shot' process. Shifts in theta-frequency oscillations over the medial frontal cortex may allow real-time measurements of memory destabilisation as these are consistently implicated in PE processing and subsequent behavioural updating (Cavanagh et al. 2009; Cavanagh et al. 2011; Cavanagh et al. 2010; Cohen 2011). Ongoing assessment of oscillations does not require the same level of trial averaging as individual ERP analysis, however it remains to be seen whether this will be sensitive enough to predict memory destabilisation following single retrieval procedures. Collection of EEG before, during and after memory retrieval sessions in combination with other proposed indices of prediction

error, such as pupillary responses, discrepancies in outcome expectancy ratings and the retrieval manipulation described in *Chapter* 5 may allow identification of potential neurobehavioural signatures of memory destabilisation (these will be the subject of ongoing work within my team).

Although highly exploratory at this stage, by modelling behavioural change observed at test as a function of candidate EEG, explicit ratings and psychophysiological signals, it may be possible to eventually derive independent metrics of memory destabilisation. Understanding when and how memories become unstable would have profound implications for our understanding of neuroplasticity in addiction, but also for psychiatry more generally. As mentioned in *Chapter 1*, many psychiatric conditions can be conceptualised as maladaptive engagement of neural plasticity. An ability to assess when memories destabilise (and are therefore plastic) will greatly enhance the efficacy of existing treatments such as behavioural, cognitive-behavioural and emotion-focussed therapy (Lane et al. 2014). Thus while measuring memory destabilisation independently is an ambitious goal, I believe it is highly worthwhile.

However, measuring destabilisation is only useful in SUDs if it can be reliably achieved for well-learned MMMs. In *Chapter 5* I reported the evidence I found for MMM destabilisation following guided expectancy violation, but this was in a relatively small sample of hazardous drinkers, and not clinical, alcohol-dependent patients. In the latter group, the history of cue-drinking episodes is likely to be greater and over a greater variety of contexts, which may confer resistance of these memory traces to destabilisation. The retrieval –counterconditioning procedure must therefore be further validated not only in a larger sample of hazardous drinkers but also in a fully-powered study of alcohol-dependent individuals. Both approaches are clinically valid as

hazardous drinkers can be conceptualised as being in a prodromal state, where effective treatment may prevent their conversion to full dependence.

It is revealing to note that, of the three studies reported here, the one that found least evidence of reconsolidation effects was the one conducted in a dependent sample of smokers. This may be taken as evidence suggesting differential susceptibility of different cue-drug memories to destabilisation, or suggesting that when MMMs reach habit-level, they become highly destabilisation-resistant. This is in keeping with the known inflexibility of habitual memory traces (Everitt and Robbins 2005). However, the retrieval procedure in that study was designed prior to the demonstration that maximising PE was important in MMM destabilisation. An important way to build on the collective work in this thesis would be to test the effects of memantine in smokers using a maximal PE reactivation procedure as per *Chapter 5*. Thus before memantine is abandoned as a potential reconsolidation-blocker, it must be shown that habitual memories are destabilised in reconsolidation interference trials in the first place. It is possible, however, that even retrieval with maximum PE through guided expectancy violation may not be sufficient for MMM destabilisation in addicted groups, leading us to a potential 'dead end' in the field, reminiscent of DCS enhanced cue exposure discussed in Chapter 1. Additionally, as discussed in *Chapter 2*, meta-analysis revealed that reinforcer type moderates effect sizes for the reconsolidation of reward memory in animals. This may be a phenomenon that is limited to preclinical research. Alternatively, it may be the case that reconsolidation is simply not effective in nicotine using populations. The results presented in this thesis (*Chapters 4* and *5*) do, however, indicate that these approaches may be efficacious in alcohol using populations.

6.3 If all else fails?

How are we to proceed if our best attempts to create PE during MMM retrieval in drug-dependent patients fail to destabilise these memories? Recent research suggests that there may be pharmacological means of increasing the destabilisation propensity of well-learned memories by priming the epigenetic pathways involved. As outlined in *Chapter 1*, reconsolidation is dependent upon the transcription of DNA and de novo protein synthesis. In its 'inactive' state, DNA is tightly packaged via proteins into spatially compact chromatin structures (Mirsky 1971). Relaxation of this chromatin packaging is necessary to allow transcription factors and enzymes access to nuclear DNA and produce mRNA. Thus chromatin remodelling is a primary mechanism for epigenetic regulation of genes (Hewish and Burgoyne 1973). The majority of the structural proteins in chromatin that directly interact with DNA (the 'joints' holding the complex together) are histone proteins (Smith 1991). As with many proteins, the ongoing function of histones is regulated by the addition and removal of acetyl groups (acetylation/deacetylation) and methyl groups (methylation/ demethylation), which modifies the bond between histones and DNA, with acetylation of histones 'freeing' the DNA so that it can be accessed for transcription and deacetylation 'repackaging' DNA to its inactive state (Grunstein 1997). These acetylation and methylation reactions are catalysed by acetylase/deacetylase and methylase/demethylase enzymes. Histone deacetylase is thus the enzyme responsible for regulation the 'switching on and off' of genes through regulating access to DNA (Richon et al. 2000).

Recent evidence has shown that by inhibiting histone deacetylase (causing histones to remain acetylated and DNA to remain available for transcription), via the same retrieval procedures that failed to destabilise these memories without drug, remote, strongly trained memories can be destabilised and undergo reconsolidation (Gräff et al., 2013). Given the molecular pathways involved in reconsolidation described in *Chapter 1*, it is clear to see why this should be the case. Histone deacetylase inhibitors (HDACis) are

already used therapeutically, with Valproic acid prescribed for epilepsy and mood disorders (Phiel et al. 2001) and Vorinostat, used in lymphoma treatment (Grant et al. 2007). Further, HDACis are the focus of ongoing drug discovery due to their promise as cancer drugs, so it is likely that more selective and potent HDACis will become available over the next decade, opening up a potential pharmacopeia of plasticity-enhancing compounds for psychiatric use.

A combination of epigenetic priming and maximising prediction error could potentially destabilise the strongest, most habitual of memories. The next decade will see the blossoming of 'epigenetic psychiatry', examining the interplay between genes and the environment and the role of these interactions in neural states of health and disease. Aberrant neural plasticity, at some level, is implicated in virtually all psychiatric conditions, so understanding the pathways underpinning plasticity from gene to protein to cell to neural network, and aberrations in these pathways in relation to phenotypes, should shed light on why psychiatric conditions occur in the first place and how they can be best treated. Variation in the genes encoding enzymes responsible for regulating epigenetic processes like methylation and acetylation may provide further insight into the aetiology of deficits in neural plasticity and inform how best to tailor treatments to disorders in specific populations.

Until then, there are other potential means of increasing the susceptibility of memory traces to destabilisation. Coming ironically full-circle (Das and Kamboj 2012), pro-glutamatergic compounds such as D-serine, glycine and D-cycloserine may be usefully employed in priming the NMDAergic activation necessary for memory destabilisation (Mamou et al. 2006). Although timing of administration and reactivation would have to be carefully considered, as there is the possibility of DCS enhancing reconsolidation (Lee 2009), it would be theoretically possible to give a dose of DCS prior to

reactivation, reactivate memories then rapidly antagonise NMDARs, either with intravenous ketamine or inhaled Nitrous Oxide. This may potentiate destabilisation and interfere with reconsolidation, allowing further reduction in strength of MMMs. Importantly, a double dissociation of the roles of GluN2a and GluN2b receptor subunits has recently been found in different stages of reconsolidation, with GluN2b critically involved in destabilisation and not restabilisation and GluN2a involved in restabilisation, but not destabilisation. Currently we do not possess drugs with sufficient specificity for these subunit receptor sites (with the exception of Ifenprodil, a selective GluN2b antagonist) to be able to manipulate the NMDAR with such precision in humans. However, drug development may identify novel NMDAergic compounds. This would essentially overcome the administration timing problem with NMDA antagonists (whereby antagonising GluN2b prior to reactivation prevents destabilisation), as GluN2a antagonists could be employed that selectively impair restabilisation.

Of course, if memory *updating* using behavioural procedures, rather than reconsolidation blockade is the aim following MMM destabilisation, such pharmacological precision will not be necessary, as pro-glutamatergic compounds such as DCS may enhance destabilisation *and* restabilisation and would not result in undesirable effects by potentiating both processes. This begs the question of whether there is any benefit of a pharmacological 'reconsolidation blockade' approach over a behavioural 'memory updating' approach at all.

*6.4 A farewell to pharms**?***

The most striking findings reported in this thesis occurred as a result of a purely behavioural procedure. In this study, there were no concerns around inadvertent pharmacological interference with destabilisation, as was the case in the study with memantine. Importantly, effects were shown for memories trained naturalistically with

alcohol reinforcement, for which there was the weakest evidence of disruption from meta-analysis of preclinical studies in *Chapter 2*, indicating that these memories can be robustly disrupted in humans. Moreover, the entire retrieval-counterconditioning procedure took around thirty minutes, compared to around 4.5 hours for the memantine intervention. The retrieval-updating approach could easily be implemented by clinical psychologists as there is no need to prescribe any drug and there are no concerns regarding drug side-effects or contraindications. However, the counterconditioning procedure is inherently aversive. As discussed in *Chapter 5*, the Bitrex-containing drinks and pictures used produced strong disgust reactions in all participants. This salient aversive experience, operating on disgust mechanisms, may have been critical to the success of the intervention. From my personal experience in conducting exposure-based interventions in cigarette smokers (Kamboj et al. 2012) and hazardous drinkers (Kamboj et al. 2011), the participant's engagement in the corrective learning procedure can be variable and hard to quantify. In MMM extinction, there is by definition no presentation of a primary reinforcer, therefore withholding alcohol or cigarettes produces a salient emotional response only in proportion to participants' strength of desire to consume the drug. In the laboratory setting and in the presence of an experimenter, this desire is markedly weaker than in a naturalistic environment. This is in stark contrast to the effects produced by counterconditioning, where disgusting UCSs produced evident and universal disgust responses.

Adherence to an aversive intervention like counterconditioning may be lower than for extinction-based therapies in a clinical setting and patients may be understandably resistant to participating in the intervention. The use of the procedure as a treatment thus warrants careful ethical consideration. It is possible that its greater efficacy warrants the brief aversive experience, or it may be shown that in clinical samples, it does not convey sufficient benefit to warrant use. However until the approach is validated in

clinical samples we will not know whether it is a viable treatment. Few direct comparisons of different forms of corrective behavioural learning (different forms of extinction, conditioned avoidance, and counterconditioning) in SUDs have been made in the context of SUD intervention, and this should be a priority for future research so that informed decisions can be made when weighing the efficacy of each against its practicability.

Pharmacological means of disrupting memory reconsolidation should potentially suffer less from this variability in engagement with therapy, as the pharmacological mechanism of action of a drug is expected to be more consistent across patients, or at least less susceptible to variations in implementation than behavioural therapies. Further, although not directly related to treatment efficacy itself, there is generally greater financial incentive and research funding for developing pharmacological treatments than behavioural treatments. Unfortunately, a 'drug to prevent relapse' is likely to garner far greater commercial financial backing for clinical trials than a novel behavioural procedure.

However, the limitation of the reconsolidation blocking pharmacopoeia has been highlighted consistently throughout this thesis. At present, there are only three NMDAergic drugs with potential in this arena; memantine, ketamine and Nitrous Oxide. *Chapter 2* found null effects of memantine in quitting smokers and its long peak latency means it must be dosed orally prior to reactivation, introducing problems with destabilisation. As mentioned above its use in the context of adequate destabilisation procedures remains to be tested and it should therefore not be abandoned as an agent for MMM weakening. As discussed in the relevant section of *Chapter 4*, N_2O, due to its rapid onset and offset, could be extremely useful in 'bridging the gap' between administration and peak effects of oral preparations of drugs administered after

reactivation. The discovery of N_2O as a potential reconsolidation blocker in its own right is exciting for this field as it possesses many of the characteristics of an ideal drug for this purpose. Further research will be required to determine the efficacy of N_2O following the reactivation of naturalistically trained MMMs, but if it is found to be fit for this purpose, it will hopefully spur an increase in human MMM reconsolidation research.

It was beyond the scope of the current thesis to examine post-retrieval ketamine, as it must be administered intravenously by a qualified anaesthetist. However, this would be the next logical step in assessing the 'pharmacological approach' to reconsolidation blockade. Ketamine has been assessed as an adjunct to cognitive behavioural therapy in treating alcohol (Krupitsky and Grinenko 1997) and heroin (Krupitsky et al. 2002) addiction, where its side effect profile was not found to hinder its utility. Its utility might be maximised through combination with memory destabilisation procedures in these groups. No other, more potent NMDA antagonist is available for use in humans, so if post-reactivation ketamine is not found to impact clinically relevant variables in a meaningful way, it may be time to search for new drug targets in MMM reconsolidation.

Although this thesis focussed on NMDAR modulators, metabotropic glutamate receptors, particularly mGluR5may be a key target for reconsolidation interference (Salinska 2006). These receptors are heavily implicated in learning and memory (Rodrigues et al. 2002) and mGluR5 antagonists have been shown to have anti-addictive properties, reducing cocaine self-administration in rats (McGeehan and Olive 2003), with knockout rats lacking mGluR5s failing to self-administer cocaine at all (Chiamulera et al. 2001). Antagonists at mGluR5 are well tolerated and currently used in humans (Berry-Kravis et al. 2009) and they are an exciting potential drug target for

reconsolidation-based therapy in addiction, although, as with NMDAR antagonists, there is a current lack of human research examining them for this purpose.

There is thus scope for both employing pharmacological and behavioural interventions in MMM reconsolidation and, along with development of independent metrics of memory destabilisation, research designed to allow a direct comparison of the two approaches will be critical to developing effective behavioural or pharmacobehavioural therapies that are ready for clinical use.

6.5 Lost in Translation: Conceptual Issues in Memory and Reconsolidation

Behavioural modelling in animals is absolutely essential to understanding and bridging the 'chasm' between neuropharmacology and behaviour (Rang et al. 2003). Indeed, apart from the current work, the field of appetitive memory reconsolidation exists almost exclusively within these models, hence their dominance seen in *Chapter 1*. Such studies aim to inform our understanding of how basic molecular mechanisms can create normal and maladaptive behaviour. Broaching this chasm is no simple task and fraught with conceptual and methodological obstacles. Consider the complexity of the pathways identified in *Figure 1.5, Chapter 1.* This represents a highly simplified schematic of a small subset of relevant signal transduction pathways within a single cell. This complexity increases exponentially when cell-to-cell interactions are taken into account and further still with the organisation of neurons into heterogeneous functional networks. Complex behaviour arises as a result of this emergent complexity (Bar-Yam 1997; Hopfield 1982). As neural networks in psychiatry are not simple linear systems (Chialvo 2010), highly variable pathologies at the cellular level can manifest in the same cognitive-behavioural deficits, while the same cellular aberrations can create divergent cognitive-behavioural effects (Huys et al. 2011b). When we administer a drug systemically, we are targeting low-level molecular processes and the drugs can

produce measurable behavioural effects, but the nature of the intermediate mechanisms in humans is unknown. Certain pharmacological systems may be implicated to a greater extent (and therefore more important to target) for psychiatric symptoms in some individuals than others and development of treatment 'menus' that allow variable approaches to pathology based on individual differences will be important (Krystal and State 2014).

There is a further 'chasm' between animal behavioural models and human clinical manifestations of the putatively modelled behaviour. SUDs are perhaps some of the most tractable of human psychiatric problems for animal modelling, as predictive computational models of behaviour based on reinforcement learning theory and dopaminergic modulation of motivation are emerging that can convincingly describe key features of addictive behaviours (Dayan and Berridge 2014; Huys et al. 2014; Montague et al. 2012). Despite this, there are key differences between rodent and human behavioural process. Some of these may be due to environmental rather than interspecies differences per se. Operant conditioning of amphetamine self-administration is much harder to demonstrate if rats are exposed to enriched (i.e. more like human) environments (Bardo et al. 2001). Other behavioural distinctions are the focus of much animal modelling for which it is difficult to see the human clinical relevance. Some of these will be briefly discussed in the light of the current work.

As discussed in *Chapter 1*, in the reconsolidation literature, rodent research has identified several putative moderators of memory reconsolidation. It is frequently proposed that certain types of associative memory are more or less susceptible to reconsolidation than others. This is non-intuitive from a neuropharmacological perspective, as all associative memory types commonly tested in lab paradigms putatively involve NMDAR activation with subsequent transcriptional cascades and

AMPA trafficking for expression and maintenance (Shimizu et al. 2000; Tsien et al. 1996; Wang et al. 2011). One of the main distinctions between memory types is that between Pavlovian and instrumental learning. Researchers often state that instrumental memories do not undergo reconsolidation e.g. (Tronson and Taylor 2007), however this is based on a single (but highly cited) null finding (Hernandez and Kelley 2004), while positive findings have been ignored (Diergaarde et al. 2006; Przybyslawski and Sara 1997). The current work challenges this distinction in reconsolidation susceptibility.

Logic would suggest that instrumental memories should undergo reconsolidation to allow efficient updating of appropriate action-outcome contingencies (which are known to be highly flexible) (Dayan and Balleine 2002) and the current work suggests that this is indeed the case. In *Chapter 2*, meta-analysis found robust evidence for both Pavlovian and instrumental appetitive memory reconsolidation blockade. Further, in *Chapter 4* I found that reconsolidation-blocking effects of post-retrieval Nitrous Oxide that appeared to operate largely on instrumental responding. This is not to say Pavlovian memories were not affected, as only pupil data were available as a continuous measure of responding to Pavlovian conditioned stimuli and pupillary responses to these were generally small. Regardless, these findings demonstrate that in terms of reconsolidation susceptibility, the putative Pavlovian –instrumental dichotomy may be false, a conclusion which is further supported by recent evidence (Exton-McGuinness et al. 2014; Tedesco et al. 2014) showing instrumental memory reconsolidation.

More generally, beyond the operationalized definition of Pavlovian outcomes as independent of action and instrumental outcomes as action-dependent, the clinical relevance of the distinction is somewhat unclear. All contemporary computational accounts of reinforcement learning differentiate Pavlovian learning, based on the values (V) of states (s) and instrumental learning, based on the values (Q) of actions in given

states (s) (O'Doherty et al. 2003). However, these distinctions are largely based upon rat experiments and there is a large discrepancy in the action and cognitive representational repertoire of rodents and humans. It is possible, therefore, that some of the observed phenomena are species-specific.

Approach behaviour in rats, for example, is generally classed as a Pavlovian response behaviour (Parkinson et al. 1999), even though approach clearly influences reward outcome, as a reward cannot be consumed if the animal cannot reach it. 'Oculomotor approach' can be reliably observed in humans (Mogg et al. 2003; Mogg et al. 2005) and is sensitive to manipulations of relevant neurotransmitter systems, i.e. dopamine (Freeman et al. 2014) but automatic approach of the entire organism to reward locations has never been shown. When humans do move towards rewards in naturalistic settings, it is undoubtedly with the goal of interacting with the reward in the appropriate way. This 'appropriateness' of responding highlights another dissociation between human and rodent approach behaviours.

Following repeated pairing of a cue with a reward (a light with food, for example), some rats, known as 'sign trackers' (Hearst and Jenkins 1974) will begin to approach the light, interact with it and attempting to consume it. Others, known as 'goal trackers' will go straight to the location of reward delivery and make preparatory consummatory responses. These two behavioural response characteristics are also dissociable in terms of the recruitment in dopamine, with greater phasic firing shifts to cue presentation in sign-trackers (Flagel et al. 2011). However, human examples of sign tracking are again lacking. Humans are rarely observed attempting to smoke tobacconists, inject heroin dealers or copulate with condom packets. The single example that is repeatedly used to suggest that sign tracking does occur in humans is the anecdotal report of crack cocaine users 'chasing ghosts', looking for crack rocks on the floor (Berridge et al. 2009; Tomie

et al. 2008) and occasionally smoking sugar or salt granules found in this manner. However, it is more likely that this represents superstitious or delusional beliefs about dropped crack than true sign-tracking, particularly given the fact that psychomotor stimulants increases delusion-like beliefs (Bartlett et al. 1997). Indeed, translational attempts to identify human sign-tracking behaviour and link it to dopaminergic activity have been abandoned due to difficulty demonstrating human sign-tracking in experimental settings (R. Koster, personal communication). If sign tracking were an important component of addiction, it seems it should be easier to demonstrate robust examples in humans.

That these effects are easily demonstrated in rats but not in humans hints at evolutionarily prepared and ethologically specific responses in the former species, likely as a result of the aforementioned difference in cognitive-behavioural repertoire and capacity for model-based goal representation between the two. In *Chapter 4* in this thesis, I demonstrated that humans readily learned to make an avoidance response to a reward-predictive CS to obtain a reward (although this was admittedly slower than the learning of an approach-to-win response). Chicks are incapable of such learning and will continue to approach a food bowl even if this causes it to recede at twice the pace of approach (Hershberger 1986). It is perhaps unsurprising then, that glutamatergic modulators (like memantine) have different effects on reconsolidation in day-old chicks (Samartgis et al. 2012) compared to rats (Popik et al. 2005) and humans (*Chapter 3*).

These differences between species are extremely important, considering that so many of our current models of reward learning (Sutton and Barto 1981; Sutton and Barto 1998), incentive salience (Berridge 2009; Berridge and Robinson 1998; Robinson and Berridge 1993) and addiction are based on experiments with rodents. What are believed to be important distinctions in behavioural processes in animals may be far less so in humans.

As highlighted in *Chapter 2*, there is virtually never an instance in human drug use where drug delivery occurs in a purely instrumental or Pavlovian fashion. Quite the opposite, a great deal of time and effort can be spent procuring drugs (indeed this is one of the hallmarks of addiction), requiring action and associative chains far more complex than lever pressing or simple approach. This is not to say that the distinction between Pavlovian and instrumental memory lacks utility *per se*, but that the insightful questions for human SUDs regard how Pavlovian and instrumental mechanisms interact to support such ongoing motivated behaviour.

In future, it will be necessary to directly and objectively appraise behavioural homologues between animal models of SUDs and clinical or preclinical substance-using human populations. More research in back-translation of human findings to animal models will expedite this process by highlighting where the latter are and are not sufficient to capture behavioural disorder in humans. One area where this would not be possible however is in demonstrating reconsolidation of explicit memories. It has been shown in human fear memory reconsolidation paradigms and *Chapter 4* that reconsolidation interference with Propranolol or Nitrous Oxide targets autonomic (that is, low level motivation or sympathetic nervous) aspects of conditioned responding (Kindt et al. 2009; Soeter and Kindt 2010; 2011), but leaves explicit knowledge of contingencies between stimuli and outcomes intact. Clearly there is no way to gauge expectancy in rats so this cannot be tested in animal models. However, further research into the levels of human amnesia that can be produced by reconsolidation blockade is warranted.

6.5. Aberrant memories & Hindsight: Regrets and Limitations

The research presented throughout this thesis paralleled my evolving (and still limited) understanding of the mechanisms involved in MMM reconsolidation. If it were possible to repeat the experiments presented herein, armed with the knowledge gained from having performed them in the first place, there are many changes I would make. Firstly, by careful inspection of the methods sections of primary research, it may be possible to determine what level of prediction error was present during reactivation sessions. By comparing the length, number and reinforcement schedules of conditioning trials to the length, available stimuli and reinforcement during reactivation, a metric of mismatch could be derived. Regressing effect sizes on such a 'prediction error' score would allow assessment of PE in memory destabilisation in these paradigms. If it were found that those trials with the smallest effects were those in which PE was likely to be weaker, this would alter the interpretation of drug efficacy on appetitive memory reconsolidation and potentially change the outcomes of the meta-analysis *in Chapter 2*.

Despite having negative results, *Study 2* with memantine in quitting smokers was by far the greatest investment of this work, with the entire study taking almost two years to complete. For the reader's amusement, a CONSORT diagram representing the recruitment difficulties in this study is presented in the appendix. The recruitment and follow-up for this study highlighted some nuances surrounding the survey figures suggesting that 50-70% express a desire to quit annually (West 2006). The expression of a desire to quit highlights a certain (not particularly great) level of motivation to stop smoking. Of those smokers expressing this desire, fewer take any kind of action to actually stop, fewer still approach cessation with a concerted plan, including a quit date, pharmacological and behavioural support, and only a subset of these achieve abstinence for longer than a week.

The sample included in *Study 2* represented a group towards the top of this 'motivation continuum' and are therefore likely not representative of all the smokers 'expressing a desire to quit'. Although a relatively small sample, assuming those who responded to the advertisements for the study represented this latter group, only 7% followed through and completed the study. This suggests that the vast majority of smokers are unlikely to engage in a quit attempt with the level of motivation or organisation (e.g. discarding leftover cigarettes, lighters and tobacco, acquiring nicotine gum) required for any kind of success. Therefore those that might engage in a novel reconsolidation-based treatment for cessation, even if it is highly effective (which this was not), will represent a small minority of smokers. The major part of reducing the health costs associated with smoking will therefore require increasing smokers' motivation to engage with treatment in the first place.

The design of studies during this thesis has reflected an evolving state-of-knowledge of the boundary conditions on reconsolidation. Throughout the research, various factors have been changed which complicates the comparison of different studies. The experimental sample changed from cigarette smokers in *Chapter 2* to hazardous drinkers subsequently and therefore from an addicted to a non-addicted (but high risk) population. The findings from these studies are therefore not directly comparable. The post-retrieval intervention was further changed in each study, from memantine to Nitrous Oxide to counterconditioning, again meaning it is not possible to tell whether variable results were due to changes in the efficacy of the intervention or due to parameters of destabilisation (which also varied from study to study). Had there been more time and resources available, this thesis would have been greatly improved by assessing memantine and Nitrous Oxide following the reactivation with PE procedure developed for *Chapter 5* in hazardous drinkers. The desire to maintain clinical applicability in the research was consistently weighed against the desire for sensitive

and well-controlled experimental protocols (and constrained by the time and resource limitations of doctoral research), but *Chapter 2's* study with memantine would have benefitted from more controlled experimental work into destabilisation procedures prior to its completion. Trialling multiple doses would also have been an efficient use of resources. One drawback of reconsolidation research is the necessity for at least three groups to ensure effects are drug + reactivation dependent. Total Ns in these studies therefore rapidly increase and adding a single extra dose group is easier in the long-term than completing an entirely new study with new control groups.

As discussed in *Chapter 1*, addiction is a complex and multivariate problem that arises as a result of a variety of genetic, epigenetic and environmental insults. Given the current state of knowledge concerning the lifespan of memory traces, the process of reconsolidation offers the best therapeutic target we know of for targeting MMMs and preventing relapse. However, this is not to say that it will work in a meaningful way as a stand-alone treatment for substance use disorders. There is a minimum amount of motivation required to achieve success in long-term drug abstinence, regardless of how effective an anti-relapse intervention is. Reconsolidation interference may help those motivated to quit to achieve permanent abstinence and even offer some protection against relapse, given observed effects of protection against reacquisition (Chapter 4 and (Monfils et al. 2009). However it will not prevent abstinent individuals who choose to begin using drugs again from doing so. Further, the reasons for drug use vary and co-morbidity of SUDs with other psychiatric conditions is highly prevalent (Volkow 2004). Many patients use drugs to self-medicate existing psychological conditions and in these groups, cessation of drug use can result in increased negative affect (Baker et al. 2004). It can be difficult to infer causality in these circumstances. Does a patient drink because he is depressed or is he depressed because he drinks? Or are both epiphenomenal to a distal causative factor such as childhood trauma? When considering treatment options it

must therefore be considered that SUDs may be secondary pathologies and treating co-morbid disorders in tandem may be the only approach that effects improvement in these populations.

The reader might criticise the research conducted here and conclusions derived of being overly behaviourist in their representation of SUDs. Given the emergent complexity of cognitive systems and lack of linear mapping from molecule to neural network to rat behaviour to human behaviour, the approach taken throughout this thesis (of looking at identified basic molecular mechanisms of memory reconsolidation and attempting to exploit them for clinical benefit in humans with a single drug) may seem reductionist and simplistic. Clinical psychopharmacologists have tended to operate with relatively simple (some might say associationist) models of learning, partly because these are what can be readily tested in laboratory animals with drug interventions. There is a long-standing and fierce debate between 'cognitive' and 'associative' accounts of learning amongst human experimental psychologists, with some going to far as to completely abandon associative accounts of learning (Brewer 1974). I am sure that psychologists with a cognitive bent would suggest that, given the human capacity for propositional thinking and cognitive modulation of learning (that far outstrip that of any laboratory animal), the simplistic animal-based models used in this thesis are an incomplete description of human behaviour.

While this is true and there are undoubtedly important higher cognitive aspects of both memory and SUDs that are neglected by the current research (Kavanagh et al. 2005). I believe it is unhelpful and ultimately incorrect to unilaterally label learning as purely 'associative' or 'cognitive'. Both putatively arise as a result of the same basic neuropharmacological processes (discussed throughout this thesis), and likely represent

different levels of the same increasingly networked hierarchical neuronal systems. One of the strengths of neuropsychopharmacology is its requirement for researchers to critically appraise how proposed models of learning and cognition might be instantiated in the molecular and electrical currency of synapses and neurons. It is in understanding where best to intervene when processing goes awry in these hierarchical structures that will hold the key for the development of effective clinical interventions. For example, it is possible that the cognitive experience of constructs such as craving arise as a result of the maladaptive reward learning processes that have been the focus of this thesis (Tiffany 1990). If this is the case, addressing these processes may reduce the negative cognitive-affective experiences associated with addiction, but this remains to be demonstrated. Alternatively, therapies that concurrently target cognitive and mnemonic aspects of SUDs (e.g. a combined MMM reconsolidation blockade and CBT therapy) may be required for optimal clinical improvement.

The positive effects identified in this thesis demonstrate that targeting basic pharmacological and learning mechanisms may be sufficient to effect meaningful clinical change, even if the precise intermediate mechanisms of change are currently poorly understood. However, much could be gained from incorporating findings and approaches from cognitive psychology with more basic associative interventions. In *Chapter 5*, a large part of the efficacy of the approach was undoubtedly due to the propositional representation of stimulus contingencies. Taking the approach used in this study forward, it would be desirable to design post-retrieval interventions to maximise the generalisation of corrective learning. Various factors, including stimulus properties and instructions to participants, have been identified that can shift learning from single elements (Rescorla and Wagner 1972) to more configural representations (Pearce 1987) of stimulus arrays (Melchers et al. 2008). Taking account of these factors would allow optimisation of behavioural reconsolidation interventions for SUDs. In the

form of an eventual therapy, such interventions would be conducted under the supervision of a therapist, so optimising both the parameters of counterconditioning and instructions from therapist to patient will be key in getting the greatest benefit from the approach. Despite the myriad unanswered questions and difficulties in translating research from pharmacology to clinical psychopharmacology, I believe clinically relevant interventions based on reconsolidation can be designed and implemented by applying this translational approach in humans.

Lastly, one could speculate whether and if so when reconsolidation-based therapies for SUDs (and potentially other psychiatric disorders) will be seen in clinics. There are barriers beyond the psychopharmacological and methodological ones discussed here. Reconsolidation research is an ethical minefield, with concerns being raised about 'brainwashing' and memory erasure (Kass 2003) and the over-medicalisation of normal aversive memories (Henry et al. 2007). It is possible that those raising concerns against this research on ethical grounds are unaware of the fact that research demonstrating 'erasure' by a drug in humans does not exist (although a small molecule, zeta inhibitory peptide, conforms to this profile when tested in rats; Shema et al. 2007) or that episodic memories are consistently re-structured and modified from actual events (Loftus 1996; Loftus and Palmer 1974). However, these concerns are likely to be widely echoed. Certainly, the potential benefits of any reconsolidation-based intervention must be weighed against the ethical costs, but in the case of SUDs and PTSD, the balance is overwhelmingly in favour of intervention. Despite its currently fictional nature, the idea of pharmacological episodic memory erasure strikes a personal chord that will inevitably provoke strong opinions on the use of such therapies (however misrepresented) in psychiatric disorders. Reconsolidation interference also provokes unfortunate reminiscences of Anthony Burgess' 'A Clockwork Orange' and its journey

from the lab to the clinic is likely to garner controversy. However, it is hoped that if sufficiently efficacious interventions for debilitating disorders like SUDs are developed, that this will overcome the resistance to the adoption of such treatments.

6.6. Concluding Remarks

The work presented in this thesis is the first, to the author's knowledge, to examine the role of NMDAR blockade and disgust counterconditioning in MMM interference in humans. The major contributions of this work include:

1) The identification of NMDAR antagonists as more robustly interfering with appetitive memory reconsolidation than β-Blockers
2) The potential of Nitrous Oxide as a novel, well tolerated reconsolidation-blocking agent
3) The use of guided expectancy violation to generate maximal prediction error during MMM retrieval
4) The use of a disgust-based counterconditioning procedure to reduce the evaluative and motivational status of alcohol cues.

Given the dearth of available human research in this field, these findings represent a significant advance in our understanding of human MMM reconsolidation mechanisms and a platform for the large volume of further research that is required in this field. The research presented here is promising and suggests that memory reconsolidation may offer a unique opportunity for lasting therapeutic improvement in SUDs.

References

Agranoff BW, Davis RE, Brink JJ (1966) Chemical studies on memory fixation in goldfish. Brain research 1: 303-309.

Akers KG, Martinez-Canabal A, Restivo L, Yiu AP, De Cristofaro A, Hsiang H-L, Wheeler AL, Guskjolen A, Niibori Y, Shoji H, Ohira K, Richards BA, Miyakawa T, Josselyn SA, Frankland PW (2014) Hippocampal Neurogenesis Regulates Forgetting During Adulthood and Infancy. Science 344: 598-602.

Alaghband Y, Marshall JF (2013) Common influences of non-competitive NMDA receptor antagonists on the consolidation and reconsolidation of cocaine-cue memory. Psychopharmacology 226: 707-719.

Alberini CM, LeDoux JE (2013) Memory reconsolidation. Current Biology 23: R746-R750.

Baddeley A (1992) Working memory. Science 255: 556-559.

Baker JD, Azorlosa JL (1996) The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. Behavioral neuroscience 110: 618.

Baker TB, Piper ME, McCarthy DE, Majeskie MR, Fiore MC (2004) Addiction motivation reformulated: an affective processing model of negative reinforcement. Psychological review 111: 33.

Bar-Yam Y (1997) Dynamics of complex systems. Addison-Wesley Reading, MA

Bardo M, Klebaur J, Valone J, Deaton C (2001) Environmental enrichment decreases intravenous self-administration of amphetamine in female and male rats. Psychopharmacology 155: 278-284.

Bartlett E, Hallin A, Chapman B, Angrist B (1997) Selective sensitization to the psychosis-inducing effects of cocaine: a possible marker for addiction relapse vulnerability? Neuropsychopharmacology 16: 77-82.

Beck AT, Steer RA, Carbin MG (1988) Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. Clinical psychology review 8: 77-100.

Beckerman MA, Ogorodnik E, Glass MJ (2013) Acute morphine associated alterations in the subcellular location of the AMPA-GluR1 receptor subunit in dendrites of neurons in the mouse central nucleus of the amygdala: Comparisons and contrasts with other glutamate receptor subunits. Synapse 67: 692-704.

Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008) High impulsivity predicts the switch to compulsive cocaine-taking. Science 320: 1352-1355.

Bellman RE, Dreyfus SE (1962) Applied dynamic programming.

Ben-Ari Y, Aniksztejn L, Bregestovski P (1992) Protein kinase C modulation of NMDA currents: an important link for LTP induction. Trends in neurosciences 15: 333-339.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B (Methodological): 289-300.

Bernier BE, Whitaker LR, Morikawa H (2011) Previous ethanol experience enhances synaptic plasticity of NMDA receptors in the ventral tegmental area. The Journal of Neuroscience 31: 5205-5212.

Berridge KC (2009) 'Liking'and 'wanting'food rewards: brain substrates and roles in eating disorders. Physiology & behavior 97: 537-550.

Berridge KC (2012) From prediction error to incentive salience: mesolimbic computation of reward motivation. European Journal of Neuroscience 35: 1124-1143.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research Reviews 28: 309-369.

Berridge KC, Robinson TE, Aldridge JW (2009) Dissecting components of reward: 'liking', 'wanting', and learning. Current Opinion in Pharmacology 9: 65-73.

Berry-Kravis E, Hessl D, Coffey S, Hervey C, Schneider A, Yuhas J, Hutchison J, Snape M, Tranfaglia M, Nguyen DV (2009) A pilot open label, single dose trial of fenobam in adults with fragile X syndrome. Journal of medical genetics 46: 266-271.

Besnard A, Caboche J, Laroche S (2012) Reconsolidation of memory: a decade of debate. Progress in neurobiology 99: 61-80.

Black M, Lanthorn T, Small D, Mealing G, Lam V, Morley P (1996) Study of potency, kinetics of block and toxicity of NMDA receptor antagonists using fura-2. Eur J Pharmacol 317: 377-81.

Blanpied TA, Boeckman FA, Aizenman E, Johnson JW (1997) Trapping channel block of NMDA-activated responses by amantadine and memantine. Journal of neurophysiology 77: 309-23.

Blundell J, Kouser M, Powell CM (2008) Systemic inhibition of mammalian target of rapamycin inhibits fear memory reconsolidation. Neurobiology of learning and memory 90: 28-35.

Bond A, Lader M (1974) The use of analogue scales in rating subjective feelings. British Journal of Medical Psychology 47: 211-218.

Botreau F, Paolone G, Stewart J (2006) d-Cycloserine facilitates extinction of a cocaine-induced conditioned place preference. Behavioural brain research 172: 173-178.

Bouton ME, Bolles RC (1979) Contextual control of the extinction of conditioned fear. Learning and Motivation 10: 445-466.

Bouton ME, Mineka S, Barlow DH (2001) A modern learning theory perspective on the etiology of panic disorder. Psychological review 108: 4.

Bradley MM, Miccoli L, Escrig MA, Lang PJ (2008) The pupil as a measure of emotional arousal and autonomic activation. Psychophysiology 45: 602-607.

Bremner JD, Krystal JH, Putnam FW, Southwick SM, Marmar C, Charney DS, Mazure CM (1998) Measurement of dissociative states with the clinician-administered dissociative states scale (CADSS). Journal of traumatic stress 11: 125-136.

Brewer WF (1974) There is no convincing evidence for operant or classical conditioning in adult humans.

Broms U, Silventoinen K, Lahelma E, Koskenvuo M, Kaprio J (2004) Smoking cessation by socioeconomic status and marital status: The contribution of smoking behavior and family background. Nicotine & Tobacco Research 6: 447-455.

Brown TE, Lee BR, Sorg BA (2008) The NMDA antagonist MK-801 disrupts reconsolidation of a cocaine-associated memory for conditioned place preference but not for self-administration in rats. Learning & Memory 15: 857-865.

Butelman ER (1989) A novel NMDA antagonist, MK-801, impairs performance in a hippocampal-dependent spatial learning task. Pharmacology Biochemistry and Behavior 34: 13-16.

Cahill L, Babinsky R, Markowitsch HJ, McGaugh JL (1995) The amygdala and emotional memory. Nature.

Cammarota M, Bevilaqua LR, Medina JH, Izquierdo I (2004) Retrieval does not induce reconsolidation of inhibitory avoidance memory. Learning & Memory 11: 572-578.

Carrera MP, Carey RJ, Dias FRC, de Mattos LW (2012) Memory re-consolidation and drug conditioning: an apomorphine conditioned locomotor stimulant response can be enhanced or reversed by a single high versus low apomorphine post-trial treatment. Psychopharmacology 220: 281-291.

Carver CS, White TL (1994) Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: the BIS/BAS scales. Journal of personality and social psychology 67: 319.

Cavanagh JF, Cohen MX, Allen JJ (2009) Prelude to and resolution of an error: EEG phase synchrony reveals cognitive control dynamics during action monitoring. The Journal of Neuroscience 29: 98-105.

Cavanagh JF, Figueroa CM, Cohen MX, Frank MJ (2011) Frontal theta reflects uncertainty and unexpectedness during exploration and exploitation. Cerebral Cortex: bhr332.

Cavanagh JF, Frank MJ, Klein TJ, Allen JJ (2010) Frontal theta links prediction errors to behavioral adaptation in reinforcement learning. Neuroimage 49: 3198-3209.

Chan WY, Leung HT, Westbrook RF, McNally GP (2010) Effects of recent exposure to a conditioned stimulus on extinction of Pavlovian fear conditioning. Learning & memory (Cold Spring Harbor, NY) 17: 512-21.

Chen L, Huang L-YM (1992) Protein kinase C reduces Mg2+ block of NMDA-receptor channels as a mechanism of modulation.

Chialvo DR (2010) Emergent complex neural dynamics. Nature Physics 6: 744-750.

Chiamulera C, Epping-Jordan MP, Zocchi A, Marcon C, Cottiny C, Tacconi S, Corsi M, Orzi F, Conquet F (2001) Reinforcing and locomotor stimulant effects of

cocaine are absent in mGluR5 null mutant mice. Nature neuroscience 4: 873-874.

Clem RL, Huganir RL (2010) Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science 330: 1108-1112.

Cohen MX (2011) Error-related medial frontal theta activity predicts cingulate-related structural connectivity. Neuroimage 55: 1373-1383.

Conklin CA, Tiffany ST (2002) Applying extinction research and theory to cue-exposure addiction treatments. Addiction 97: 155-167.

Corlett PR, Cambridge V, Gardner JM, Piggot JS, Turner DC, Everitt JC, Arana FS, Morgan HL, Milton AL, Lee JL (2013) Ketamine Effects on Memory Reconsolidation Favor a Learning Model of Delusions. PloS one 8: e65088.

Costanzi M, Cannas S, Saraulli D, Rossi-Arnaud C, Cestari V (2011) Extinction after retrieval: Effects on the associative and nonassociative components of remote contextual fear memory. Learning & Memory 18: 508-518.

Creeley C, Wozniak DF, Labruyere J, Taylor GT, Olney JW (2006) Low Doses of Memantine Disrupt Memory in Adult Rats. The Journal of Neuroscience 26: 3923-3932.

Das RK, Freeman TP, Kamboj SK (2013a) The effects of N-methyl d-aspartate and B-adrenergic receptor antagonists on the reconsolidation of reward memory: A meta-analysis. Neuroscience & Biobehavioral Reviews 37: 240-255.

Das RK, Kamboj SK (2012) Maintaining clinical relevance: considerations for the future of research into D-cycloserine and cue exposure therapy for addiction. Biological psychiatry 72: e29-e30.

Das RK, Kamboj SK, Ramadas M, Yogan K, Gupta V, Redman E, Curran HV, Morgan CJ (2013b) Cannabidiol enhances consolidation of explicit fear extinction in humans. Psychopharmacology 226: 781-792.

Davy H (1800) Researches, Chemical and Philosophical, Chiefly Concerning Nitrous Oxide, Or Dephlogisticated Nitrous Air, and Its Respiration, by Humphry Davy. J. Johnson

Dawe S, Powell J, Richards D, Gossop M, Marks I, Strang J, Gray JA (1993) Does post-withdrawal cue exposure improve outcome in opiate addiction? A controlled trial. Addiction 88: 1233-1245.

Dayan P, Balleine BW (2002) Reward, motivation, and reinforcement learning. Neuron 36: 285-298.

Dayan P, Berridge KC (2014) Model-based and model-free Pavlovian reward learning: Revaluation, revision, and revelation. Cognitive, Affective, & Behavioral Neuroscience: 1-20.

de Oliveira Alvares L, Pasqualini Genro B, Diehl F, Molina V, Quillfeldt J (2008) Opposite action of hippocampal CB1 receptors in memory reconsolidation and extinction. Neuroscience 154: 1648-1655.

Debiec J, LeDoux JE, Nader K (2002) Cellular and systems reconsolidation in the hippocampus. Neuron 36: 527-538.

Dhonnchadha BN, Szalay JJ, Achat-Mendes C, Platt DM, Otto MW, Spealman RD, Kantak KM (2010) D-cycloserine deters reacquisition of cocaine self-administration by augmenting extinction learning. Neuropsychopharmacology 35: 357-367.

Díaz-Mataix L, Ruiz Martinez RC, Schafe GE, LeDoux JE, Doyère V (2013) Detection of a temporal error triggers reconsolidation of amygdala-dependent memories. Current Biology 23: 467-472.

Diergaarde L, Schoffelmeer AN, De Vries TJ (2006) β-Adrenoceptor mediated inhibition of long-term reward-related memory reconsolidation. Behavioural brain research 170: 333-336.

Drummond D, Tiffany ST, Glautier SE, Remington BE (1995) Addictive behaviour: Cue exposure theory and practice. John Wiley & Sons

Drummond DC, Cooper T, Glautier SP (1990) Conditioned learning in alcohol dependence: implications for cue exposure treatment. British Journal of Addiction 85: 725-743.

Drummond DC, Glautier S (1994) A controlled trial of cue exposure treatment in alcohol dependence. Journal of consulting and clinical psychology 62: 809.

Dudai Y (2006) Reconsolidation: the advantage of being refocused. Current opinion in neurobiology 16: 174-178.

Dudai Y, Eisenberg M (2004) Rites of passage of the engram: reconsolidation and the lingering consolidation hypothesis. Neuron 44: 93-100.

Duncan CP (1949) The retroactive effect of electroshock on learning. Journal of comparative and physiological psychology 42: 32.

Dunlosky J, Domoto PK, Wang M-L, Ishikawa T, Roberson I, Nelson TO, Ramsay DS (1998) Inhalation of 30% nitrous oxide impairs people's learning without impairing people's judgments of what will be remembered. Experimental and clinical psychopharmacology 6: 77.

Ebbinghaus H (1913) Memory: A contribution to experimental psychology. Teachers college, Columbia university

Eisenberg M, Dudai Y (2004) Reconsolidation of fresh, remote, and extinguished fear memory in Medaka: old fears don't die. European Journal of Neuroscience 20: 3397-3403.

Eisenberg M, Kobilo T, Berman DE, Dudai Y (2003) Stability of retrieved memory: inverse correlation with trace dominance. Science 301: 1102-1104.

Engelhard IM, Leer A, Lange E, Olatunji BO (2014) Shaking That Icky Feeling: Effects of Extinction and Counterconditioning on Disgust-Related Evaluative Learning. Behavior Therapy.

Esteban JA, Shi S-H, Wilson C, Nuriya M, Huganir RL, Malinow R (2003) PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nature neuroscience 6: 136-143.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nature neuroscience 8: 1481-1489.

Exton-McGuinness MT, Patton RC, Sacco LB, Lee JL (2014) Reconsolidation of a well-learned instrumental memory. Learning & Memory 21: 468-477.

Fields HL, Hjelmstad GO, Margolis EB, Nicola SM (2007) Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. Annu Rev Neurosci 30: 289-316.

Finnie PS, Nader K (2012) The role of metaplasticity mechanisms in regulating memory destabilization and reconsolidation. Neuroscience & Biobehavioral Reviews 36: 1667-1707.

Fiore M (2008) Treating tobacco use and dependence: 2008 update: Clinical practice guideline. DIANE Publishing

Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA, Clinton SM, Phillips PE, Akil H (2011) A selective role for dopamine in stimulus-reward learning. Nature 469: 53-57.

Forcato C, Argibay P, Pedreira M, Maldonado H (2009) Human reconsolidation does not always occur when a memory is retrieved: the relevance of the reminder structure. Neurobiology of learning and memory 91: 50-57.

Forcato C, Burgos VL, Argibay PF, Molina VA, Pedreira ME, Maldonado H (2007) Reconsolidation of declarative memory in humans. Learning & Memory 14: 295-303.

Forcato C, Rodríguez ML, Pedreira ME, Maldonado H (2010) Reconsolidation in humans opens up declarative memory to the entrance of new information. Neurobiology of learning and memory 93: 77-84.

Forsythe ID, Westbrook GL, Mayer ML (1988) Modulation of excitatory synaptic transmission by glycine and zinc in cultures of mouse hippocampal neurons. The Journal of Neuroscience 8: 3733-3741.

Frankland PW, Ding H-K, Takahashi E, Suzuki A, Kida S, Silva AJ (2006) Stability of recent and remote contextual fear memory. Learning & Memory 13: 451-457.

Freeman TP, Das RK, Kamboj SK, Curran HV (2014) Dopamine, urges to smoke, and the relative salience of drug versus non-drug reward. Social cognitive and affective neuroscience: nsu026.

Gard DE, Gard MG, Kring AM, John OP (2006) Anticipatory and consummatory components of the experience of pleasure: a scale development study. Journal of Research in Personality 40: 1086-1102.

Ghahremani DG, Lee B, Robertson CL, Tabibnia G, Morgan AT, De Shetler N, Brown AK, Monterosso JR, Aron AR, Mandelkern MA (2012) Striatal dopamine D2/D3 receptors mediate response inhibition and related activity in frontostriatal neural circuitry in humans. The Journal of Neuroscience 32: 7316-7324.

Glauser SC, Glauser EM, Reideriberg MM, Rusy BF, Tallarida RJ (1970) Metabolic changes associated with the cessation of cigarette smoking. Archives of Environmental Health: An International Journal 20: 377-381.

Goerig M, Schulte am Esch J (2001) History of nitrous oxide—with special reference to its early use in Germany. Best Practice & Research Clinical Anaesthesiology 15: 313-338.

Gordon HW (2002) Early environmental stress and biological vulnerability to drug abuse. Psychoneuroendocrinology 27: 115-126.

Gräff J, Joseph Nadine F, Horn Meryl E, Samiei A, Meng J, Seo J, Rei D, Bero Adam W, Phan Trongha X, Wagner F, Holson E, Xu J, Sun J, Neve Rachael L, Mach Robert H, Haggarty Stephen J, Tsai L-H Epigenetic Priming of Memory Updating during Reconsolidation to Attenuate Remote Fear Memories. Cell 156: 261-276.

Grant S, Easley C, Kirkpatrick P (2007) Vorinostat. Nature Reviews Drug Discovery 6: 21-22.

Grillon C (2009) D-cycloserine facilitation of fear extinction and exposure-based therapy might rely on lower-level, automatic mechanisms. Biological psychiatry 66: 636-641.

Grunstein M (1997) Histone acetylation in chromatin structure and transcription. Nature 389: 349-352.

Guitart-Masip M, Economides M, Huys QM, Frank M, Chowdhury R, Duzel E, Dayan P, Dolan R (2014) Differential, but not opponent, effects of l-DOPA and citalopram on action learning with reward and punishment. Psychopharmacology 231: 955-966.

Guitart-Masip M, Huys QJ, Fuentemilla L, Dayan P, Duzel E, Dolan RJ (2012) Go and no-go learning in reward and punishment: interactions between affect and effect. Neuroimage 62: 154-166.

Hearst E, Jenkins HM (1974) Sign-tracking: The stimulus-reinforcer relation and directed action. Psychonomic Society

Heatherton TF, Kozlowski LT, Frecker RC, FAGERSTROM KO (1991) The Fagerström test for nicotine dependence: a revision of the Fagerstrom Tolerance Questionnaire. British journal of addiction 86: 1119-1127.

Henry M, Fishman JR, Youngner SJ (2007) Propranolol and the prevention of post-traumatic stress disorder: Is it wrong to erase the "sting" of bad memories? The American Journal of Bioethics 7: 12-20.

Heresco-Levy U, Javitt DC, Ebstein R, Vass A, Lichtenberg P, Bar G, Catinari S, Ermilov M (2005) D-serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. Biological psychiatry 57: 577-585.

Hernandez L, Hoebel BG (1988) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life sciences 42: 1705-1712.

Hernandez PJ, Kelley AE (2004) Long-term memory for instrumental responses does not undergo protein synthesis-dependent reconsolidation upon retrieval. Learning & Memory 11: 748-754.

Hershberger WA (1986) An approach through the looking-glass. Animal Learning & Behavior 14: 443-451.

Hewish DR, Burgoyne LA (1973) Chromatin sub-structure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. Biochemical and Biophysical Research Communications 52: 504-510.

Hofmann SG, Hüweler R, MacKillop J, Kantak KM (2011) Effects of d-Cycloserine on Craving to Alcohol Cues in Problem Drinkers: Preliminary Findings. The American Journal of Drug and Alcohol Abuse 38: 101-107.

Holroyd CB, Nieuwenhuis S, Yeung N, Cohen JD (2003) Errors in reward prediction are reflected in the event-related brain potential. NeuroReport 14: 2481-2484.

Hopfield JJ (1982) Neural networks and physical systems with emergent collective computational abilities. Proceedings of the national academy of sciences 79: 2554-2558.

Huys QJ, Cools R, Gölzer M, Friedel E, Heinz A, Dolan RJ, Dayan P (2011a) Disentangling the roles of approach, activation and valence in instrumental and pavlovian responding. PLoS computational biology 7: e1002028.

Huys QJ, Moutoussis M, Williams J (2011b) Are computational models of any use to psychiatry? Neural Networks 24: 544-551.

Huys QJ, Tobler P, Hasler G, Flagel SB (2014) The role of learning-related dopamine signals in addiction vulnerability. Prog Neurobiol.

Inda MC, Muravieva EV, Alberini CM (2011) Memory retrieval and the passage of time: from reconsolidation and strengthening to extinction. The Journal of Neuroscience 31: 1635-1643.

Jackson A, Nesic J, Groombridge C, Clowry O, Rusted J, Duka T (2008) Differential Involvement of Glutamatergic Mechanisms in the Cognitive and Subjective Effects of Smoking. Neuropsychopharmacology 34: 257-265.

Jevtović-Todorović V, Todorovć S, Mennerick S, Powell S, Dikranian K, Benshoff N, Zorumski C, Olney J (1998) Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nature medicine 4: 460-463.

Jevtovic-Todorovic V, Wozniak DF, Benshoff ND, Olney JW (2001) A comparative evaluation of the neurotoxic properties of ketamine and nitrous oxide. Brain research 895: 264-267.

Jones M, Errington M, French P, Fine A, Bliss T, Garel S, Charnay P, Bozon B, Laroche S, Davis S (2001) A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. Nature neuroscience 4: 289-296.

Jorenby DE, Hays JT, Rigotti NA, Azoulay S, Watsky EJ, Williams KE, Billing CB, Gong J, Reeves KR, Group VPS (2006) Efficacy of varenicline, an α4β2 nicotinic acetylcholine receptor partial agonist, vs placebo or sustained-release bupropion for smoking cessation: a randomized controlled trial. Jama 296: 56-63.

Judge ME, Quartermain D (1982) Characteristics of retrograde amnesia following reactivation of memory in mice. Physiology & Behavior 28: 585-590.

Kalisch R, Holt B, Petrovic P, De Martino B, Klöppel S, Büchel C, Dolan RJ (2009) The NMDA agonist D-cycloserine facilitates fear memory consolidation in humans. Cerebral cortex 19: 187-196.

Kamboj SK, Joye A, Das RK, Gibson AJ, Morgan CJ, Curran HV (2012) Cue exposure and response prevention with heavy smokers: a laboratory-based randomised placebo-controlled trial examining the effects of D-cycloserine on cue reactivity and attentional bias. Psychopharmacology 221: 273-284.

Kamboj SK, Massey-Chase R, Rodney L, Das R, Almahdi B, Curran HV, Morgan CJ (2011) Changes in cue reactivity and attentional bias following experimental cue exposure and response prevention: a laboratory study of the effects of D-cycloserine in heavy drinkers. Psychopharmacology 217: 25-37.

Karpicke JD, Roediger HL (2008) The critical importance of retrieval for learning. science 319: 966-968.

Karpicke JD, Roediger III HL (2007) Repeated retrieval during learning is the key to long-term retention. Journal of Memory and Language 57: 151-162.

Kass L (2003) Beyond therapy: biotechnology and the pursuit of happiness. Harper Perennial

Kavanagh DJ, Andrade J, May J (2005) Imaginary relish and exquisite torture: the elaborated intrusion theory of desire. Psychological review 112: 446.

Kelley AE (2004) Memory and Addiction: Shared Neural Circuitry and Molecular Mechanisms. Neuron 44: 161-179.

Kindt M, Soeter M, Vervliet B (2009) Beyond extinction: erasing human fear responses and preventing the return of fear. Nature neuroscience 12: 256-258.

Kippin TE, Szumlinski KK, Kapasova Z, Rezner B, See RE (2007) Prenatal stress enhances responsiveness to cocaine. Neuropsychopharmacology 33: 769-782.

Kirtley A, Thomas KL (2010) The exclusive induction of extinction is gated by BDNF. Learning & Memory 17: 612-619.

Koob GF (1992a) Dopamine, addiction and reward Seminars in Neuroscience. Elsevier, pp 139-148

Koob GF (1992b) Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends in Pharmacological Sciences 13: 177-184.

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24: 97-129.

Kotz D, Brown J, West R (2013) Predictive validity of the Motivation To Stop Scale (MTSS): A single-item measure of motivation to stop smoking. Drug and alcohol dependence 128: 15-19.

Kreek MJ, Nielsen DA, Butelman ER, LaForge KS (2005) Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nat Neurosci 8: 1450-1457.

Krug M, Lössner B, Ott T (1984) Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. Brain Research Bulletin 13: 39-42.

Krupitsky E, Burakov A, Romanova T, Dunaevsky I, Strassman R, Grinenko A (2002) Ketamine psychotherapy for heroin addiction: immediate effects and two-year follow-up. Journal of substance abuse treatment 23: 273-283.

Krupitsky E, Grinenko A (1997) Ketamine psychedelic therapy (KPT): a review of the results of ten years of research. Journal of psychoactive drugs 29: 165-183.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Charney DS (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Archives of general psychiatry 51: 199-214.

Krystal John H, State Matthew W (2014) Psychiatric Disorders: Diagnosis to Therapy. Cell 157: 201-214.

Lane RD, Ryan L, Nadel L, Greenberg L (2014) Memory Reconsolidation, Emotional Arousal and the Process of Change in Psychotherapy: New Insights from Brain Science. Behavioral and Brain Sciences: 1-80.

Lang PJ, Bradley MM, Cuthbert BN (2005) International affective picture system (IAPS): Affective ratings of pictures and instruction manual. NIMH, Center for the Study of Emotion & Attention

Lee B, London ED, Poldrack RA, Farahi J, Nacca A, Monterosso JR, Mumford JA, Bokarius AV, Dahlbom M, Mukherjee J (2009) Striatal dopamine d2/d3 receptor availability is reduced in methamphetamine dependence and is linked to impulsivity. The Journal of Neuroscience 29: 14734-14740.

Lee JL (2008) Memory reconsolidation mediates the strengthening of memories by additional learning. Nature neuroscience 11: 1264.

Lee JL (2009) Reconsolidation: maintaining memory relevance. Trends in neurosciences 32: 413-420.

Lee JL, Everitt BJ (2008a) Appetitive memory reconsolidation depends upon NMDA receptor-mediated neurotransmission. Neurobiology of learning and memory 90: 147-154.

Lee JL, Everitt BJ, Thomas KL (2004) Independent cellular processes for hippocampal memory consolidation and reconsolidation. Science 304: 839-843.

Lee JL, Hynds RE (2013a) Divergent cellular pathways of hippocampal memory consolidation and reconsolidation. Hippocampus 23: 233-244.

Lee JL, Milton AL, Everitt BJ (2006) Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. The Journal of neuroscience 26: 5881-5887.

Lee JLC, Everitt BJ (2008b) Reactivation-dependent amnesia in Pavlovian approach and instrumental transfer. Learning & Memory 15: 597-602.

Lee JLC, Hynds RE (2013b) Divergent cellular pathways of hippocampal memory consolidation and reconsolidation. Hippocampus 23: 233-244.

Lee S-H, Choi J-H, Lee N, Lee H-R, Kim J-I, Yu N-K, Choi S-L, Lee S-H, Kim H, Kaang B-K (2008) Synaptic protein degradation underlies destabilization of retrieved fear memory. Science 319: 1253-1256.

Lewis DJ, Maher BA (1965) Neural consolidation and electroconvulsive shock. Psychological review 72: 225.

Lions C, Carrieri MP, Michel L, Mora M, Marcellin F, Morel A, Spire B, Roux P (2014) Predictors of non-prescribed opioid use after one year of methadone treatment: An attributable-risk approach (ANRS-Methaville trial). Drug and alcohol dependence 135: 1-8.

Liu J, Zhao L, Xue Y, Shi J, Suo L, Luo Y, Chai B, Yang C, Fang Q, Zhang Y (2014) An Unconditioned Stimulus Retrieval Extinction Procedure to Prevent the Return of Fear Memory. Biological psychiatry.

Loeber S, Croissant B, Heinz A, Mann K, Flor H (2006) Cue exposure in the treatment of alcohol dependence: Effects on drinking outcome, craving and self-efficacy. British Journal of Clinical Psychology 45: 515-529.

Loftus EF (1996) Eyewitness testimony. Harvard University Press

Loftus EF, Palmer JC (1974) Reconstruction of automobile destruction: An example of the interaction between language and memory. Journal of verbal learning and verbal behavior 13: 585-589.

Makkar SR, Zhang SQ, Cranney J (2010) Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory. Neuropsychopharmacology 35: 1625-1652.

Malinow R, Madison DV, Tsien RW (1988) Persistent protein kinase activity underlying long-term potentiation. Nature 335: 820-824.

Mamou CB, Gamache K, Nader K (2006) NMDA receptors are critical for unleashing consolidated auditory fear memories. Nat Neurosci 9: 1237-1239.

Mansvelder HD, McGehee DS (2000) Long-term potentiation of excitatory inputs to brain reward areas by nicotine. Neuron 27: 349-357.

Marissen MA, Franken IH, Blanken P, van den Brink W, Hendriks VM (2005) Cue exposure therapy for opiate dependent clients. Journal of Substance Use 10: 97-105.

Maskell PD, Speder P, Newberry NR, Bermudez I (2003) Inhibition of human α7 nicotinic acetylcholine receptors by open channel blockers of N-methyl-D-aspartate receptors. British Journal of Pharmacology 140: 1313-1319.

Matthies H, Frey U, Reymann K, Krug M, Jork R, Schroeder H (1990) Different mechanisms and multiple stages of LTP. Advances in Experimental Medicine and Biology 268: 359-368.

McCambridge J, Day M (2008) Randomized controlled trial of the effects of completing the Alcohol Use Disorders Identification Test questionnaire on self-reported hazardous drinking. Addiction 103: 241-248.

McGaugh JL (1966) Time-dependent processes in memory storage. Science 153: 1351-1358.

McGaugh JL (2000) Memory--a Century of Consolidation. Science 287: 248-251.

McGeehan AJ, Olive MF (2003) The mGluR5 antagonist MPEP reduces the conditioned rewarding effects of cocaine but not other drugs of abuse. Synapse 47: 240-242.

McMahon J, Jones B (1993) The negative alcohol expectancy questionnaire. Journal of the Association of Nurses in Substance Abuse 12: 17.

Melchers KG, Shanks DR, Lachnit H (2008) Stimulus coding in human associative learning: Flexible representations of parts and wholes. Behavioural Processes 77: 413-427.

Merlo E, Milton AL, Goozée ZY, Theobald DE, Everitt BJ (2014) Reconsolidation and Extinction Are Dissociable and Mutually Exclusive Processes: Behavioral and Molecular Evidence. The Journal of Neuroscience 34: 2422-2431.

Mickiewicz AL, Napier TC (2011) Repeated exposure to morphine alters surface expression of AMPA receptors in the rat medial prefrontal cortex. European Journal of Neuroscience 33: 259-265.

Milekic MH, Alberini CM (2002) Temporally graded requirement for protein synthesis following memory reactivation. Neuron 36: 521-525.

Millan EZ, Milligan-Saville J, McNally GP (2013) Memory retrieval, extinction, and reinstatement of alcohol seeking. Neurobiol Learn Mem 101: 26-32.

Miller CA, Marshall JF (2005) Molecular Substrates for Retrieval and Reconsolidation of Cocaine-Associated Contextual Memory. Neuron 47: 873-884.

Miller WR, Tonigan JS (1996) Assessing drinkers' motivation for change: the Stages of Change Readiness and Treatment Eagerness Scale (SOCRATES). Psychology of Addictive Behaviors 10: 81.

Milton AL, Everitt BJ (2012) The persistence of maladaptive memory: addiction, drug memories and anti-relapse treatments. Neuroscience & Biobehavioral Reviews 36: 1119-1139.

Milton AL, Lee JL, Butler VJ, Gardner R, Everitt BJ (2008a) Intra-amygdala and systemic antagonism of NMDA receptors prevents the reconsolidation of drug-associated memory and impairs subsequently both novel and previously acquired drug-seeking behaviors. The Journal of Neuroscience 28: 8230-8237.

Milton AL, Lee JL, Everitt BJ (2008b) Reconsolidation of appetitive memories for both natural and drug reinforcement is dependent on β-adrenergic receptors. Learning & Memory 15: 88-92.

Milton AL, Merlo E, Ratano P, Gregory BL, Dumbreck JK, Everitt BJ (2013) Double dissociation of the requirement for GluN2B-and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory. The Journal of Neuroscience 33: 1109-1115.

Milton AL, Schramm MJ, Wawrzynski JR, Gore F, Oikonomou-Mpegeti F, Wang NQ, Samuel D, Economidou D, Everitt BJ (2012) Antagonism at NMDA receptors, but not β-adrenergic receptors, disrupts the reconsolidation of pavlovian conditioned approach and instrumental transfer for ethanol-associated conditioned stimuli. Psychopharmacology 219: 751-761.

Mirsky AE (1971) The structure of chromatin. Proceedings of the National Academy of Sciences of the United States of America 68: 2945-2948.

Misanin JR, Miller RR, Lewis DJ (1968) Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. Science 160: 554-555.

Mogg K, Bradley BP, Field M, De Houwer J (2003) Eye movements to smoking-related pictures in smokers: relationship between attentional biases and implicit and explicit measures of stimulus valence. Addiction 98: 825-836.

Mogg K, Field M, Bradley B (2005) Attentional and approach biases for smoking cues in smokers: an investigation of competing theoretical views of addiction. Psychopharmacology 180: 333-341.

Monfils M-H, Cowansage KK, Klann E, LeDoux JE (2009) Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. Science 324: 951-955.

Montague PR, Dolan RJ, Friston KJ, Dayan P (2012) Computational psychiatry. Trends in cognitive sciences 16: 72-80.

Morgan CJ, Curran HV (2006) Acute and chronic effects of ketamine upon human memory: a review. Psychopharmacology 188: 408-424.

Morgan CJ, Mofeez A, Brandner B, Bromley L, Curran HV (2004) Acute effects of ketamine on memory systems and psychotic symptoms in healthy volunteers. Neuropsychopharmacology.

Morris RGM (2006) Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. European Journal of Neuroscience 23: 2829-2846.

Muetzelfeldt L, Kamboj S, Rees H, Taylor J, Morgan C, Curran H (2008) Journey through the K-hole: phenomenological aspects of ketamine use. Drug and alcohol dependence 95: 219-229.

Muller D, JoLY M, Lynch G (1988) Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. Science 242: 1694-1697.

Müller GE, Pilzecker A (1900) Experimentelle beiträge zur lehre vom gedächtniss. JA Barth

Myers KM, Carlezon Jr WA (2012) D-cycloserine effects on extinction of conditioned responses to drug-related cues. Biological psychiatry 71: 947-955.

Nadel L, Moscovitch M (1997) Memory consolidation, retrograde amnesia and the hippocampal complex. Current opinion in neurobiology 7: 217-227.

Nader K, Schafe GE, Le Doux JE (2000) Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. Nature 406: 722-726.

Nevins MA (1980) Neuropathy after nitrous oxide abuse. JAMA 244: 2264-2264.

Nguyen PV, Abel T, Kandel ER (1994) Requirement of a critical period of transcription for induction of a late phase of LTP. Science 265: 1104-1107.

Niaura R, Abrams DB, Shadel WG, Rohsenow DJ, Monti PM, Sirota AD (1999) Cue exposure treatment for smoking relapse prevention: a controlled clinical trial. Addiction 94: 685-695.

Nutt DJ, King LA, Phillips LD (2010) Drug harms in the UK: a multicriteria decision analysis. The Lancet 376: 1558-1565.

O'Brien CP (1997) A range of research-based pharmacotherapies for addiction. Science 278: 66-70.

O'Doherty JP, Dayan P, Friston K, Critchley H, Dolan RJ (2003) Temporal Difference Models and Reward-Related Learning in the Human Brain. Neuron 38: 329-337.

O'Farrell TJ, Hutton VV, Murphy CM (1999) Domestic violence before and after alcoholism treatment: a two-year longitudinal study. Journal of Studies on Alcohol and Drugs 60: 317.

Olatunji BO, Cisler JM, Deacon BJ, Connolly K, Lohr JM (2007a) The Disgust Propensity and Sensitivity Scale-Revised: Psychometric properties and specificity in relation to anxiety disorder symptoms. J Anxiety Disord 21: 918-930.

Olatunji BO, Forsyth JP, Cherian A (2007b) Evaluative differential conditioning of disgust: A sticky form of relational learning that is resistant to extinction. J Anxiety Disord 21: 820-834.

Olatunji BO, Sawchuk CN (2005) Disgust: Characteristic features, social manifestations, and clinical implications. Journal of Social and Clinical Psychology 24: 932-962.

Olney JW, Labruyere J, Price MT (1989) Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244: 1360-1362.

Osan R, Tort AB, Amaral OB (2011) A mismatch-based model for memory reconsolidation and extinction in attractor networks. PloS one 6: e23113.

Parbrook GD (1967) The levels of nitrous oxide analgesia. British journal of anaesthesia 39: 974-982.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity byd-amphetamine. The Journal of Neuroscience 19: 2401-2411.

Parsons CG, Danysz W, Quack G (1999) Memantine is a clinically well tolerated N-methyl-d-aspartate (NMDA) receptor antagonist—a review of preclinical data. Neuropharmacology 38: 735-767.

Pavlov IP (1927) Conditioned reflexes. An Investigation of the Physiological Activity of the Cerebral Cortex, London.

Pearce JM (1987) A model for stimulus generalization in Pavlovian conditioning. Psychological review 94: 61.

Pedreira ME, Pérez-Cuesta LM, Maldonado H (2004) Mismatch between what is expected and what actually occurs triggers memory reconsolidation or extinction. Learning & Memory 11: 579-585.

Pérez-Cuesta LM, Maldonado H (2009) Memory reconsolidation and extinction in the crab: Mutual exclusion or coexistence? Learning & Memory 16: 714-721.

Periclou A, Ventura D, Rao N, Abramowitz W (2006) Pharmacokinetic study of memantine in healthy and renally impaired subjects[ast]. Clin Pharmacol Ther 79: 134-143.

Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS (2001) Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. Journal of Biological Chemistry 276: 36734-36741.

Pierce RC, Kumaresan V (2006) The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? Neuroscience & Biobehavioral Reviews 30: 215-238.

Piñeyro ME, Monti RIF, Alfei JM, Bueno AM, Urcelay GP (2014) Memory destabilization is critical for the success of the reactivation–extinction procedure. Learning & Memory 21: 46-54.

Popik P, Wrobel M, Bisaga A (2005) Reinstatement of morphine-conditioned reward is blocked by memantine. Neuropsychopharmacology 31: 160-170.

Popik P, Wrobel M, Bisaga A (2006) Reinstatement of morphine-conditioned reward is blocked by memantine. Neuropsychopharmacology 31: 160-170.

Potts GF, Martin LE, Burton P, Montague PR (2006) When Things Are Better or Worse than Expected: The Medial Frontal Cortex and the Allocation of Processing Resources. Journal of Cognitive Neuroscience 18: 1112-1119.

Potts R, Shanks DR (2012) Can testing immunize memories against interference? Journal of Experimental Psychology: Learning, Memory, and Cognition 38: 1780.

Preuschoff K, Marius't Hart B, Einhäuser W (2011) Pupil dilation signals surprise: evidence for noradrenaline's role in decision making. Frontiers in neuroscience 5.

Price KL, Baker NL, McRae-Clark AL, Saladin ME, DeSantis SM, Santa Ana EJ, Brady KT (2013) A randomized, placebo-controlled laboratory study of the effects of d-cycloserine on craving in cocaine-dependent individuals. Psychopharmacology 226: 739-746.

Procidano ME, Heller K (1983) Measures of perceived social support from friends and from family: Three validation studies. American journal of community psychology 11: 1-24.

Przybyslawski J, Sara SJ (1997) Reconsolidation of memory after its reactivation. Behavioural brain research 84: 241-246.

Rammes G, Danysz W, Parsons C (2008) Pharmacodynamics of memantine: an update. Current neuropharmacology 6: 55.

Rang H, Dale MM, Ritter J, Moore P (2003) Pharmacology Churchill Livingstone. New York.

Redgrave P, Gurney K (2006) The short-latency dopamine signal: a role in discovering novel actions? Nature reviews neuroscience 7: 967-975.

Reitan RM (1958) Validity of the Trail Making Test as an indicator of organic brain damage. Perceptual and motor skills 8: 271-276.

Rescorla RA, Wagner AR (1972) A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. Classical conditioning II: Current research and theory 2: 64-99.

Richon VM, Sandhoff TW, Rifkind RA, Marks PA (2000) Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proceedings of the National Academy of Sciences 97: 10014-10019.

Robinson M, Franklin K (2010) Reconsolidation of a morphine place preference: impact of the strength and age of memory on disruption by propranolol and midazolam. Behavioural brain research 213: 201-207.

Robinson MJ, Armson M, Franklin KB (2011) The effect of propranolol and midazolam on the reconsolidation of a morphine place preference in chronically treated rats. Frontiers in behavioral neuroscience 5.

Robinson MJ, Berridge KC (2013) Instant transformation of learned repulsion into motivational "wanting". Current Biology 23: 282-289.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain research reviews 18: 247-291.

Robinson TE, Berridge KC (2001) Incentive-sensitization and addiction. Addiction 96: 103-114.

Robson JG, Burns BD, Welt PJL (1960) The effect of inhaling dilute nitrous oxide upon recent memory and time estimation. Can Anaes Soc J 7: 399-410.

Rodrigues SM, Bauer EP, Farb CR, Schafe GE, LeDoux JE (2002) The group I metabotropic glutamate receptor mGluR5 is required for fear memory formation and long-term potentiation in the lateral amygdala. The Journal of neuroscience 22: 5219-5229.

Rose JE, Behm FM, Westman EC, Johnson M (2000) Dissociating nicotine and nonnicotine components of cigarette smoking. Pharmacology Biochemistry and Behavior 67: 71-81.

Rudy JW, O'Reilly RC (1999) Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus. Behavioral neuroscience 113: 867.

Saladin ME, Gray KM, McRae-Clark AL, LaRowe SD, Yeatts SD, Baker NL, Hartwell KJ, Brady KT (2013) A double blind, placebo-controlled study of the effects of post-retrieval propranolol on reconsolidation of memory for craving and cue reactivity in cocaine dependent humans. Psychopharmacology 226: 721-737.

Salinska E (2006) The role of group I metabotropic glutamate receptors in memory consolidation and reconsolidation in the passive avoidance task in 1-day-old chicks. Neurochemistry International 48: 447-452.

Samartgis JR, Schachte L, Hazi A, Crowe SF (2012) Memantine facilitates memory consolidation and reconsolidation in the day-old chick. Neurobiol Learn Mem 97: 380-5.

Sara SJ, Hars B (2006) In memory of consolidation. Learning & Memory 13: 515-521.

Saunders JB, Aasland OG, Babor TF, Delafuente JR, Grant M (1993) DEVELOPMENT OF THE ALCOHOL-USE DISORDERS IDENTIFICATION TEST (AUDIT) - WHO COLLABORATIVE PROJECT ON EARLY DETECTION OF PERSONS WITH HARMFUL ALCOHOL-CONSUMPTION .2. Addiction 88: 791-804.

Schell MJ, Molliver ME, Snyder SH (1995) D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proceedings of the National Academy of Sciences 92: 3948-3952.

Schiller D, Monfils M-H, Raio CM, Johnson DC, LeDoux JE, Phelps EA (2009) Preventing the return of fear in humans using reconsolidation update mechanisms. Nature 463: 49-53.

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275: 1593-1599.

Schwabe L, Nader K, Pruessner JC (2014) Reconsolidation of Human Memory: Brain Mechanisms and Clinical Relevance. Biological Psychiatry 76: 274-280.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. Journal of neurology, neurosurgery, and psychiatry 20: 11.

Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 138: 32.

Selye H (1973) The Evolution of the Stress Concept: The originator of the concept traces its development from the discovery in 1936 of the alarm reaction to modern therapeutic applications of syntoxic and catatoxic hormones. American scientist: 692-699.

Sevenster D, Beckers T, Kindt M (2012) Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. Neurobiology of learning and memory 97: 338-345.

Sevenster D, Beckers T, Kindt M (2013) Prediction error governs pharmacologically induced amnesia for learned fear. Science 339: 830-833.

Sheinin A, Shavit S, Benveniste M (2001) Subunit specificity and mechanism of action of NMDA partial agonist D-cycloserine. Neuropharmacology 41: 151-158.

Shema R, Sacktor TC, Dudai Y (2007) Rapid erasure of long-term memory associations in the cortex by an inhibitor of PKMζ. Science 317: 951-953.

Shimizu E, Tang Y-P, Rampon C, Tsien JZ (2000) NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. Science 290: 1170-1174.

Singleton E, Henningfield J, Tiffany S (1994) Alcohol craving questionnaire: ACQ-Now: background and administration manual. Baltimore: NIDA Addiction Research Centre.

Skinner BF (1938) The behavior of organisms: An experimental analysis.

Smith MM (1991) Histone structure and function. Current Opinion in Cell Biology 3: 429-437.

Smits JA, Rosenfield D, Otto MW, Marques L, Davis ML, Meuret AE, Simon NM, Pollack MH, Hofmann SG (2013) D-cycloserine enhancement of exposure therapy for social anxiety disorder depends on the success of exposure sessions. Journal of psychiatric research 47: 1455-1461.

Smolka M, Schmidt LG (1999) The influence of heroin dose and route of administration on the severity of the opiate withdrawal syndrome. Addiction 94: 1191-1198.

Sobell LC, Sobell MB (1992) Timeline follow-back Measuring alcohol consumption. Springer, pp 41-72

Soeter M, Kindt M (2010) Dissociating response systems: erasing fear from memory. Neurobiology of learning and memory 94: 30-41.

Soeter M, Kindt M (2011) Disrupting reconsolidation: pharmacological and behavioral manipulations. Learning & Memory 18: 357-366.

Song I, Huganir RL (2002) Regulation of AMPA receptors during synaptic plasticity. Trends in neurosciences 25: 578-588.

Sorg BA (2012) Reconsolidation of drug memories. Neuroscience & Biobehavioral Reviews 36: 1400-1417.

Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the state-trait anxiety inventory.

Sutton RS, Barto AG (1981) Toward a modern theory of adaptive networks: Expectation and prediction. Psychological Review 88: 135-170.

Sutton RS, Barto AG (1998) Introduction to reinforcement learning. MIT Press

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. The Journal of neuroscience 24: 4787-4795.

Tedesco V, Mutti A, Auber A, Chiamulera C (2014) Nicotine-seeking reinstatement is reduced by inhibition of instrumental memory reconsolidation. Behavioural pharmacology.

Thanos PK, Bermeo C, Wang GJ, Volkow ND (2011) D-cycloserine facilitates extinction of cocaine self-administration in rats. Synapse 65: 938-944.

Thuerauf N, Lunkenheimer J, Lunkenheimer B, Sperling W, Bleich S, Schlabeck M, Wiltfang J, Kornhuber J (2007) Memantine fails to facilitate partial cigarette deprivation in smokers--no role of Memantine in the treatment of nicotine dependency? Journal of neural transmission (Vienna, Austria : 1996) 114: 351-7.

Tiffany ST (1990) A cognitive model of drug urges and drug-use behavior: role of automatic and nonautomatic processes. Psychological review 97: 147.

Tiffany ST, Drobes DJ (1991) The development and initial validation of a questionnaire on smoking urges. British journal of addiction 86: 1467-1476.

Tomie A, Grimes KL, Pohorecky LA (2008) Behavioral characteristics and neurobiological substrates shared by Pavlovian sign-tracking and drug abuse. Brain research reviews 58: 121-135.

Tong C, Bovbjerg DH, Erblich J (2007) Smoking-related videos for use in cue-induced craving paradigms. Addictive behaviors 32: 3034-3044.

Torregrossa MM, Taylor JR (2013) Learning to forget: manipulating extinction and reconsolidation processes to treat addiction. Psychopharmacology 226: 659-672.

Treadway MT, Buckholtz JW, Schwartzman AN, Lambert WE, Zald DH (2009) Worth the 'EEfRT'? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. PLoS One 4: e6598.

Tronel S, Milekic MH, Alberini CM (2005) Linking new information to a reactivated memory requires consolidation and not reconsolidation mechanisms. PLoS biology 3: e293.

Tronson NC, Taylor JR (2007) Molecular mechanisms of memory reconsolidation. Nature Reviews Neuroscience 8: 262-275.

Tronson NC, Wiseman SL, Olausson P, Taylor JR (2006) Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. Nat Neurosci 9: 167-169.

Tsai G, Yang P, Chung L-C, Lange N, Coyle JT (1998) D-serine added to antipsychotics for the treatment of schizophrenia. Biological psychiatry 44: 1081-1089.

Tsien JZ, Huerta PT, Tonegawa S (1996) The essential role of hippocampal CA1 NMDA receptor–dependent synaptic plasticity in spatial memory. Cell 87: 1327-1338.

Valjent E, Corbillé A-G, Bertran-Gonzalez J, Hervé D, Girault J-A (2006) Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. Proceedings of the National Academy of Sciences of the United States of America 103: 2932-2937.

Volkow N, Wang G-J, Fischman M, Foltin R, Fowler J, Abumrad N, Vitkun S, Logan J, Gatley S, Pappas N (1997) Relationship between subjective effects of cocaine and dopamine transporter occupancy.

Volkow ND (2004) The reality of comorbidity: depression and drug abuse. Biological psychiatry 56: 714-717.

Volkow ND, Wang G-J, Fowler JS, Logan J, Gatley SJ, Wong C, Hitzemann R, Pappas NR (1999) Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D2receptors. Journal of Pharmacology and Experimental Therapeutics 291: 409-415.

Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Childress A-R, Jayne M, Ma Y, Wong C (2006) Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. The Journal of Neuroscience 26: 6583-6588.

Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Jayne M, Ma Y, Pradhan K, Wong C (2007) Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. The Journal of Neuroscience 27: 12700-12706.

Waelti P, Dickinson A, Schultz W (2001) Dopamine responses comply with basic assumptions of formal learning theory. Nature 412: 43-48.

Wang LP, Li F, Wang D, Xie K, Wang D, Shen X, Tsien JZ (2011) NMDA receptors in dopaminergic neurons are crucial for habit learning. Neuron 72: 1055-1066.

Wang S-H, de Oliveira Alvares L, Nader K (2009) Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. Nature neuroscience 12: 905-912.

Watson GB, Bolanowski MA, Baganoff MP, Deppeler CL, Lanthorn TH (1990) d-Cycloserine acts as a partial agonist at the glycine modulatory site of the NMDA receptor expressed in< i> Xenopus</i> oocytes. Brain research 510: 158-160.

Watt AJ, Sjöström PJ, Häusser M, Nelson SB, Turrigiano GG (2004) A proportional but slower NMDA potentiation follows AMPA potentiation in LTP. Nature neuroscience 7: 518-524.

Wechsler D (2008) Wechsler Adult Intelligence Scale–Fourth Edition (WAIS–IV). San Antonio, TX: NCS Pearson.

West R (2006) Smoking and smoking cessation in England, 2006.

West R, Hajek P (2004) Evaluation of the mood and physical symptoms scale (MPSS) to assess cigarette withdrawal. Psychopharmacology 177: 195-199.

Weybrew BB, Stark JE (1967) Psychological and physiological changes associated with deprivation from smoking. US Naval Submarine Medical Center

Wise RA (2009) Roles for nigrostriatal—not just mesocorticolimbic—dopamine in reward and addiction. Trends in neurosciences 32: 517-524.

Wong DF, Kuwabara H, Schretlen DJ, Bonson KR, Zhou Y, Nandi A, Brašić JR, Kimes AS, Maris MA, Kumar A (2006) Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. Neuropsychopharmacology 31: 2716-2727.

Wu Y, Li Y, Gao J, Sui N (2012) Differential effect of NMDA receptor antagonist in the nucleus accumbens on reconsolidation of morphine -related positive and aversive memory in rats. European Journal of Pharmacology 674: 321-326.

Xia P, Chen H-sV, Zhang D, Lipton SA (2010) Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. The Journal of Neuroscience 30: 11246-11250.

Xue Y-X, Luo Y-X, Wu P, Shi H-S, Xue L-F, Chen C, Zhu W-L, Ding Z-B, Bao Y-p, Shi J (2012) A memory retrieval-extinction procedure to prevent drug craving and relapse. Science 336: 241-245.

Yamakura T, Harris RA (2000) Effects of Gaseous Anesthetics Nitrous Oxide and Xenon on Ligand-gated Ion Channels: Comparison with Isoflurane and Ethanol. Anesthesiology 93: 1095-1101.

Yang C-H, Huang C-C, Hsu K-S (2011a) Generalization of Fear Inhibition by Disrupting Hippocampal Protein Synthesis-Dependent Reconsolidation Process. Neuropsychopharmacology 36: 1992-2008.

Yang J, Yu J, Jia X, Zhu W, Zhao L, Li S, Xu C, Yang C, Wu P, Lu L (2011b) Inhibition of nuclear factor-κB impairs reconsolidation of morphine reward memory in rats. Behavioural Brain Research 216: 592-596.

Ying S-W, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TV, Bramham CR (2002) Brain-derived neurotrophic factor induces long-term potentiation in intact adult hippocampus: requirement for ERK activation coupled to CREB and upregulation of Arc synthesis. The Journal of neuroscience 22: 1532-1540.

Yoon JH, Newton TF, Haile CN, Bordnick PS, Fintzy RE, Culbertson C, Mahoney III JJ, Hawkins RY, LaBounty KR, Ross EL (2013) Effects of D-cycloserine on cue-induced craving and cigarette smoking among concurrent cocaine-and nicotine-dependent volunteers. Addictive behaviors 38: 1518-1526.

Young NS, Ioannidis JPA, Al-Ubaydli O (2008) Why Current Publication Practices May Distort Science. PLoS Med 5: e201.

Zhai H, Wu P, Chen S, Li F, Liu Y, Lu L (2008) Effects of scopolamine and ketamine on reconsolidation of morphine conditioned place preference in rats. Behavioural Pharmacology 19: 211-216 10.1097/FBP.0b013e3282fe88a0.

Zola-Morgan S, Squire LR, Amaral D (1986) Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. The Journal of Neuroscience 6: 2950-2967.

Zweifel LS, Parker JG, Lobb CJ, Rainwater A, Wall VZ, Fadok JP, Darvas M, Kim MJ, Mizumori SJ, Paladini CA (2009) Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. Proceedings of the national academy of sciences 106: 7281-7288.

Appendix 1

Chapter 2 Study Quality Assessment Instrument

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

Is the paradigm used a valid measure of reward responding? (1 = yes, 0 = no)

Is the design appropriate to assess drug effects on reward reconsolidation? (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 (if appropriate) described? (1 = described, 1 = randomisation not appropriate & not described, 0= should have randomised and haven't described)

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

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

Are the outcome measures appropriate to assess the pharmacological intervetntion in reconsolidation? (lever presses, nose pokes, time on drug-paired floor, acquistion of new instrumental response etc. 1 = yes, 0 = no)

Did the subjects meet the inclusion/exclusion criteria (where these were present?) (1 = exclusions explained in terms of criteria, 1 = no exclusions, 0 = exclusions made without reference to criteria)

Are demographics (i.e. species/weight) for all 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 pre-experiment variables e.g. species, weight, home conditions, (1 = yes, 0 = no)

Where outcome measure was experimenter rated, was inter-rater reliability achieved and evaluated? (1 = automated, 1 = experimenter inter-rated and reliability assessed, 0 = experimenter rated but not inter-rating or reliability testing)

Were the laboratory methods known to be accurate and are they still considered valid? (Any standard measure of reward conditioning such as CPP, nose poke, self-administration = 1. If non validated test = 0)

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

Was drug given before reactivation or after? (0 for before, 1 for before & after, 1 for simultaneously and 1 for after.)

Were all assays done in the same laboratory using the same methods? If not, what steps were taken to assure inter-assay reliability? (1 = all same methods and lab, 1 = different labs, same methods and reliability procedures described, 0 = different labs/methods, reliability not described)

Is administration route of drug given? (1 = yes, 0 = no)

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

Where drugs are given intracerebrally, are the implantation procedure and site clearly described? (1 = IC and procedures fully described, 1 = systemic or intraperitoneal, 0 = IC and poor description)

Are all relevant treatment schedules clearly described? (no. trials, no. reinforcers, timing relative to CSs: 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)

Ethical Approval for Chapters 3 and 4

UCL RESEARCH ETHICS COMMITTEE
GRADUATE SCHOOL OFFICE

→ May '14

UCL

Dr Surjeev Kamboj
Clinical, Educational and Health Psychology
1-19 Torrington Place
UCL

16 May 2012

Dear Dr Kamboj

Notification of Ethical Approval
Project ID: 3928/002: Reconsolidation of automatic stimulus-response memory in hazardous drinkers and cigarette smokers

I am pleased to confirm that your study has been approved by the UCL Research Ethics Committee for the duration of the project i.e. until May 2013 on condition that a more comprehensive list of exclusion criteria are provided. It was recommended that propranolol should be avoided in participants with low blood pressure (e.g. those with a systolic blood pressure of less than 100 mmHg) and those on other drugs (e.g. calcium channel blockers) that should not be taken with propranolol. Memantine should not be administered to participants with epilepsy, pregnant women or young women of child-bearing potential

Approval is also subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form'.

The form identified above can be accessed by logging on to the ethics website homepage: http://www.grad.ucl.ac.uk/ethics/ and clicking on the button marked 'Key Responsibilities of the Researcher Following Approval'.

2. It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

   Reporting Non-Serious Adverse Events
   For non-serious adverse events you will need to inform Helen Dougal, Ethics Committee Administrator (ethics@ucl.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you.

   Reporting Serious Adverse Events
   The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an

Participant Information sheet for *Chapter 3*

Who are we recruiting?

We would like to recruit smokers (> 10 cigarettes per day and meeting screening eligibility criteria) aged 18-45 to participate in this research project. You must be seriously looking to quit smoking in the near future, and agree to attempt to quit as part of the study.

Details of Study:

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 UCL Clinical Psychopharmacology Unit (CPU).

Why are we doing this study?

Heavy smoking is associated with a variety of psychological and physical health issues. Many people find it difficult to cut down their smoking despite repeated attempts to do so. While some treatments exist to help quitting, none of them are very effective at helping people quit in the long-term. Previous research suggests that a certain brain chemical involved in memory is important in causing relapse after quitting smoking. This study aims to test a specific theory about how this chemical affects processes related to smoking and affects relapse after quitting. We hope that this study will inform new and more effective therapies to help people stay quit. Volunteers will be given a single dose of a medication (memantine) which blocks this chemical, or an inactive placebo (sugar pill). By taking part in this study you will contribute to the scientific knowledge of tobacco addiction and help with the development of better future treatments. 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. Please note, while this study aims to inform a cutting-edge smoking cessation treatment, it is *not* a validated smoking cessation treatment in its own right. Therefore, while your smoking habits may change over the course of the study, this is not guaranteed. Be aware that, should you choose to take part, you will be randomised to a group and neither you nor the experimenter will know whether you have received drug or placebo.

What will I have to do?

If you agree to participate in this study you must email CPUexperiments@gmail.com

with a contact telephone number 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 smoking, physical and mental health history. This should take around 15 minutes. Please note that, based on you answers to these questions; you may not be eligible to take part in the study, in which case we will stop the screening. If you *are* eligible to take part, you will be sent some questionnaires to fill out at home and be required to complete an online daily smoking diary for a week before coming to the Clinical Psychopharmacology Unit at UCL, at a time convenient for you. Please note that if you do not fill out the questionnaires and return them, or do not fill out the diary every day (it should only take 2-3 minutes a day), your testing sessions will be cancelled.

In total, you will have to come in on two occasions about 7-10 days apart. You will be required to abstain from drinking alcohol or using any illicit drugs for the 24 hours before testing. You must also not eat or drink any caffeinated drink in the 3 hours before the first session.

A central part of this study is that you attempt to abstain from smoking after the end of the first session for as long as possible. You should view the end of the first session as a target 'quit' day, from which point on you will not smoke. While we appreciate that it may not be possible to stop smoking completely, you should only take part if you want to quit smoking in the near future and are willing to seriously attempt to do so as part of the study.

We will measure breath alcohol and carbon monoxide levels with a smokerlyzer test at the beginning of every testing session. If the breathalyser test shows that you have a high blood alcohol concentration, testing will have to be cancelled and you will not be paid. We will also assess your recent smoking with a salivary sample that measures cotinine, a metabolite of nicotine.

Day 1

You will first receive 10mg memantine or a placebo. In order for the experiment to be 'blind', you will not know which drug you have taken, nor will the experimenter, as all the pills are identical. If you wish to find out which drug you took, this information can be sent to you by a third party after full completion of the study. You will then go through some questionnaires that measure general mood and some personality factors and complete come computer-based tasks. These will involve measuring your reaction times and eye movements to various pictures. There will be a rest period of between 30 and 50 minutes after this until 2.5 hours has passed since taking the pill. Finally you will complete some brief computer and questionnaire tasks. Depending on what group you are assigned to, you may be exposed to some items that remind you of smoking. One task will involve you watching a video while we measure your heart rate, blood pressure and skin conductance. This will involve fitting an electrode belt

against your skin (i.e. under your top). You will be shown how to fit the belt and then allowed to put the belt on in private. All of these procedures are completely safe and non-painful and the belt should be very comfortable when you are wearing it. If you are not comfortable wearing the belt against your skin, you should not take part in this study. After this, you *may or may not* be required to smoke a cigarette. In total, day 1 will take around 3 hours After day 1, you *must* not smoke or drink for the rest of the day after the end of testing, and you should attempt to abstain from smoking (stay quit) for as long as you possibly can.

Between the first and second testing sessions, you must continue filling out the online diary at the end of *every day*. It is vital that you fill this out daily. If you do not fill out the diary, your second session will be cancelled and you will not receive payment.

Day 2

On day 2, one week later, you will repeat the tasks from day 1, but you will not be required to take any pills. This session should last around 1 hour in total.

Follow Up

After day 2, you will not have to come to UCL again, but *must* continue filling in the electronic smoking diary every day for 3 weeks. Continuing to fill out the diary critical to study, as it will allow us to assess whether the treatment affects your ability to stay quit in the long-term. As such, you will not receive full payment unless you continue to fill out the diary every day for 3 weeks after the study. After this, we ask you to fill out the diary only once a week for 4 weeks. You will be sent reminders about when to do this. We will contact you once at 3 months, 6 months, 9 months and 1 year to ask you some quick follow-up questions and to check in on how your smoking has changed.

How will I be paid?

You will be reimbursed for time spent in UCL at the standard rate of £7.50 per hour. In total, testing time should be around 4 hours, so you will receive ~£30 payment (there is the opportunity to win slightly more money in some of the tasks). Full payment is contingent upon completing both the testing days and diary. Therefore. You will receive half of the payment (£15) upon completion of Day 2 (as long as the diary has been completed up to that point). The rest of the payment (£15-20) will be paid directly into your account or by cheque after completion of the diary for 3 weeks following day 2.

What are these drugs and what are the possible risks?

Memantine is used in the treatment of moderate to severe Alzheimer's disease, as it protects against some of the loss of cognitive function associated with the disease.

Memantine is generally very well tolerated, especially at the low doses that will be used in this study. It is unlikely that you will notice any subjective effects of the drug if you receive it. In a small minority (~1%) people, the drug can cause dizziness, agitation or confusion; however, this is highly unlikely at the doses used in this study. As memantine is metabolised by the kidneys, if you have any history of, or current kidney dysfunction, you will not be able to take part in the study.

As memantine can cause mild drowsiness, you must not operate any heavy machinery, drink or drive after taking the drug.

What are the benefits of taking part?

By taking part in the study, you are contributing to the developments of better treatments for quitting smoking. In answering the questions and tracking your smoking behaviour over the course of the study you may gain new insight into the drivers and triggers for your smoking. As quitting smoking is part of the study, you will experience all the health benefits of quitting (*reduced risk of all cancers, improved cardiovascular function and respiration reduced risk of heart disease, increased life expectancy, significant monetary savings, more free time etc.)* for as long as you stay quit. The treatment itself *may* help you stay quitted longer or reduce your cravings for cigarettes while you are abstinent, however we *cannot* guarantee that this will be the case. Everyone involved in the study will be directed to NHS support material for quitting and is encouraged to use nicotine replacement therapy (NRT), both of which can help manage cravings during the first week of quitting.

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 by emailing CPUexperiments@gmail.com.

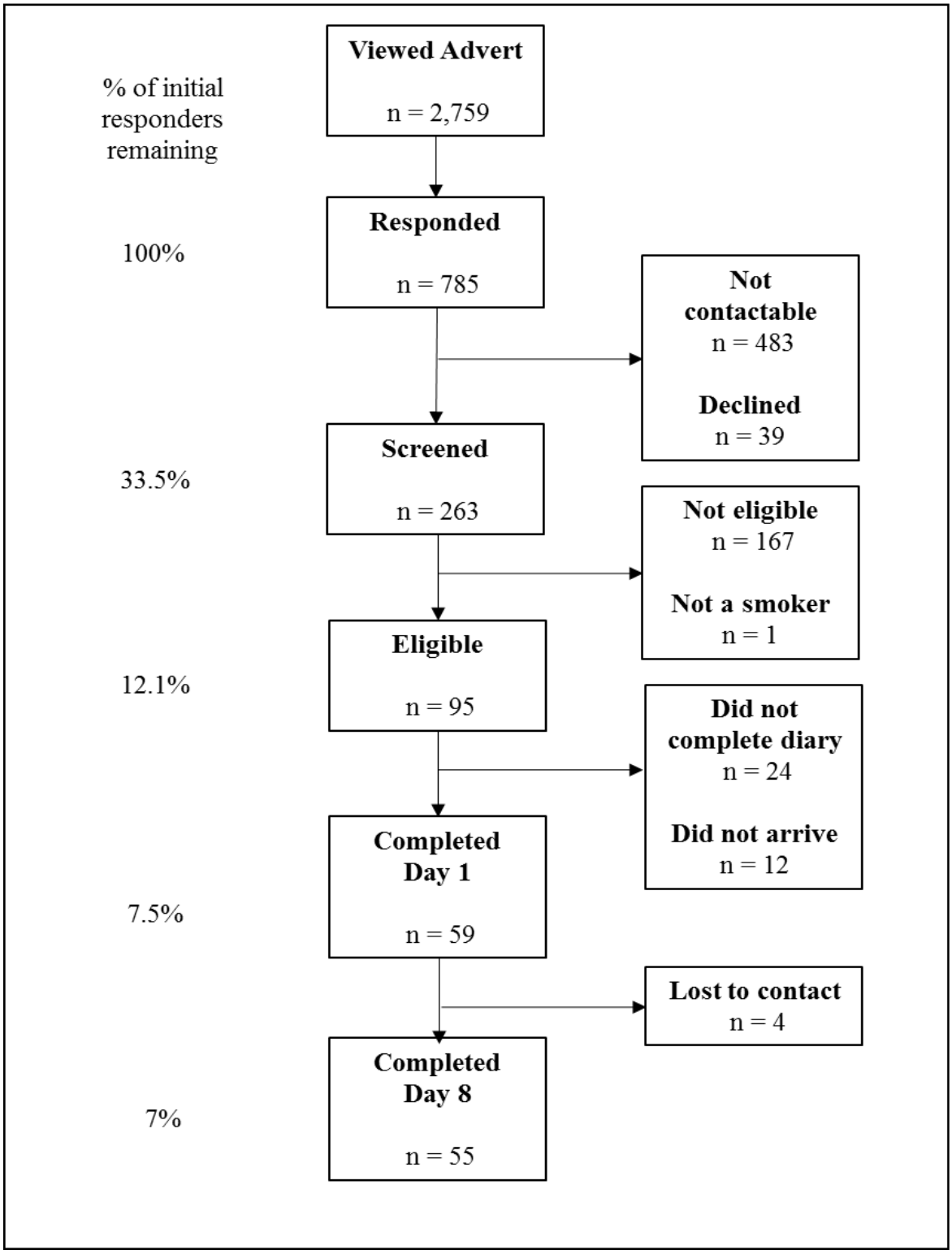
This study has been approved by the UCL ethics committee

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 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.

CONSORT Diagram of participant recruitment for *Chapter 3*



Information Sheet *Chapter 4*

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 3 days out of every 7. The governmental daily guidelines are 2-3 units per day for women and 3-4 units for men.

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.

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 N_2O are quite similar to being drunk, in that it can make people quite giggly, uncoordinated or dissociated. There will be a standard dose of N_2O 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 N_2O very quickly disappear and you will feel normal again within a few minutes. You will be randomly assigned to breathe N_2O or a placebo (normal air) and will not be told which you receive.

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 short series of questions to check your eligibility for the study. Please note that, 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 at a time convenient for you on 3 occasions, around 48 hours apart. 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 is above the cut-off point for the study, testing will have to be re-arranged or you will be excluded from the study.

Day 1: After going through some short questionnaires that measure general mood and attitudes you will complete two computer tasks that will involve learning about the relationships between different stimuli, either pictures or words. In one of these tasks, you will be playing to win points for beer that you consume at the end of the session. **As such, you should organise this session at a time when you would want to drink beer and feel motivated to win beer during the task**. In the other task, you will be paid for the number of word pairs you can remember and can earn up to an extra £7.20 in this task over the course of the 3 days. In total, this session will last around 1.5 hours.

Day 2(Day 1 + 2 -3 days): You will come into the UCL again to the Department of Pharmacology (Cruciform building), where you will inhale either Nitrous Oxide gas (N_2O) or normal air and repeat the tasks from Day 1, playing to win beer or money. Whether you receive N2O or normal air will depend on random allocation to an experimental group and will be 'blind'. That is, you will not be told which group you are in. Note that the concentration of N_2O may make you feel quite 'drunk' while you are breathing it and you should not take part if you would not be comfortable feeling this way for several minutes. In total, this day will last 30-40 minutes. Again, this should be organised at a time **when you would want to drink beer and feel motivated to win beer during the task**

Day 3 (Day 2 + 2 – 3 days): You will again come to the CPU to repeat some computer tasks measuring you mood, attitudes and repeat the learning tasks from day 1 and day 2 to win beer or money, so this session must also be **when you would want to drink beer and feel motivated to win beer during the task**. At the end of this day, you will not have to come into UCL again but you may be contacted by the experimenters to ask some follow-up questions about the study. This session will last around 45 minutes to 1 hour.

How will I be paid?

You will receive payment of £7.50 per hour for your participation upon completion of all three days of the study. In total, the basic testing should last ~2.5 - 3 hours, so you can expect to earn around £15 to £22 basic pay. You can also win up to £7.20 depending on your performance on one of the memory tasks.

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 below.

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 Z6364106/2013/05/27**

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 experimenters:

Project coordinator: Ravi Das

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

Email: CPUexperiments@gmail.com

Telephone: 02076798225

Ethical Approval for Chapter 5

UCL RESEARCH ETHICS COMMITTEE
GRADUATE SCHOOL OFFICE

Dr Sunjeev Kamboj
Clinical Psychopharmacology Unit
1-19 Torrington Place
UCL

1 July 2013

Dear Dr Kamboj

Notification of Ethical Approval
Project ID: 3901/001: Understanding destabilisation and updating of drug memories

I am pleased to confirm that your study has been approved by the UCL Research Ethics Committee for the duration of the project i.e. until February 2015

Approval is subject to the following conditions:

1. You must seek Chair's approval for proposed amendments to the research for which this approval has been given. Ethical approval is specific to this project and must not be treated as applicable to research of a similar nature. Each research project is reviewed separately and if there are significant changes to the research protocol you should seek confirmation of continued ethical approval by completing the 'Amendment Approval Request Form'.

The form identified above can be accessed by logging on to the ethics website homepage: http://www.grad.ucl.ac.uk/ethics/ and clicking on the button marked 'Key Responsibilities of the Researcher Following Approval'.

2 It is your responsibility to report to the Committee any unanticipated problems or adverse events involving risks to participants or others. Both non-serious and serious adverse events must be reported.

Reporting Non-Serious Adverse Events
For non-serious adverse events you will need to inform Helen Dougal, Ethics Committee Administrator (ethics@uc.ac.uk), within ten days of an adverse incident occurring and provide a full written report that should include any amendments to the participant information sheet and study protocol. The Chair or Vice-Chair of the Ethics Committee will confirm that the incident is non-serious and report to the Committee at the next meeting. The final view of the Committee will be communicated to you

Reporting Serious Adverse Events
The Ethics Committee should be notified of all serious adverse events via the Ethics Committee Administrator immediately the incident occurs. Where the adverse incident is unexpected and serious, the Chair or Vice-Chair will decide whether the study should be terminated pending the opinion of an independent expert. The adverse event will be considered at the next Committee meeting and a decision will be made on the need to change the information leaflet and/or study protocol.

Participant Information Sheet Chapter 5

Who are we recruiting?

We would like to invite heavy drinkers, defined as people who often drink twice (or more than twice) the governmental daily recommendation of alcohol. The governmental daily guidelines are 2-3 units per day for women and 3-4 units for men.

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?

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 taste perception in heavy drinkers and how this can be changed. To test this, you will be asked to consume different drinks and rate how much you like the taste of them after viewing certain pictures. 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 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, 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) at UCL on two occasions several 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.

Day 1:

On the first day, after going through some questionnaires that measure general mood and personality factors, you will take part in a 'taste test' of some drinks. The drinks you receive will depend on the group to which you are randomly allocated. You will rate the drinks for how much you like them and also rate some pictures for how they affect your perception of the drink's taste.

You will then complete a computer tasks that will involve making some ratings, measuring your reaction times and eye movements in response to some pictures. In this task, you may see some pictures that are unpleasant and designed to evoke an emotional reaction. You will be required to consume drinks at certain points during this task. These drinks will vary in how pleasant people generally think they are. Some will be pleasant, but some may be very bitter. You will be required to drink all the drinks samples you are given.

Day 2:

A few days alter later, you will come into the CPU again for a follow-up test where we will again measure your response to various pictures and complete some questionnaires and your taste ratings of different drinks. After the end of this day, you will not need to come in again, but we will contact you to ask some quick follow-up questions after the study.

How will I be paid?

You will receive payment for your participation upon completion of the second day. You will be reimbursed at the rate of £7.50 per hour. Unfortunately we cannot reimburse extra travel expenses.

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.

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.

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.

All data will be 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: CPUTrackerLab@gmail.com