NEURAL MECHANISMS OF MEMORY RECONSOLIDATION

By

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ABSTRACT

This thesis investigates the mechanisms of memory reconsolidation, with a particular focus on instrumental memories. Memories are dynamic in nature and can destabilise and re-stabilise in order to strengthen and update with new information. Destabilisation renders memories labile and vulnerable to amnestic intervention, requiring a reconsolidation phase in order to return to a stable form. Reconsolidation has been demonstrated in a great many memory settings, however the memories underpinning instrumental behaviours have not yet been shown to undergo reconsolidation. Starting from the hypothesis that reconsolidation mediates memory updating, this thesis investigates the reconsolidation of instrumental memories using primarily lever pressing in rats as a model, but also a novel active avoidance paradigm; reconsolidation is also investigated in a place aversion setting. Instrumental memories are found to destabilise following a suitable change in contingency, and their reconsolidation is shown to require activity at the N-methyl-D-aspartate receptor (NMDAR), and in the case of goal-directed memory, co-activation of dopamine-1 and NMDARs. Consideration of the conditions under which instrumental memories will and will not destabilise suggests certain boundary conditions on reconsolidation and this thesis proposes that a change in incentive outcome is required in order for memories to be destabilised.

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CHAPTER 1

GENERAL INTRODUCTION

Memories are known to be dynamic in nature. They alternate between phases of stability and malleability which enable them to maintain their relevance by being updated with new information. When memories are first learned they are unstable, and vulnerable to amnestic insult; a period of consolidation is required for the memory to become stable and long lasting. When memories are retrieved under appropriate conditions, they destabilise and then require a phase of reconsolidation to return to their stable form. This reconsolidation process enables new salient information to be incorporated into a memory, keeping them updated; however this process may also underpin the phenomenon of false memory. Reconsolidation has also received a great deal of attention as a potential treatment target for maladaptive memory disorders, as disrupting it can result in amnesia for that memory. The reconsolidation process has been demonstrated in a variety of contexts, and across many species; however there is a large gap in our knowledge for the reconsolidation of instrumental memories, those that underpin behavioural action. There are currently no published studies successfully demonstrating the reconsolidation of instrumental memories, in fact the current literature suggests instrumental memories do not undergo reconsolidation. This thesis sets out to demonstrate the existence of the reconsolidation process in instrumental memories, and thus address this gap in our understanding of reconsolidation. This would have great importance for the treatment of maladaptive memory disorders such as drug addiction and obsessive compulsive disorder, as instrumental habit memories are believed to support these behaviours.

The nature of memory

Aristotle proposed a distinction between sensing and thinking (Aristotle *et al.*, 1931). The link between the two was imagination. He suggested that sensory images leave imprints on the mind, in

the same way that a signet ring leaves an imprint in hot wax. These imprints (memories) are perceived by the soul through imagination. In the same way that one can imagine an object with their eyes closed (without any sensory perception), memory was the re-imagination of past impressions, which could be perceived by the soul in the mind's eye. Aristotle uses the analogy of a portrait painting. The painting is both a physical object and a likeness of a real person, which we can use as a framework to imagine what the real person looks like. In the case of the signet ring analogy, the impression the ring leaves behind is both a tangible object in its own right and a likeness of the original ring, to be used as a mould for reconstructing the original image. This theory recognised memory as a two part process, requiring both storage of a physical 'impression' and retrieval of the original sensory stimulus.

Memory as a constructive process

Frederic Bartlett proposed that memories were recalled based upon a *schema*, a framework for the memory which was interpreted in the present by existing cultural preconceptions and understanding of the world (Bartlett, 1932). This was most notably demonstrated in his experiment involving the retelling of "The War of the Ghosts" (Bartlett, 1920). In this experiment he asked subjects to retell a Native-American folk-tale. During recall subjects were able to convey the broad outline of the plot and often place names (the basic *schema* for the memory); however various details would be changed or even omitted in the retelling. Bartlett noted that these changes often took the form of omitting irrelevant information and modifying parts of the story to be more internally consistent with the teller's existing life and culture. For example "canoe" was often changed to "boat", and "paddling" to "rowing". As the tale was repeatedly retold the subjects' recollection would often become more and more influenced by their earlier interpretations of the story. These results make an important contribution to our interpretation of memory as they reject the hypothesis that the brain functions as a storehouse of exact accounts of events, but rather memories are approximate outlines (or *schemata*) of events which are re-imagined when we recollect them.

Memory is inherently vulnerable to distortion, by misconstruction of information that fits into the *schema* for the memory but may not have been part of the original telling. A well-researched example of this is the Deese–Roediger–McDermott task (Deese, 1959; Roediger & McDermott, 1995). In this paradigm subjects are presented with a list of related words, for example: “thread”, “thimble”, “sharp”; memory was then tested using a list composed of the original words, new unrelated-words and lure-words which were associated with those on the original list. For example when presented with a list of “bread”, “needle” and “sewing”, subjects often falsely identify the latter two words as coming from the original list, despite neither being presented previously. Another method of experimentally producing false memories is to deliberately prompt subjects with false information (Loftus & Greene, 1980) or suggestive language (Loftus & Palmer, 1974). The power of outside influences to distort memories has significant implications for society, particularly in solving crime where eyewitness testimony is highly valued (Loftus, 2003). In some cases memories may not be true at all, but entirely false confabulations formed from suggestion and imagination. For example, researchers have successfully created false memories for being lost in a shopping mall (Loftus & Pickrell, 1995; Pezdek *et al.*, 1997). False memories are believed to underpin the recovery of alleged repressed memories, a condition now referred to as false memory syndrome (Merskey, 1998).

While the creation of false memories may suggest our memory is unreliable and malleable it has been argued that the constructive nature of memory serves an adaptive function in the imagination and prediction of future events (Atance & O’Neill, 2001; Buckner & Carroll, 2007), a process which appears to draw on memories of the past (Szpunar & McDermott, 2008). The roman orator Cicero advocated artificial creation of a false memory in his “Method of Loci” to improve memory recollection (Yates, 1966), and this method has been used to successfully recite π to over 65,536 decimal places (Raz *et al.*, 2009). Thus, memory *schemata* may function to aid recall in healthy individuals (Schacter & Addis, 2007).

Associative conditioning

Theories of memory have come to be defined by the eponymous Ivan Pavlov and his experiments in dogs (Pavlov, 1927). Pavlov presented a beating metronome, the conditioned stimulus (CS) immediately prior to the appearance of food, the unconditioned stimulus (US), such that the metronome predicted the appearance of food. Before conditioning took place, a naïve animal would salivate in response to food being inserted into the mouth, an unconditioned response (UR). During training presentation of the CS immediately before delivery of the US would cause the dog to pair together the CS and US in a CS–US memory of the event. Pavlov followed up his experiments by presenting food in front of the dog, rather than directly in the mouth. This still elicited a conditioned salivation response, however only if the animals had previously tasted the dog-food. This suggested that the motivational properties of the food were also learned. While Pavlov favoured a conditioned reflex interpretation of his data (where the salivation response itself became paired with the CS) more recent theories have suggested CS–US (i.e. metronome–food) and incentive learning (i.e. dog-food is tasty) are distinct processes (Dickinson & Balleine, 1994, 2002).

Another example of a conditioned reflex was demonstrated by Edward Thorndike (Thorndike, 1898, 1911), who placed cats in custom-build "puzzle-boxes" which required a certain response to escape from. Typically a small food reward was placed just outside the box in order to motivate escape. Thorndike proposed that the positive feelings of escape and freedom reinforced the response that lead to that feeling. This *Law of Effect* strengthened an association between the context, or the stimulus (S), and the response (R) that lead to escape. The next time the cat was placed in the box, Thorndike proposed the context elicited this S–R memory, causing the cat to feel an urge to perform the escape response again. This form of conditioning was deemed to be distinct from that described by Pavlov (Skinner, 1935, 1937; Konorski & Miller, 1937) and termed instrumental, or operant, behaviour. One problem with a purely conditioned reflex interpretation of behaviour was that it could not explain intentional, goal-directed behaviours. It was generally agreed that the S–R model proposed by Thorndike could explain habitual behaviour, however goal-directed actions were distinct

in that they required a belief the action would have a certain consequence and a desire to obtain (or avoid) that consequence (James, 1890; Heyes & Dickinson, 1990; Stock & Stock, 2004). That is to say that in order for behaviour to be considered goal-directed it must be demonstrated that there is a belief that a certain outcome (or consequence) is contingent upon a certain action, and that the outcome is a desired behavioural goal; if the outcome is not a goal, then behaviour cannot be considered goal-directed (Dickinson & Balleine, 1994). In order to accommodate these criteria, goal-directed behaviour was suggested to be mediated by an Action–Outcome (A–O) association. Actions are fundamentally equivalent to habitual responses, but are conceptually distinct in that actions are classified as intentional (Dickinson, 1985). A–O associations encode the contingency between the action and its consequences (thus satisfying the belief criterion) and the consequence is represented as a behavioural outcome, or goal (satisfying the desire criterion).

Instrumental conditioning

The criteria of belief and desire distinguished goal-directed behaviour from automated habitual responses; both behaviours were agreed to coexist, with habits forming after extended repetition of a response (James, 1890; Dickinson & Balleine, 1993; Stock & Stock, 2004), however this was primarily based upon introspective reasoning. It was several years before these different mechanisms of instrumental behaviour were distinguished in animals (Dickinson, 1985). Working from the principle that goal-directed memory encoded the consequences of actions, it was demonstrated that reducing the value of the reinforcer (i.e. the goal) by pairing it with sickness would also impair instrumental responding provided training was limited; however once a behaviour was well-trained and likely habitual, changes in reward value had no effect, presumably because the S–R association did not encode the outcome of the response (Adams, 1982). The implication was that goal-directed associations must possess a representation of the outcome as a behavioural goal, existing as A–O memories, and satisfying the criterion of desire. The lack of any devaluation effect in the well-trained habitual animals confirmed that S–R responding was distinct from A–O behaviour, as

either habitual animals did not believe the action had the, now devalued, consequence (lack of belief) or did not encode the outcome as a goal (lack of desire).

Goal-directed memories also show high sensitivity to changes in reinforcer contingency. The first demonstration of this trained rats to lever press for water (Hammond, 1980). Rats learned to press the lever, with each lever press enabling a certain probability of water presentation each second; however when the probability of non-contingent presentations of water was increased, without reducing the probability of contingent reinforcement, rats would reduce their pressing. This result cannot be explained by a conditioned reflex S–R model as responses were still reinforced just as in the training phase (Balleine & Dickinson, 1998a). By implication there must have existed a memory encoding the contingency between the behavioural response and the outcome, thus satisfying the belief criterion for goal-directed behaviour.

It has been suggested that in order to be considered goal-directed behaviour must be sensitive to both outcome value and response contingency (Balleine & Dickinson, 1998a); however while it has been suggested that habits are less sensitive to contingency degradation (Balleine & Dickinson, 1998a; Yin *et al.*, 2006; Derusso *et al.*, 2010), they are not insensitive to the omission schedules used in these studies, and data are not yet conclusive. Interestingly Yin *et al.* (2006) found no difference in performance on an omission schedule test between rats given muscimol-inactivation of the DLS (presumed goal-directed) and control-infused habitual responders, differences only emerged on a following extinction test; although this may have been due to reduced responding during the omission test causing minimal levels of responding. Habitual behaviour has not yet been tested in a true contingency degradation (increasing non-contingent reward, with consistent contingent reinforcement), and as such the precise sensitivity (or lack) of habits to contingency change degradation is unknown, although conventional learning theory would predict they are completely insensitive. Currently outcome devaluation remains the only reliable way to distinguish goal-directed from habitual responding. The sensitivity of goal-directed (and likely also habitual) behaviour to omission schedules does, however, distinguish instrumental from pavlovian behaviours.

Actions and habits are also dissociable by their dependance on different brain regions. Lesions (Yin *et al.*, 2004) or inactivation (Packard & McGaugh, 1996) of the dorsolateral striatum (DLS) causes behaviour to remain (or become) sensitive reinforcer value, regardless of training. Furthermore DLS inactivation renders behaviour more sensitive to contingency change (Yin *et al.*, 2006). On the other hand lesions or inactivation of the dorsomedial striatum (DMS) causes behaviour to remain insensitive to reward devaluation treatment (Yin *et al.*, 2005). In line with the double dissociation between the roles of the DMS and DLS in instrumental conditioning, behaviour shifts from DMS to DLS control as training is increased (Murray *et al.*, 2012). Further evidence for a neural dissociation of A–O and S–R memories comes from lesions of the medial prefrontal cortex (mPFC); lesions of the prelimbic (PL) mPFC prevent reinforcer devaluation effects (Killcross & Coutureau, 2003), while lesions of the infralimbic (IL) mPFC restore them following overtraining (Coutureau & Killcross, 2003; Killcross & Coutureau, 2003). Interestingly the PL does have a strong projection to the DMS (Berendse *et al.*, 1992), and the nucleus accumbens (NAc) (Berendse *et al.*, 1992; Ding *et al.*, 2001); however, while the NAc does not appear to play a role in encoding of response contingency (Balleine & Killcross, 1994; Corbit *et al.*, 2001), it is critical for mediating the motivational effects of pavlovian cues on behaviour (Shiflett & Balleine, 2010), and acquiring instrumental contingency when reward is delayed (Cardinal & Cheung, 2005). Furthermore pre-training, but not post-training, lesions of the mPFC impair goal-directed learning, suggesting the role of the mPFC may be in initial encoding of the contingency rather than behavioural expression (Ostlund & Balleine, 2005).

Pavlovian conditioning

The distinction between pavlovian and instrumental behaviour is perhaps best demonstrated by example. In one experiment by Wayne Hershberger, inspired by Alice in Wonderland and Through the Looking Glass, chicks were trained to approach a food bowl; however there was a twist, during testing the bowl would move away from the chicks twice as fast as they approached, such that they needed to turn around and “walk the other way” in order to reach the food bowl (Hershberger,

1986) much like Alice approaching the Red Queen. Were the behaviour mediating approach to the bowl instrumental the chicks should have understood the consequences of their actions (i.e. the contingency between their responses and reinforcement) and learned to move away from the bowl in order to obtain the reward. This did not happen and chicks continued to approach the bowl (which of course they could never reach), their behaviour severely hindered by the bizarre environment. In contrast to approach behaviour, lever pressing for food appears to be primarily an instrumental behaviour, demonstrated by sensitivity to omission schedules (see above).

CS–US learning

Several lines of evidence suggest that an association is formed between the CS and a specific sensory representation of the US. Selective devaluation of a US (similar to as in instrumental conditioning, above) leads to a reduction in responding in the presence of a previously paired CS (Colwill & Motzkin, 1994). Interestingly these types of pavlovian association are similar to instrumental A–O memories in that they are sensitive to both reinforcer value (Holland & Rescorla, 1975; Dickinson & Balleine, 1990; Colwill & Motzkin, 1994; Holland, 2004) and contingency degradation (Colwill & Motzkin, 1994; Delamater, 1995; Ostlund & Balleine, 2008a). The only way behaviour could be sensitive to these effects is if there existed an association to the US (much the same argument as for instrumental A–O behaviour).

Interestingly, presentation of a CS appears to elicit a sensory representation of the US which can lead to misperception. For example a sucrose–paired CS can cause water to be misperceived as sweet (Delamater *et al.*, 1986), while devaluation of the sucrose results in the CS causing water to be perceived as equally distasteful (Kerfoot *et al.*, 2007); however this effect appears to diminish with increased training (Holland *et al.*, 2008). Presentation of a sucrose-paired CS appears to activate many of the same neurons as the sucrose alone, and may explain misperception effects as the CS may activate a population of neurons responsible for perception of the US (Saddoris *et al.*, 2009; Desgranges *et al.*, 2010). It has been suggested this phenomenon may underpin hallucination

(Delamater, 2012). Interestingly, the diminished ability of the CS to elicit this misperception effect with increased training does not affect the sensitivity of approach behaviour to reward devaluation (Holland *et al.*, 2008), and it has been suggested this indicates the presence of two US representations within the brain encoding expectation and sensory experience (Delamater, 2012). There is some support for the idea of parallel processing of the US in the different roles of the amygdala in pavlovian learning. While the central nucleus (CeN) appears to encode the motivational affective value of the US, the basolateral amygdala (BLA) mediates an association between the CS and the sensory properties of the US (Cardinal *et al.*, 2003; Balleine, 2005). Additionally, while lesions of the NAc core do not impair pavlovian learning, they do impair the ability to use sensory CS–US associations to modulate behaviour (Corbit *et al.*, 2001). Similar impairments can also be obtained by disconnecting the NAc core and BLA (Shiflett & Balleine, 2010).

Motivational learning

In addition to being paired to a representation of the US, a CS can also acquire motivational properties through association. This is perhaps best demonstrated through the phenomenon of sign-tracking of a conditioned response, versus goal-tracking (Robinson & Flagel, 2009). In this paradigm rats learned a retracting lever (the CS) predicted delivery of a food pellet. Interestingly some rats focus their responding on making contact with the CS (sign-trackers), while others make conditioned response towards the food-delivery port (goal-trackers); furthermore sign-tracking rats would now work to acquire the CS, suggesting it has acquired motivational properties and become a conditioned reinforcer. This experiment demonstrates that a CS can either elicit a representation of the US (goal-tracking) or activate an affective motivational process (sign-tracking).

A further example of this comes from the demonstration of dissociable general and US-specific pavlovian-to-instrumental transfer (PIT) effects. Typically a CS paired with the same outcome as an instrumental response causes responding to become potentiated for the outcome-sharing response (Estes, 1948; Baxter & Zamble, 1982; Colwill & Motzkin, 1994; Corbit *et al.*, 2007), in addition this is

often also mediated in part by a reduction in responding for alternative responses (Colwill & Motzkin, 1994; Rescorla, 1994; Corbit & Balleine, 2005). Outcome specific transfer is not sensitive to reinforcer devaluation (Rescorla, 1994), and thus is likely mediated via a motivational process; however a caveat to this is that sensitivity of transfer to reinforcer devaluation appears to change with number of different types of reinforcer used and amount of training (Holland, 2004). It may be in cases with two reinforcers that the CS acts purely as a cue (Rescorla, 1994; Holland, 2004). In experiments that have used different reinforcers to those used to condition instrumental responses it has been possible to observe general transfer (Dickinson & Balleine, 1990; Corbit & Balleine, 2005; Corbit *et al.*, 2007), where performance is increased regardless of response outcome; this is consistent with the view that pavlovian cues can activate a motivational system directly (Dickinson & Dearing, 1979; Dickinson & Balleine, 2002). Interestingly the magnitude of general transfer does appear to be sensitive to the current value of the US (Dickinson & Balleine, 1990); this may be related to the existence of dissociable anticipated and experienced reward systems (c.f. expected vs. experienced US representations, above) within the brain (Blundell *et al.*, 2001; Corbit & Balleine, 2011).

These representations of reinforcer anticipation and sensory experience parallel earlier theories of Pavlovian conditioning which dissociated between preparatory and consummatory conditioning. By way of demonstration, in eye blink conditioning pairing a CS with a puff of air to the eye (the US) elicits a defensive eye blink reflex when the CS is presented; however this is also accompanied by an increase in heart rate. It was proposed that the increase in heart rate represented preparatory conditioning, while the defensive reflex was consummatory (Konorski, 1967; Dickinson & Dearing, 1979; Dickinson & Balleine, 2002). The preparatory incentive memory directly activated an aversive motivational system which could then potentiate consummatory responses in the same motivational system. A demonstration of this effect was done by pairing a CS with footshock, but also conditioning another CS to an air-puff to the eye (Bombace *et al.*, 1991). The footshock-paired CS could then enhance the eyeblink reflex. Past theories suggested this is because the footshock-paired CS directly activated an aversive motivational system (Dickinson & Dearing, 1979; Dickinson & Balleine, 2002), however if the sensory (US_S) and motivational (US_M) properties of the US are encoded separately

then this may imply preparatory conditioning is mediated via a motivational representation of the US (i.e. a CS–US_M association), while consummatory conditioning is driven by CS–US_S memory (Blundell *et al.*, 2001).

A notable phenomenon in Pavlovian conditioning is blocking. This occurs when a compound stimulus CS1/CS2 is paired with a reinforcer. If CS1 is pretrained to the US before compound conditioning, then during compound training the conditioning of CS2 to the US will be weakened, or blocked by the pre-existing CS1–US association. Early explanations for this phenomenon suggested that since CS1 already accurately predicted the US, that CS2 was ignored or did not produce any salient learning signal (Rescorla & Wagner, 1972). Further investigation of blocking revealed transreinforcer blocking, an effect by which a CS1–US1 association could block formation of a CS2–US2 association provided US1 and US2 were of a similar motivational value (i.e. either appetitive, or both aversive). For example if US1 were footshock, and US2 a loud aversive noise (Bakal *et al.*, 1974); transreinforcer blocking has also been demonstrated with food and water in an appetitive setting (Ganesan & Pearce, 1988). It was suggested that the reason for this blocking was that reinforcers with a common affective valence activated a common appetitive or aversive system (Konorski, 1967; Dickinson & Dearing, 1979). Interestingly a CS which predicted omission of food could block conditioning to an aversive US, and a CS which predicted omission of punishment could block appetitive conditioning (Dickinson & Dearing, 1979; Goodman & Fowler, 1983; Dickinson & Balleine, 2002); this supports that a CS predicting omission of an aversive stimulus takes on an appetitive affective valence, and one predicting reward omission an aversive value. These findings were explained with the framework of competing appetitive and aversive systems. Sudden removal of a US leads to rebound excitation of the opposing valence system, leading to formation of “frustrative” CSs and “hopeful” CSs; this has been taken as providing further evidence for opposing appetitive and aversive motivational systems (Dickinson & Balleine, 2002).

Motivational CS–US_M pavlovian memories seem to require the CeN (Corbit & Balleine, 2005; Purgert *et al.*, 2012). While it has been suggested the NAc shell is required to mediate general motivational

transfer effects (Shiflett & Balleine, 2010), it appears the NAc shell is required to use motivational memory. For example, disconnection of the shell and hippocampus prevents learning of conditioned place preference (Ito *et al.*, 2008a). Interestingly it appears the CeN can interact indirectly with the DLS to mediate learning and expression of habit behaviour (Lingawi & Balleine, 2012), however the CeN does not project directly to the DLS but does so via a projection to the substantia nigra pars compacta (SNc), the source of a great many midbrain dopamine neurons which are required for habit learning (Faure *et al.*, 2005; Wang *et al.*, 2011). Whether this indicates an interaction between habits and motivational pavlovian influences is unclear, although habits do appear to be more sensitive to instrumental transfer effects (Holland, 2004; Wiltgen *et al.*, 2012).

Incentive learning

One final form of learning concerns how stimuli acquire their value. This form of learning has sometimes been termed instrumental incentive, in contrast to the pavlovian incentive (motivational learning) described above (Dickinson & Balleine, 2002), or evaluative conditioning (Balleine, 2011). Pavlov noted that dogs which had not previously experienced the dog-food would not condition to his metronome; a similar finding was observed later, that new born rat pups would not immediately seek food or water when hungry or thirsty (Hall *et al.*, 2000; Changizi *et al.*, 2002). In fact, it appears that seeking behaviours for natural rewards when in a relevant motivational state are learned rather than innate. Consistent with this finding it has been demonstrated that rats trained to make an instrumental response to acquire one of two liquid reinforcers in a thirsty state do not reduce their instrumental responding when they are not water-deprived, unless they have previous experience with the reinforcer when non water-deprived (Lopez *et al.*, 1992). The implication is that rats must learn the liquids are not valuable when not thirsty. Similarly, reward-specific satiety causes a reduction in incentive value for that reward, and interestingly an increase for alternatives (Balleine & Dickinson, 1998b). In essence, the hunger-sating properties of food are learned, as are is thirst-

quenching of water (Dickinson & Balleine, 2002); for example, food is more valuable when hungry than when full.

This form of learning appears to underlie the process by which reinforcers are devalued (or revalued). This has particular relevance to instrumental behaviours as actions and habits are frequently distinguished by their differential sensitivity to reward devaluation. In one example of this rats were trained to lever press for sucrose solution (Balleine & Dickinson, 1991) which was then devalued by LiCl injection immediately after reward re-exposure, inducing gastric malaise. Interestingly the instrumental response was not devalued unless rats were re-exposed to the sucrose solution again between devaluation treatment and testing. The implication is that devaluation of a reward requires a feedback process that signals the reward is no longer "liked", updating the desire, or "wanting" for the reward (Balleine, 2011).

The BLA is believed to play a central role in this form of conditioning (Balleine & O'Doherty, 2010; Balleine, 2011) as lesions of the BLA prevent reward devaluation (Málková *et al.*, 1997; Blundell *et al.*, 2001), and this region also mediates the effect of food associations on feeding behaviour (Holland *et al.*, 2002; Petrovich *et al.*, 2002). The NAc and ventral pallidum (VP) also believed to play a key role in this form of learning, interacting to mediate the hedonic reactions to rewards and encoding their value (Smith & Berridge, 2007). This appears to involve endogenous opioid signalling in the VP (Smith & Berridge, 2005; Peciña *et al.*, 2006). Interestingly infusion of the μ-opioid antagonist, naloxone into the VP or NAc shell blocks the increase in sucrose palatability (aka reward "liking") following a change in food-deprivation, but does not impair reward seeking behaviour; however naloxone infused into the BLA does not prevent increases in "liking", but does impair the ability of this incentive learning to influence instrumental seeking performance (Wassum *et al.*, 2009). This may suggest a dissociation between VP "liking" and BLA "wanting" systems within the brain (Balleine, 2011).

Memory as a physiological process

In the late 19th Century Théodule Ribot described what he called the *Loi de Regression* (Ribot, 1882): the observation that patients with head insult often suffer amnesia for both the event during which the injury occurred and memories from immediately prior; however older memories were left intact producing a temporal gradient of retrograde amnesia. Perhaps the most famous example of this was the case study of Henry Molaison (also known as Patient H.M.), who had received a medial temporal lobe (MTL) resection in an attempt to treat his epilepsy. Following the surgery to remove his epileptic focus, H.M. became the embodiment of Ribot's Law, suffering severe temporally graded retrograde amnesia (Scoville & Milner, 1957). His short-term memory (STM) for the immediate present was intact, however would not be maintained as a long-term memory (LTM) and he was unable to form any new episodic memories (a phenomenon known as anterograde amnesia).

The idea that a physiological process underpinned memory storage became formalised by Georg Müller and Alfons Pilzecker. They presented paired nonsense syllables to volunteers and measured the frequency of errors they made during recall (Müller & Pilzecker, 1900; Lechner *et al.*, 1999). During the experiment participants noted that they would involuntarily remember pairs they had just learned in the time between training trials (an effect they described as 'perseveration'). Müller and Pilzecker proposed this involuntary recall represented an active ongoing physiological process of consolidation, by which the association between the syllables (i.e. the memory) was being strengthened; they hypothesised that disrupting this consolidation process would weaken the memory trace, impairing recall. They tested this theory by presenting new syllable pairs at various intervals following training on the original pairs, finding that recall of the original pairings was impaired if a second training session occurred within a minute of the first. While Müller and Pilzecker's results would now be interpreted as retroactive interference (Lechner *et al.*, 1999), their hypothesis that there was a physiological memory consolidation process was invoked in order to explain Ribot's Law and retrograde amnesia (Burnham, 1903; Squire & Alvarez, 1995). This link strongly suggested a physical consolidation process in the brain was required for the storage of

memories. While many patients with brain damage, particularly to the MTL (such as H.M.), suffer retrograde amnesia over a long time period of a few years (Squire & Alvarez, 1995) patients who only suffer trauma (without lasting damage) typically only suffer retrograde amnesia for a period of time less than 30 minutes (Russel & Nathan, 1946). This briefer clinical amnesia is more consistent with the experimental findings of a short window during which the memory consolidation can be disrupted.

The reason for the differences in the magnitude of amnesia in patients with differing degrees of brain injury may be due to the precise nature of their deficit. Remember that memory is a two part process requiring both storage and retrieval; impairments in either of these two processes could result in an amnesia. As such memory loss may represent a failure of memory to be consolidated (a storage deficit) or a failure in recall (a retrieval deficit). Following traumatic brain injury there are often a large deficits in memory; however this mostly recovers with time, leaving only a brief period of memory loss, often coinciding with the time of the injury (Russel & Nathan, 1946). It is highly likely that transient amnesias represent retrieval deficits, as memory is later successfully recalled, and the less severe amnesias, storage impairments. In the case of amnesic patients with longer periods of lasting retrograde amnesia, these too may represent retrieval deficits, as recall can be successful under appropriate conditions (Warrington & Weiskrantz, 1970; Diamond *et al.*, 1997).

Of note are another category of amnesic patients with basal forebrain damage, typically following an aneurysm of the anterior communicating artery (ACoA). Amnesia in these patients is distinct from that of patients with MTL damage, and shares many commonalities with Korsakoff's syndrome (Damasio *et al.*, 1985). Importantly, memory can often be improved in certain patients through use of cognitive organisational strategies, regardless of whether their amnesia resulted from ACoA aneurysm (Diamond *et al.*, 1997), Korsakoff's or MTL damage (Warrington & Weiskrantz, 1970), suggesting that while some patients may have impaired storage processing, others may have amnesia due to an impairment in retrieval. In line with this finding is the phenomenon of shrinkage, where the period of temporally graded retrograde amnesia shrinks following brain injury (Russel &

Nathan, 1946); suggesting memory was intact but simply inaccessible. It is important to remember that memory is a two part process, requiring both storage and retrieval, and amnesia can manifest as a result of impairments in either the storage or retrieval processes.

Cellular consolidation

The end of the 19th Century saw great advances in our understanding brain, namely that its activity was electrical in nature (Caton, 1875, 1877; Berger, 1927) and that it was made up of clusters of neurons connected by synapses (Cajal, 1906). This lead to electroconvulsive shock (ECS) gaining wide acceptance and popularity as a treatment for psychological disorders (Endler, 1988). This therapy was an electrical counterpart to insulin shock therapy; however one curious side-effect of ECS was a temporally graded retrograde amnesia (Squire *et al.*, 1975).

Carl Duncan theorised that delivering ECS to the head after learning would disrupt the physiological process of learning and memory (consolidation) and cause amnesia. Duncan trained groups of rats to turn left in a T-maze in order to obtain a food reward. After fifteen days of training he then trained them to turn right, delivering ECS immediately after this session (Duncan, 1948). When placed back in the maze, the behaviour of shocked rats reverted to that of the original, older memory. The implication was that the ECS prevented consolidation of the newly learned memory into LTM, such that ECS treated rats displayed amnesia. In follow-up work, Duncan showed that ECS was effective at disrupting memory if given up to fifteen minutes after learning, but not after one hour had elapsed (Duncan, 1949). The diminishing effect of ECS the longer it was given after training produced a pattern which greatly resembled the retrograde amnesia seen in human patients who had suffered injury to the brain (recall Ribot's Law). Duncan's account of the retrograde amnestic effect of ECS also corresponded to the diminishing influence of interference with time in Müller and Pilzecker's experiment, in which they indicated their interference procedure was ineffective after approximately fifteen minutes. These findings implied the existence of a short-lived period of instability (a 'consolidation window' of opportunity) during which memory could be interfered with or disrupted;

after this window had closed memories could not be disrupted, and by implication the consolidation process had reached completion. This accounted for Duncan's finding that the newer, more recent T-maze memory could be interfered with, but the older memory could not (presumably because the consolidation process was no longer ongoing, thus could not be disrupted). Duncan's artificial induction of a retrograde amnesia lead to the acceptance of memory consolidation as the physiological process underpinning learning and memory (Glickman, 1961).

While these results were consistent with memory disruption, the amnesia could also have been due to counter-conditioning to the ECS (Lewis & Adams, 1963) or the lasting physiological and motivational effects of repeated ECS exposure and convulsive seizures (Lewis & Maher, 1965); however the amnestic effect could also be obtained with a single ECS (Leonard & Zavala, 1964) and also under ether anaesthesia, thus preventing the induction of convulsive seizures (McGaugh & Alpern, 1966) implying the seizures themselves were not responsible for the memory loss.

Further study of consolidation revealed initial learning could also be disrupted by hypothermia (Riccio *et al.*, 1968; Hinderliter *et al.*, 1975; Blozovski & Buser, 1988) or treatment with protein synthesis inhibitors, such as puromycin (Flexner *et al.*, 1962, 1963; Barondes & Cohen, 1966), anisomycin (Squire & Davis, 1975; Hernandez *et al.*, 2002) and cycloheximide (Squire, 1979; Mierzejewski *et al.*, 2008). These findings provided strong support that consolidation of new memories was a physiological process. In particular, sensitivity to protein synthesis inhibition is believed by many to be the canonical demonstration of consolidation, as typically acquisition and STM are intact, and only LTM is impaired; this demonstrates the impairment was only caused in LTM storage, and provides a direct link to physiological processes and neuronal growth (Tronson & Taylor, 2007). Interestingly, the consolidation process could also be enhanced, such as by adrenaline (Gold & McGaugh, 1975; Gold *et al.*, 1975; Cahill & Alkire, 2003), improving memory (Cahill & McGaugh, 1996); the conclusion was that memories initially exist in a labile state, which can be either enhanced or impaired. Once the consolidation process had completed, memories became fixed, unchanging and permanent (McGaugh, 2000).

Long-term potentiation

Contributing to the appeal of consolidation as a mechanism of memory storage was the dual-trace theory conceived by Donald Hebb at about the same time as Duncan's original experiments (Hebb, 1949). In his work, Hebb proposed that oscillations in a neural circuit created a STM of an event; repeated reverberation of this circuit kept the memory in an unstable form of temporary storage. Over time, the connections in the reverberating circuit strengthened and stabilized into a persistent LTM. Dual-trace theory predicted that memory is formed by strengthening of these connections. While this theory predicts a serial dependence of LTM upon STM, it is unclear whether this is the case, and STM and LTM may be doubly dissociable based on their neural substrates (Barker *et al.*, 2006; Bannerman & Sprengel, 2010). Current theories of STM recognise its limited capacity (Miller, 1956; Cowan, 2001) and studies are typically interpreted within the framework of "working memory" (Baddeley & Hitch, 1974; Pezzulo, 2007) in which information is kept online and available to the mind on a sensory-specific "sketch-pad", however its precise contribution to LTM is unknown.

Anatomically, the brain is made up of neurons which consist of many dendritic spines (inputs) and a single axon (output). The axon then terminates in synapses to the dendrites of other cells (Cajal, 1906). Axons transmit signals electrically via the entry of sodium ions (Na^+). Normally the cell maintains a resting potential of approximately -70mV. Mild perturbations to the resting potential are only temporary, and the cell returns to its resting potential; however once the cell membrane potential is raised above a certain threshold, voltage-gated ion channels open allowing rapid entry of Na^+ and depolarising the membrane. This dramatic depolarisation of the membrane opens neighbouring ion channels which then propagate the signal along the axon (Hodgkin & Huxley, 1952), called an action potential. The cell then repolarises through the opening of potassium ion (K^+) channels returning to the resting potential, awaiting propagation of the next action potential. Incoming action potentials to synapses cause the opening of voltage-gated calcium channels (VGCCs) which allow calcium (Ca^{2+}) entry, leading to the release of chemical neurotransmitter into the synaptic cleft. The neurotransmitter then activates a neighbouring neuron, causing a small, sub-

threshold excitatory (EPSP), or inhibitory (IPSP), postsynaptic potential. If sufficient EPSPs occur in close spatial or temporal proximity and the neighbouring post-synaptic cell reaches threshold, an action potential is triggered in this cell which will then signal to its synaptic partners; alternatively sufficient IPSPs can inhibit an action potential from occurring, temporarily silencing the neuron. Neurons are connected in large networks (Cajal, 1906; Thomas & Bornstein, 2003), and in Hebb's model it was the formation and strengthening of these synaptic connections between neurons that were key to the formation of LTM; given evidence of a metabolic process occurring during memory consolidation (see above), dual-trace theory provided an attractive mechanism for memory storage.

The strengthening of synapses was later observed in the rabbit hippocampus. High frequency pre-stimulation of the perforant pathway would amplify future EPSPs in the dentate gyrus (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973; Bliss & Collingridge, 1993). This process of long-term potentiation (LTP) was theorised to be the synaptic mechanism of learning and memory. The induction of LTP was later found to be dependent upon Ca^{2+} entry and activation of Ca^{2+}/calmodulin-dependant kinase II (CaMKII) (Fukunaga *et al.*, 1995, 1996; Liu *et al.*, 1999) protein kinase A (PKA) activation (Nayak *et al.*, 1998; Huang *et al.*, 2000), the mitogen-activated protein kinase (MAPK) pathway (Liu *et al.*, 1999; Roberson *et al.*, 1999; Huang *et al.*, 2000) and cyclic adenosine-monophosphate (cAMP) response-element binding protein (CREB) (Silva *et al.*, 1998; Kaleem *et al.*, 2011).

Synaptic strengthening has been most notably demonstrated in the gill-withdrawal reflex of the sea slug, *Aplysia*; learning leads to changes in the number and strength of synapses (Frost *et al.*, 1985; Bailey & Chen, 1989). These changes in synaptic plasticity in *Aplysia* were found to require many of the same processes implicated in LTP (Martin *et al.*, 1997; Pittenger & Kandel, 2003). Continued investigation of memory consolidation has shown LTM formation to require protein synthesis (McGaugh, 1966, 2000; Schafe & LeDoux, 2000; Hernandez *et al.*, 2002), PKA (Schafe & LeDoux, 2000; Baldwin, Sadeghian, Holahan, *et al.*, 2002), MAPK (Bozon *et al.*, 2003; Kelly *et al.*, 2003; Ribeiro *et al.*, 2005) and CREB activation (Silva *et al.*, 1998; Bozon *et al.*, 2003; Chen *et al.*, 2012). CREB leads

to gene expression and synthesis of new receptors which strengthen synaptic connections (Kittler & Moss, 2001), much as in Hebb's prediction. Furthermore, LTP-like synaptic strengthening appears to occur in the amygdala during pavlovian fear learning (Rogan *et al.*, 1997; Sigurdsson *et al.*, 2007). Given the commonalities between LTP and LTM (Abraham & Williams, 2008), it is likely LTP is the neurobiological correlate of LTM formation.

Dependence of learning upon NMDARs

One of the key synaptic receptors identified in the establishment of both LTP and LTM was the N-methyl-D-aspartate receptor (NMDAR). This was a glutamate activated Na^+/Ca^{2+} channel; since the NMDAR allowed Ca^{2+} entry it was considered a prime synaptic target for investigation of LTP and memory consolidation (Morris *et al.*, 1986; Davis *et al.*, 1992). NMDARs exist as heteromers consisting of 4 subunits, typically 2 NR1s with the remaining two subunits made of up NR2 and NR3 subunits. There are several variations in these subunits with NR2A, NR2B, NR2C, NR2D, NR3A and NR3B units having been identified, in addition to eight alternative splicing patterns of the NR1 subunit (Paoletti *et al.*, 2013). Subunits differ in their carboxyl-terminals which affects their interaction with cell-signalling and trafficking mechanisms (Traynelis *et al.*, 2010) and different subunits may have distinct roles in learning and memory processes (Milton *et al.*, 2013).

Activation of NMDARs requires the presence of glutamate (binding the NR2 subunits) and a glycine or D-serine co-agonist (binding to the NR1 and 3 subunits). NMDARs also have an additional gating mechanism which distinguishes it from other ionotropic glutamate receptors. On the inactive neuron, the ion channel is also blocked by a magnesium ion (Mg^{2+}), which prevents entry of Na^+ and Ca^{2+} even in the presence of glutamate. In order for the channel to allow cation entry the neuron first has to become depolarised, repelling the Mg^{2+}-block; the channel can then open in the presence of glutamate, allowing Na^+/Ca^{2+} entry. As such activation of the NMDAR requires coincident depolarisation in both the post-synaptic (to relieve the Mg^{2+} block) and pre-synaptic (to release glutamate) neurons, and was termed a "molecular co-incidence detector"; it was suggested that it

was these receptors which were responsible for learning and memory by detecting stimuli in close proximity (i.e. a CS and a US) and strengthening the connections between them (Malenka & Nicoll, 1993). Evidence for this co-incidence detector function was the finding that NMDARs contribute to synaptic summation, the process by which multiple sub-threshold EPSPs within close temporal proximity can summate to reach the synapses depolarisation threshold (Malenka & Nicoll, 1993; Hunt & Castillo, 2012). The ability of NMDARs to facilitate temporal summation of EPSPs may be key to information processing in synaptic networks (Larkum & Nevian, 2008), and NMDAR activity may also maintain prolonged firing of the network (Major & Tank, 2004).

The presence of the Mg^{2+} block on the NMDAR is required for learning and memory in Drosophila (Miyashita *et al.*, 2012). Furthermore, the NR2A and NR2B subunits are highly sensitive to this Mg^{2+} block, while NR2C and NR2D have a much reduced Mg^{2+} sensitivity (Paoletti *et al.*, 2013). The NR2A and B subunits have been strongly implicated in memory maintenance (Milton *et al.*, 2013), further reinforcing the likely importance of the Mg^{2+} block and coincidence-detecting properties of NMDARs in learning and memory.

Investigation of the role of NMDARs in LTP lead to the understanding that repeated activations of a synapse in close temporal proximity enables the NMDAR to signal, allowing Ca^{2+} entry; this Ca^{2+} activated CaMKII, which strengthened the synapse through insertion of additional receptors (Rongo & Kaplan, 1999; Hayashi, 2000), allowing it to be more easily depolarised in the future. Interactions between NMDARs and CaMKII may also be involved in "tagging" synapses for growth and long-term maintenance (Sanhueza & Lisman, 2013). In behavioural studies NMDAR activation is typically inhibited using 2-amino-pentanoic acid (AP-5) an NR2-glutamate-binding site competitive inhibitor (Laube *et al.*, 1997), or the open-channel binding, non-competitive MK-801 (Foster & Wong, 1987). MK-801 is frequently used systemically and also intra-cerebrally; however AP-5 cannot cross the blood-brain barrier, and thus can only be administered intra-cranially. AP-5 is highly selective for NMDARs, but does not discriminate between different variants of the NR2 subunit. Blockade of NMDARs with either AP-5 (Morris *et al.*, 1986; Liang *et al.*, 1994; Kelley *et al.*, 1997; Smith-Roe *et al.*,

1999; Dalley *et al.*, 2005; Zellner *et al.*, 2009; McKee *et al.*, 2010) or MK-801 (Heale & Harley, 1990; Gould *et al.*, 2002; Mackes & Willner, 2006; Flint *et al.*, 2013) has demonstrated that activity at these receptors is universally required for acquisition of long-term memory across behavioural tasks, cementing a role for NMDARs in learning.

Memory Reconsolidation

Following initial learning, memories exist as labile, short-lived traces which are susceptible to disruption. In order to persist, memories are consolidated into stable engrams, requiring protein synthesis and changes in synaptic plasticity (McGaugh, 2000). Given the establishment of LTP as a hypothesised mechanism of memory storage and the resistance of memory to amnestic agents outside of the consolidation window, it became broadly believed that, once established, memories were permanent and unchanging. This is, however, untrue; memory is a constructive process and the act of remembering can lead to changes and distortions of memory content (Bartlett, 1932).

The dynamic nature of memory in the field of consolidation was first observed in auditory fear conditioning (Misanin *et al.*, 1968) and in inhibitory avoidance (Schneider & Sherman, 1968). In the first of these experiments, an auditory stimulus was paired with electric foot-shock, forming an aversive CS–Shock memory. Memory was assessed by the ability of the CS to inhibit licking for water (known as conditioned suppression, as the CS suppresses a behavioural response via its motivational properties). Confirming previous findings, they showed that ECS delivered immediately after training prevented later expression of the CS–Shock memory. Additionally, they demonstrated that ECS was ineffective if given 24 hours after training (after the consolidation process had completed); however if the CS was re-presented prior to treatment, the memory was once again vulnerable to disruption by ECS. The researchers theorised that presentation of the CS caused the stable, consolidated memory to transition into an unstable state which was once again vulnerable to ECS treatment, an effect the researchers termed “cue-dependant amnesia” (Lewis, 1979). The second of these early studies found that ECS did not disrupt inhibitory avoidance memory once the memory was learned,

however a reminder immediately prior to ECS resulted in amnesia (Schneider & Sherman, 1968); this supported the idea that memories were not permanently stable, but in fact dynamic.

The idea that ECS produced amnesia in more recently active memories initially struggled to gain mainstream acceptance, owing to failed attempts at reproducing the result (Dawson & McGaugh, 1969; Gold & King, 1972); however the effect was replicated in an appetitive setting (Lewis *et al.*, 1972) and using hypothermia (Mactutus *et al.*, 1979) or protein synthesis inhibition (Judge & Quartermain, 1982) as amnestic agents. As such the theory of memory was expanded, suggested that LTMs could exist in either an active or inactive state (Lewis, 1979). The active state is that following initial learning, in which memories are unstable and malleable, susceptible to disruption or enhancement. The process of consolidation stabilises memories, converting them to an inactive form; however memories can be reactivated by re-exposure to relevant cues, returning to the active state and becoming again vulnerable to manipulation (Figure 1.1A). Experimentally, memories are returned to their unstable, active form in a "reactivation" session. This session typically consists of CS or context exposure, although frequently US re-exposure is given in conjunction with CS/Context exposure. The reactivation session parameters are intended to destabilise an already established memory and return it to its unstable, active form in which it can be disrupted by amnestic agents. A restabilisation process then returns memory to the stable, inactive form. This restabilisation process was later termed reconsolidation (Przybyslawski & Sara, 1997; Nader *et al.*, 2000; Nader, 2003), owing to it being similar to the initial consolidation of memory. For example, reconsolidation appeared sensitive to ECS (Misanin *et al.*, 1968), hypothermia (Mactutus *et al.*, 1979), and protein synthesis inhibition (Judge & Quartermain, 1982; Nader *et al.*, 2000).

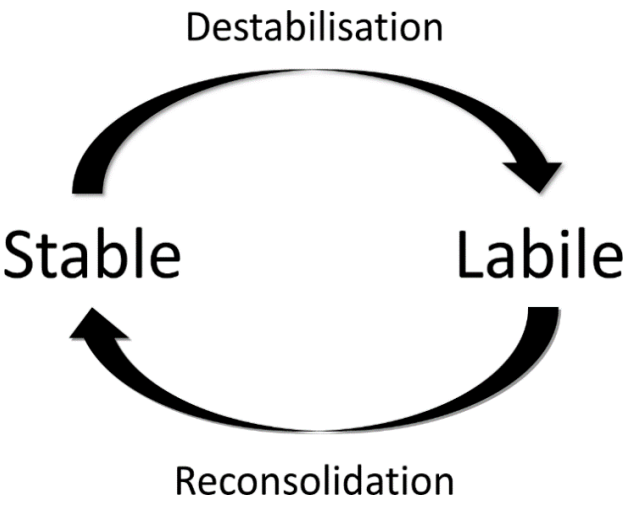

Figure 1.1: Representation of the dynamic nature of memory. During initial learning, consolidation converts memory into its stable, inactive form which is resistant to amnestic intervention. A, a memory reactivation session destabilises memories, returning them to the active state in which it is labile and vulnerable to amnestic treatment. Once destabilised, memories require a reconsolidation process to return to the inactive, stable state and be maintained in the long-term. Adapted from Nader (2003).

A variety of cellular processes have since been implicated in the reconsolidation process. Some of these mechanisms are also common with consolidation. For example both processes possess a requirement for protein synthesis (Nader *et al.*, 2000; Schafe & LeDoux, 2000; Hernandez *et al.*, 2002; Milekic *et al.*, 2006; Abraham & Williams, 2008; Rodriguez-Ortiz *et al.*, 2008), in addition signalling via the NMDAR is generally involved in both memory acquisition (Kelley *et al.*, 1997; Baldwin *et al.*, 2000; Goosens & Maren, 2004; Mackes & Willner, 2006; Zellner *et al.*, 2009; McKee *et al.*, 2010) and reconsolidation (Lee *et al.*, 2006a; Brown *et al.*, 2008; Milton, Lee, Butler, *et al.*, 2008; Flint *et al.*, 2013). The requirement for NMDAR in consolidation and reconsolidation less clear than for protein synthesis inhibition, as NMDAR antagonism sometimes impairs STM performance in addition to LTM (Lee & Hynds, 2012). As such, whether NMDAR are important for encoding and re-encoding, as well as the physiological processes of consolidation and reconsolidation is unclear. Certainly the period of NMDAR-dependence appears to be very limited, and pre- rather than post-

session treatment is often (although not always) required to be effective in disrupting consolidation (Hernandez *et al.*, 2005; Schenberg & Oliveira, 2008) and reconsolidation (Lee & Everitt, 2008a).

As with the study of initial, memory could also be enhanced, rather than impaired, once the memory was rendered unstable by reactivation. In one such study, strychnine was used to enhance performance (Gordon & Spear, 1973), however this facilitation of memory only occurred if the memory was reactivated prior treatment and was time limited (Gordon, 1977). Interestingly the window of memory instability was shorter than that observed for memory consolidation (Gordon, 1977), an effect also observed when using an amnestic intervention such as hypothermia or protein synthesis inhibition (Mactutus *et al.*, 1979; Judge & Quartermain, 1982), suggesting the process of restabilisation was different to that of initial learning. Later research would demonstrate consolidation and reconsolidation in the hippocampus of rats to depend upon different molecular signalling pathways. The consolidation of a contextual fear memory was shown to depend upon expression of brain-derived neurotrophic factor (BDNF) and MAPK kinase (MEK), but not the alpha inhibitor of nuclear factor kappa-β kinase (IKKα) or the immediate-early gene *zif268* (Lee *et al.*, 2004; Lee & Hynds, 2012). One the other hand, reconsolidation was shown to require *zif268* expression and IKKα, but not BDNF or MEK; this demonstrated that, at least in the hippocampus, consolidation and reconsolidation are doubly dissociable processes, although the BDNF/*zif268* dissociation may not extend to other brain areas (Bozon *et al.*, 2003; Lonergan *et al.*, 2010). Notably nuclear factor kappa-β (the target of IKKα) may be involved in both the consolidation (Freudenthal *et al.*, 2005) and reconsolidation of inhibitory avoidance (Boccia *et al.*, 2007); however the time-course of activation differs reinforcing the idea that consolidation and reconsolidation are distinct cellular processes.

Memory destabilisation

Noteworthy to the study of reconsolidation is the process of destabilisation, the means by which memories return to a labile form. In the traditional model of reconsolidation (Figure 1.1), the destabilisation process produces amnesia (by rendering memory unstable); however memory is

normally preserved by the reconsolidation process, which returns the memory to a stable form. Memory destabilisation has been shown to require protein degradation in the hippocampus (Lee, 2008; Lee *et al.*, 2008) and nucleus accumbens (Ren *et al.*, 2013). By inhibiting protein degradation memory remains stable and is protected from the effect of amnestic agents; however retrieval can still occur unhindered. Furthermore, preventing behavioural expression is not sufficient to prevent destabilisation (Milton *et al.*, 2013), suggesting the processes of retrieval and destabilisation (with subsequent reconsolidation) are distinct neural processes. Destabilisation can also be prevented by blockade of cannabinoid-1 receptors (Suzuki *et al.*, 2008; Kim *et al.*, 2011) or L-type voltage-gated calcium channels (VGCCs) in the hippocampus (Suzuki *et al.*, 2008; Flavell *et al.*, 2011; Kim *et al.*, 2011). Interestingly different NMDAR subunits appear to be involved in consolidation and reconsolidation of fear memory in the amygdala (Milton *et al.*, 2013), although it is unclear whether this generalises to other brain regions.

The reconsolidation process only occurs if memory is first destabilised, and this destabilisation process is distinct from retrieval. This finding is important as memories will only undergo reconsolidation under certain conditions (Suzuki *et al.*, 2004; Lee *et al.*, 2006a; Robinson & Franklin, 2007, 2010; Lee & Everitt, 2008b; Bustos *et al.*, 2010; Kirtley & Thomas, 2010; de la Fuente *et al.*, 2011; Reichelt & Lee, 2012; Flavell & Lee, 2013). The sensitivity of destabilisation to the precise reactivation conditions may explain the initial failures to replicate the cue-dependant amnesia effect (Dawson & McGaugh, 1969; Gold & King, 1972), and other more recent negative findings (Biedenkapp & Rudy, 2004; Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). Since destabilisation and reconsolidation are typically induced by memory retrieval under extinction conditions (i.e. in the absence of the reinforcer) this has given rise to the often observed boundary condition between reconsolidation and extinction (Lee *et al.*, 2006a; Tronson & Taylor, 2007), and under extinction conditions they may be competing processes (Eisenberg *et al.*, 2003); however this is not always the case (Duvarci *et al.*, 2006). Typically, briefer reactivation sessions, or fewer stimulus exposures, trigger destabilisation and reconsolidation. On the other hand longer sessions with extended exposure to stimuli lead to new extinction learning; however the precise boundary

condition appears to change with memory strength and age (Eisenberg & Dudai, 2004; Suzuki *et al.*, 2004; Reichelt & Lee, 2012).

Extinction represents new learning (Bouton, 2002, 2004), which suppresses the original memory (rather than erasing or replacing it); however disruptions to reconsolidation seem to be impairments of the underlying trace. It has been suggested that reconsolidation functions to update memories with new information, in order to maintain their functional relevance to behaviour (Lee, 2009); in support of this theory, reconsolidation has been shown to mediate hippocampal memory updating (Lee, 2010). This fits in with studies of false memory, which have suggested imagination and memory serve to predict future events (Atance & O'Neill, 2001, 2005; Buckner & Carroll, 2007; Szpunar & McDermott, 2008); thus memories must remain relevant in order to accurately predict the future.

Prediction errors in reconsolidation

Assuming reconsolidation normally serves to update memories (see above) it has been suggested that new information is required in the reactivation session (Finnie & Nader, 2012), and that destabilisation occurs in response to a prediction error signal leading to subsequent reconsolidation and updating of memory (Lee, 2009). Prediction errors are believed to play a key role in memory by providing a learning signal (Rescorla & Wagner, 1972), or by redirecting attention in order to modulate associative strength (Pearce & Hall, 1980). In the first of these roles, a difference between expected versus actual reinforcement provides a learning signal with which to increase (or decrease) associative strength, resulting in a large error signal during initial learning (presumably driving the initial memory consolidation process) and culminating in no error once the outcome is accurately predicted (and thus cause learning to plateau). In the latter role, unexpected (or "surprising") outcomes cause a redirection of attention to allow learning, while stimuli that are accurately predicted are not attended to (preventing new learning, but also protecting them from modification). It appears learning can involve both these processes (Dickinson & Hall, 1976; Schultz & Dickinson, 2000).

There is some evidence of a role for prediction error in driving the reconsolidation process from examination of the precise parameters under which reconsolidation will occur. Reconsolidation of a well learned memory has been shown to occur when the reactivation contingency differs from the conditions used in training (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013); however memory is not destabilised if the contingency is the same as in training. It is inferred that contingency change would produce an error signal, however it is worth noting that training reactivations have been used in the past to successfully destabilise memory (Duvarci & Nader, 2004; Eisenberg & Dudai, 2004; Milekic *et al.*, 2006; Valjent *et al.*, 2006). The reason for this discrepancy may be due to the amount of training used in these experiments. With fewer training trials it may be easier for a conditioning trial to produce an error signal as the outcome is likely not accurately predicted yet, allowing for a Rescorla-Wagner-like learning signal (see above). This is consistent with the idea that reconsolidation represents a "lingering" consolidation process which mediates memory strengthening (Alberini, 2011). This account is not entirely satisfactory, however, as reconsolidation mediates updating of memory content (Lee, 2010) as well as strength (Lee, 2008). Additionally, memories that are well-learned to asymptotic performance can undergo reconsolidation under certain conditions (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013), although these conditions likely differ from those that permit reconsolidation following a reduced amount of training (Reichelt & Lee, 2012).

A further theoretical consideration is that a purely Rescorla-Wagner-like prediction error could not explain extinction learning. Firstly, a Rescorla-Wagner learning signal should cause "unlearning" (or weakening) of the memory association. While this is consistent with a reconsolidation-mediated updating process, extinction typically appears to involve new learning, rather than "unlearning" (Bouton, 2002). Secondly, an extinction session should produce a Rescorla-Wagner-like prediction error regardless of the session length, as there is a difference between the predicted and acquired (now zero) reinforcement; however the duration of extinction appears to affect whether memory is destabilised or extinguished, with reconsolidation and extinction appearing to be competing processes (Eisenberg *et al.*, 2003). Thus a purely Rescorla-Wagner learning signal appears to be involved in both the reconsolidation of existing memories, and consolidation of new extinction

memories. It may be that attentional redirection (or "surprise", see above) also plays a key role in determining whether extinction or reconsolidation processes are engaged during a reactivation session. It is possible that a reactivation session must be both "surprising", as well as possessing a sufficient change in reinforcement as to produce a learning signal, in order to trigger destabilisation and reconsolidation.

Further evidence for the role of prediction error signals in memory destabilisation comes from work demonstrating dysregulation of ventral tegmental area (VTA) signalling protects against reactivation-dependant amnesia (Reichelt *et al.*, 2013). The VTA, along with the SNc, is a major source of midbrain dopamine neurons; the midbrain and dopaminergic signalling have been strongly linked to the generation of prediction error signals (Fiorillo *et al.*, 2003; Schultz, 2007; Takahashi *et al.*, 2009). Dopamine neurons appear to respond to unexpected reward, or unexpected omission or delay of reward (Schultz, 1997; Roesch *et al.*, 2010) consistent with a bi-directional Rescola-Wagner-like prediction error. With extended training dopamine neurons will cease responding to the reinforcer but instead fire in response to the stimulus that predicts the reinforcer, a feature that emerges gradually as training progresses (Pan *et al.*, 2005). Thus dopamine neurons are critically implicated in signalling of reward prediction errors (i.e. putative Rescorla-Wagner learning signals). Notably, the VTA appears to modulate orientation responding to a CS (El-Amamy & Holland, 2007; Lee *et al.*, 2011), likely shifting attention via cross inhibition of the contralateral SNc (Lee *et al.*, 2011). Furthermore, dopamine signalling has been strongly implicated in the modulation of attention (Kähkönen *et al.*, 2002; Nieoullon, 2002; Chudasama & Robbins, 2004). Thus dopaminergic neurons in the VTA and SNc are seemingly capable of signalling both changes in expected reward and "surprise". Therefore dopaminergic prediction error signals may play a key role in destabilisation and updating of memories under normal behavioural conditions.

Dependence of reconsolidation on NMDARs

Much like acquisition/consolidation, reconsolidation has been shown to depend upon NMDAR transmission in almost all memory settings that have been tested. A notable exception is inhibitory avoidance in which NMDAR antagonism in the hippocampus during memory reactivation produces only a transient impairment of memory (Luft *et al.*, 2008), although this may reflect the limited role of the hippocampus in the reconsolidation of inhibitory avoidance (Power *et al.*, 2006; Milekic *et al.*, 2007). Dependence of reconsolidation on NMDARs has been demonstrated in several other aversive memories (Suzuki *et al.*, 2004; Lee *et al.*, 2006a; Charlier & Tirelli, 2011).

In appetitive settings, all memories that have been tested have been shown to require NMDAR activity in order to reconsolidate. Among them, pavlovian approach (Lee & Everitt, 2008c; Milton *et al.*, 2012), PIT (Lee & Everitt, 2008c; Milton *et al.*, 2012), conditioned reinforcement (Milton, Lee, Butler, *et al.*, 2008), stimulus–reward (Lee & Everitt, 2008b), and goal-tracking memories (Reichelt & Lee, 2012), in addition to conditioned place preference (Brown *et al.*, 2008; Alaghband & Marshall, 2013). Given the seemingly universal requirement for NMDAR transmission in appetitive memory reconsolidation, it seems likely that disrupting the function of the NMDAR during an appropriate reactivation session will disrupt the memories underlying appetitive instrumental behaviour.

It is also worth noting the contribution of monoamine transmission to the reconsolidation process. It has been argued that the adrenergic system makes a large contribution to memory formation and strength by interacting with the initial consolidation process (Cahill & McGaugh, 1998; McGaugh, 2000). In line with this contribution to memory, the reconsolidation of pavlovian fear (Muravieva & Alberini, 2010; Soeter & Kindt, 2011) and food (Milton, Lee, & Everitt, 2008) memories have been shown to depend upon activity at the β-adrenergic receptor (β-AR); however this is not a universal requirement for reconsolidation. Reconsolidation of pavlovian autoshaping and PIT has been shown not to require β-ARs (Lee & Everitt, 2008c; Milton *et al.*, 2012), however reconsolidation of the conditioned reinforcing properties of CSs does appear dependent upon β-ARs (Milton, Lee, & Everitt, 2008). Furthermore the β-AR antagonist, propranolol, has been shown to disrupt pavlovian footshock

memory, but not inhibitory avoidance (Muravieva & Alberini, 2010). Thus the relative involvement of β-ARs in reconsolidation may depend on the type of behaviour being studied. There may also be involvement of dopamine-1 receptors (D1Rs) in some forms of memory reconsolidation (Sherry *et al.*, 2005; Maroun & Akirav, 2009); however study of the potential role of D1Rs is limited, and again involvement of D1Rs may vary depending upon the experimental protocol (Maroun & Akirav, 2009). It has recently been suggested that there may be individual differences in an individual's sensitivity to reconsolidation manipulation, resulting from genetic polymorphisms in dopamine and serotonin transmission systems (Agren *et al.*, 2012); however it is unknown whether serotonin plays a role in memory reconsolidation.

Reconsolidation of instrumental memories

Initially demonstrated in conditioned suppression (Misanin *et al.*, 1968), the phenomenon of reconsolidation has since been shown in a variety of other memory paradigms since (Table 1.1).

Pure context memory	Lee, 2010
Episodic memory	Hupbach *et al.*, 2007, 2009
Declarative memory	Forcato *et al.*, 2007; Chan & LaPaglia, 2013
Object recognition	Akirav & Maroun, 2006; Maroun & Akirav, 2008; Winters *et al.*, 2009
Spatial memory	Lewis *et al.*, 1972; Rossato *et al.*, 2006
Contextual fear	Lee *et al.*, 2004; Lee & Hynds, 2012
Cued fear	Nader *et al.*, 2000; Lee *et al.*, 2006a
Inhibitory avoidance	Boccia *et al.*, 2004; Milekic *et al.*, 2007; Baratti *et al.*, 2008
Passive avoidance	Mactutus *et al.*, 1979; Flint *et al.*, 2013
Autoshaping	Lee & Everitt, 2008c; Milton *et al.*, 2012
Pavlovian-instrumental transfer	Lee & Everitt, 2008c; Milton *et al.*, 2012
Incentive value memory	Wang *et al.*, 2005
Conditioned reinforcement	Lee *et al.*, 2005; Milton, Lee, & Everitt, 2008; Milton, Lee, Butler, *et al.*, 2008
Goal-tracking	Reichelt & Lee, 2012
Conditioned suppression	Misanin *et al.*, 1968; Judge & Quartermain, 1982
Conditioned withdrawal	Hellemans *et al.*, 2006
Conditioned place preference	Milekic *et al.*, 2006; Valjent *et al.*, 2006; Brown *et al.*, 2008; Robinson & Franklin, 2010; Wu *et al.*, 2012
Extinction memory	Eisenberg & Dudai, 2004; Garcia-Delatorre *et al.*, 2010; Rossato *et al.*, 2010

Table 1.1: Table of memories which have been shown to undergo reconsolidation.

There is, however, one major omission from the above list. Reconsolidation has not been demonstrated for the memories which underpin instrumental behaviour, in fact existing literature suggests the memories underlying instrumental memory do not undergo reconsolidation (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). This may be due to instrumental memories not undergoing reconsolidation due to being fundamentally different from other types of memory; alternatively instrumental memory may have been destabilised in these studies, but was not disrupted by the amnesic treatment used (protein synthesis inhibition). A final explanation is that the precise conditions required to destabilise instrumental memories have not yet been found.

On the first point, while animal studies have not demonstrated reconsolidation of instrumental memories, given the suggested role of reconsolidation as an updating mechanism it is reasonable to suggest that they do. It has been hypothesised that reconsolidation serves to strengthen memories (Lee, 2008) and update their content (Lee, 2009, 2010). Thus one can ask the question: do instrumental memories require updating in our daily lives? Instrumental memories encode the consequences of our actions, and with training become habitual and automated. An example is driving a car: initially behaviour requires thought, but eventually the car can be driven automatically (for example the sound of the engine triggers the response to change gear). This extends to navigation. You may drive to work via a certain route because of an understanding of the spatial relationship between your house and workplace; however after repeated experience with the route behaviour becomes habitual. A common occurrence is a "slip-of-action" whereby, when navigating to another location, the habitual response unintentionally triggers a wrong turn towards the workplace rather than the intended destination. Hypothetically, if there were a change in outcome contingency this should require an updating of instrumental habit memory. Using the example of navigating to work, let us say outside one's house you can turn either left or right. Based upon the spatial relationship between your house and workplace turning right provides the fastest route, and you take this route to work for many months becoming a creature of habit. After months of commuting bliss, the right-hand route is suddenly subjected to roadworks, traffic lights and new traffic-calming speed bumps (due to new local council intervention, let us say). The result of this is the right-hand route is now plagued by congestion and journey time is vastly increased. As such, turning left outside your house now provides a quicker route to work; however, having taken the same route for many months, you habitually turn right when leaving for work. How then do you learn to turn left? One possibility is to learn a new left-turn instrumental memory, however this would not allow a trouble-free journey. You would now possess two opposing habits triggered by the same stimulus, and behaviour would become random with only a 50% chance of making a left-hand turn. These two competing habits could co-exist if, for example, you departed for work earlier (when there was no traffic around). One could then carry on turning right early in the morning, but left at rush-hour;

however this would require existing memory to be updated in order to become state-dependant (i.e. time-of-day). Another alternative is to update your instrumental memory. If you were to suppress the old habit memory and solve the task in a goal-directed way, then this would require you to update your memory to incorporate the new outcome of a right-turn (a traffic jam). Alternatively, you could update the habit memory content such that the junction outside your house now triggers a left-turn response. In these latter cases a reconsolidation mechanism would be required to update memory content (Lee, 2009, 2010).

If instrumental memories can theoretically undergo reconsolidation, then why has it not been demonstrated in past experiments? The second possibility we considered was that the amnestic treatment was not effective at disrupting instrumental reconsolidation. Both past studies used protein synthesis inhibitors: anisomycin (Hernandez & Kelley, 2004) or cycloheximide (Mierzejewski *et al.*, 2009). Inhibition of protein synthesis has previously been shown to disrupt both consolidation (Schafe & LeDoux, 2000; Hernandez *et al.*, 2002; Robinson & Franklin, 2007) and reconsolidation (Nader *et al.*, 2000; Milekic & Alberini, 2002; Wang *et al.*, 2005; Robinson & Franklin, 2007) in both appetitive and aversive settings. Protein synthesis inhibition has also been successful in disrupting reconsolidation when administered in conjunction with a training trial for both appetitive and aversive memory (Duvarci & Nader, 2004; Milekic *et al.*, 2006). Furthermore, dependence upon protein synthesis is considered to be the canonical demonstration of consolidation and reconsolidation (Tronson & Taylor, 2007). Thus, it seems unlikely that the lack of an effect was due to instrumental memory reconsolidation not being dependent upon protein synthesis.

A final possibility is the correct reactivation parameters to trigger instrumental memory reconsolidation may not yet have been found. Both previous studies which explicitly studied instrumental reconsolidation used well-trained animals, which were likely habitual (Adams, 1982; Dickinson, 1985). This raises an additional confound that behaviour could have been controlled by either an S–R habit or A–O action. Weakening or erasure of one of these memories would not necessarily lead to a behavioural impairment if the remaining association could compensate (Packard

& McGaugh, 1996; Coutureau & Killcross, 2003; Yin *et al.*, 2004). Both studies used a training session (Hernandez & Kelley, 2004), or brief training session (Mierzejewski *et al.*, 2009), to destabilise the memory trace. Training sessions have been shown to be capable of destabilising memory (Duvarci & Nader, 2004; Eisenberg & Dudai, 2004; Milekic *et al.*, 2006; Valjent *et al.*, 2006), and it has also been possible to destabilise and impair pavlovian outcome (Lee *et al.*, 2005, 2006b; Lee & Everitt, 2008b) and incentive (Wang *et al.*, 2005) memory, without impairing operant memory, in instrumental settings; it may be the conditions for destabilisation of instrumental memory differ from those sufficient to destabilise pavlovian memory. Further evidence comes from aversive settings, in which training reactivations are ineffective at destabilising pavlovian memory if the contingency is well-learned (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013). Since previous studies used a well-trained memory and a training trial to reactivate the memory (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009) it seems highly likely these conditions were insufficient to destabilise instrumental memory. This thesis takes the position that if the appropriate conditions can be found, then the associations underpinning instrumental behaviour could be impaired.

Treatment of maladaptive memories

Reactivation-dependant amnesia has received a great deal of attention for its potential application as a treatment for maladaptive memory disorders (Debiec & Altemus, 2006; Milton, 2013), such as post-traumatic stress, drug addiction, compulsive food seeking and obsessive compulsive disorder (OCD). Theoretically the correct conditions during memory reactivation would destabilise the maladaptive trace, rendering it vulnerable to a suitable amnestic agent.

In particular much research has focused on the retrieval-extinction paradigm (Kindt *et al.*, 2009; Monfils *et al.*, 2009; Clem & Huganir, 2010; Xue *et al.*, 2012). In this setup memory is retrieved in a brief reactivation session, and putatively destabilised. This is followed after a short delay by a long extinction session. While extinction is typically believed to result in new learning (Bouton, 2002, 2004), in this paradigm it appears to cause weakening of the underlying memory trace (Monfils *et al.*,

2009; Quirk *et al.*, 2010); although some have suggested this represents enhancement of extinction learning (Ishii *et al.*, 2012). The advantage of this particular paradigm is it does not require the use of amnestic agents, and therefore has great translational potential for development of clinical treatments.

Relevant to the application of reconsolidation-based therapies is the precise nature of reactivation-dependent amnesia. It has been suggested that reconsolidation impairments may represent enhanced extinction and/or an impairment of retrieval (Lattal & Abel, 2004); alternatively reactivation-dependent amnesia may result from a storage impairment in which case memories are truly erased. The exact nature of reconsolidation impairments is unknown, and may be unknowable as a irretrievable memory should theoretically be indistinguishable experimentally from a memory whose storage has been disrupted. Interestingly reactivation-dependant amnesia appears to be specific to the reactivated trace (Debiec *et al.*, 2006), and certain components of behaviour are left intact following intervention. For example, behaviour is often mediated by multiple memory associations, and instrumental memories are often left intact in appetitive reconsolidation studies (Wang *et al.*, 2005; Lee & Everitt, 2008a). An interesting question is whether, given the constructive nature of memory, memories can be reconstructed from incomplete associative networks; however from the perspective of clinical treatment the ability to produce any lasting behavioural effect is significant.

Summary

Instrumental behaviour can be mediated by either goal-directed or habitual process and these have a significant interaction with pavlovian outcome and incentive processes. Goal-directed A–O associations can be distinguished from habitual S–R memory by reward devaluation, typically achieved by pairing the reinforcer with LiCl injection. Past studies investigating the reconsolidation of instrumental memory have not demonstrated any effect of protein synthesis inhibition on the maintenance of these memories; however the reactivation parameters used in these studies were

likely inappropriate to trigger the reconsolidation process. These past studies also used well-trained animals which were likely habitual and thus it is unclear whether a reconsolidation deficit was compensated for by the goal-directed trace in these studies.

This thesis hypothesises that instrumental memories will undergo reconsolidation provided appropriate reactivation parameters are used, primarily using appetitive lever pressing reinforced by sucrose in rats as a model. A variety of reactivation parameters are tested based on the assumption that memory reconsolidation mediates memory updating. The sufficiency for the reactivation parameters to trigger memory destabilisation is assessed using systemic administration of the NMDAR antagonist, MK-801. NMDAR antagonism has been shown to be universally required for appetitive memory reconsolidation and thus it is highly likely that NMDAR activity is also required for reconsolidation in the lever pressing task. Notably, protein synthesis inhibition is considered the canonical demonstration of reconsolidation (Tronson & Taylor, 2007); however this thesis uses the NMDAR antagonist, MK-801. Use of protein synthesis inhibition in appetitive settings is problematic in that inhibition of protein synthesis appears to cause illness, and occasionally death (Hernandez & Kelley, 2004). This illness can pair to the reinforcer, forming a conditioned taste aversion (Hernandez & Kelley, 2004). This effect can also occur using intra-cerebral, rather than systemic, inhibition of protein synthesis (Jonkman & Everitt, 2009, 2011). Importantly this aversion can impair the incentive value of the reinforcer such that rats are not motivated to acquire. This presents a particular confound for appetitive studies as they must dissociate between loss of reward value, and impairment of memory. The use of MK-801 was intended to circumvent this problem with protein synthesis inhibitors, and the dose of MK-801 used has previously been demonstrated to be effective in both appetitive (Lee & Everitt, 2008c; Milton *et al.*, 2012) and aversive (Lee *et al.*, 2006a) paradigms.

The reconsolidation of goal-directed memories is first investigated in Chapter 2, using brief extinction reactivation sessions in an attempt to destabilise the memory, similar to past literature on pavlovian memory reconsolidation. Both MK-801 and a retrieval-extinction paradigm are used in order to

disrupt memory reconsolidation; however these treatments were without effect on memory. Chapter 3 investigates the ability of MK-801 to disrupt reconsolidation in two novel aversive paradigms. This was done primarily to confirm the ability of the MK-801 to disrupt reconsolidation, but the active avoidance model used in this chapter may also represent a form of aversive instrumental learning. Following confirmation of the potency of the MK-801 in Chapter 3, Chapter 4 continues the search for appropriate parameters with which to destabilise a goal-directed instrumental memory. This search finds that goal-directed memories can be destabilised by a change in reinforcer contingency, and goes on to investigate the role of NMDARs and D1Rs the NAc in the reconsolidation of this memory. Finally Chapter 5 tests the hypothesis that changes in contingency will also destabilise instrumental habit memory, ultimately successfully. The thesis reaches the conclusion that reconsolidation process is a likely to be universal feature of memory maintenance and updating. The finding that both goal-directed and habitual memories undergo reconsolidation has significant implications for the development of novel reconsolidation-based therapies for maladaptive memory disorders.

CHAPTER 2

DISRUPTION OF AN INSTRUMENTAL TASK WITH TWO LEVERS

Introduction

When learning first occurs memories exist in an unstable state, vulnerable to disruption by various amnestic agents (McGaugh, 2000). A process of consolidation establishes memories in a stable, long-lasting form; however they remain dynamic (Alberini *et al.*, 2006). Under certain conditions memories can be destabilised, returning to an unstable, labile state (Nader, 2003) in which memories can be modified and updated with new information (Lee, 2009). In order to persist in the long term, updated memories must return to their stable form; this is achieved via a process of reconsolidation. Disrupting the reconsolidation process prevents memory persistence, producing reactivation-dependant amnesia, and provides an avenue by which memories might be erased.

Inducing amnesia for pavlovian memories using reconsolidation-disruption has been demonstrated in both rat models (Nader *et al.*, 2000; Brown *et al.*, 2008; Lee & Everitt, 2008c) and human volunteers (Kindt *et al.*, 2009; Oyarzún *et al.*, 2012; Xue *et al.*, 2012); however existing literature has suggested instrumental memories do not undergo reconsolidation (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). Both these past studies used discrete stimuli to signal a successful response and subsequent reinforcement. However, in pairing the response to a signal, the signal also becomes paired to the reward. This pavlovian stimulus–reward memory can influence operant responding via pavlovian-motivational processes, potentiating responses (Davey *et al.*, 1981; Dickinson & Balleine, 1994). Appetitive pavlovian memories are already known to undergo reconsolidation under conditions which preserve any underlying instrumental behaviour (Wang *et al.*, 2005; Lee & Everitt, 2008b), further supporting the hypothesis that instrumental memories do not undergo reconsolidation. Any attempt to demonstrate reconsolidation of an instrumental memory must distinguish between impairments of instrumental versus pavlovian memories. We attempted to

address this issue by not presenting any feedback signals during training. While associations to the context cannot be prevented this minimised any potential instrumental transfer effect.

A further confounding factor in these two prior experiments is that they both utilised extended amounts of training; ten days in the case of Hernandez and Kelley, and fifteen by Mierzejewski and colleagues. It is well documented that instrumental memories can be mediated by goal-directed, Action–Outcome (A–O) and habitual, Stimulus–Response (S–R) memories (Dickinson, 1985). With limited training, instrumental responding is typically goal-directed, but as training is extended behaviour shifts to habitual control which is insensitive to changes in reward value (Adams, 1982). Importantly A–O associations can compensate for behaviour if the S–R memory is prevented from being expressed (Packard & McGaugh, 1996; Yin *et al.*, 2004). Thus, if either of the past studies on instrumental reconsolidation had disrupted the S–R memory, the remaining goal-directed trace could compensate for behaviour, preventing amnesia from being observed. Our solution was to provide minimal instrumental training, in order to produce a goal-directed behaviour. Rats could only obtain a maximum of 100 reinforcements, an amount previously shown to produce behaviour predominantly under A–O control (Adams, 1982).

A final criticism of past studies on instrumental reconsolidation is that responses were reinforced on every session, thus it is conceivable that an effect could have been masked by rats re-learning the task during the test sessions. Our experiments were conducted without any post-conditioning reward presentations, thus rats could not re-learn the task outside of the initial training phase.

We first attempted to disrupt reconsolidation using the N-methyl-D-aspartate receptor (NMDAR) antagonist MK-801. This departed from past literature on instrumental reconsolidation using protein synthesis inhibitors (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009) as they have been shown to impair instrumental responding via conditioned taste aversion (Hernandez & Kelley, 2004) even when given intra-cranially (Jonkman & Everitt, 2009, 2011). MK-801 has been well-established to impair the reconsolidation process across a variety of settings (Lee *et al.*, 2006a; Brown *et al.*, 2008; Milton, Lee, Butler, *et al.*, 2008) and thus was deemed well suited for our experiments. MK-801 was

administered prior to two reactivation procedures: a brief non-reinforced session (similar to that conventionally used to reactivate pavlovian memories) or an interrupted-exposure reactivation, where rats were removed from the experimental chambers between each lever press. The rationale for the removal reactivation was that unexpected termination of the session would maximise surprise during the session and cause an error signal, the proposed trigger for memory destabilisation (Lee, 2009).

Following a lack of effect using MK-801, we investigated whether the failure to disrupt the reconsolidation of instrumental memories was due to NMDARs not being functionally important in the instrumental reconsolidation process by using a drug-free approach; this used a retrieval-extinction paradigm already shown to be effective in pavlovian settings (Monfils *et al.*, 2009; Clem & Huganir, 2010; Schiller *et al.*, 2010; Oyarzún *et al.*, 2012; Xue *et al.*, 2012). This procedure involves reactivating the memory trace, then exposing rats to a long extinction session. As memory should be rendered labile by the reactivation session, the long extinction is then believed to impair the original memory rather than trigger new learning. The removal reactivation successfully impaired lever pressing, but may have caused high levels of stress due to repeated handling; stress has been shown to impair the reconsolidation of a conditioned place preference (Wang *et al.*, 2008). To investigate this, an additional non-removal reactivation was used to control for any influence of repeated handling, consisting of discrete lever pressing exposure, but without removal from the experimental chamber.

Materials and Methods

Subjects

Subjects were 82 experimentally naïve Lister-Hooded rats (Harlan Laboratories, UK), weighing 200-225g at the start of experimental procedures. Rats were housed in groups of 4, maintained at 21^{o}C on a 12hr light-dark cycle (lights on at 0700). Subjects were food restricted (15 g per day) in order to

motivate responding. Access to water was unrestricted. All procedures were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 (PPL 40/3205).

For the systemic MK-801 studies 16 rats were used in the brief non-reinforced reactivation, and 16 in the removal reactivation study. 3 rats were excluded from the systemic non-reinforced reactivation analysis due in insufficient performance (less than 30 active lever presses on the second training day).

In the retrieval-extinction paradigm, 18 rats were used for the initial removal reactivation study. The final non-removal control study used a further 32 rats; 8 per group each for the standard, extinction-only, reverse, and 24-hour reverse groups. 1 rat from the extinction-only group, and 3 from the 24-hour reverse, were excluded from analysis owing to insufficient training performance (fewer than 30 active lever presses on the second day of training).

Drugs and Behavioural Apparatus

MK-801 (AbCam, UK) was dissolved in saline to a concentration of 0.1mg/ml. Rats received an intraperitoneal injection of MK-801 (0.1mg/kg) or equivalent saline vehicle control. Drug was administered 30 minutes prior to the reactivation session in the NMDAR antagonism investigation. The dose and time window were chosen based upon previous appetitive reconsolidation studies (Lee & Everitt, 2008a).

Training, reactivation and testing sessions took place in 8 operant boxes (MedAssociates, VT), measuring 25x32x25.5cm and each housed individually in a soundproof chamber. The rear wall and door were made of Perspex, the other walls of metal. The boxes contained a grid floor of 19 stainless-steel bars (4.8mm diameter), underneath which was a removable tray. A reward-delivery magazine was mounted on the right-hand wall, flanked by two retractable levers. The magazine contained an infrared detector which recorded magazine entries (nosepokes). The box was

illuminated by a small white houselight mounted on the upper left hand wall, which came on at the start of each experimental session, switching off at the end.

Behavioural Training

Rats were initially pre-trained to collect 45mg sucrose pellets (TestDiet) from the magazine, on a random-interval schedule (mean 60 seconds) for 30 minutes. This pre-training procedure was implemented to increase the number of rats that successfully acquired the lever pressing response in the limited time frame. Instrumental training began the next day. Rats received two training sessions over two consecutive days during which both levers were extended and one was assigned the active lever (R+), responses upon which were rewarded with a sucrose pellet. Following an R+ response, both levers retracted for 10 seconds in order to maximise learning by allowing time to retrieve and eat the pellet. Inactive lever presses (R-) had no programmed consequence. Training sessions lasted 30 minutes and a maximum of 50 pellets could be obtained per session. This restricted the total number of pellets acquirable during training to 100, a number known to produce goal-directed responding (Adams, 1982).

Systemic Studies

Reactivation took place 24 hours after the final day of training. As the precise parameters sufficient to destabilise instrumental memories are unknown, rats first received one of two different reactivation procedures. In all reactivations, R- responses had no programmed consequence, and no pellets were delivered.

Brief non-reinforced reactivation: The session consisted of a 5-minute session in which both levers were extended into the operant box. An active lever press resulted in retraction of both levers; as in training levers re-extended after 10 seconds.

Removal Reactivation: Both levers were extended for 60 seconds. A single R+ response retracted both levers and ended the session. Rats were then returned to their home cages, which had been temporarily relocated to the testing room. Rats were then placed back in the operant boxes and given another reactivation session. This was repeated for a total of 10 reactivations, each spaced 2 minutes apart. Only R+ responses were recorded during this reactivation.

Each reactivation of these sessions was assessed in its ability to destabilise A–O memory based on the ability of systemic MK-801, administered 30 minutes prior to reactivation, to disrupt the reconsolidation of that memory. Memory strength was assessed in a 30-minute extinction test 24 hours after reactivation, in which neither lever had any programmed consequence; if reconsolidation was impaired, then lever pressing should be reduced compared to saline-injected controls.

Retrieval-extinction paradigm

Owing to a lack of effect with systemic MK-801, the ability of the removal reactivation to render the memory labile was also tested using a retrieval-extinction paradigm. In this procedure, the removal reactivation session was followed 15-minutes later by a 1-hour extinction session, a protocol believed to weaken the underlying memory trace by rendering it labile prior to the extinction (Monfils *et al.*, 2009). If MK-801 was ineffective at blocking reconsolidation owing to NMDARs not being required for A–O memory reconsolidation, then a retrieval-extinction paradigm may have more success, as it does not make any assumptions about the neural substrates of reconsolidation. Given the complexity of the removal reactivation, a control group was used which received both the reactivation and extinction sessions, but in a reversed order (extinction→15-minutes→reactivation). The reversed order should not weaken the A–O memory as it would not be labile during the long extinction session. As before, memory was tested in a 30-minute extinction session 24 hours after reactivation.

The reduction in lever pressing caused by retrieval-extinction was further investigated using a non-removal reactivation. This new reactivation procedure was intended to control for any restraint-stress caused by repeated handling during the removal reactivation.

Non-removal Reactivation: Both levers were initially extended and an R+ response caused both levers to retract. The levers re-extended every 3 minutes, retracting again on another R+; this repeated for 10 cycles over a 30-minute session (allowing for a maximum of 10 R+ responses). If no R+ was made then levers remained extended into the next 3-minute 'cycle'. Rats were not removed from the boxes and the houselight remained on throughout the 30-minutes.

This final experiment also included an extinction-only control group, and a delayed reactivation condition in which extinction was followed 24-hours later by the non-removal reactivation. This was done to control for any non-specific effects of the reactivation procedure. Again, lever pressing was tested 24 hours later in a 30-minute extinction test.

Statistical analysis

After the second day of training, rats were assigned to equally performing groups, matched for both R+ and R- responses. The lever assigned R+ was counter-balanced between groups. Rats which made fewer than 30 R+ responses on the second day of training were excluded from the final analysis, due to insufficient learning.

Lever pressing during training sessions was analysed using three-way repeated measures analysis of variance (ANOVA) with drug Treatment, response Lever and Training session as factors. Reactivation and test data were analysed separately using two-way ANOVA with drug Treatment and response Lever as factors. Results with $p<0.05$ were deemed significant. For the final retrieval-extinction experiment using the interval reactivation, post-hoc pair-wise comparisons were performed in order to determine the source of significant effects using Fisher's Least Squared Difference (LSD) method (Results with $p<0.05$ deemed significant).

Results

Systemic Studies

Brief non-reinforced reactivation

Lever pressing increased with Training ($F_{(1,11)}$=9.80, p=0.010) and became focused on the R+ Lever (Lever: $F_{(1,11)}$=172.6, p<0.001; Training x Lever: $F_{(1,11)}$=27.45, p<0.001), demonstrating rats learned the task (Figure 2.1A). There was no difference between treatment groups during training (Treatment: $F_{(1,11)}$=1.15, p=0.306; Training x Treatment: $F_{(1,11)}$=0.51, p=0.492; Treatment x Lever: $F_{(1,11)}$=0.19, p=0.674; Training x Treatment x Lever: $F_{(1,11)}$=0.19, p=0.669), indicating experimental groups were well-matched prior to reactivation. At reactivation, ANOVA revealed a significant bias towards R+ ($F_{(1,11)}$=61.72, p<0.001), signifying retrieval of the instrumental contingency (Figure 2.1B); however without any main effect of Treatment ($F_{(1,11)}$=3.26, p=0.098; Treatment x Lever: $F_{(1,11)}$=1.70, p=0.218). During the test session (Figure 2.1C) rats responded more on the R+ Lever ($F_{(1,11)}$=104.02, p<0.001) with no significant difference between drug groups (Treatment: $F_{(1,11)}$=0.16, p=0.694; Treatment x Lever: $F_{(1,11)}$=0.44, p=0.520). Thus, MK-801 did not disrupt reconsolidation when administered prior to the brief extinction session.

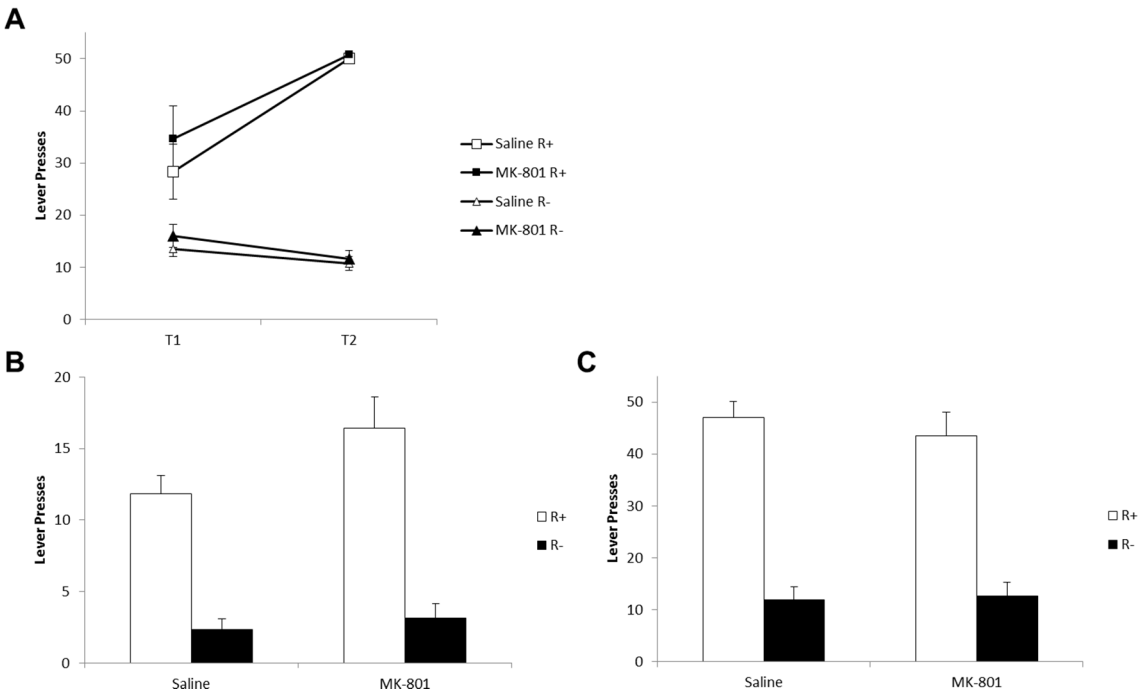

Figure 2.1: Systemic MK-801 does not disrupt the reconsolidation of a lever pressing memory when administered prior to a brief non-reinforced retrieval session. **A**, all rats learnt to press the lever for sucrose pellets and bias responding to the active lever, with no differences in the active (squares) or inactive (triangles) lever presses of saline (white) or MK-801 (black) groups on either the first (T1) or second (T2) day of the training phase. A maximum of 50 reinforcements could be obtained on either training day. **B**, during the brief non-reinforced reactivation session MK-801 treated (n=7) rats do not significantly differ in either active (white bars) or inactive (black bars) from saline controls (n=6). This session lasted 5-minutes and lever pressing was not restricted. Both groups significantly bias their responding towards the active lever, indicating successful memory retrieval. **C**, both groups of rats responded at equivalent levels on both the active (white bars) and inactive (black bars) levers in a 30-minute extinction test of long-term memory conducted 24 hours after reactivation; thus instrumental reconsolidation was not impaired. Data represent mean ± SEM. R+ denotes active lever presses, R- inactive.

Removal Reactivation

Lever pressing increased during Training ($F_{(1,14)}$=6.83, p=0.020) becoming biased towards the R+ response (Lever: $F_{(1,14)}$=80.93, p<0.001: Training x Lever: $F_{(1,14)}$=28.94, p<0.001), demonstrating acquisition of the task. No main effect of Treatment ($F_{(1,14)}$=0.06, p > 0.8), Training x Treatment ($F_{(1,14)}$=0.11, p=0.748), Lever x Treatment ($F_{(1,14)}$=0.05, p=0.835) or Training x Lever x Treatment ($F_{(1,14)}$=0.35, p=0.566) was observed, indicating experimental groups performed equally during training (Figure 2.2A). At reactivation, MK-801-treated rats made significantly greater active responses ($F_{(1,14)}$=6.03, p=0.028) compared to saline controls (Figure 2.2B); R- presses were not recorded during the reactivation. When tested all rats showed a significant preference for the R+ Lever ($F_{(1,14)}$=58.91, p<0.001) with no group differences (Treatment: $F_{(1,14)}$=0.30, p=0.590; Lever x Treatment: $F_{(1,14)}$=0.44, p=0.519). Therefore MK-801 had no effect on reconsolidation following the repeated reactivation procedure (Figure 2.2C).

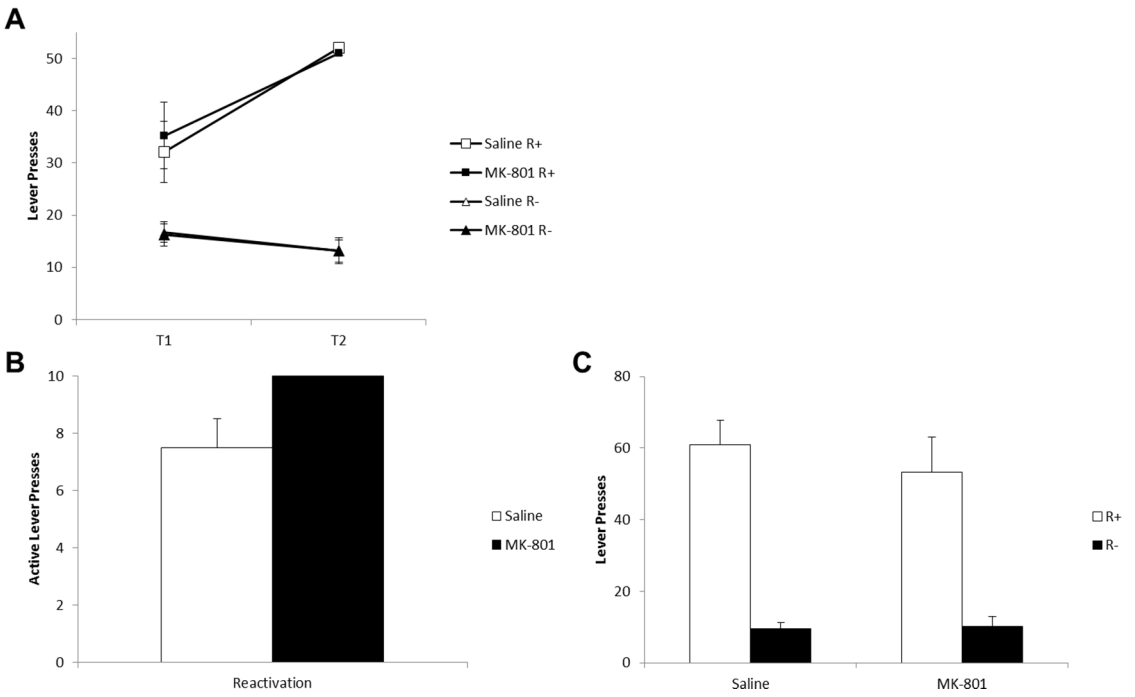

Figure 2.2: Injection of MK-801 prior to the removal reactivation protocol did not disrupt later expression of an instrumental lever pressing memory. **A**, saline (white) and MK-801-injected (black) groups show similar levels of performance on both the active (squares) and inactive (triangles) levers during both the first (T1) and second (T2) training days. A maximum of 50-pellets could be obtained on either session. **B**, during the removal reactivation session, rats were removed from the experimental chambers following each active lever press and returned 2-minutes later. This continued for 10 'cycles, allowing for a maximum of 10 active lever presses. All MK-801 (black bar, n=8) injected rats made the maximum number of 10 responses on the active lever, significantly more than saline controls (white bar, n=8); inactive lever presses were not recorded during this session. **C**, 24 hours after reactivation there was no difference in either active (white bars) or inactive (black bars) lever pressing between treatment groups during a 30-minute extinction test; therefore reconsolidation of the instrumental memory was not impaired. Data expressed as mean number of lever presses ± SEM. R+ denotes active responses, R- inactive.

Retrieval-Extinction paradigm

Removal reactivation

Following the lack of effect with systemic MK-801, the ability of the removal reactivation to destabilise instrumental memory was tested using the retrieval-extinction paradigm. During training (Figure 2.3A) rats successfully acquired the task (Training: $F_{(1,16)}$=5.12, p=0.038; Lever: $F_{(1,16)}$=100.25, p<0.001; Training x Lever: $F_{(1,16)}$=24.30, p<0.001). No significant differences between experimental groups were observed during training (Treatment: $F_{(1,16)}$=0.86, p=0.369; Training x Treatment: $F_{(1,16)}$=0.09, p=0.768; Lever x Treatment: $F_{(1,16)}$=0.35, p=0.565; Training x Lever x Treatment: $F_{(1,16)}$=0.05, p=0.832). During the reactivation session (Figure 2.3B) all rats made similar R+ responses ($F_{(1,16)}$=0.10, p=0.755) regardless of whether the reactivation was presented in the standard or reverse control condition. During extinction (Figure 2.3C), ANOVA revealed a significant bias towards the R+ Lever ($F_{(1,16)}$=40.61, p<0.001) with no Lever x Treatment interaction ($F_{(1,16)}$=1.85, p=0.193). While rats which received reactivation followed by extinction (standard-ordered group) showed a reduction in lever pressing, there was no significant effect of Treatment ($F_{(1,16)}$=4.48, p=0.050). At test (Figure 2.3D), rats given a standard-ordered procedure significantly reduced their overall lever pressing (Treatment: $F_{(1,16)}$=6.74, p=0.020) with a significant Lever x Treatment interaction ($F_{(1,16)}$=5.71, p=0.030). There was also an overall bias towards the active lever in both experimental conditions (Lever: $F_{(1,16)}$=37.18, p<0.001). Analysis of simple main effects revealed a significant effect of Treatment on active ($F_{(1,16)}$=7.16, p=0.017), but not inactive lever presses ($F_{(1,16)}$=0.25, p=0.626); thus R+ presses were significantly reduced in the standard reactivation condition.

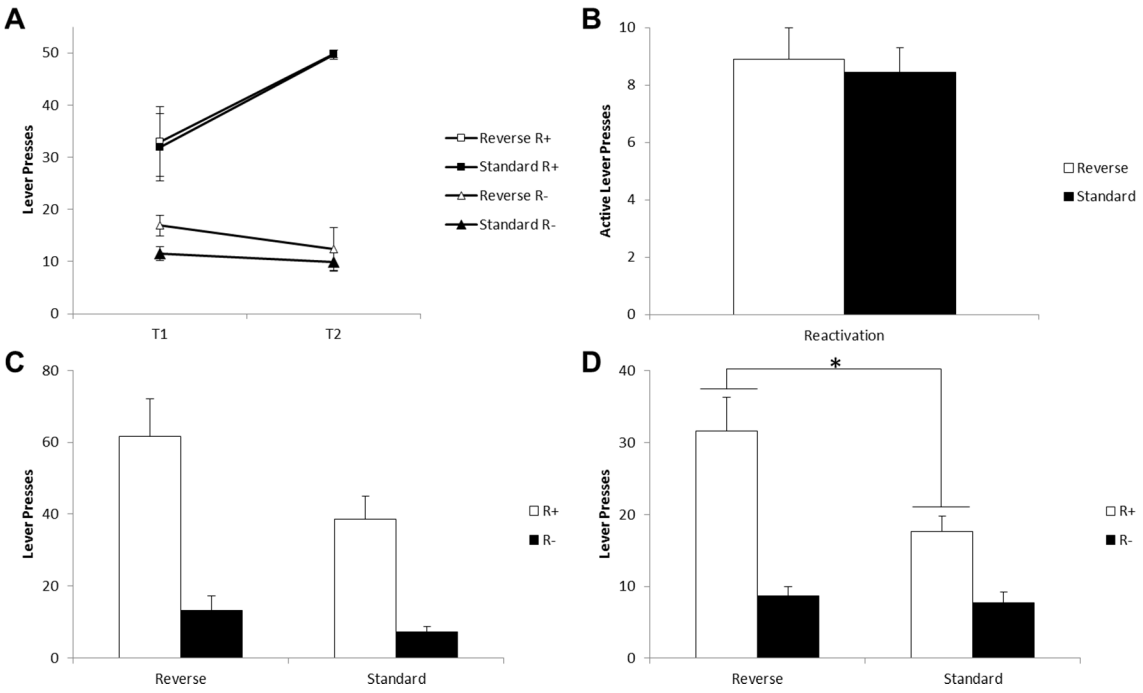

Figure 2.3: Removal reactivation prior to a long extinction session significantly impaired active lever presses at test. **A**, reverse (white) and standard-ordered (black) groups learned the task equally during training, making equivalent active (squares) and inactive (triangles) lever presses on both the first (T1) and second (T2) training day. A max of 50 rewards could be obtained on any one training session. **B**, during the removal reactivation rats were removed from the experimental chambers between each active lever press. Rats were returned to the boxes after 2-minutes, up to a maximum of 10 active lever presses. Active lever press performance remained similar regardless of whether the reactivation was given in the standard (black, n=9) or reverse (white, n=9) order. **C**, during the 1-hour extinction session there is a moderate reduction in overall lever pressing in those rats which had already received reactivation (standard group); however this was not statistically conclusive between groups (p=0.05). Performance on this session remained biased towards the active lever (white bars) over the inactive lever (black bars). **D**, 24 hours after the retrieval-extinction procedure performance was tested in a 30-minute extinction session. Rats given the removal reactivation prior to extinction (standard order) make significantly fewer active lever responses (white bars) than reverse ordered controls (black bars). Data expressed as mean number of lever presses ± SEM. R+ denotes active responses, R- inactive. (*) indicates significant differences $p<0.05$.

Non-removal Reactivation

A non-removal reactivation condition was next used, in order to further investigate the effect found with the removal reactivation in the retrieval-extinction paradigm. After training rats were divided into four experimental groups that would receive either the non-removal reactivation followed 15 minutes later by a 1-hour extinction (standard group), the extinction followed 15 minutes later by reactivation (the reversed order procedure), the reverse order procedure with a 24 hour delay between extinction and reactivation sessions, or the 1-hour extinction alone (without reactivation). ANOVA revealed lever presses significantly increased with Training ($F_{(1,24)}$=21.08, p<0.001) and bias to the R+ Lever (Lever: $F_{(1,24)}$=156.83, p<0.001; Training x Lever interaction: $F_{(1,24)}$=42.94, p<0.001), demonstrating learning for the task (Figure 2.4A). Experimental groups showed no significant differences (Treatment: $F_{(3,24)}$=0.22, p=0.883; Training x Treatment: $F_{(3,24)}$=0.50, p=0.685; Lever x Treatment: $F_{(3,24)}$=0.18, p=0.912; Training x Lever x Treatment: $F_{(3,24)}$=0.82, p=0.494).

At reactivation (Figure 2.4B), rats preferred the R+ lever ($F_{(1,24)}$=15.97, p=0.001) with no difference in responding between Treatment groups (Treatment: $F_{(3,24)}$=0.32, p=0.732; Lever x Treatment: $F_{(3,24)}$=0.10, p=0.907). During the extinction session (Figure 2.4C) ANOVA revealed a significant Lever x Treatment interaction ($F_{(3,24)}$=6.32, p=0.003) with main effects of Lever ($F_{(1,24)}$=41.84, p<0.001) and Treatment ($F_{(3,24)}$=3.62, p=0.028). Analysis of simple main effects revealed a significant difference in R+ ($F_{(3,24)}$=6.63, p=0.002), but not R- ($F_{(3,24)}$=1.68, p=0.198) responses between treatment groups. Post-hoc pair-wise comparisons (LSD, p<0.05) of active responses revealed a significant reduction in rats given the standard procedure compared to extinction-only and rats given a reversed order with a 15-minute or 24-hour delay. Extinction-only controls did not significantly differ from reversed order rats with a 15-minute or 24-hour delay, and the reversed controls did not significantly differ from each other. Therefore, the interaction was driven by a significant reduction in the active lever responding of rats which received the standard protocol.

At test (Figure 2.4D) ANOVA revealed a significant Lever x Treatment interaction ($F_{(3,24)}$=4.68, p=0.010) with main effects of Lever ($F_{(1,24)}$=75.88, p<0.001) and Treatment ($F_{(3,24)}$=3.44, p=0.033).

Analysis of simple main effects revealed a significant effect of Treatment on active lever presses ($F_{(3,24)}$=4.75, p=0.010), but not inactive responses ($F_{(3,24)}$=0.84, p=0.486). Post-hoc comparisons (LSD, p<0.05) of R+ presses revealed a significant difference between the R+ responses of the standard group and those rats which received reactivation after extinction, with either a 15-minute or 24-hour delay; however the standard group did not differ from rats given extinction-only. Rats given reactivation after extinction also made significantly more R+ responses than extinction-only controls, regardless of whether the reactivation was delayed by 15-minutes or 24-hours. Finally, the two reversed ordered groups did not significantly differ from each other, demonstrating that the effect was driven by a reduction in R+ pressing of both the standard and extinction-only groups compared to reversed-order controls.

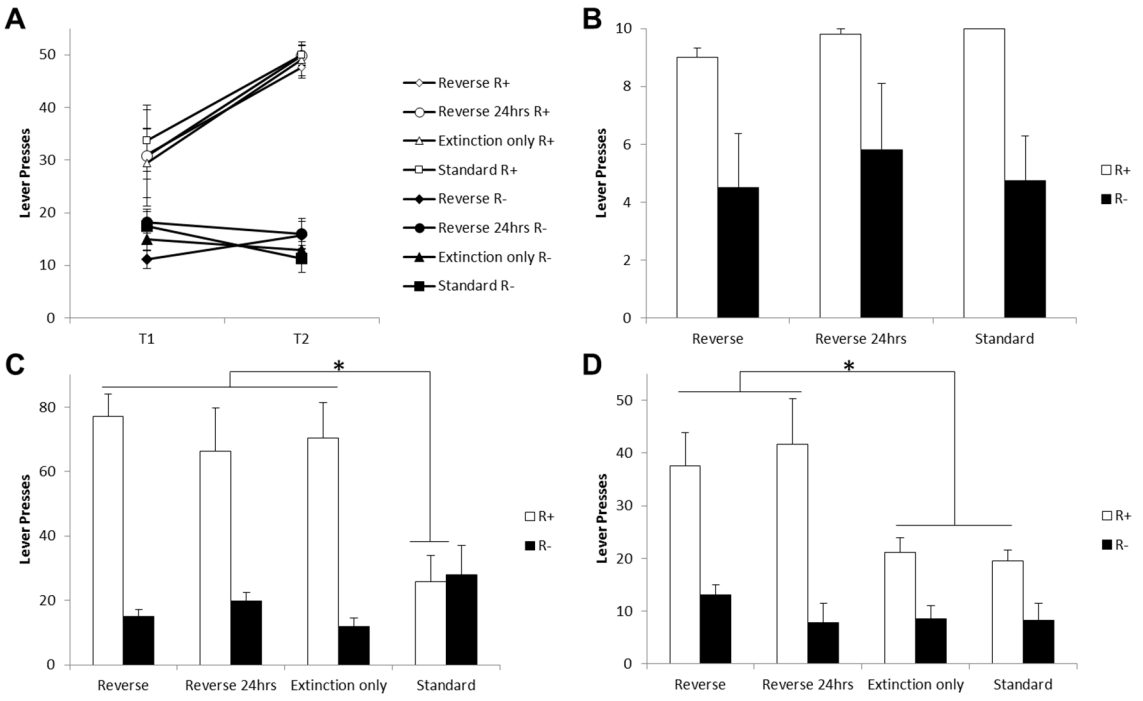

Figure 2.4: Memory reactivation session improves later responding when given after the extinction session. **A**, rats in each of the reverse (diamonds), reverse with 24 hour delay (circles), extinction only (triangles) and standard-order (squares) acquire the lever pressing response to equivalent levels. All rats make significantly more active (white) than inactive (black) responses. A maximum of 50 rewards could be obtained on either the first (T1) or second (T2) training session. **B**, during the non-removal reactivation rats remained in the experimental boxes, however both levers were retracted following each active lever press (white bars). Lever were re-extended every 3 minutes over the 30-minute session, allowing a maximum of 10 active lever presses. Inactive lever presses (black bars) had no programmed consequence. There were no significant differences in the lever pressing of any group. All rats in the standard-ordered group achieved the maximum of 10 active lever presses. **C**, during the 1-hour extinction, rats which had already received a reactivation (standard condition, n=8), make significantly fewer active lever presses than reverse (n=8), reverse-24-hour-delay (n=5) or extinction only (n=7) controls. **D**, in a 30-minute extinction test conducted 24 hours after the retrieval-extinction protocol, groups which received a reversed order procedure (reverse and reverse 24hrs groups) make significantly greater active lever presses compared to standard-ordered and extinction-only groups. Data expressed as mean number of lever presses ± SEM. R+ denotes active responses, R- inactive. (*) indicates significant post-hoc differences, $p<0.05$.

Discussion

These early experiments show that under the present experimental conditions systemic MK-801 does not disrupt the reconsolidation of an instrumental lever-pressing memory when administered prior to a brief non-reinforced session, or a removal reactivation procedure. While the initial use of the removal reactivation in a retrieval-extinction paradigm produced a significant reduction in responding, investigation of this effect using appropriate controls revealed there to be a response augmenting effect of receiving a reactivation session post-extinction.

Systemic MK-801 administered prior to a brief non-reinforced reactivation did not disrupt reconsolidation of the instrumental trace, despite brief extinction sessions being commonly used to (successfully) destabilise pavlovian memories (Lee *et al.*, 2006a; Milton, Lee, Butler, *et al.*, 2008; Flavell & Lee, 2013). However, the finding is consistent with past literature suggesting instrumental memories do not undergo reconsolidation (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). While this would seemingly imply instrumental memories do not undergo reconsolidation, it may be that none of the reactivation parameters used either here or previously were appropriate.

Memory age (Milekic & Alberini, 2002; Suzuki *et al.*, 2004) and strength (Suzuki *et al.*, 2004; Flavell & Lee, 2013) can both influence whether a memory will destabilise given certain reactivation parameters. For example, older memories may require an increased length (Suzuki *et al.*, 2004) or frequency (Reichelt & Lee, 2012) of stimulus presentation in order to destabilise. With this in mind, it may be that instrumental memories do undergo reconsolidation; however the present data and previous literature used ineffectual conditions for destabilising instrumental memory.

Rather than adjust the length or maximum responses of the reactivation session, the later experiments in this chapter, using the removal and non-removal reactivations, attempted to maximise the “surprise” experienced during the reactivation session. This was intended to maximise a prediction-error signal that is believed to be necessary to trigger memory updating (Lee, 2009; Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013).

MK-801-injection prior to the removal reactivation procedure was also without any impairment of long-term behavioural performance. Again this lack of effect could be due to inappropriate reactivation parameters, or simply that instrumental memories do not undergo reconsolidation. An alternative explanation is that the memory trace was successfully destabilised by both reactivation conditions; however that NMDARs are not functionally necessary for the reconsolidation of instrumental memories. This hypothesis was tested using the retrieval-extinction paradigm, first using the removal reactivation procedure. These reactivation parameters were used as they were judged most likely to maximise the putative error signal required to destabilise memory (Lee, 2009; Sevenster *et al.*, 2013), as the duration was minimal and a lever press resulted in a new outcome (termination of the houselight and removal from the box).

It is worth noting the acute increase in responding at reactivation in MK-801 treated rats given the removal reactivation. This is consistent with the mild hyperactivity caused by the dose used here (Hargreaves & Cain, 1995). While MK-801-injection prior to the brief non-reinforced reactivation did cause an acute increase in responding, this was not significant. A possible reason for this is that the group sizes were too small to observe significant hyperactivity. Alternatively, the session may have engaged extinction processes, reducing responding and masking any increase caused by MK-801. It seems unlikely that extinction occurred however, as interestingly following the brief non-reinforced reactivation, MK-801 neither impaired new extinction learning nor disrupted reconsolidation; notable given the frequently observed boundary condition between extinction and reconsolidation (Tronson & Taylor, 2007). It may be that the brief extinction used here was too long to trigger reconsolidation and too short to enable extinction. This circumstance has been observed before in past literature in which intermediate numbers of CS exposures, between those that cause destabilisation and extinction, engage neither reconsolidation or extinction processes (Flavell & Lee, 2013). This may imply that even briefer non-reinforced extinction sessions could be sufficient to destabilise A–O memories.

Given administration of MK-801 was without long-term effect on behaviour, we then utilised a retrieval-extinction paradigm (Monfils *et al.*, 2009; Clem & Huganir, 2010) which does not use drugs and therefore does not make any assumptions for the requirement of specific receptors in the reconsolidation process. Retrieval prior to a long extinction did impair responding the next day compared to a reversed order control, as predicted if reconsolidation was impaired. It was felt, however, that the removal reactivation procedure caused significant restraint-stress to the rats through repeated handling and perhaps this was the source of the effect. Stress has been reported to disrupt reconsolidation of object recognition memory (Maroun & Akirav, 2008) and conditioned place preference (Wang *et al.*, 2008); it has also been shown to variably impair (Schwabe *et al.*, 2009) or enhance memory consolidation (Roozendaal, 2002; Cahill *et al.*, 2003). It is possible that the effect observed with the removal reactivation prior to the extinction session was due to these potential stress influences. Excessive stress during reactivation may have enhanced extinction in the standard group, or stress may have impaired extinction in the reversed control. Either possibility could explain the pattern of results. The non-removal reactivation tested this by removing the need for repeated handling.

The non-removal reactivation was fundamentally equivalent to the removal reactivation, however rats were not removed from the boxes after each R+ response, rather there was a brief inter-response-interval before the lever was re-extended. This minimised handling required, reducing any potential restraint-stress; however this should still have resulted in an error signal as the delay between responses would be dramatically increased compared to training (Díaz-Mataix *et al.*, 2013). Rats given the non-removal reactivation prior to extinction reduced their responding compared to reverse-order controls, replicating the effect of the removal reactivation; however an equivalent reduction in responding was also obtained in the extinction-only group, suggesting the impairment was not due to manipulation of reconsolidation. Instead it appears the performance of rats given a reversed-order procedure was enhanced by receiving the reactivation after the extinction. Notably, when performance has been tested 24-hours after the intervention in past studies the putatively reconsolidation-impaired subjects respond at similar levels to controls which received only extinction

(Monfils *et al.*, 2009; Clem & Huganir, 2010; Schiller *et al.*, 2010), as was observed in the present study. The reconsolidation impairment in these past studies typically only becomes apparent following tests of spontaneous recovery and reinstatement. It is unknown whether, had a spontaneous recovery or reinstatement test been done, performance would have recovered in the standard treatment group; if responding does not recover this supports a reconsolidation interpretation. Importantly, deficits in behavioural performance have been observed regardless of whether the reactivation session was presented 10-minutes before or after the extinction exposure (Baker *et al.*, 2013). Since we observed different effects of pre- and post-extinction reactivation sessions this would strongly suggest the effect was not due to any impairment of reconsolidation. In line with the results of Baker *et al.* (2013) our data would support the conclusion that reactivation prior to a long extinction session does not impair reconsolidation.

The final control group received the non-removal reactivation 24-hours after the extinction to test whether the reversed-order enhancement was due to interference with extinction learning. This delay was chosen as it is far outside any hypothesised window of consolidation or reconsolidation (Judge & Quartermain, 1982); six-hour delayed amnestic treatment does not impair initial learning (Schafe & LeDoux, 2000; Hernandez *et al.*, 2002) nor reactivation (Schiller *et al.*, 2010; Xue *et al.*, 2012). Test performance in the reversed-order condition was increased regardless of whether the reactivation was delayed by 15 minutes, or 24 hours after extinction. This strongly suggests the effect of the non-removal reactivation was not due to an effect on consolidation or reconsolidation, but rather it acted to reinstate the memory from extinction.

It is well established that extinction represents new learning, rather than forgetting (Bouton, 2002). Following extinction, behaviour can be recovered by changes in context or re-expose to reward or reward-paired stimuli (Bouton, 2002, 2004; Bouton *et al.*, 2011). If the reactivation somehow reminded rats of the rewarded action, thus reducing the impact of the extinction on behaviour, then this could explain the resurgence of responding of rats given a reversed-order procedure. One possibility is that rats conditioned to the retraction of the levers. Both the training sessions and non-

removal reactivation involved retraction of the lever when pressed; however the extinction session did not. Thus, it is possible that during training rats conditioned to the sound, or sight, of lever-retraction and paired it with reward delivery (which immediately followed retraction); the rats would then possess a pavlovian retraction-reward memory. Presentation of the lever-retraction again during reactivation could then recover behaviour from extinction. Previous literature has shown that lever-retraction can act as a conditioned stimulus (Davey *et al.*, 1981), supporting this hypothesis. Since the initial removal reactivation protocol also included lever-retraction this hypothesis may also explain the data from that experiment. A visual comparison of the data supports this theory; rats given a reversed-order protocol, regardless of reactivation condition or delay after extinction, appear enhanced compared to extinction-only and standard experimental groups. As the enhancing effect of the reactivation is observed with both the removal and non-removal conditions, it would appear highly improbable that stress was responsible for the effect seen in the repeated reactivation protocol.

The present data also do not conclusively demonstrate reconsolidation was not impaired in the standard retrieval-extinction groups. It remains a possibility that if given a reactivation following their extinction, the standard ordered and extinction-only groups could have recovered. Had the forward group not reinstated this would have implied a reconsolidation impairment (Oyarzún *et al.*, 2012); however this would be indistinguishable from enhancement of extinction (Lattal & Wood, 2013). The nature of impairments caused by the retrieval-extinction paradigm have recently been brought into question, as retrieval-extinction appears to enhance renewal in a new context (Chan *et al.*, 2010) and may still impair performance even when the retrieval trial is given after extinction (Baker *et al.*, 2013), suggesting the impairments are caused by modulation of extinction rather than reconsolidation. If retrieval-extinction deficits do not reflect reconsolidation impairments then it is possible reconsolidation would never have been impaired in the present study, regardless of whether the memory was successfully destabilised during reactivation. Furthermore, combined with the earlier results showing MK-801 did not impair responding when given prior to the removal or non-removal reactivations, it seems highly likely that neither of these conditions destabilised the

instrumental memory. If the parameters were sufficient, then one would have expected MK-801 to have impaired responding at test. Thus neither the brief non-reinforced, removal nor non-removal reactivations appear sufficient to destabilisation instrumental memory.

The present study did not demonstrate that a goal-directed instrumental memory can be destabilised, consistent with past suggestions that operant memories do not undergo reconsolidation (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). These findings add to existing literature, failing to demonstrate an instrumental reconsolidation effect in a weakly-trained, non-habitual memory. The present study also attempted to destabilise the A–O memory using a brief non-reinforced session and a novel removal procedure designed to maximise prediction error, without success. Taken together with existing literature it appears instrumental memories are not destabilised by brief extinction sessions (Lee & Everitt, 2008b), reward exposure (Wang *et al.*, 2005), brief training trials (Mierzejewski *et al.*, 2009), full length training sessions (Hernandez & Kelley, 2004) or the removal and non-removal reactivations presented here. While together they present strong evidence that neither goal-directed nor habitual instrumental memories undergo reconsolidation, this list is by no means exhaustive and differences in the training parameters of these studies may have influences the appropriateness of the reactivation parameters. It still remains possible that instrumental memories can be destabilised under suitable conditions.

CHAPTER 3

RECONSOLIDATION IN NOVEL AVERSIVE SETTINGS

Introduction

Following the lack of any amnestic effect of MK-801 in Chapter 2, the ability of MK-801 to act as an amnestic agent was tested in two novel aversive paradigms in order to confirm its efficacy. Reconsolidation has been successfully demonstrated in both contextual (Lee *et al.*, 2004; Lee, 2008) and auditory (Nader *et al.*, 2000) fear conditioning, and shown to be dependent upon N-methyl-D-aspartate receptor (NMDAR) activity (Lee *et al.*, 2006a; Charlier & Tirelli, 2011; Lee & Hynds, 2012). Reconsolidation has also been demonstrated in inhibitory avoidance (IA) paradigms (Boccia *et al.*, 2004; Milekic *et al.*, 2007; Baratti *et al.*, 2008); however while initial consolidation of IA requires NMDAR activity in the amygdala (Liang *et al.*, 1994), whether its reconsolidation is NMDAR-dependant has not been tested (Reichelt & Lee, 2013a). Here we developed two novel experimental paradigms testing both conditioned place aversion (CPA) and active avoidance (AA). The ability of MK-801 to disrupt reconsolidation in these settings was then tested.

Morphine withdrawal-induced CPA has previously been shown to undergo reconsolidation, dependent upon protein synthesis in the hippocampus (Taubenfeld *et al.*, 2010) and basolateral amygdala (BLA) complex (Wu, Li, Yang, *et al.*, 2012). Reconsolidation of CPA has also been shown to require β-adrenergic receptors in the BLA (Wu, Li, Yang, *et al.*, 2012), but not NMDAR activity in the nucleus accumbens (Wu, Li, Gao, *et al.*, 2012); however it is unclear whether NMDARs are necessary in other brain regions. NMDAR activity is required in the amygdala for the initial consolidation of IA (Liang *et al.*, 1994), and while the NMDAR-dependence of IA reconsolidation is unknown, the BLA appears to play a role (Milekic *et al.*, 2007). This neuroanatomy is similar to cued fear, which also depends upon the BLA (Nader *et al.*, 2000; Si *et al.*, 2012), suggesting there may be commonality in the neural processing of aversive learning between fear paradigms. Furthermore, reconsolidation has

been consistently demonstrated for appetitive conditioned place preference (CPP) memory (Milekic *et al.*, 2006; Kelley *et al.*, 2007; Robinson, Ross, *et al.*, 2011), and this does appear to be NMDAR-dependant (Brown *et al.*, 2008; Itzhak, 2008).

A key difference between past studies of CPA and the present experiments is our use of mild footshock to generate an aversion. As this paradigm was relatively novel, it was unknown what parameters would be required to destabilise the memory. Past CPP studies have used reactivation sessions in which rats are either confined to the previously reinforced compartment (Milekic *et al.*, 2006; Valjent *et al.*, 2006; Brown *et al.*, 2008; Wu, Li, Yang, *et al.*, 2012), or non-confined and allowed to explore the entire experimental context (Kelley *et al.*, 2007; Itzhak, 2008; Robinson & Franklin, 2010; Robinson, Ross, *et al.*, 2011). While reinforcer-exposure is typically used during a confined reactivation in order to destabilise CPP memory, whether this is necessary for destabilisation of CPA memory is unknown. A non-confined, non-reinforced reactivation has previously been shown to destabilise morphine-withdrawal CPA (Wu, Li, Yang, *et al.*, 2012). Additionally brief extinction exposure has successfully been shown to destabilise IA memory (Boccia *et al.*, 2004; Milekic *et al.*, 2007; Baratti *et al.*, 2008). As such, two different reactivation sessions were used: a confined (as in conditioning) and non-confined (as in initial habituation). We reasoned that at least one, or both, of these reactivations would be sufficient to destabilise CPA memory.

The reconsolidation of AA memories has not previously been studied. AA differs from CPA in that a response is required by the animal to evade the aversive outcome. In CPA a single compartment is paired with an aversive outcome and an animal can simply spend time in other compartments to avoid the outcome, while in AA an animal must continually make a response (typically compartment switching or lever pressing) in order to evade the aversive outcome. Existing research has shown the acquisition and performance of AA to require the BLA (Choi *et al.*, 2010; Lázaro-Muñoz *et al.*, 2010). Interestingly the central nucleus (CeN) of the amygdala may mediate a competing pavlovian freezing response which inhibits learning of avoidance (Choi *et al.*, 2010; Lázaro-Muñoz *et al.*, 2010), consistent with the hypothesis that competing pavlovian (defensive) and instrumental (avoidance)

responses are learned during avoidance training (Moscarello & LeDoux, 2013). Notably, dopamine in the striatum (dorsal and ventral) and amygdala (Darvas *et al.*, 2011) is required in the acquisition of AA, but behaviour may become solely dependent upon striatal dopamine with extended training (Darvas *et al.*, 2011). Both the CeN (Baldwin *et al.*, 2000) and striatum (Yin *et al.*, 2004, 2005) are implicated in instrumental behaviour, and these regions may interact in the expression of habits (Lingawi & Balleine, 2012); thus there may be a role for certain CeN circuitry in AA learning and in mediating of the instrumental component of the avoidance. The different contributions of amygdala subnuclei to different behaviours may reflect the nature of the task (i.e. pavlovian versus instrumental); it may also affect the parameters required for memory destabilisation to occur. We generated an avoidance memory by training rats to switch compartments in order to avoid an un-signalled mildly aversive clicker, deviating from past studies which have typically used signalled (Choi *et al.*, 2010; Darvas *et al.*, 2011) or un-signalled (Lázaro-Muñoz *et al.*, 2010) footshock. Memory was then reactivated in a brief context-exposure session.

AA differs from past studies of fear memory reconsolidation in that rats are enabled to perform an operant response in order to avoid the aversive outcome. The aversive unconditioned stimulus (US) is unavoidable in contextual- and cued-fear paradigms; however rats can minimise their US exposure in AA settings by interacting with the environment (Paré & Holser, 1973). In the case of IA, rats can avoid the US in principle, however rarely do. In IA conditioning rats step down to a darkened grid floor (rats step down purely out of a natural preference for dark areas), the grid floor then delivers a footshock. The next day rats delay stepping down, however will eventually move into the dark area. Whether this represents an understanding of A–O contingency, or simply suppression of responding is unclear. IA is seemingly the closest fear conditioning paradigm to AA, and does undergo reconsolidation (Milekic & Alberini, 2002; Boccia *et al.*, 2004; Baratti *et al.*, 2008). Thus, while IA and AA are opposite in their behavioural outputs (inhibited responding in IA versus continuous responding in AA) it seems likely that AA memories can undergo reconsolidation.

Materials and Methods

Subjects

Subjects were 105 Lister-Hooded rats (Charles River, UK), weighing 200-225g at the start of experimental procedures. Rats were housed in cages of 4, maintained at 21^{o}C on a 12hr light-dark cycle (lights on at 0700). Access to food and water was unrestricted. All procedures were conducted in accordance with the United Kingdom Animals (Scientific Procedures) Act 1986 (PPL 40/3205).

For the CPA study, 68 rats were divided into three groups. 18 for the confined reactivation group, 18 to the non-confined reactivation, and 32 were used as non-reactivated controls. 2 rats were excluded from the confined group and 10 from the non-reactivated due to failed learning (>50% of time spent in shock-paired compartment during short-term memory test).

In the AA experiment, 37 rats were used, split into four groups: those that received memory reactivation following an injection of MK-801 (n=9) or saline (n=9), and those that received MK-801 (n=10) or saline (n=9) in the absence of explicit memory reactivation. No rats were excluded from the AA dataset.

Drugs and Behavioural Apparatus

MK-801 (AbCam) was dissolved in sterile-saline to a concentration of 0.1mg/ml and injected intraperitoneally 30 minutes prior to reactivation at a dose of 0.1mg/kg.

The boxes used were as described in Chapter 2, however levers were retracted and the boxes each divided into two compartments with a wooden divider (custom-made). Two sets of dividers were fashioned to 23.5x24x0.5cm, one solid wood, the other open-divider had an 11cm^2 hole through which rats could jump to swap compartments. In order to distinguish each compartment the left side of the divider was painted ‘Subtle Ivory’, the right a custom ‘Plum’ blend (Homebase, UK) and two white lights illuminated the right chamber.

Behavioural procedures

Conditioned place aversion

Rats were first habituated to the context in a 15-minute session. They were initially placed in the right hand side (RHS) and then were able to freely move throughout the box. The next day the solid divider was used to confine rats to first the left hand side (LHS) for 7½ minutes after which they were transferred to the RHS for a further 7½ minutes (for a total of 15 minutes in the context). After 5 minutes in the RHS, rats received a mild 2-second, 0.5mA electric footshock. A second identical conditioning session was given the next day.

The day after the second conditioning session, rats were injected with MK-801 or saline vehicle 30 minutes prior to one of three, 5-minute reactivation sessions. No shocks were delivered during the reactivations.

Confined reactivation: Rats were confined using the solid divider first to the LHS, then the RHS for half the session each.

Non-confined reactivation: Other rats were initially placed in the LHS with the open-divider, and could freely move between compartments. After 2½ minutes rats were removed and placed in the RHS in order to mimic the protocol of the confined reactivation.

No reactivation: A final group of rats received injection without memory reactivation.

Three hours after reactivation, memory was tested in a 5-minute post-reactivation short-term memory (pr-STM) test. Rats were initially placed in the RHS with the open-divider and could move freely between compartments. The next day long-term memory was assessed in a similar 15-minute non-confined extinction session (pr-LTM). A second pr-LTM test was conducted 3 days after the first.

Active avoidance

Boxes were divided in two using the open-divider; rats could freely move between compartments. A mildly aversive mechanical clicker (25 Hz) was presented every 10 seconds for a maximum of 20 seconds; however rats could avoid the clicker by switching compartments. Performing an avoidance response terminated the clicker and delayed it for an additional 30 seconds. Responses reset the delay timer such that continuous responding could prevent activation of the clicker entirely. Rats were trained for 20 minutes each day for 5 days.

At reactivation rats received injection prior to a 5-minute context re-exposure; no clickers were presented during reactivation. A non-reactivated control group received drug injection, but without any behavioural session in order to test whether the effect of the drug was dependent upon memory reactivation. The next day rats were allowed to freely explore the context for 20 minutes, no clickers were presented during testing. The number and timings of jumps was recorded for each session.

Statistical Analysis

For CPA, total time spent in each compartment was measured (calculated as time with hindlimbs in each compartment) for pr-STM and two tests of pr-LTM. Time spent perching on the divider was excluded from analysis. Data for pr-LTM tests was divided into 5-minute bins (to enable comparison to the pr-STM test). Data for each test were analysed separately using repeated measures analysis of variance (ANOVA) using Side of the divider and drug Treatment as factors. For pr-LTM tests, data were analysed with the additional factor of time Bin. Rats which spent >50% of their time at pr-STM in the shock-paired side were excluded from analysis due to a lack of learning. In order to assess spatial-discrimination bonferroni-corrected planned comparisons were carried out for each drug treatment group comparing time spent in each compartment at each test (effective $p<0.025$). In the AA study the number of jump responses (compartment changes) was analysed using three-way ANOVA, with Training session, drug Treatment and Reactivation as factors. Reactivation data was

analysed separately with drug Treatment as a factor. Test data were divided into 5-minute bins and analysed separately with Extinction bin, drug Treatment and Reactivation as factors. An equivalent analysis was performed for the number of clickers rats were exposed to on each session. For reactivation and test data, a hypothetical number of clickers was calculated using response timings to create a measure of response contingency. Results with $p<0.05$ were deemed significant. Where appropriate, a Greenhouse-Geisser correction was used to correct for non-spherical data.

Results

Conditioned Place Aversion

Confined Reactivation

Habituation and conditioning sessions proceeded without incident. Following conditioning rats were administered MK-801, or saline vehicle, prior to the confined reactivation session, and were then tested 3, 24 and 96 hours later in order to assess short and long-term memory. During the pr-STM test overall ANOVA revealed a significant bias towards the non-shock paired Side ($F_{(1,14)}=26.44$, $p<0.001$) with no effect of Treatment ($F_{(1,14)}=0.01$, $p=0.914$) or Side x Treatment interaction ($F_{(1,14)}=0.10$, $p=0.754$). Planned comparisons (effective $p<0.025$) of both Saline ($F_{(1,7)}=10.36$, $p=0.015$) and MK-801-injected ($F_{(1,7)}=16.99$, $p=0.004$) rats demonstrated a significant preference for the un-shocked compartment, indicating both groups learned the avoidance strategy. In a test of pr-LTM overall ANOVA revealed a significant preference for the non-shocked Side ($F_{(1,14)}=45.14$, $p<0.001$), with no main effect of Treatment ($F_{(1,14)}=0.16$, $p=0.693$) or session Bin ($F_{(2,28)}=0.62$, $p=0.543$) and no interactions (Side x Treatment: $F_{(1,14)}=1.15$, $p=0.301$; Side x Bin: $F_{(2,28)}=0.20$, $p=0.819$; Bin x Treatment: $F_{(2,28)}=1.62$, $p=0.215$; Side x Bin x Treatment: $F_{(2,28)}=2.13$, $p=0.138$). Planned comparisons showed MK-801-treated animals continued to avoid the shock-paired compartment ($F_{(1,7)}=11.86$, $p=0.011$), with no difference across session (Bin: $F_{(2,14)}=0.26$, $p=0.775$; Side x Bin: $F_{(2,14)}=2.14$, $p=0.155$); Saline controls also showed significant preference for the neutral side (Figure 3.1A) throughout the first test

(Side: $F_{(1,7)}$=46.27, p<0.001; Bin: $F_{(2,14)}$=4.32, p=0.035; Side x Bin: $F_{(2,14)}$=0.48, p=0.626). In a second test of pr-LTM, overall ANOVA revealed a significant preference for the non-shocked Side ($F_{(1,14)}$=18.03, p=0.001), with no main effect of Treatment ($F_{(1,14)}$=0.03, p=0.862) or session Bin ($F_{(2,28)}$=1.33, p=0.280), however there was a significant Side x Bin x Treatment interaction ($F_{(2,28)}$=3.43, p=0.046). No other interactions were significant (Side x Treatment: $F_{(1,14)}$=0.69, p=0.422; Side x Bin: $F_{(2,28)}$=2.02, p=0.152; Bin x Treatment: $F_{(2,28)}$=0.003, p=0.997). Planned comparisons showed saline-treated controls continued to avoid the shock-paired compartment (Side: $F_{(1,7)}$=19.34, p=0.003; Bin: $F_{(2,14)}$=0.51, p=0.612; Side x Bin: $F_{(2,14)}$=0.38, p=0.694); however MK-801-injected rats showed no significant compartment preference ($F_{(1,7)}$=4.38, p=0.075). The MK-801 group did recover their discrimination between compartments (Figure 3.1B) as the session progressed (Bin: $F_{(2,14)}$=1.02, p=0.387; Side x Bin: $F_{(2,14)}$=5.59, p=0.016), showing little discrimination initially ($F_{(1,7)}$=0.532, p=0.490), however establishing a clear trend of avoidance to the shocked-paired compartment over the 2nd ($F_{(1,7)}$=3.88, p=0.090) and 3rd ($F_{(1,7)}$=11.79, p=0.011) time bins.

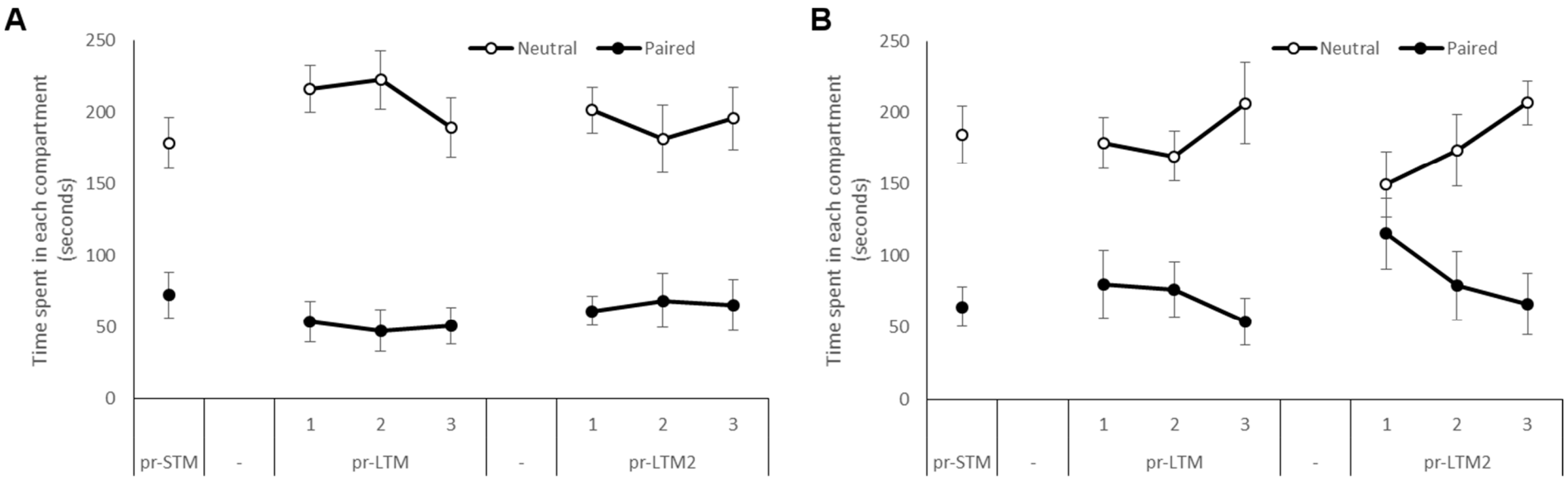

Figure 3.1: MK-801 administered prior to the confined reactivation causes a delayed impairment of CPA to the shock-paired compartment. **A**, saline-treated controls (n=8) show a significant preference for the neutral, un-paired compartment (white circles) throughout all test sessions. **B**, rats given MK-801 (n=8) prior to the confined reactivation show intact CPA 3 hours after reactivation during pr-STM, and 24 hours later at pr-LTM; however avoidance of the shock-paired compartment (black circles) is diminished at the beginning of a second test of pr-LTM 48 hours after reactivation (pr-LTM2). Discrimination between compartments does recover by the end of the session and the deficit is only transient. Data presented as mean ± SEM. 1, 2 and 3 signify 5-minute within-session time bins.

Non-Confined Reactivation

Habituation and conditioning sessions proceeded without incident, after which rats were administered MK-801 or equivalent vehicle prior to the non-confined reactivation session, then pr-STM was tested 3 hours, with two pr-LTM tests 24 and 96 hours after reactivation. Overall ANOVA of pr-STM revealed a significant preference for the non-shocked compartment ($F_{(1,16)}$=23.24, p<0.001) with no difference between groups (Treatment: $F_{(1,16)}$=0.02, p=0.889; Side x Treatment: $F_{(1,16)}$=0.26, p=0.615). Planned comparisons (effective p<0.025) of pr-STM performance showed Saline ($F_{(1,8)}$=10.10, p=0.013) and MK-801-treated ($F_{(1,8)}$=13.16, p=0.007) rats avoided the shock-paired compartment, demonstrating groups learned the avoidance strategy. In the pr-LTM test overall ANOVA revealed rats avoided the shocked Side ($F_{(1,16)}$=5.70, p=0.030) throughout the session (Bin: $F_{(2,32)}$=1.66, p=0.207) with no group differences (Treatment: $F_{(1,16)}$=0.001, p=0.974; Side x Treatment: $F_{(1,16)}$=1.58, p=0.226; Bin x Treatment: $F_{(2,32)}$=0.139, p=0.207; Side x Bin: $F_{(2,32)}$=2.51, p=0.098); Side x Bin x Treatment: $F_{(2,32)}$=0.41, p=0.665); however while there was no effect in the overall ANOVA, planned comparisons showed MK-801-treated animals failed to demonstrate any discrimination (Figure 3.2B) between the shock-paired and neutral compartments ($F_{(1,8)}$=0.387, p=0.551), with no difference across the session (Bin: $F_{(2,16)}$=0.81, p=0.463; Side x Bin: $F_{(2,16)}$=1.47, p=0.259). Planned comparisons for saline-injected controls again showed significant preference for the neutral side (Figure 3.2A) throughout the first pr-LTM test session (Side: $F_{(1,8)}$=18.87, p=0.002; Bin: $F_{(2,16)}$=1.40, p=0.275; Side x Bin: $F_{(2,16)}$=1.42, p=0.270). In the second pr-LTM test overall ANOVA again revealed a significant avoidance of the shock-paired Side ($F_{(1,16)}$=10.14, p=0.006). No other effects or interactions were significant (Treatment: $F_{(1,16)}$=0.35,p=0.560; Bin: $F_{(1.47,23.57)}$=0.93, p=0.381; Side x Treatment: $F_{(1,16)}$=1.86, p=0.191; Bin x Treatment: $F_{(1.47,23.57)}$=2.81, p=0.094; Side x Bin: $F_{(1.32,21.05)}$=2.32, p=0.137; Side x Bin x Treatment: $F_{(1.32,21.05)}$=0.57, p=0.503). Planned comparisons showed the impaired discrimination of the MK-801 group persisted into the second pr-LTM test (Side: $F_{(1,8)}$=1.00, p=0.348; Bin: $F_{(2,16)}$=0.45, p=0.644; Side x Bin: $F_{(1.12,8.92)}$=0.644, p=0.460); however the CPA of the shock-paired side remained intact in Saline-injected controls (Side: $F_{(1,8)}$=30.87, p=0.001; Bin: $F_{(2,16)}$=3.71, p=0.048; Side x Bin: $F_{(2,16)}$=4.10, p=0.036).

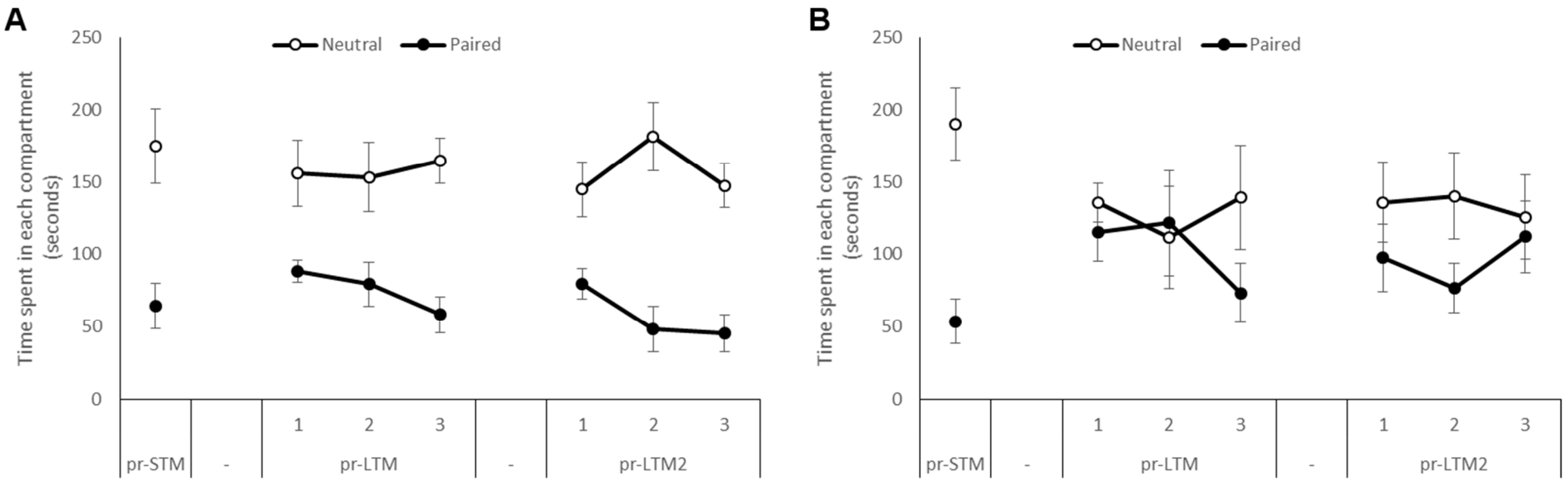

Figure 3.2: Administration of MK-801 prior to a non-confined reactivation session significantly impairs future CPA. **A**, saline controls (n=9) spent less time in the shock-paired compartment (black circles) during all test sessions. **B**, MK-801-injected rats (n=9) demonstrate intact pr-STM 3 hours after reactivation; however discrimination between the neutral (white circles) and shock-paired compartments (black circles) is impaired 24 (pr-LTM) and 48 hours later (pr-LTM2) in two test of pr-LTM. The persistent nature of the deficit strongly suggests memory was impaired by the amnestic treatment. Data presented as mean ± SEM. 1, 2 and 3 signify 5-minute within-session time bins.

No Reactivation

Habituation and conditioning sessions proceeded without incident, after which rats were administered saline or MK-801 in the absence of any reactivation session. As previously, performance was tested 3 hours, 24 and 96 hours later. At pr-STM overall ANOVA revealed significant avoidance of the shocked Side ($F_{(1,13)}$=25.97, p<0.001) by both treatment groups (Treatment: $F_{(1,13)}$=0.83, p=0.380; Side x Treatment: $F_{(1,13)}$=0.004, p=0.949). Planned comparisons (effective p<0.025) showed both Saline ($F_{(1,10)}$=46.33, p<0.001) and MK-801-treated ($F_{(1,10)}$=15.58, p=0.003) animals demonstrated a significant CPA of the shock-paired side. In the first test of pr-LTM overall ANOVA indicated significant avoidance of the shock-paired Side ($F_{(1,13)}$=13.20, p=0.003) by both treatment groups (Treatment: $F_{(1,13)}$=3.84, p=0.072; Side x Treatment: $F_{(1,13)}$=0.09, p=0.775) throughout the session (Side x Bin x Treatment: $F_{(2,26)}$=0.11, p=0.896); however both groups decreased the overall amount of time spent in either compartment as the session progressed (Bin: $F_{(2,26)}$=8.10, p=0.002; Bin x Treatment: $F_{(2,26)}$=1.23, p=0.308; Side x Bin: $F_{(2,26)}$=1.23, p=0.308), indicating more time was spent perching on the divider at the end of the session. Planned comparisons showed MK-801-treated subjects successfully avoided (Figure 3.3B) the shock-paired compartment ($F_{(1,10)}$=31.29, p<0.001), with no significant difference across the session (Bin: $F_{(2,20)}$=3.74, p=0.042; Side x Bin: $F_{(2,20)}$=0.06, p=0.941). While saline-injected controls spent more time in the neutral compared to the shocked compartment (Figure 3.3A), this was not significant ($F_{(1,10)}$=6.25, p=0.031), and expression of CPA did not improve over the session (Bin: $F_{(2,20)}$=1.65, p=0.218; Side x Bin: $F_{(2,20)}$=0.37, p=0.697). Overall ANOVA revealed avoidance of the shock-paired side was maintained in the second pr-LTM test ($F_{(1,13)}$=9.78, p=0.008) by both treatment groups (Treatment: $F_{(1,13)}$=0.48, p=0.499; Side x Treatment: $F_{(1,13)}$=0.43, p=0.521) throughout the session (Bin: $F_{(1.27,16.52)}$=0.69, p=0.451; Bin x Treatment: $F_{(1.27,16.52)}$=1.15, p=0.315; Side x Bin: $F_{(1.16,15.08)}$=0.14, p=0.748; Side x Bin x Treatment: $F_{(1.16,15.08)}$=0.08, p=0.816). Planned comparisons of discrimination showed saline controls ($F_{(1,10)}$=16.70, p=0.002) avoided the shock-paired compartment, with preference for the neutral compartment increasing over the session (Bin: $F_{(2,20)}$=0.252, p=0.779; Side x Bin: $F_{(2,20)}$=4.80, p=0.020).

MK-801-treated rats also continued to avoid the shock-paired compartment (Side: $F_{(1,10)}$=7.92, p=0.018; Bin: $F_{(1.23,12.01)}$=1.06, p=0.340; Side x Bin: $F_{(1.20,12.02)}$=0.09, p=0.819).

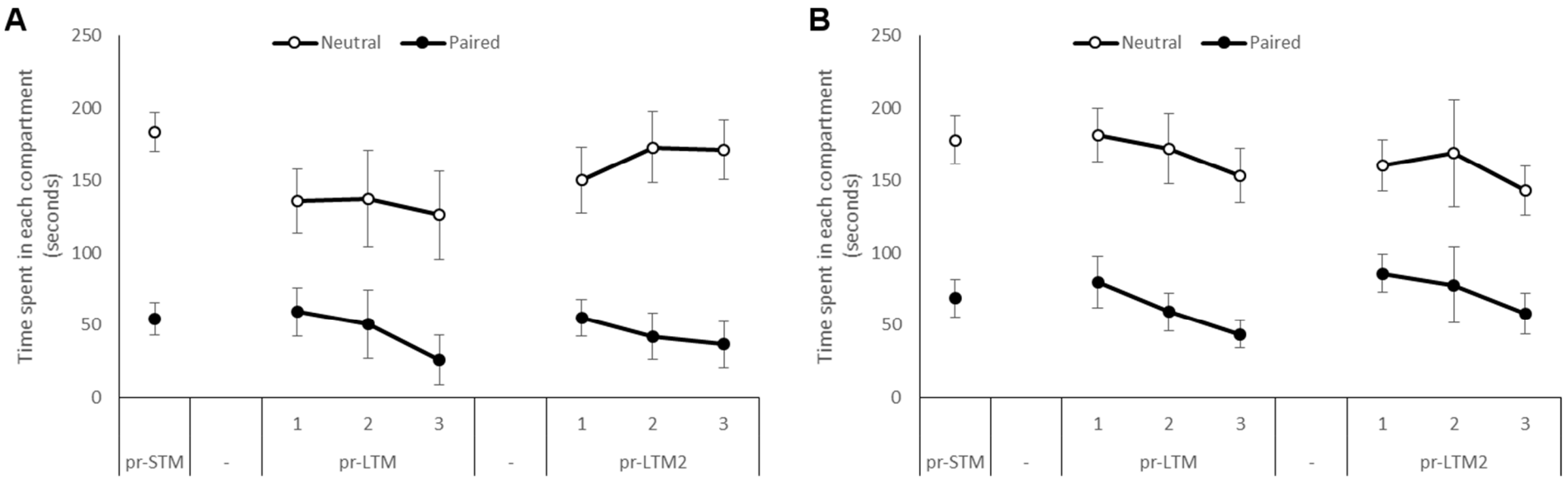

Figure 3.3: Administration of MK-801 in the absence of any reactivation session did not impair CPA. **A**, saline-treated animals (n=11) show a significant avoidance of the shock-paired side (black circles) in a test of pr-STM and pr-LTM2, 3 hours and 48 hours after reactivation respectively. In the first test of pr-LTM, 24 hours after reactivation, there is a strong preference for the unpaired compartment (white circles), however this is not significant overall. **B**, rats given MK-801 (n=11) in the absence of a behavioural session avoid the shock-paired side (black circles) in all three memory tests, 3 (pr-STM), 24 (pr-LTM) and 48 hours (pr-LTM2) after reactivation. Data presented as mean ± SEM. 1, 2 and 3 signify 5-minute within-session time bins.

Active avoidance

ANOVA revealed a significant increase in number of jump responses over Training ($F_{(4,132)}$=11.38, p<0.001), with no main effects of Treatment ($F_{(1,33)}$=0.01, p=0.906) or Reactivation ($F_{(1,33)}$=3.00, p=0.093) and no interactions (Treatment x Reactivation: $F_{(1,33)}$=0.08, p=0.774; Training x Treatment: $F_{(2.53,83.45)}$=0.11, p=0.934; Training x Reactivation: $F_{(2.53, 83.45)}$=1.19, p=0.316; Training x Treatment x Reactivation: $F_{(2.53, 83.45)}$=0.24, p=0.837; Figure 3.4A). The number of clicker exposures also decreased with Training ($F_{(4,132)}$=16.05, p<0.001), indicating rats learned successfully to avoid the clicker (Figure 3.5A). ANOVA also revealed a significant main effect of Reactivation ($F_{(1,33)}$=7.62, p=0.009) during training sessions, showing increased clicker exposure in the non-reactivated groups during training. There were no other significant effects (Treatment: $F_{(1,33)}$=0.12, p=0.733) or interactions (Treatment x Reactivation: $F_{(1,33)}$=0.001, p=0.973; Training x Treatment: $F_{(2.77,91.27)}$=0.58, p=0.616; Training x Reactivation: $F_{(2.77,91.27)}$=0.76, p=0.509; Training x Treatment x Reactivation: $F_{(2.77,91.27)}$=0.31, p=0.801). Analysis of clicker exposure on the final day of training showed no significant effect of Treatment ($F_{(1,33)}$=0.13, p=0.722), Reactivation ($F_{(1,33)}$=0.45, p=0.505) or Treatment x Reactivation interaction ($F_{(1,33)}$=0.29, p=0.592) indicating that rats were equally performing by the end of training (Figure 3.5A). At reactivation, there were no significant differences in number of responses (Figure 3.4B) made ($F_{(1,16)}$=0.11, p=0.745). The number of clickers each rat would have been hypothetically exposed to was calculated for this session, based upon response timings; however all rats would have successfully evaded the clicker had the presentation contingency been the same as in training.

Analysis of compartment changes at test (Figure 3.4C) revealed a significant reduction with Extinction ($F_{(3,99)}$=70.98, p<0.001) with no main effects of Treatment ($F_{(1,33)}$=3.28, p=0.079) or Reactivation ($F_{(1,33)}$=2.11, p=0.156) and no interactions (Treatment x Reactivation: $F_{(1,33)}$=3.65, p=0.065; Extinction x Treatment: $F_{(3,99)}$=0.24, p=0.870; Extinction x Reactivation: $F_{(3,99)}$=0.549, p=0.650; Extinction x Treatment x Reactivation: $F_{(3,99)}$=0.87, p=0.458).

Based on the timing of responses a number of hypothetical clicker presentations were calculated in order to assess the ability of rats to successfully recall the contingency (Figure 3.5B). Analysis of this

data at test revealed a significant Treatment x Reactivation interaction ($F_{(1,33)}$=4.44, p=0.043) with no main effect of Treatment ($F_{(1,33)}$=2.27, p=0.141) or Reactivation ($F_{(1,33)}$=3.17, p=0.084), however there was a general overall increase in theoretical-clickers with Extinction ($F_{(3,99)}$=37.24, p<0.001). No other results were significant (Extinction x Treatment: $F_{(3,99)}$=1.02, p=0.389; Extinction x Reactivation: $F_{(3,99)}$=0.50, p=0.684; Extinction x Treatment x Reactivation: $F_{(3,99)}$=1.16, p=0.331). Analysis of simple main effects at test revealed a significant effect of Treatment in reactivated rats ($F_{(1,16)}$=5.33, p=0.035), with MK-801-injected animals showing a poorer understanding of the contingency (more theoretical-clickers) compared to saline controls; however there was no significant difference between treatment groups in non-reactivated controls ($F_{(1,17)}$=0.23, p=0.641). Importantly, orthogonal simple main effects revealed reactivated, saline-treated rats showed significantly better understanding of the clicker contingency compared to their non-reactivated equivalents ($F_{(1,16)}$=8.12, p=0.012); however MK-801-treated groups showed no difference in hypothetical-clicker avoidance at test ($F_{(1,17)}$=0.05, p=0.825), suggesting the deficit may not be in reconsolidation.

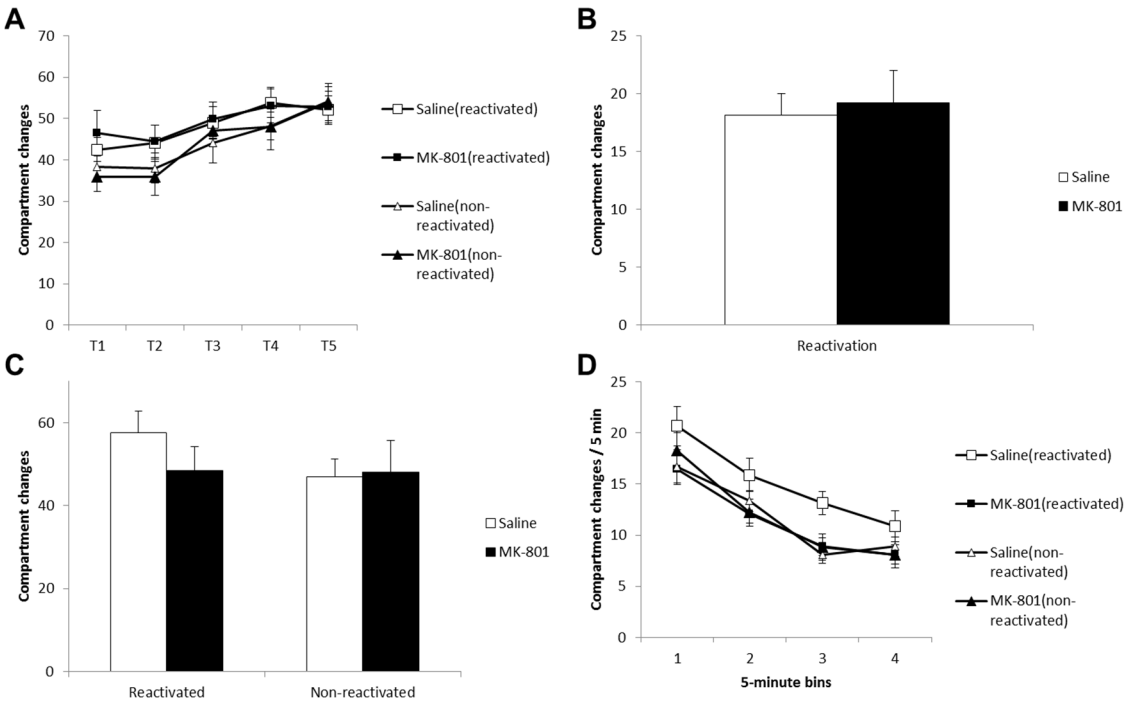

Figure 3.4: There were no significant differences in frequency of avoidance responses made between treatment groups. **A**, during training, reactivated (squares) and non-reactivated (triangles) learned to increase their jumping response frequency over the first (T1), second (T2), third (T3), fourth (T4) and fifth (T5) day of training, regardless of whether they were to receive MK-801 (black) or saline (white) prior to reactivation. **B**, at reactivation rats were re-exposed to the training context in the absence of the clicker for 5 minutes. Rats treated with MK-801 (black bar, n=9) or saline (white bar, n=9) made similar number of compartment change responses during reactivation. **C**, when tested 24 hours after reactivation, the overall number of compartment changes made by previously reactivated rats did not significantly differ from non-reactivated MK-801 (black bars, n=10) or saline (white bars, n=9) injected counterparts, although there was a trend towards increased responding in the reactivated vehicle rats. **D**, test session data represented as 5-minute, within-session bins. All groups showed similar rates of extinction. White = saline, black = MK-801, squares = reactivated, triangles = non-reactivated. Data represented as mean ± SEM.

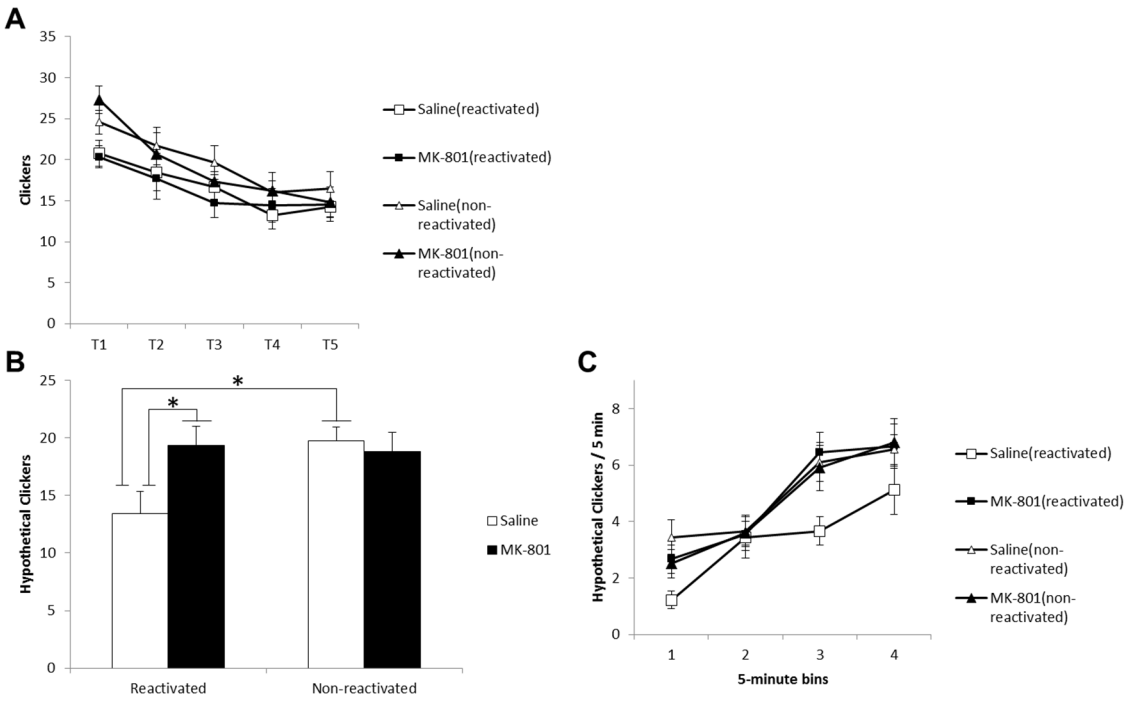

Figure 3.5: Vehicle treated rats given a reactivation show significantly improved avoidance of hypothetical-clickers at test. **A**, rats successfully learn the clicker-avoidance contingency during training. Non-reactivated groups (triangles) received more clicker exposure on the first (T1), second (T2) and third (T3) training days, however performance appears similar to reactivated (squares) groups on the fourth (T4) and fifth (T5) days of training. There were no significant differences in clicker exposure between saline (white) and MK-801 (black) groups prior to reactivation. At reactivation rats were re-exposed to the chamber in the absence of the clicker for 5 minutes. For the reactivation session a hypothetical number of clicker exposures was calculated based on response timing, however all rats would have successfully evaded all clickers had they been presented on the same schedule as in training. **B**, when tested 24 hours after reactivation rats which received a saline injection (white bars) combined with reactivation (n=9) show a significantly improved expression of the avoidance contingency compared with their MK-801-treated (black bars, n=9) counterparts and non-reactivated (Saline, n=9; MK-801, n=10) controls based upon the hypothetical number of clicker presentations. **C**, hypothetical clicker exposure data from the test session represented as 5-minute within session bins (saline = white, MK-801 = black, squares = reactivated, triangles = non-reactivated). Data represented as mean ± SEM. (*) indicates significant differences, $p<0.05$.

Discussion

The data presented here show a significant effect of MK-801 in both CPA and AA settings. In the CPA study, MK-801 produced a delayed impairment of discrimination between the two compartments when given prior to a confined-reactivation. Interestingly, while MK-801 produced no overall significant effect with the non-confined reactivation, this intervention appeared to cause a much more dramatic and long-lasting impairment of CPA. Moreover, MK-801 had no effect in the absence of memory reactivation. In the AA paradigm MK-801 also produced a reactivation-dependant impairment of behaviour, although this did not impair performance compared to non-reactivated controls. As such this may represent an impairment in consolidation of new learning, rather than reconsolidation. These data suggest MK-801 is capable of disrupting consolidation and reconsolidation memory processes.

The effect of MK-801 in the CPA paradigm was reactivation-dependant, as it did not produce any reduction in compartment discrimination when given without reactivation. This strongly implies the memory deficits were caused by disruption of reconsolidation. Given we were uncertain of the exact parameters required to trigger destabilisation, two different reactivations were used; one in which rats were confined to the shock-paired compartment, the other non-confined. Interestingly the effect of MK-801 on long-term behaviour differed between reactivations. In the case of the non-confined reactivation, overall analysis showed no difference between treatment groups, however MK-801-treated animals did not show any significant avoidance in the planned comparisons. This apparent deficit in pr-LTM tests demonstrated a long-lasting impairment of CPA. Lasting memory deficits have been proposed to be important for demonstrating reconsolidation impairments (Dudai, 2006). As such it seems highly likely that MK-801 prior to the non-confined reactivation session impaired the reconsolidation of CPA memory, although the lack of an effect in the overall ANOVA means there is insufficient data to state this conclusively. In the case of the confined reactivation, MK-801 did not produce any CPA reduction in the first pr-LTM test. An impairment only emerged on a later test; however this effect was transient, with discrimination between compartments re-

emerging as the session progressed. Thus, it seems unlikely this reflects impaired reconsolidation, more likely there was a diminished capacity for the context to cue memory retrieval.

Administration of MK-801 without any reactivation session did not produce any long-term effect on CPA; however saline controls did not demonstrate significant CPA in planned comparisons of the first pr-LTM test, although visually they did appear to avoid the shock-paired compartment. Avoidance in saline-treated, non-reactivated controls strengthened over the course of the second pr-LTM test and the group demonstrated significant CPA. From a visual inspection of the data there also appears to be a similar pattern in saline-injected, non-confined-reactivated rats, and in rats given MK-801 prior to the confined session (discussed above). This general improvement in CPA with time may reflect the presence of two strategies with which the task can be solved. Rats could understand the spatial relationship between the context and the shock (model-based strategy) and move to the neutral side; alternatively, rats could adapt their location based upon the incentive valence of each compartment (model-free strategy). This latter strategy is akin to the somatic-marker hypothesis (Damasio, 1996) in which the shock-paired compartment elicits a negative emotional response leading to avoidance. Incentive learning requires re-exposure to the stimulus or context (Dickinson & Balleine, 2002), perhaps explaining why CPA appears to improve with continued exposure to the context. The development of a model-free avoidance strategy may also explain the general reduction of time spent in either compartment by non-reactivated rats in the first pr-LTM test. It may be this represents the learning of an incentive-based strategy during the test session. Notably, this did not appear to occur in reactivated rats, however this may be due to these groups having had more overall context exposure prior to the test compared to non-reactivated controls (as reactivated rats received context exposure during the reactivation). This increase in perching time may be similar to cued- or context-fear memory in which rats learn an association between the conditioned stimulus (CS) and both the sensory (US_S) and motivational (US_M) properties of the US. This allows the CS to caused freezing behaviour, presumably by activation of an aversive system. While CPA does not appear to be mediated by a similar mechanism, as freezing would inhibit avoidance, this not necessarily the case. In cued-fear paradigms rats are unable to avoid the CS or US, and may freeze

out of inability to escape. In the present study a sensory CS–US_S association between the grid-floor and the shock could cause rats to perch on the divider as both compartments contain the grid-floor. Indeed this may explain the initial increase in perching time in non-reactivated rats on the first test. On the other hand a CS–US_M memory should allow CPA by eliciting negative emotions in the shock-paired compartment. In this respect the mechanisms of cued-fear and CPA may be similar as acquisition of a negative incentive by one compartment likely occurs by activation of an aversive motivational system, which likely also mediates freezing behaviour in cued-fear (LeDoux, 2003).

A visual inspection of non-reactivated rats also appears to show an improvement in discrimination as testing continues, driven in part by spending more time in the non-shocked compartment rather than less in the shock-paired side. Thus, CPA may depend on both learning the shocked-side is aversive, and the non-paired compartment is safe; possibly via a CS–US_M memory assigning incentive value to the compartments. Learning that a specific compartment is safe or dangerous may be similar to IA learning, in which rats must avoid stepping into a dark-compartment. In IA there may be a mechanism by which re-entry of the shocked-compartment is suppressed, possibly by an incentive mechanism which may be similar to CPA. IA memory has been shown to undergo reconsolidation (Boccia *et al.*, 2004; Milekic *et al.*, 2007) and given the similarities to CPA, this may support that the loss of discrimination in the non-confined, MK-801-injected group was due to an impairment of reconsolidation.

The presence of both CS–US_S and CS–US_M memories may explain the different effects of MK-801-injection in the confined and non-confined reactivations. It may be that one reactivation condition caused the loss of context–incentive memory (thereby preventing learning of an incentive CPA strategy), while the other impaired a model-based context–shock memory. This is not an entirely satisfactory explanation. The re-emergence of avoidance in the confined-MK-801 group may imply intact incentive memory (and thus impaired memory of the spatial contingency), however the MK-801-impairment does not emerge until the second pr-LTM test, suggesting memory was intact during the first test. Additionally, the impairment with the non-confined reactivation appears to be long-

lasting and present from the first test. As such, it is more likely that only the non-confined reactivation destabilised fear memory (given the lasting impairment), and the confined reactivation did not (as the impairment was both delayed and only transient).

The finding that non-confined context exposure appeared to destabilise CPA memory is consistent with a previous demonstration that CPA memories undergo reconsolidation (Wu, Li, Yang, *et al.*, 2012); however memory reconsolidation did not appear to be disrupted in the confined condition. A possible reason for this is that novelty is required at reactivation to destabilise CPA and CPP memory (Robinson, Ross, *et al.*, 2011); this is consistent with a role of reconsolidation in memory updating (Lee, 2009). While nice theoretically this interpretation cannot explain the present data, as the non-confined condition is not novel since it is equivalent to the habituation session; nor is the novelty hypothesis satisfactory to explain the results of Robinson *et al.* (2011) as they also used a habituation session prior to conditioning which was functionally equivalent their reactivation session. An alternative explanation of the present data is that memory destabilises to update the non-confined context with a shock contingency. On day 1 of the present experiment rats were exposed to the non-confined context (nCtx) and presumably formed spatial memory for this. Rats were then conditioned in a confined context (cCtx) in which they learned the RHS delivered footshock. At reactivation, rats re-exposed to the nCtx now need to update their neutral nCtx memory to include a footshock contingency for the RHS. Rats given cCtx-reactivation or no reactivation do not update their memory as they have not been re-exposed to the nCtx; instead these groups likely update their nCtx memories on the pr-STM test (functionally equivalent to the non-confined reactivation), however this is outside the behaviourally-effective window for MK-801 (Hargreaves & Cain, 1995). This hypothesis would re-interpret the findings from Robinson *et al.* (2011) as updating of context–incentive memory, as they used a very similar setup to the present study with non-confined habituation and reactivation sessions and confined conditioning trials; however they used morphine to elicit CPP rather than aversion. Together with the present results, it seems likely that the key trigger for memory destabilisation is updating of the neutral-context memory with a new incentive value. Other studies have also used non-reinforced, non-confined reactivations to successfully

destabilise CPP (Kelley *et al.*, 2007; Itzhak, 2008; Robinson & Franklin, 2010). A key commonality in these studies is the use of non-confined habituation, and confined conditioning sessions; thus it is conceivable that reconsolidation is triggered during non-confined re-exposure in order to update the non-confined context with the new spatial incentive contingency (leading to expression of preference or avoidance in the non-confined context). The role of incentive updating in triggering reconsolidation may also explain cases when multiple reactivation sessions have been required for deficits in CPP memory (Brown *et al.*, 2007, 2008; Sadler *et al.*, 2007; Fricks-Gleason & Marshall, 2008; Robinson, Armson, *et al.*, 2011), as incentive value only updates upon stimulus re-exposure (Balleine, 2011). This raises a key issue over whether the reinforcer–incentive memory was disrupted in these past studies which have used multiple reactivations, as incentive memory is known to undergo reconsolidation (Wang *et al.*, 2005); this presents a key confound as the behavioural deficit cannot be directly attributed to an impairment of CPP. This reinforces the argument that reconsolidation deficits should be obtainable with a single reactivation (Dudai, 2006).

Notable is the lack of any lasting memory impairment with the confined reactivation. This is consistent with findings in CPP studies, in which confined context-exposure does not destabilise memory (Milekic *et al.*, 2006; Valjent *et al.*, 2006). Interestingly, exposure to the reinforcer in combination with confined context-exposure does destabilise CPP memory (Milekic *et al.*, 2006; Valjent *et al.*, 2006; Wu, Li, Gao, *et al.*, 2012; Wu, Li, Yang, *et al.*, 2012). Given the parallels between CPA and CPP with non-confined reactivations it seems likely that the confined reactivation could have been effective in the present study if combined with a brief footshock (the reinforcer). The requirement for the reinforcer may indicate that reconsolidation is mediating memory strengthening (Lee, 2008). Thus it appears CPP/CPA memories undergo reconsolidation in order to strengthen or update context–incentive contingency; however reconsolidation will only occur under appropriate conditions. Past literature has shown both non-reinforced, unconfined reactivation sessions (Kelley *et al.*, 2007; Itzhak, 2008; Robinson & Franklin, 2010; Robinson, Ross, *et al.*, 2011) and confined, reinforced trials (Milekic *et al.*, 2006; Valjent *et al.*, 2006; Wu, Li, Gao, *et al.*, 2012; Wu, Li, Yang, *et al.*, 2012) to be effective in destabilising CPP memory. Furthermore, previous work (Wu, Li, Yang, *et al.*,

2012) and the present study demonstrate non-confined reactivations are sufficient for destabilisation of CPA memory; however it remains to be determined if reinforced, confined reactivations will be equally effective.

Previous literature has shown NMDAR activity to be required to reconsolidate aversive context (Lee & Hynds, 2012), appetitive CPP (Brown *et al.*, 2008; Itzhak, 2008) and spatial memories (Kim *et al.*, 2011). Given the present study implicates NMDAR activity in the reconsolidation of CPA, it appears NMDAR activity is a universal requirement for spatial memory reconsolidation. The present study contrasts with a past study of drug-withdrawal-induced CPA which did not show any effect of NMDAR antagonism in the nucleus accumbens on reconsolidation (Wu, Li, Gao, *et al.*, 2012); however this may be due to the use of local rather than systemic NMDAR blockade. Given their role in the reconsolidation of CPP, the hippocampus and amygdala may be more likely central loci of NMDAR activity (Milekic *et al.*, 2006).

Treatment with MK-801 also reduced expression of AA compared to saline controls. This manifested as a significant impairment of response contingency (measured by number of theoretical-clicker exposures). Additionally this effect was reactivation-dependant with no effect of MK-801 in non-reactivated controls; however this may not represent a reconsolidation deficit. Non-reactivated controls show similar response frequency and contingency to MK-801-injected reactivated rats, while reactivated-saline animals appear augmented compared to non-reactivated controls. Were there a reconsolidation-impairment, reactivated MK-801 rats should have performed worse than non-reactivated controls, but this was not the case. Given that the effect appears to be driven by augmentation of the reactivated-saline group it may be that new learning occurred during reactivation.

Traditionally brief extinction sessions are used to destabilise memory (Lee *et al.*, 2006a; Milton, Lee, & Everitt, 2008), and it is interesting there appeared to be new learning in the present experiment. Perhaps the simplest explanation is that extinction occurred at reactivation, the consolidation of which was impaired by MK-801. If this extinction suppressed competing behaviours, then AA could

theoretically improve (Moscarello & LeDoux, 2013). Lesions of the CeN, which appear to remove competing responses, tend to improve acquisition of AA but importantly there appears to be no significant facilitatory effect once avoidance is learned (Lázaro-Muñoz *et al.*, 2010). Given AA appears well acquired in the present study, it seems unlikely this can explain the data. Furthermore one might also have expected any facilitatory extinction learning (which suppressed competing responses) to have occurred at the beginning of the test session for remaining groups, leading to similar responding between reactivated-saline and non-reactivated controls at the end of the test. This did not appear to be the case. One final problem for an extinction interpretation is that a visual comparison of total responses during reactivation versus the first bin of the test session appears to show saline-treated animals maintaining their response frequency across sessions, while MK-801-injected rats appear to reduce their response frequency. Thus it seems highly unlikely that extinction learning can explain the data. An interesting alternative is that reactivation acted as a brief training trial. Analysis of response contingency revealed all rats would have successfully avoided the clicker at reactivation; thus rats may have perceived the reactivation as a training session. This may explain the augmentation of vehicle-treated rats, as reactivation acted as an additional training session. If new learning did occur during reactivation, then there is a question of whether this may have occurred via a reconsolidation mechanism, given reconsolidation has previously been shown to mediate strengthening of context memory (Lee, 2008). Notably, training trials have successfully destabilised memory in other settings (Duvarci & Nader, 2004; Eisenberg & Dudai, 2004; Milekic *et al.*, 2006; Rodriguez-Ortiz *et al.*, 2008), although a training trial may be insufficient to trigger reconsolidation when the memory is well-trained (Hernandez & Kelley, 2004; Díaz-Mataix *et al.*, 2013). This may be because when memory is learned to asymptotic levels of performance it does not require updating. One problem with the interpretation that reconsolidation-mediated new learning occurred at reactivation is that training performance appears asymptotic in the reactivated groups; previous studies have shown training trials not to engage reconsolidation when the contingency of an aversive US is well-learned (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013). As such it would seem unlikely reactivation caused updating of the response contingency, as it was already well-learned by the end

of training. Given changes in US contingency appear sufficient to induce reconsolidation of well-trained memory in other aversive settings (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013), it may be that presentation of a non-contingent clicker would have been sufficient to trigger reconsolidation in the present study; alternatively clicker presentation may have been necessary for reconsolidation to occur, much as in studies of CPP (see above). A key problem with this interpretation is that performance of non-reactivated groups may not be asymptotic at the end of training. This may indicate that non-reactivated rats did not learn as well as other groups, certainly they perform poorer at the beginning of training. Consequently performance of the non-reactivated groups may have been lower at test due to poorer learning, masking any reconsolidation impairment in the MK-801-reactivated rats. However given all groups performed equally at the end of training it seems unlikely test performance was affected. Thus the most likely explanation for the pattern of data is that new learning occurred in the reactivated-saline group. Whether this learning occurred via a consolidation or reconsolidation mechanism is unknown and would require doubly dissociable neurobiological mechanisms to be identified for this task. Currently, doubly dissociable consolidation and reconsolidation mechanisms have only been demonstrated for hippocampal learning (Lee *et al.*, 2004; Lee & Hynds, 2012).

If AA did not undergo any form of reconsolidation-mediated updating, then how else might the improvement of the reactivated-saline group be explained? One possibility is that the absence of the clicker at reactivation was reinforcing, via a positive hedonic state such as “relief” (Dickinson & Balleine, 2002). This could theoretically form an appetitive motivational context memory which could enhance the vigour of responding at test, leading to an increased frequency of responding. While the reactivated-saline group generally respond more frequently throughout the session, this was not significant, and the effect of MK-801 manifested in the response contingency which should theoretically not have been altered by a change in motivation; although theoretically a general increase in vigour could have reduced clicker exposure, not via understanding of contingency, but by simply responding with sufficient frequency. Another problem for this interpretation is that the same incentive learning which may have occurred during reactivation should have occurred during the test

for the non-reactivated controls, although this may be related to the length of the session, with the briefer reactivation triggering encoding of appetitive incentive learning and the longer test session causing extinction. This parallels the finding that brief extinction sessions trigger reconsolidation, while longer sessions do not (Lee *et al.*, 2006a; Reichelt & Lee, 2012). This interpretation would suggest that brief sessions lead to changes in hedonic value of stimuli causing updating via reconsolidation, while longer sessions provide time to realise the reinforcer is truly absent causing extinction and suppressing responding to the expectation of the reinforcer rather than modifying the value of the CS. Certainly it seems that over-expectation can be used to trigger the reconsolidation process (Reichelt & Lee, 2013b). This would interpret the increased AA of reactivated-saline rats as reconsolidation-mediated updating of incentive. The production of a positive hedonic state in the reactivation, through the absence of clicker presentation, may also explain why MK-801 did not produce any hyperactive effect during the reactivation session. Typically MK-801 causes acute mild hyperactivity (Hargreaves & Cain, 1995), however this was not observed in the current study. It may be that the hyperactive effect of MK-801 is mediated by a change in hedonic state. MK-801 does alter the hedonic experience of natural reinforcers (Vardigan *et al.*, 2010) and possesses reinforcing properties as measured by its ability to induce CPP (Layer *et al.*, 1993). If the reactivation did induce an appetitive affective state due to the absence of the clicker this may have masked any positive motivational effect of MK-801, preventing the observation of any MK-801-induced hyperactivity.

One final interpretation that follows from the above hypothesis, is that the positive hedonic state created by clicker absence may have reinforced the compartment-switching response during the reactivation, forming a Stimulus–Response (S–R) habit much as envisaged by Thordike's Law of Effect (Thorndike, 1911). This raises a key issue of how rats are solving the avoidance task. Do they learn the contingency between their actions and the aversive outcome forming a goal-directed Action–Outcome (A–O) memory, or do they perform the response when in the context via S –R habit? In the present study, whether behaviour is mediated by A–O or S–R associations cannot be determined as there are no assays to determine this in AA paradigms. Furthermore the clicker cannot be devalued in the same manner as rewards in appetitive studies (Dickinson, 1985). It is believed A–O memories

encode contingency (Dickinson & Balleine, 1994; Yin *et al.*, 2006) and since the improvement in reactivated-saline group performance manifested significantly in response contingency, rather response frequency (although responses were generally increased), then this might suggest the contingency-encoding A–O memory was updated/strengthened via reconsolidation. A key problem for an A–O-updating interpretation is that the contingency was well-learned and thus should not have needed updating (see earlier). Another possibility is that responding was goal-directed during training, then at reactivation the absence of the clicker reinforced the jump response forming, and consolidating, a new S–R memory. One problem for this interpretation is that behaviour was at, or near, plateau by the end of training; typically well trained memories are habitual (Dickinson, 1985; Yin & Knowlton, 2006). If S–R memory was already learned during training then it may have undergone reconsolidation-mediated strengthening during reactivation. One problem with a habit-strengthening interpretation is that the behavioural effect of reactivation could be mediated by formation (or strengthening) of pavlovian context–incentive, which would increase vigour. Either or both of these learning processes could explain the pattern of data, and seem the most likely interpretations of the present results.

In summary the present data demonstrate that MK-801 can successfully impair expression of aversive memory. In the CPA study administration of MK-801 prior to the non-confined reactivation appeared to disrupt the reconsolidation of place aversion memory; however the transient effect with the confined reactivation did not appear to be due to impairment of reconsolidation. The likely ability of the non-confined condition to trigger destabilisation of the memory trace may have been linked to a requirement for memory updating in this condition. MK-801 also impaired learning in the AA paradigm; however it is unclear whether this was due to impairment of either consolidation or reconsolidation processes. Furthermore, whether this learning deficit occurred in pavlovian or instrumental memory is also unclear, however the potency of MK-801 was successfully demonstrated. One outstanding question is whether the presence of the reinforcer during the reactivation of AA memory would have been required to trigger destabilisation and reconsolidation, or whether this would have provided a “stronger” reactivation, enhancing the observed effect.

Presentation of the clicker could signal a change in contingency of reinforcer delivery, and this has been shown to trigger destabilisation of memory in studies of well-learned cued fear. Reinforcer-presentation may also have been necessary to elicit reconsolidation in the confined CPA reactivation. It is hypothesised here that changes in incentive motivation are sufficient to trigger destabilisation and reconsolidation-mediated updating; however the dependence of this upon reinforcer presentation during the reactivation session is unclear.

CHAPTER 4

RECONSOLIDATION OF A GOAL-DIRECTED INSTRUMENTAL MEMORY

Introduction

Following initial learning memories are unstable and vulnerable to amnesia, requiring a phase of consolidation in order to become stable (McGaugh, 2000). Once consolidated memories become fixed, but are not immutable. Memories are in fact dynamic in nature and undergo cycles of destabilisation and reconsolidation (Nader, 2003) in order to be strengthened (Lee, 2008) and updated with new information (Lee, 2010). Memory reconsolidation has been shown to occur in a multitude of experimental settings (see general introduction); however, despite the incentive learning that modulates instrumental responding undergoing reconsolidation (Wang *et al.*, 2005), no published studies have yet demonstrated the reconsolidation of the memories underlying instrumental behaviour (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009).

One confound in interpreting past studies of instrumental reconsolidation is that operant behaviour can be supported by both goal-directed Action–Outcome (A–O) or habitual Stimulus–Response (S–R) learning (Dickinson, 1985). If the reconsolidation of one of these traces disrupted, theoretically the remaining association could compensate, preventing amnesia from being observed. To avoid this issue we produced a weakly-learned lever pressing memory, using restricted amounts of training that was likely to produce A–O-mediated behaviour (Adams, 1982). This used a similar method to Chapter 2; however the lever-retraction was removed, owing to its ability to act as a conditioned stimulus (Davey *et al.*, 1981). The inactive lever was also removed to bring the training conditions in line with past research which created a goal-directed lever pressing memory (Adams, 1982). Whether responding was goal-directed following training was tested using reward devaluation, as A–O and S–R memories can be distinguished by their sensitivity to reward value (Adams, 1982; Dickinson, 1985).

Based upon current literature instrumental memories do not appear to destabilise following a brief extinction reactivation (Lee & Everitt, 2008b), reinforcer exposure (Wang *et al.*, 2005) or a training trial (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). This contrasts with literature from other settings in which brief extinction (Nader *et al.*, 2000; Milton, Lee, & Everitt, 2008), outcome exposure (Wang *et al.*, 2005) and training trials (Duvarci & Nader, 2004; Eisenberg & Dudai, 2004; Milekic *et al.*, 2006) have all been shown to destabilise memory traces. As we were unsure what reactivation parameters would be sufficient to induce destabilisation of the instrumental trace, we tested a variety of reactivation parameters, using past work (Chapter 2) and the hypothesis that reconsolidation mediates memory updating (Lee, 2009) as a starting point. The efficacy of these behavioural conditions to destabilise the instrumental memory was initially verified using systemic injections of the non-competitive N-methyl-D-aspartate receptor (NMDAR) antagonist MK-801; given the seemingly universal dependence of reconsolidation on NMDARs (Lee *et al.*, 2006a; Brown *et al.*, 2008; Milton, Lee, Butler, *et al.*, 2008). If memory was successfully destabilised then MK-801 should impair its reconsolidation, resulting in amnesia and a behavioural reduction in lever pressing.

Following the successful determination of reactivation parameters to destabilise the lever pressing memory we then progressed to intra-cerebral infusions targeted at the nucleus accumbens (NAc). Initial consolidation of instrumental memories has been shown to depend upon protein synthesis (Hernandez *et al.*, 2002) and activation of c-AMP dependent protein kinase (Baldwin, Sadeghian, Holahan, *et al.*, 2002) within the NAc core; furthermore NMDAR transmission (Kelley *et al.*, 1997) and dopamine-1 receptor (D1R) activity (Smith-Roe & Kelley, 2000; Hernandez *et al.*, 2005) are necessary in the NAc in order for lever pressing to be acquired. While the striatum has been strongly linked to the behavioural expression of instrumental behaviours (Balleine & O'Doherty, 2010), protein synthesis in the dorsolateral striatum is not required for initial consolidation of a lever pressing memory (Hernandez *et al.*, 2002). Based on Hernandez *et al.* (2002), we selected the NAc as the target of intra-cerebral infusions, as sensitivity to protein synthesis is frequently viewed as the canonical demonstration of consolidation. Furthermore our choice of infusion drug and dose was heavily influenced by Smith-Roe & Kelley (2000), past research which was carried out in the NAc.

We chose to infuse the competitive NMDAR antagonist AP-5 and the D1R antagonist SCH23390 in order to assess any potential role for local activation of D1Rs and NMDARs in the reconsolidation of instrumental memories, as accumbal activity via these receptors is implicated in the acquisition of lever pressing (Smith-Roe & Kelley, 2000). Furthermore, we co-infused low doses of AP-5 and SCH23390 based upon Smith-Roe & Kelley (2000), in order to investigate any potential role for co-activation of D1Rs and NMDARs in the reconsolidation of a lever pressing memory. Finally, we also infused MK-801, in order to determine whether the NAc was the central locus of action of systemic MK-801 used in the earlier experiments.

Materials and Methods

Subjects

Subjects were 188 male Lister-Hooded rats (Charles River), weighing 200-350g at the start of the experiment. Rats were housed in cages of 4 at 21^{o}C on a 12-hour light-dark cycle (lights on at 0700) and fed a restricted diet of 15g per day. Water was freely available except during experimental procedures. All procedures were carried out in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act (PPL 40/3205).

Initially 16 rats were used in the reward devaluation study in order to confirm behaviour was goal-directed. 3 rats were excluded due to insufficient performance (less than 30 lever presses on the second training session).

92 rats were used for the systemic studies, however 19 were excluded due to insufficient training performance (fewer than 30 lever presses on the second training day).

For the intra-cranial study, 80 rats were used with 25 excluded due to insufficient learning. An additional 3 rats were killed following surgical complications during the recovery phase and did not start the experiment. A further 11 rats had bent or blocked cannulae and were excluded from the analysis.

Drugs

LiCl (Sigma-Aldrich) was dissolved in deionised water to a concentration of 0.12M. During devaluation rats were administered a dose of 10ml/kg paired with the reward pellets.

In the systemic studies, MK-801 (AbCam) was dissolved in sterile saline to a concentration of 0.1mg/ml. 30 minutes prior to the memory reactivation session, rats were administered intraperitoneally with 0.1mg/kg of MK-801 or equivalent volume of saline vehicle.

For intra-cerebral infusions, all drugs were dissolved in sterile PBS. AP-5 (AbCam), SCH23390 (RBI, USA) and MK-801 were made to a concentration of 1µg/0.5µl. The combined AP-5/SCH23390 solution was made up to 0.1µg/0.5µl of each AP-5 and SCH23390. These doses were chosen based on previous literature (Kelley *et al.*, 1997; Smith-Roe & Kelley, 2000). Immediately prior to the memory reactivation session, injectors were inserted into the guide cannulae and 0.5µl of drug or PBS vehicle was infused at a rate of 0.5µl/min using a microdrive syringe pump (Harvard Apparatus). Injectors were left in place for one minute after the infusion to allow diffusion of the drug. Infusions were given immediately before, rather than after reactivation as past work has shown that pre- (Kelley *et al.*, 1997; Smith-Roe & Kelley, 2000) but not post-session (Hernandez *et al.*, 2005) infusions of AP-5 or SCH23390 into the NAc Core impair instrumental acquisition.

Instrumental training

Training, memory reactivation and testing sessions took place in 8 operant boxes (MedAssociates) as described in Chapter 2. During experimental sessions the houselight was on and only the left lever was extended into the box.

Rats were initially trained to collect 45mg sucrose reward pellets (TestDiet) from the magazine. Initially, pellets were delivered at random intervals (mean 60 seconds) for 15 minutes with the lever retracted. This pre-training facilitated instrumental learning over the limited training schedule. Instrumental training began immediately after the pre-training session. On the first training day (T1)

the left lever was extended into the box and delivered a sucrose pellet into the magazine when pressed, on a fixed-ratio (FR1) schedule; the lever did not retract and no discrete stimuli were presented at any point during training. The session ended when a maximum of 30 pellets had been obtained or 30-minutes had elapsed. Rats received a second 30-minute training session the next day (T2) with a maximum of 60 pellets obtainable. The limited duration of training was intended to produce a weak goal-directed memory as a maximum of only 90 rewards could be obtained, fewer than the 100 used previously to produce goal-directed responding (Adams, 1982).

Reward Devaluation

In order to confirm behaviour was goal-directed following training, the reward pellets were devalued in a preliminary cohort of rats by pairing the pellets with lithium-induced gastric malaise. Rats received two devaluation sessions over two days, during which they were placed individually in a holding cage with free access to sucrose pellets. Following a 5-minute free-feeding period rats received intraperitoneal injections of LiCl or saline, after which they were allowed a further 10 minutes of unrestricted access to sucrose pellets. The next day instrumental performance was assessed in a 30-minute extinction session.

Systemic Studies

In the systemic studies a variety of behavioural conditions were tested for their efficacy to cause memory destabilisation. Rats were trained for two days (as above), then the day after training given systemic MK-801 30 minutes prior to one of three reactivation sessions.

Brief non-reinforced reactivation: A very brief 2-minute extinction session; no rewards were delivered. The duration of this session was reduced compared to Chapter 2 as systemic MK-801 did not have any effect previously with 5-minutes of extinction.

Brief FR1 reactivation: A brief 20-minute FR1 session, identical to training but curtailed to a maximum of 20 pellets. This session was intended to test the ability of a training trial to destabilise memory in a goal-directed setting, for comparison to past instrumental reconsolidation studies of well-trained memory (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). Reinforcer-exposure has also sometimes been necessary for memory to destabilise in other appetitive settings (Milekic *et al.*, 2006; Valjent *et al.*, 2006).

VR5 reactivation: A 20-minute session in which lever presses were rewarded on a variable-ratio (mean: 5, range: 1-9) schedule (VR5). Changes in temporal contingency have been shown to destabilise pavlovian fear memory (Díaz-Mataix *et al.*, 2013); the VR5 condition was intended to test whether changes in instrumental A–O contingency would also engage reconsolidation.

Non-reactivation controls: Additional rats were used which received systemic injection of MK-801 or co-infusion of AP-5/SCH23390 (or appropriate vehicle control), but without any behavioural session.

Instrumental performance was tested the day after drug treatment in a 30-minute extinction session. The lever was extended, but no rewards were delivered.

Short-term memory testing: One additional group of rats received its test session 3 hours after reactivation. This was carried out to assess any effect of the MK-801 on post-reactivation short-term memory (pr-STM).

Intra-cranial infusions

80 rats were anesthetised using isoflurane (5% for induction of anaesthesia, 3% for maintenance), fixed within a stereotaxic frame (RWD Life Science), and implanted bilaterally with stainless steel cannulae (11mm, 22-gauge, Coopers Needleworks) directed at the NAc region of the brain (AP +1.5mm, ML ±1.8mm from bregma, DV -1.8mm from skull surface, based on the rat brain atlas by Paxinos & Watson, 2009). Cannulae were fixed in place using dental cement attached to three jewellers screws affixed to the skull, and stainless steel stylets extending 1mm past the end of the

cannulae were inserted into the cannulae post-surgery in order to maintain their patency until infusion. Rats were administered peri-operative buprenorphine. A minimum of five days recovery was allowed before experimental procedures began.

Rats were trained for two days (as above) to press the lever for sucrose pellets. The day after training stylets were removed and injectors (Plastics One) inserted into the guide cannulae (28-gauge, extending 6mm past the end of the cannulae to a final DV -7.8mm). Rats received a bilateral infusion of PBS, AP-5, SCH23390, a combined AP-5/SCH23390 infusion, or MK-801 into the NAc. Immediately following the infusion rats were returned to the experimental boxes and their memory reactivated using the VR5 reactivation procedure (as in the previous systemic studies). The following day memory performance was tested in a 30-minute extinction test.

Non-reactivation control: In order to confirm whether the effect of AP-5/SCH23390 co-infusion was reactivation-dependant (and thus due to an effect on reconsolidation), and additional group of rats received surgery and training as before; however they were returned to the home cages following co-infusion of AP-5/SCH23390 and were not given any behavioural reactivation session. Performance was tested the next day in a 30-minute extinction test.

At the end of the experiment cannulated rats were killed, their brains extracted freshly and fixed in 4% paraformaldehyde. Brains were sectioned then stained using cresyl violet and the locations of injectors confirmed using light microscopy.

Statistical Analysis

Experimental groups were matched for number of lever presses made during training. Rats which failed to obtain at least 30 rewards on the second day of training were excluded from analysis due to lack of learning. Training data were analysed using repeated measures Analysis of Variance (ANOVA) with Training day as a factor to assess whether the task was learned and whether groups were equally performing at the end of training. Results with $p<0.05$ were deemed significant.

For the devaluation study, test data were analysed using one-way ANOVA with Treatment as a factor. In the systemic experiments, reactivation and test sessions were analysed separately using one-way ANOVA for rats given brief extinction or training sessions. For intra-cerebral infusion groups reactivation and test data was compared to a single control using bonferroni-corrected planned-comparisons (effective $p<0.0125$). Non-reactivated rats were compared to their respective reactivated counterparts using two-way ANOVA with Reactivation and drug Treatment as factors.

Additional analysis was performed for each test session in which the data were divided into 5-minute bins to assess rates of extinction. Data were analysed with a two-way ANOVA with Extinction bin and drug Treatment as factors. For this analysis a Greenhouse-Geisser correction was used, where appropriate, to correct for non-spherical data.

Similar analysis was performed on nosepoking behaviour. This was done as a measure of overall motivation during sessions.

Results

Reward Devaluation

Reward devaluation was used to determine whether rats were goal-directed following training. During training (Figure 4.1A), ANOVA revealed a significant increase in lever pressing ($F_{(1,11)}=368.5$, $P<0.001$) indicating that rats learned successfully. There were no significant differences between treatment groups, indicating they were well matched (Treatment: $F_{(1,11)}=1.12$, $p=0.312$, Training x Treatment: $F_{(1,11)}=0.495$, $p=0.496$). Following devaluation, ANOVA revealed a significant reduction in lever pressing (Figure 4.1B) in the LiCl-paired group at test ($F_{(1,11)}=5.48$, $p=0.039$).

Analysis of nosepokes during training (Figure 4.2A) revealed a significant increase over Training ($F_{(1,11)}=14.61$, $p=0.003$) with no difference between groups (Treatment: $F_{(1,11)}=0.56$, $p=0.470$; Training x Treatment: $F_{(1,11)}=0.07$, $p=0.801$). At test nosepokes were significantly reduced ($F_{(1,11)}=14.74$,

p=0.003) in rats which received LiCl-pairings (Figure 4.2B), consistent with devaluation of the reward pellets.

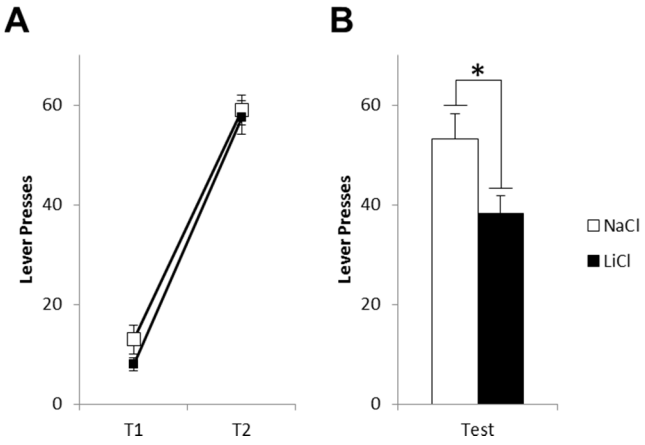

Figure 4.1: Reward-devaluation caused a significant reduction in responding at test. **A**, the lever pressing response was acquired to equivalent levels by both LiCl-paired (black squares) and NaCl-control (white squares) groups during training. A maximum of 30 pellets could be obtained on training day 1 (T1), and 60 on training day 2 (T2). **B**, pairing of the reinforcer with LiCl (black, n=6) significantly reduced lever pressing performance at test compared to NaCl-paired controls (white, n=7). This demonstrated that the training protocol produced behaviour that was goal-directed. Data are represented as mean number of lever presses ± SEM. (*) indicates significant differences, $p<0.05$.

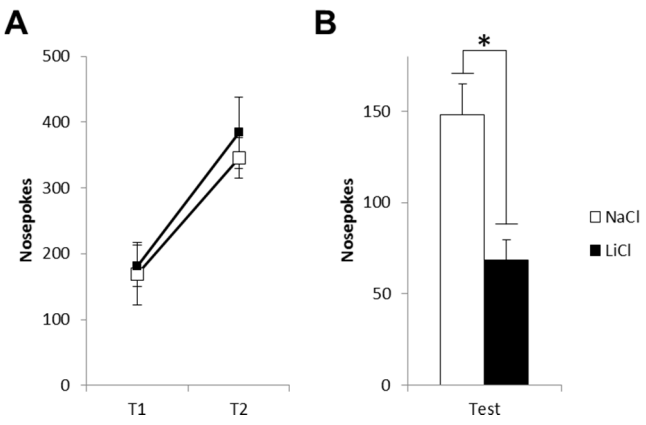

Figure 4.2: Pairing of the reward with LiCl impairs nosepoking behaviour. **A**, during training both LiCl (black squares) and NaCl (white squares) groups nosepoked at equivalent levels on both the first (T1) and second (T2) training session. **B**, when tested, the LiCl-devalued group (black bar, n=6) significantly decreased their nosepoke responses compared to NaCl-controls (white bar, n=7). This reduction is likely due to a lack of motivation to acquire the pellets, consistent devaluation of the reward. Data are represented as mean ± SEM. (*) indicates significant differences, $p<0.05$.

Systemic Studies

Brief non-reinforced reactivation

Initially, we tested the effect of MK-801 using a non-reinforced reactivation procedure analogous to that which is commonly employed in pavlovian reconsolidation studies. Lever pressing increased over Training ($F_{(1,12)}=698.0$, $p<0.001$) indicating rats learned (Figure 4.3A). A Training x Treatment interaction ($F_{(1,12)}=5.04$, $p=0.044$) was revealed with no main effect of Treatment ($F_{(1,12)}=0.06$, $p=0.815$). Analysis of simple effects on the second training day showed no significant difference in performance ($F_{(1,12)}=1.49$, $p=0.245$), indicating groups were matched at the end of training. The next day, rats were administered MK-801 or vehicle and given 2-minutes of extinction intended to destabilise their memory, and then tested on the next day. Analysis revealed significantly increased responding in MK-801-injected rats at both reactivation ($F_{(1,12)}=14.19$, $p=0.003$; Figure 4.3B) and test ($F_{(1,12)}=8.23$, $p=0.014$; Figure 4.3C). There was also a significant effect of Extinction ($F_{(5,60)}=9.27$,

p<0.001) and also an Extinction x Treatment interaction ($F_{(2.82,33.87)}$=4.76, p=0.008) during the test. Thus, the brief extinction reactivation did not appear to destabilise A–O memory.

Rats increased their nosepoking (Figure 4.4A) over Training ($F_{(1,12)}$=31.45, p<0.001) with no group differences (Treatment: $F_{(1,12)}$=2.62, p=0.132; Training x Treatment: $F_{(1,12)}$=0.09, p=0.765). During reactivation (Figure 4.4B) MK-801-injected rats nosepoke significantly more than saline controls ($F_{(1,12)}$=6.44, p=0.026). The effect of MK-801 had dissipated the next day (Figure 4.4C) with no significant effect of Treatment at test ($F_{(1,12)}$=1.37, p=0.264).

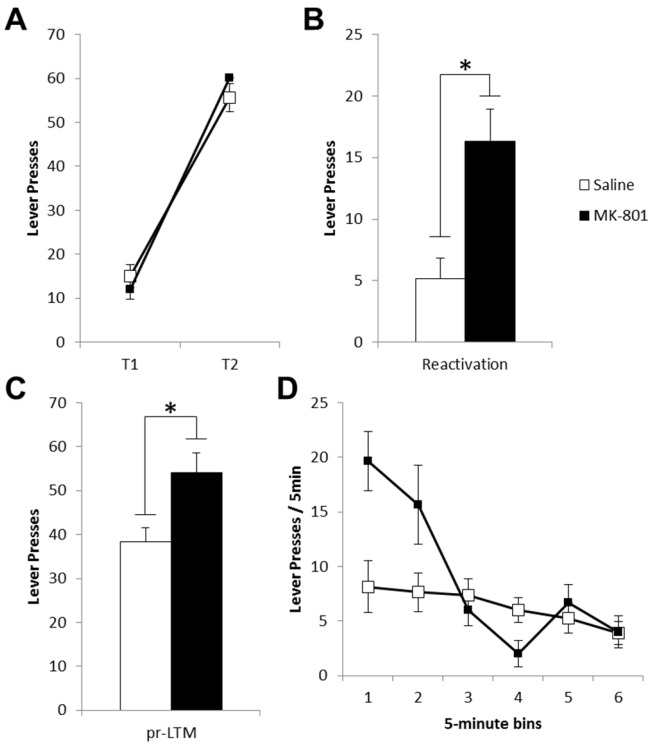

Figure 4.3: MK-801 did not impair instrumental reconsolidation when administered prior to a brief non-reinforced reactivation. **A**, rats were trained to press a lever for food over two sessions. A maximum of 30 rewards could be obtained during the first session (T1), and 60 on the second (T2). Both the MK-801 (black squares) and saline control (white squares) groups acquired the task to similar levels by the end of the training phase. **B**, MK-801 treated rats (black bar, n=6) lever pressed significantly more during the 2-minute non-reinforced reactivation session than saline vehicle controls (white bar, n=8). **C**, MK-801-injected (black bar) rats also lever pressed significantly more than saline controls (white bar) the next day at test. **D**, within-session extinction data during the test session showed saline-treated animals (white squares) showed impaired responding from the beginning of the session, compared to MK-801 rats (black squares). Data are represented as mean number of lever presses ± SEM. (*) indicates significant differences, $p<0.05$.

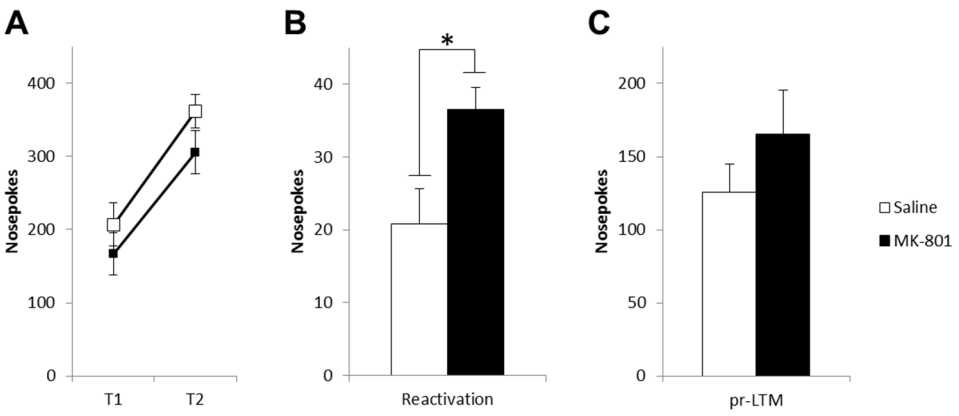

Figure 4.4: MK-801 caused an acute increase in nosepokes during the brief non-reinforced reactivation. **A**, MK-801 (black squares) and saline (white squares) treatment groups did not differ in nosepoke behaviour during training, on either the first (T1) or second (T2) day of training. **B**, at reactivation MK-801 (black bar, n=6) significantly augmented nosepoke responses compared to saline vehicle (white bar, n=8). **C**, the effect of MK-801 did not persist over 24 hours as both MK-801 (black bar) and saline-injected (white bar) groups return to equivalent levels of nosepokes at test. Data are represented as mean ± SEM. (*) indicates significant differences, $p<0.05$.

Brief FR1 reactivation

Groups learned similarly during instrumental training (Figure 4.5A; Training: $F_{(1,10)}=341.8$, $p<0.001$; Treatment: $F_{(1,10)}=0.54$, $p=0.481$; Training x Treatment interaction: $F_{(1,10)}=0.57$, $p=0.468$). At reactivation, all rats made the maximum of 20 lever presses (Figure 4.5B). At test (Figure 4.5C), rats extinguished during the session (Extinction: $F_{(5,50)}=14.37$, $p<0.001$) but there were no group differences (Treatment: $F_{(1,10)}=0.90$, $p=0.365$; Extinction x Treatment: $F_{(1.90,19.03)}=0.91$, $p=0.414$). Therefore, the FR1 reactivation was also not effective in destabilising the A–O trace.

ANOVA of nosepoking revealed a significant increase with Training ($F_{(1,10)}=41.53$, $p<0.001$; Figure 4.6A) with no group differences (Treatment: $F_{(1,10)}=0.46$, $p=0.514$; Training x Treatment: $F_{(1,10)}=1.18$, $p=0.304$). MK-801-injection caused a moderate, but non-significant rise in nokespokes (Figure 4.6B)

during the brief FR1 session ($F_{(1,10)}$=3.92, p=0.076); however had no long-term effect on nosepokes at test ($F_{(1,10)}$=0.004, p=0.949; Figure 4.6C).

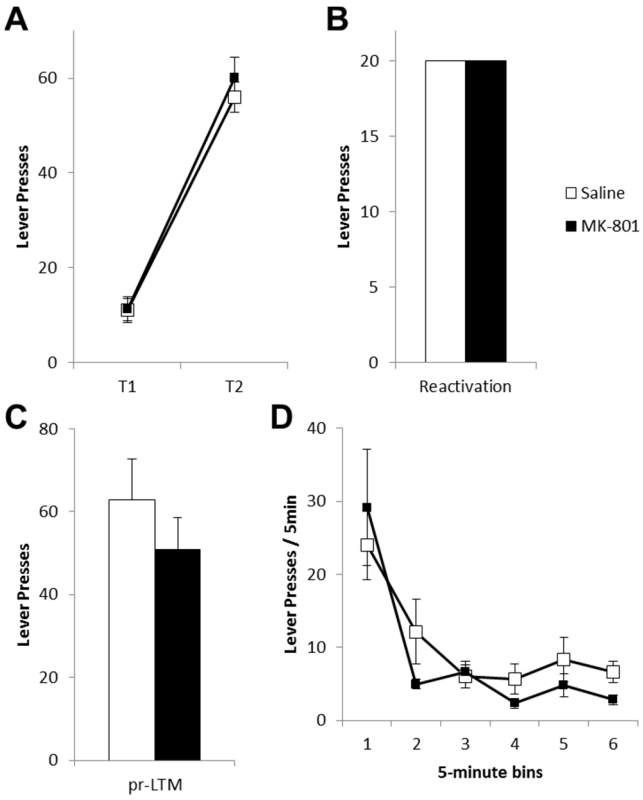

Figure 4.5: MK-801 did not impair instrumental reconsolidation when administered prior to a brief FR1 session. **A**, rats in both the saline (white squares) and MK-801 (black squares) acquire the lever press response to similar levels over the first (T1) and second (T2) days of training. A maximum of 30 rewards could be obtained on T1, 60 on T2. **B**, at memory reactivation all rats in both the MK-801 treated (black bar, n=6) and saline vehicle (white bar, n=6) groups obtained the maximum number of 20 pellets available during the reactivation session. Pellets were delivered on the same FR1 schedule as in the training phase. **C**, when tested 24 hours after reactivation, MK-801-injected (black bar) rats had no significant impairment of instrumental performance compared to saline (white bar) controls. **D**, both MK-801 (black squares) and saline-injected (white squares) groups extinguished at similar rates during the test session. Data are represented as mean ± SEM.

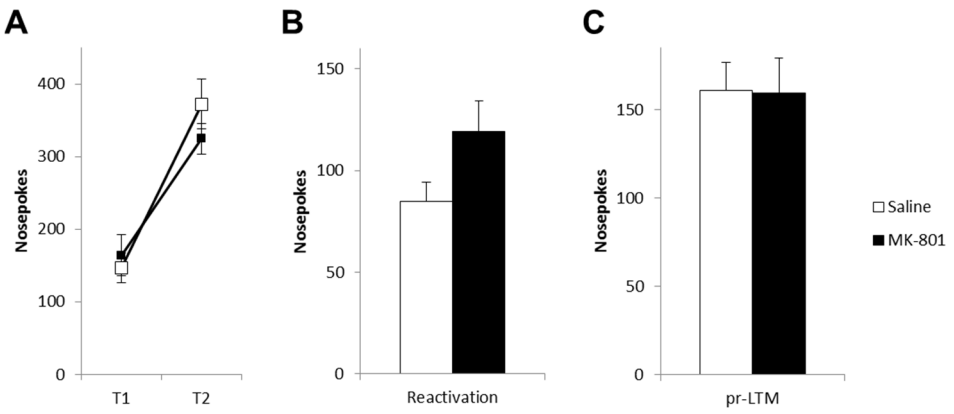

Figure 4.6: There was no significant difference in nosepoking on any session in rats given a brief FR1 training reminder reactivation. **A**, nosepokes increased over both the first (T1) and second (T2) training days, with no differences between saline (white squares) and MK-801 (black squares) groups. **B**, at reactivation MK-801 (black bar, n=6) produced a moderate but non-significant rise in nosepokes compared to saline controls (white bar, n=6). **C**, when tested 24 hours later, both MK-801 (black bar) and saline-injected (white bar) groups showed equivalent nosepoke behaviour. Data are represented as mean ± SEM.

VR5 reactivation

As reconsolidation is hypothesised to mediate memory updating (Lee, 2009), we next used a VR5 reactivation in which the reinforcement contingency was altered; we also included a non-reactivation control condition. There were no group differences during training (Figure 4.7A), with all groups showing an increase in responding (Training: $F_{(1,27)}$=1244.0, p<0.001; Treatment: $F_{(1,27)}$=0.02, p=0.895; Reactivation: $F_{(1,27)}$=1.61, p=0.215; Treatment x Reactivation: $F_{(1,27)}$=0.17, p=0.684; Training x Treatment: $F_{(1,27)}$=2.48, p=0.128; Training x Reactivation: $F_{(1,27)}$=0.64, p=0.430; Training x Treatment x Reactivation: $F_{(1,27)}$=1.20, p=0.282). There was also no significant difference between MK-801 and saline-treated reactivated groups during the VR5 (Figure 4.7B) session ($F_{(1,14)}$=0.56, p=0.468). At test (Figure 4.7C), however, there was a reactivation-dependent effect of MK-801 (Treatment x Reactivation: $F_{(1,27)}$=4.53, p=0.042), but with no significant effect of Treatment ($F_{(1,27)}$=0.55, p=0.464) or Reactivation ($F_{(1,27)}$=0.01, p=0.908). Analysis of simple main effects showed a significant effect of

MK-801 in reactivated rats ($F_{(1,14)}$=6.02, p=0.028), but not in non-reactivated controls ($F_{(1,13)}$=0.71, p=0.415). This reactivation-dependent amnestic effect indicated that MK-801 impaired instrumental memory reconsolidation under these experimental conditions. All groups extinguished at similar rates during the test (Extinction: $F_{(5,135)}$=30.42, p<0.001; Extinction x Treatment: $F_{(3.26,88.09)}$=1.19, p=0.319; Extinction x Reactivation: $F_{(3.26,88.09)}$=1.85, p=0.138; Extinction x Treatment x Reactivation: $F_{(3.26,88.09)}$=0.87, p=0.465).

Nosepokes significantly increased (Figure 4.8A) during training ($F_{(1,27)}$=126.28, p<0.001). There was also a significant main effect of Reactivation ($F_{(1,27)}$=4.69, p=0.039) with a significant Treatment x Reactivation interaction ($F_{(1,27)}$=6.21, p=0.019) . There were no other significant effects during training (Treatment: $F_{(1,27)}$=1.24, p=2.76; Training x Treatment: $F_{(1,27)}$=2.35, p=0.137; Training x Reactivation: $F_{(1,27)}$=0.40, p=0.532; Training x Treatment x Reactivation: $F_{(1,27)}$=0.03, p=0.869). Analysis of T2 did not reveal any group differences in nosepoking indicating group behaved similarly at the end of training (Treatment: $F_{(1,27)}$=0.20, p=0.655; Reactivation: $F_{(1,27)}$=0.74, p=0.396; Treatment x Reactivation: $F_{(1,27)}$=2.74, p=0.109). No acute effect of MK-801 was observed (Figure 4.8B) during the VR5 reactivation ($F_{(1,14)}$=0.99, p=0.336). Nosepokes at test (Figure 4.8C) did not significantly differ between groups (Treatment: $F_{(1,27)}$=1.01, p=0.323; Reactivation: $F_{(1,27)}$=1.03, p=0.320; Treatment x Reactivation: $F_{(1,27)}$=0.10, p=0.755).

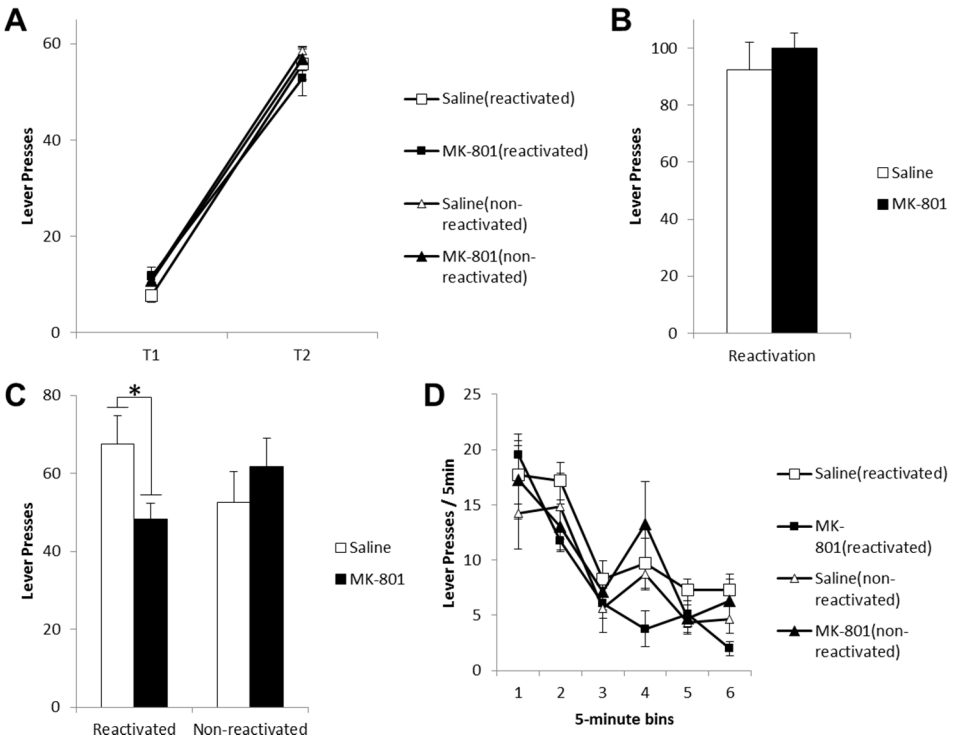

Figure 4.7: Systemic MK-801 impaired long-term instrumental performance in a reactivation-dependant manner. **A**, rats from reactivated (black squares) and non-reactivated MK-801 (black triangles) groups acquired the lever pressing response to similar levels as reactivated (white squares) and non-reactivated saline (white triangles) controls during the training phase. A maximum of 30 pellets could be obtained on the first day of training (T1), 60 on the second (T2). **B**, at reactivation rats were returned to the experimental chambers and allowed to press the lever for pellets, as in training; however the reward contingency was changed to a VR5 schedule, with a maximum of 20 rewards available during the session. MK-801 treated rats (black bar, n=9) performed at a similar level to saline vehicle controls (white bar, n=7). Non-reactivated control rats were given saline (n=8) or MK-801 (n=7) in the absence of any reactivation session. **C**, when tested 24 hours after reactivation, MK-801-injected rats (black bars) showed significantly impaired overall instrumental performance compared to saline controls (white bars). This effect only occurred when MK-801 was given in conjunction with a VR5 reactivation session (left), but not in the absence of a memory reactivation session (right). **D**, examination of within-session extinction from the test session suggests the effect was mostly driven by reduced performance of the reactivated, MK-801 treated (black squares) group in the latter half of the session, compared to reactivated saline (white squares),

non-reactivated saline (white triangles) and non-reactivated MK-801 (black triangles) controls. Data are represented as mean ± SEM. (*) indicates significant differences, p<0.05.

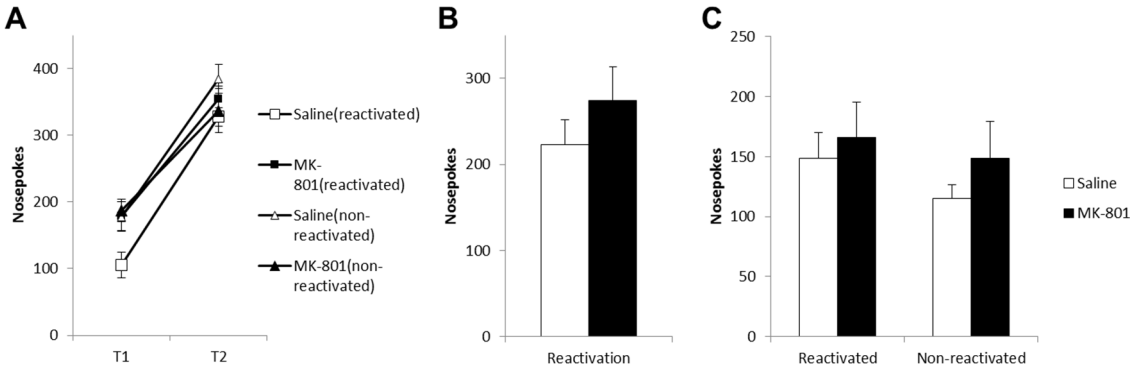

Figure 4.8: There were no significant differences in nosepoking between rats given VR5 or no reactivation. **A**, reactivated MK-801 (black squares, n=9), non-reactivated saline (white triangles, n=8) and non-reactivated MK-801 (black triangles, n=7) groups demonstrated similar nosepoking performance on the first day of training (T1), while the nokespoking of reactivated saline (white squares, n=7) group was significantly lower on this first day; however all groups demonstrated similar levels of nosepoke performance on the final day of training (T2). **B**, there was no significant difference in nosepoking between saline (white bar) and MK-801 (black bar) treatment groups during the VR5 reactivation session. **C**, at test, 24 hours after reactivation, previously MK-801-treated (black bars) showed similar levels of nosepoking to saline controls, regardless of whether rats were reactivated (left) or non-reactivated (right). Data are represented as mean ± SEM.

Short-term memory testing

As injections were given prior to reactivation, an additional group of rats were trained and tested 3 hours after receiving the VR5 reactivation. During training (Figure 4.9A), groups showed a similar increase in responding (Training: $F_{(1,14)}$=461.37, p<0.001; Treatment: $F_{(1,14)}$=1.08, p=0.317; Training x Treatment: $F_{(1,14)}$=0.58, p=0.461). There was no overall effect of Treatment on lever pressing at either Reactivation ($F_{(1,14)}$=2.24, p=0.157; Figure 4.9B), or pr-STM ($F_{(1,14)}$=0.38, p=0.548; Figure 4.9C). Groups

did not shown any difference in extinction rate at pr-STM (Extinction: $F_{(5,70)}$=14.94, p<0.001; Extinction x Treatment: $F_{(1.74,24.30)}$=1.29, p=0.289).

Analysis of nosepoking showed groups similarly increased their nosepokes (Figure 4.10A) with training (Training: $F_{(1,14)}$=9.47, p=0.008; Treatment: $F_{(1,14)}$=0.001, p=0.974; Training x Treatment ($F_{(1,14)}$=0.01, p=0.927). MK-801 did not affect nosepoking at either reactivation ($F_{(1,14)}$=0.07, p=0.797; Figure 4.10B) or on the pr-STM test ($F_{(1,14)}$=2.47, p=0.139; Figure 4.10C).

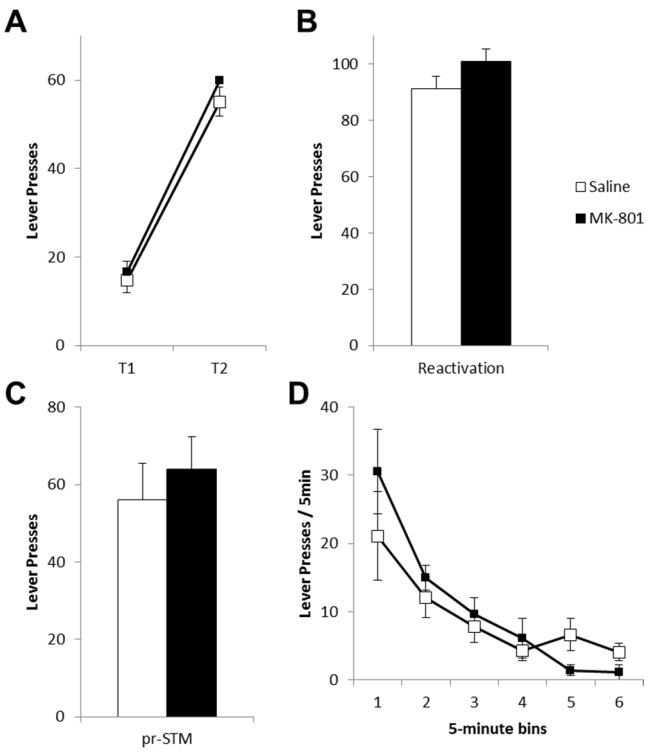

Figure 4.9: Treatment with systemic MK-801 was without effect on pr-STM. **A**, rats in both the saline (white squares) and MK-801 (black squares) treatment groups learned to press the lever successfully, and performed at similar levels on both the first (T1) and second (T2) day of training. A maximum of 30 reinforcements could be obtained on T1, 60 on T2. **B**, during the VR5 reactivation session, MK-801 treated (black bar, n=7) rats showed no difference in responding from vehicle controls (white bar, n=9). **C**, there was no lasting effect of drug treatment 3 hours after reactivation, with MK-801-injected (black bar) rats responding similarly to saline controls (white bar) in a test of pr-STM. **D**, within-session extinction bins during the pr-STM test showed MK-801 (black squares) and saline (white squares) groups extinguished at similar rates throughout the session. Data are represented as mean number of lever presses ± SEM.

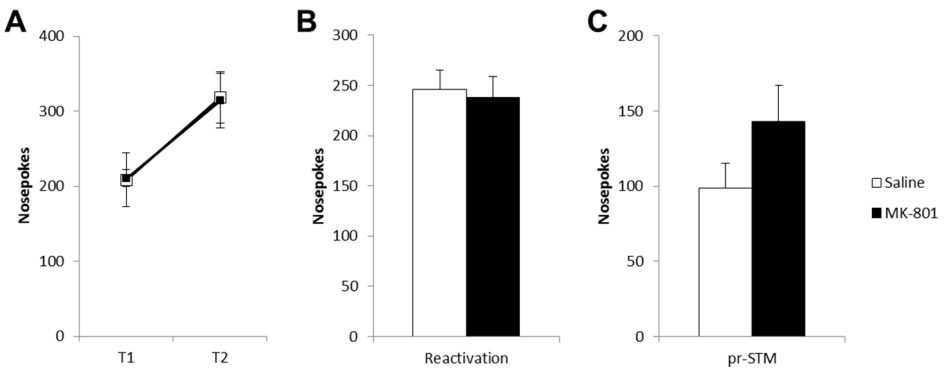

Figure 4.10: Systemic MK-801 did not significantly alter nosepoking behaviour at the pr-STM test. **A**, nosepoking behaviour did not differ between saline (white squares) or MK-801 (black squares) treatment groups during either the first (T1) or second (T2) training day. **B**, MK-801 (black bar, n=7) and saline-treated (white bar, n=9) groups nosepoked at similar levels during the VR5 reactivation session. **C**, during the pr-STM test (3 hours after reactivation) nosepoke responses of MK-801-injected rats (black bar) appear elevated compared to saline controls (white bar), however this difference was not significant. Data are represented as mean ± SEM.

Intra-cranial Infusions

Drug infusions

As the NAc is known to play a role in acquisition of lever pressing (Smith-Roe & Kelley, 2000; Hernandez *et al.*, 2002), we next infused NMDAR antagonists and/or the D1R antagonist SCH23390 directly into the NAc prior to the VR5 memory reactivation session. There were no group differences during training (Figure 4.11A; Training: $F_{(1,24)}$=711.3, p<0.001; Treatment: $F_{(4,24)}$=0.96, p=0.447; Training x Treatment: $F_{(4,24)}$=0.78, p=0.550).

As there was a single common PBS control, we conducted bonferroni-corrected planned-comparisons between the vehicle group and each drug group (effective p<0.0125). No groups significantly differed from the PBS control on the final day of training: MK-801 ($F_{(1,10)}$=0.16, p=0.700), AP-5 ($F_{(1,9)}$=0.82, p=0.389), SCH23390 ($F_{(1,10)}$=0.03, p=0.860), AP-5/SCH23390 ($F_{(1,10)}$=1.13, p=0.314). At reactivation (overall ANOVA: $F_{(4,24)}$=10.69, p<0.001) co-infusion of AP-5 and SCH23390 had a

significant acute effect (Figure 4.11B) to reduce instrumental performance ($F_{(1,10)}$=27.7, p<0.001), however no reduction in performance was observed with infusion of MK-801 ($F_{(1,10)}$=0.13, p=0.725), AP-5 ($F_{(1,9)}$=0.45, p=0.519) or SCH23390 ($F_{(1,10)}$=0.28, p=0.611) alone.

When tested 24-hours later, rats previously given the combined AP-5/SCH23390 infusion showed significantly impaired instrumental responding (Figure 4.11C). While there was no significant effect of infusion in the overall ANOVA ($F_{(4,24)}$=2.60, p=0.06), bonferroni-corrected planned comparison of the PBS and AP-5/SCH23390 groups revealed a significant reduction in lever pressing ($F_{(1,10)}$=14.1, p=0.004). In contrast, similar planned-comparisons revealed no long-term memory impairment in rats given infusions of MK-801 ($F_{(1,10)}$=0.05, p=0.823), AP-5 ($F_{(1,9)}$=0.65, p=0.441) or SCH23390 ($F_{(1,10)}$=0.03, p=0.871). Overall ANOVA of extinction bins at test revealed a significant effect of Extinction ($F_{(5,120)}$=20.08, p<0.001) with no interaction (Extinction x Treatment: $F_{(10.38,62.30)}$=1.35, p=0.224). Pair-wise comparisons showed all groups extinguished during the test (all F>5, p<0.01) with no interactions (all F<1.2, p>0.3).

ANOVA of nosepoke responses revealed a significant increase (Figure 4.12A) with training ($F_{(1,24)}$=42.00, p<0.001), with no group differences (Treatment: $F_{(4,24)}$=1.46, p=0.245; Training x Treatment: $F_{(4,24)}$=0.259, p=0.901). Overall ANOVA of the reactivation session (Figure 4.12B) revealed a significant effect of Treatment ($F_{(4,24)}$=8.14, p<0.001). Planned pair-wise comparisons showed co-infusion of AP-5/SCH23390 acutely impaired nosepokes ($F_{(1,10)}$=23.68, p<0.001). There was no acute effect with infusion of MK-801 ($F_{(1,10)}$=1.02, p=0.335), AP-5 ($F_{(1,9)}$=2.51, p=0.147) or SCH23390 ($F_{(1,10)}$=0.30, p=0.596). At test (Figure 4.12C) overall ANOVA did not show any evidence the treatments affected long-term nosepoking behaviour ($F_{(4,24)}$=1.42, p=0.257). Planned-comparisons compared to PBS vehicle controls did not reveal any significant long-term effect of MK-801 ($F_{(1,10)}$=0.03, p=0.875), AP-5 ($F_{(1,9)}$=5.198, p=0.049), SCH23390 ($F_{(1,10)}$=0.13, p=0.728) or combined AP-5/SCH23390 ($F_{(1,10)}$=4.51, p=0.060) at test.

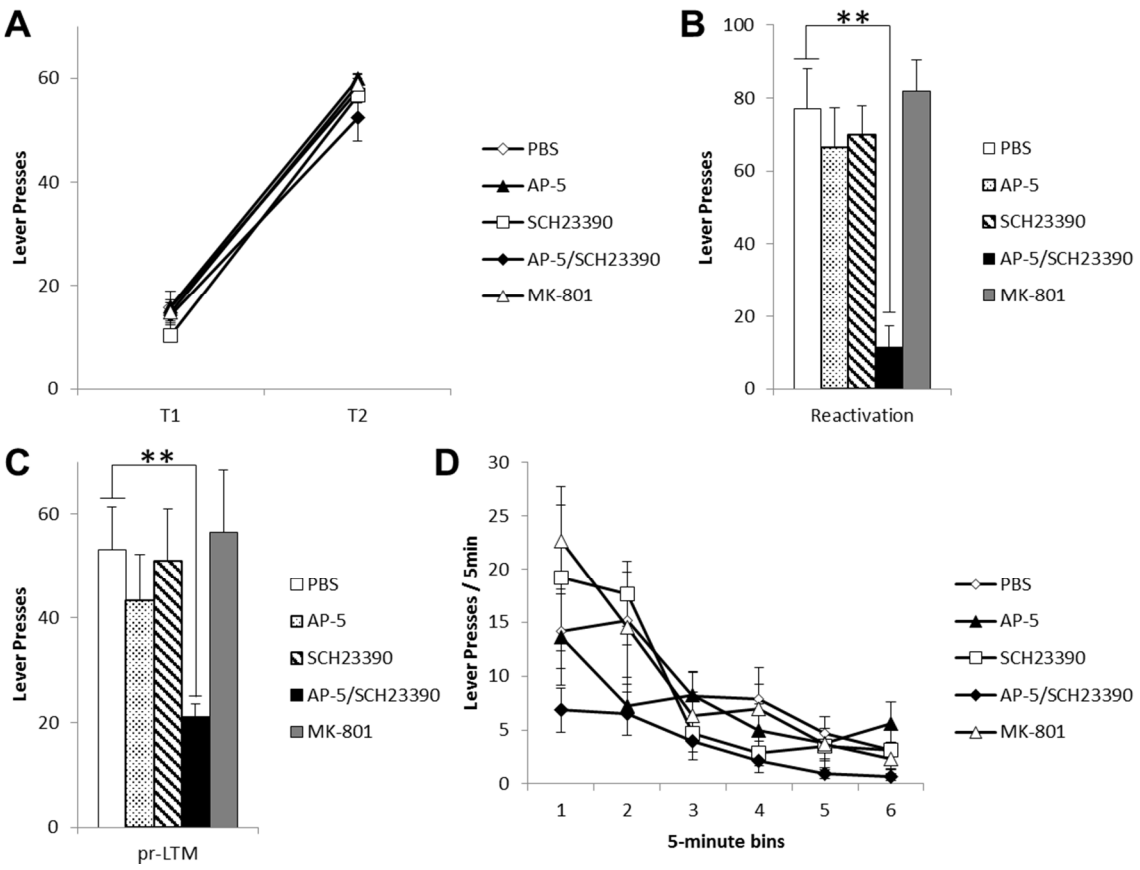

Figure 4.11: Combined infusion of AP-5/SCH23390 significantly impaired instrumental behaviour at test compared to PBS infused controls. **A**, the lever pressing response was learned over days 1 (T1) and 2 (T2) of the training phase. 30 rewards were available on T1, 60 on T2. PBS (white diamonds), AP-5 (black triangles), SCH23390 (white squares), AP-5/SCH23390 (black diamonds) and MK-801 (white triangles) all acquired the task to similar levels of performance. **B**, immediately before the VR5 reactivation rats were given an infusion of either PBS (white bar, n=6), MK-801 (grey bar, n=6), AP-5 (dotted bar, n=5), SCH23390 (striped bar, n=6) or co-infusion of AP-5 and SCH23390 (black bar, n=6); co-infusion of AP-5/SCH23390 acutely impaired responding during the reactivation session compared to the PBS control group. **C**, the co-infusion of AP-5 and SCH23390 (black bar) also significantly impaired instrumental performance at test the next day compared to rats given PBS (white bar), MK-801 (grey bar) or infusions of AP-5 (dotted bar) and SCH23390 (striped bar) alone. **D**, within-session extinction bins of the test session showed the impairment caused by co-infusion of AP-5/SCH23390 (black diamonds) was present throughout the test. The remaining PBS (white diamonds), AP-5 (black triangles), SCH23390 (white squares) and MK-801 (white triangles) groups did not significantly differ

in their rates of extinction from both each other and the AP-5/SCH23390 infused group. Data are represented as mean ± SEM. (**) indicates the outcome of planned pairwise comparisons to the PBS control group, effective p<0.0125.

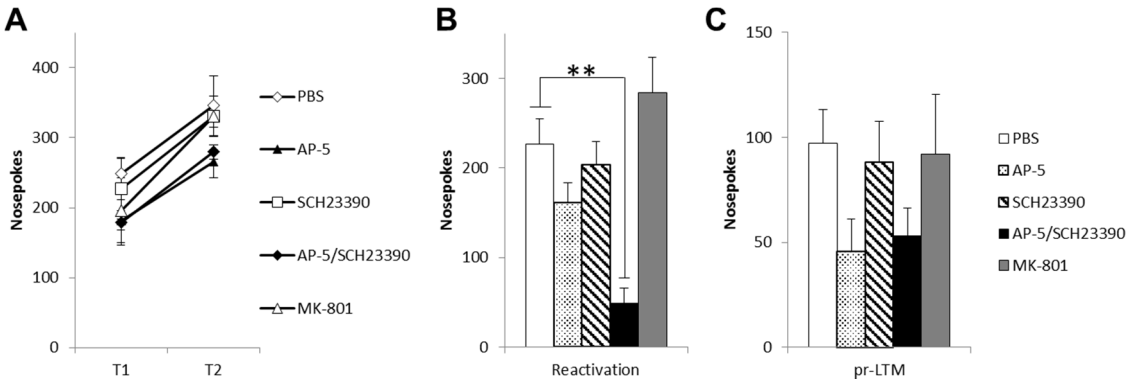

Figure 4.12: Combined infusion of AP-5/SCH23390 produced an acute deficit in nosepoke responses, with no long-term impairment. **A**, PBS (white diamonds), AP-5 (black triangles), SCH23390 (white squares), AP-5/SCH23390 (black diamonds) and MK-801 (white triangles) nosepoked at similar levels on both the first (T1) and second (T2) day of training. **B**, combined infusion of AP-5/SCH23390 (black bar, n=6) acutely impaired nosepoking at reactivation compared to the PBS control group (white bar, n=6). AP-5 (dotted bar, n=5), SCH23390 (striped bar, n=6) and MK-801 (grey bar, n=6) infused rats did not show any significant reduction in nosepokes compared to the PBS group. **C**, the effect of AP-5/SCH23390 co-infusion had dissipated 24 hours later, with no significant differences in the nosepokes of any group during the long-term memory test session. Data are represented as mean ± SEM. (**) indicates the outcome of planned pairwise comparisons to the PBS control group, effective p<0.0125.

Non-reactivation control

To test the reactivation dependence of AP-5/SCH23390 co-infusion, an additional group of rats were trained and given infusions in the absence of any behavioural session. Results were compared to the

reactivated PBS and AP-5/SCH23390 groups from the previous experiment. All rats acquired the task during Training ($F_{(1,20)}$=950.7, p<0.001). No significant differences were detected in Treatment ($F_{(1,20)}$=1.93, p=0.180), Reactivation ($F_{(1,20)}$=0.20, p=0.659), Treatment x Reactivation ($F_{(1,20)}$=0.178, p=0.678), Training x Treatment ($F_{(1,20)}$=0.05, p<0.824) or Training x Treatment x Reactivation ($F_{(1,20)}$=1.11, p=0.305); however a significant Training x Reactivation interaction ($F_{(1,20)}$=8.56, p=0.008) was revealed. Analysis of simple effects on the second day of training showed no significant differences between Treatment ($F_{(1,20)}$=1.40, p=0.250), Reactivation ($F_{(1,20)}$=3.96, p=0.060) or Training x Treatment ($F_{(1,20)}$=0.84, p=0.371), thus groups were equally performing at the end of instrumental training (Figure 4.13A).

At test (Figure 4.13B), there was a reactivation-dependent effect of AP-5/SCH23390 upon instrumental performance. ANOVA revealed a significant Treatment x Reactivation interaction ($F_{(1,20)}$=6.82, p=0.017), with no main effects of Treatment ($F_{(1,20)}$=1.03, p=0.323) or Reactivation ($F_{(1,20)}$=0.03, p=0.868). Analysis of simple effects showed a significant difference between reactivated PBS and AP-5/SCH23390 infused rats ($F_{(1,10)}$=14.1, p=0.004), but no effect of AP-5/SCH23390 in non-reactivated rats ($F_{(1,10)}$=0.83, p=0.385). Therefore, co-infusion of AP-5 and SCH23390 into the NAc impaired instrumental memory reconsolidation. All groups extinguished similarly during the test (Extinction: $F_{(5,100)}$=9.95, p<0.001: Extinction x Treatment: $F_{(2.39,47.69)}$=0.21, p=0.848; Extinction x Reactivation: $F_{(2.39,47.69)}$=0.44, p=0.679; Extinction x Treatment x Reactivation: $F_{(2.39,47.69)}$=1.72, p=0.184).

Analysis of nosepoking revealed a significant increase (Figure 4.14A) during training ($F_{(1,20)}$=40.44, p<0.001); no other significant effects were observed in training (Treatment: $F_{(1,20)}$=3.33, p=0.083; Reactivation: $F_{(1,20)}$=1.59, p=0.222; Training x Treatment: $F_{(1,20)}$=1.26, p=0.275; Training x Reactivation: $F_{(1,20)}$=0.05, p=0.833; Training x Treatment x Reactivation: $F_{(1,20)}$=1.06, p=0.315). At test (Figure 4.14B) ANOVA revealed a significant Treatment x Reactivation interaction ($F_{(1,20)}$=4.71, p=0.042), with no main effects (Treatment: $F_{(1,20)}$=0.41, p=0.531; Reactivation: $F_{(1,20)}$=0.34, p=0.567). Analysis of simple main effects did not reveal any significant differences between AP-5/SCH23390 and PBS-infused

reactivated groups ($F_{(1,10)}$=4.51, p=0.060) or in non-reactivated controls ($F_{(1,10)}$=2.24, p=0.165). Analysis of orthogonal simple effects revealed a significant difference in nosepoking between reactivated and non-reactivated PBS-infused controls ($F_{(1,9)}$=6.58, p=0.030). This comparison was not significant for rats given combined AP-5/SCH23390 ($F_{(1,11)}$=2.47, p=0.144). This indicated that the primary contributor to the overall interaction was the reduction in nosepoking by non-reactivated PBS-infused animals, compared to their reactivated counterparts.

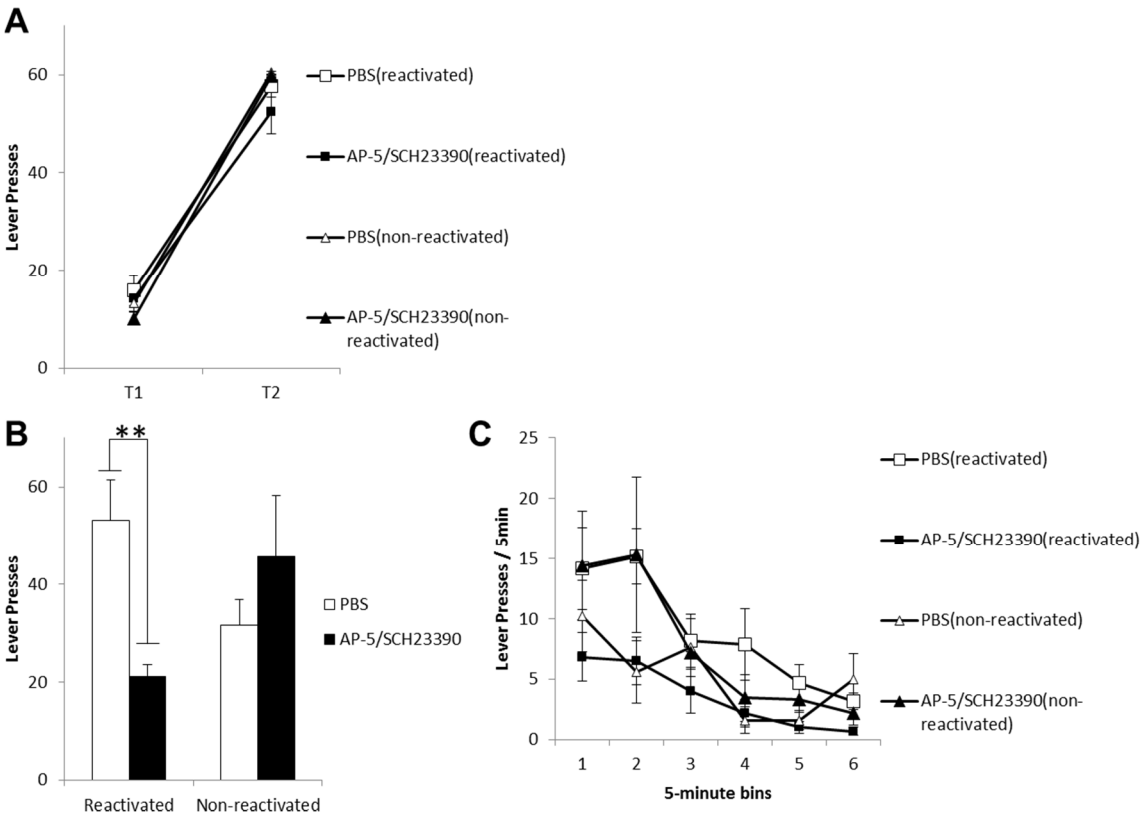

Figure 4.13: The effect of combined AP-5/SCH23390 infusion on lever pressing depended upon memory reactivation. **A**, reactivated PBS (white squares) and AP-5/SCH23390 (black squares) groups performed the lever pressing response at similar levels to non-reactivated PBS (white triangles) and AP-5/SCH23390 (black triangles) controls during training. A maximum of 30 reinforcements could be obtained on the first day of training (T1), 60 on the second (T2). **B**, the combined infusion (black bars) significantly impaired instrumental lever pressing performance 24 hours after reactivation at test (left), as previously shown in Experiment 4 (presented again here for clarity); however co-administration of AP-5/SCH23390 (black bars, n=7) did not reduce lever pressing during the 30-minute test session compared to PBS controls (white bars, n=5) if given in the absence of memory reactivation (right). **C**, within-session extinction during the test session showed no significant differences in rate of extinction of reactivated PBS (white squares) and AP-5/SCH23390 (black squares) groups compared to their non-reactivated PBS (white triangles) and AP-5/SCH23390 (black triangles) counterparts. Data are represented as mean ± SEM. (**) indicates significant differences, $p<0.01$.

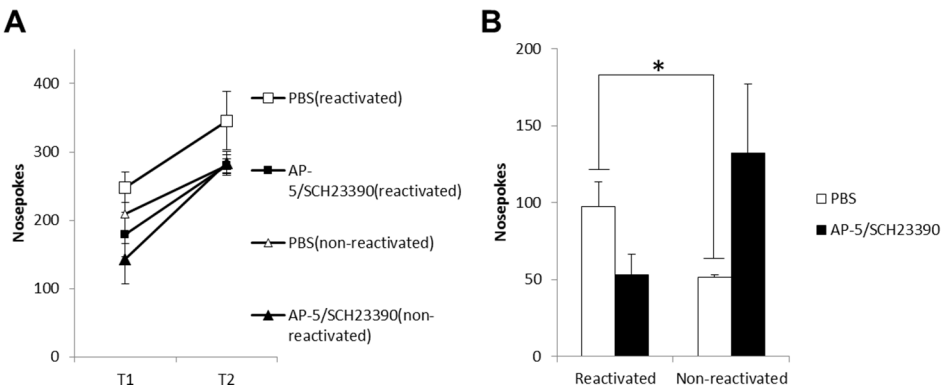

Figure 4.14: Infusion of AP-5/SCH23390 had a reactivation-dependant effect on long-term nosepoke responses. **A**, reactivated PBS (white squares) and AP-5/SCH23390 (black squares) groups showed similar levels of nosepoking to non-reactivated PBS (white triangles) and AP-5/SCH23390 (black triangles) groups during both the first (T1) and second (T2) days of the training phase. **B**, when tested 24 hours after infusion, nosepokes were significantly decreased in non-reactivated PBS controls (right, white bar, n=5) compared to their reactivated counterparts (left, white bar, n=6). Nosepoking in reactivated AP-5/SCH23390-infused rats (left, black bar, n=6) were not significantly lower than their non-reactivated counterpart (right, black bar, n=7). Data are represented as mean ± SEM. (*) indicates significant differences, $p<0.05$.

Cannula placements

At the conclusion of the intra-accumbal study, brains were sectioned to confirm the infusions were localised within the NAc (Figure 4.15).

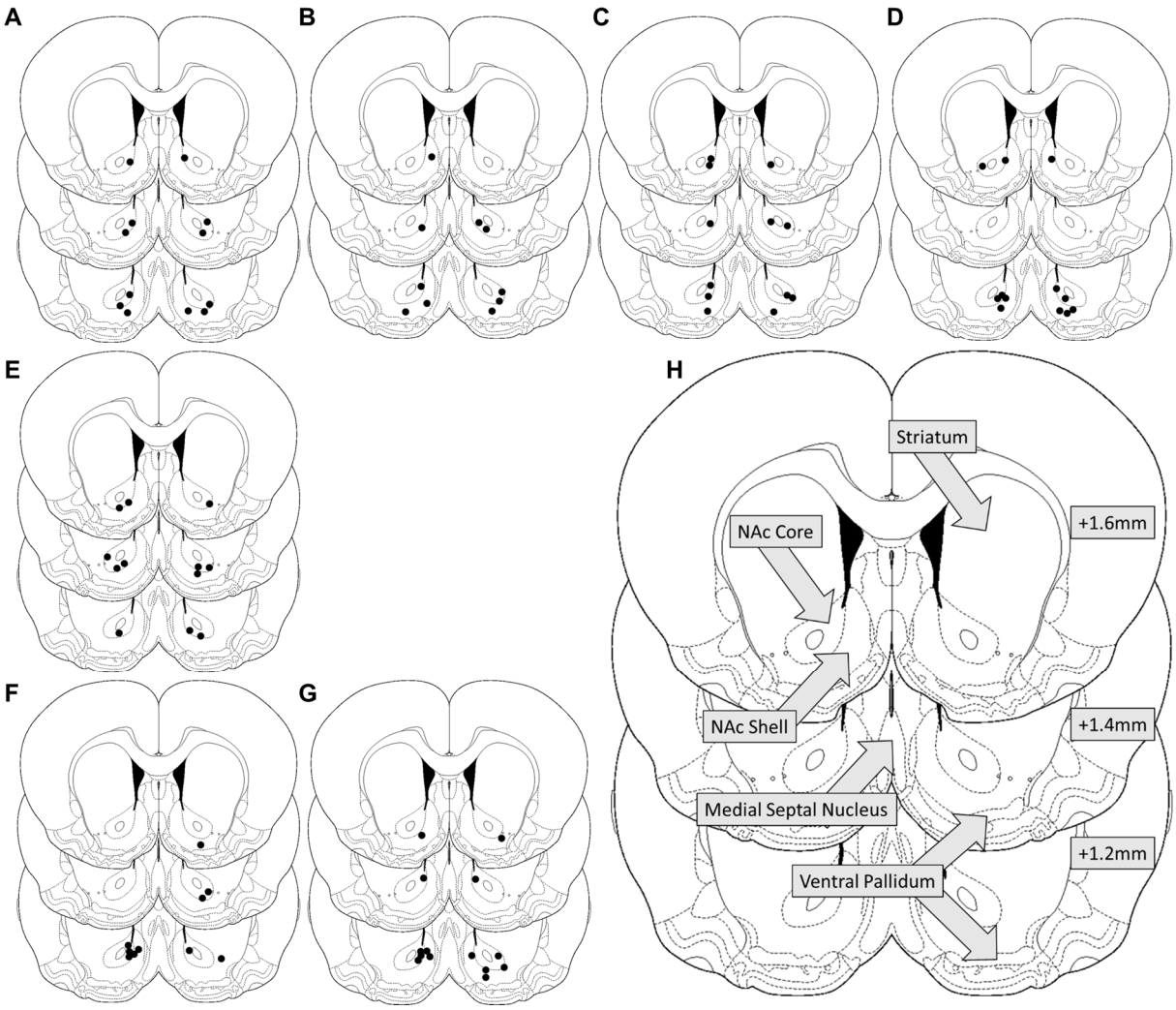

Figure 4.15: Schematic of the brain showing location of injector tips. **A**, location of injector tips for reactivated PBS-infused rats. **B**, location of injector tips for reactivated AP-5-infused rats. **C**, location of injector tips for reactivated SCH23390-infused rats. **D**, location of injector tips for reactivated AP-5/SCH23390 co-infused rats. **E**, location of injector tips for reactivated MK-801-infused rats. **F**, location of injector tips for non-reactivated PBS-infused rats. **G**, location of injector tips for non-reactivated AP-5/SCH23390 co-infused rats. **H**, labelled schematic of the brain indicating key regions surrounding the infusion site. Numbers on right signify millimetres from bregma. Schematic based upon rat brain atlas by (Paxinos & Watson, 2009. Black dots indicate the location of injector tips. All injectors were located within the NAc.

Discussion

The present results show that a weakly trained, goal-directed instrumental memory was not destabilised by a brief extinction or training reminder session; however, instrumental performance was impaired by pharmacological intervention prior to a VR5 reactivation, suggesting that a change in reward contingency is sufficient to trigger destabilisation of an appetitive instrumental A–O memory. Reconsolidation of A–O memory was found to be disrupted by systemic, but not intra-NAc, MK-801; this effect was reactivation-dependant indicating the amnestic effect was due to an impairment of reconsolidation. A–O-reconsolidation could also be disrupted by combined intra-NAc infusion of AP-5/SCH23390, indicating a role for co-activation of accumbal D1Rs and NMDARs in the memory reconsolidation process.

Our work provides evidence that goal-directed instrumental memories do undergo reactivation-induced destabilisation and subsequent reconsolidation. The reconsolidation of instrumental memories is shown here to be impaired by systemic MK-801 and intra-NAc AP-5/SCH23390 administered shortly prior to a VR5 reactivation. The amnestic effect of these treatments was critically dependent upon memory reactivation; treatment under inappropriate reactivation parameters or in the absence of any reactivation session produced no impairment in instrumental responding. Therefore, the behavioural impairments at test were not due to any non-specific effects of either treatment.

While the VR5 session might be expected to enhance responding via new learning, there was only weak evidence for this. Taking into account both the systemic and intra-cerebral studies, reactivated vehicle-treated groups did respond moderately above the level of non-reactivated controls, suggesting some level of new learning; however this is not sufficient to explain fully the pattern of data observed, as non-reactivated drug-treated groups respond at a similar level to reactivated-vehicle groups. Importantly, the deficits observed at test in reactivated treatment-impaired groups appears to be driven at least in part by a reduction in responding compared to non-reactivated animals. This is interpretatively important as the observed deficit cannot be attributed simply to an

impairment of new learning, but must be at least partly driven by disruption of reconsolidation. The intact pr-STM responding further supports this interpretation, as any impairment in acquisition of new learning at reactivation should also show on this test.

The reactivation-dependant nature of the lever pressing deficit confirms the effect of treatment was to impair reconsolidation; however the present results could be explained by impairments in the reconsolidation of non-instrumental performance-enhancing memories. For example, motivational pavlovian memories can invigorate instrumental responding (Dickinson & Balleine, 1994), and context–reward memories are known to undergo reconsolidation (Diergaarde *et al.*, 2006). Were the observed deficits due to a disruption of context–sucrose memories one would expect a loss of response vigour, resulting in reductions in both lever pressing and nosepoking; however, a significant reduction was only observed in lever pressing following treatment with systemic MK-801 or co-infusion of AP-5/SCH23390, indicating that the pavlovian associations motivating responses were intact. Interestingly there is a reactivation-dependant effect on nosepoking in cannulated rats; however this appears to be driven by a reduction in the nosepokes of the non-reactivated PBS control group compared to reactivated PBS-infused rats. This does not appear to represent new learning in the reactivated PBS group as they show similar levels of responding to the non-reactivated AP-5/SCH23390 group; instead it may represent a non-specific loss of vigour in non-reactivated PBS control rats. This does not invalidate the conclusion that A–O reconsolidation was impaired by AP-5/SCH23390, as this infusion only reduces long-term lever pressing when given in conjunction with memory reactivation. Furthermore, as the nosepoking of reactivated AP-5/SCH23390 infused rats is not significantly reduced compared to PBS controls, this would suggest both groups were equally invigorated at test.

Supporting the hypothesis that the reconsolidation impairment observed with the VR5 reactivation spared pavlovian motivational memories is the lack of a reconsolidation effect in the other reactivation conditions. One would expect context–reward memories to be destabilised by the brief extinction and/or brief training reactivations, given that these sessions comprised of re-exposure to

the context with or without concomitant sucrose presentation. Therefore, the selective nature of the impairment, following only the VR5 reactivation session, is inconsistent with an interpretation focussing on context–reward memory reconsolidation. Moreover, had these associations been disrupted there should have been a reduction in nosepoke responses (Diergaarde *et al.*, 2006); however there was no reduction in nosepoking in any drug-treated group at test following a brief training session or a VR5 reactivation, and MK-801 enhanced nosepoking after 2-minutes of extinction. Thus, it is reasonable to conclude that it was instrumental, and not pavlovian, memory that was destabilised by the VR5 reactivation.

The present results demonstrate that goal-directed instrumental memories undergo reconsolidation. Importantly, we used limited training intended to produce a weak A–O memory; this was confirmed by the reduction in responding when the reinforcer was paired with LiCl injection. Sensitivity of behaviour to reward devaluation is a well-documented indicator of goal-directed responding (Adams, 1982; Dickinson, 1985). Our work differs from past studies which used well trained, likely habitual, animals (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). It remains to be determined whether instrumental S–R associations also destabilise following a suitable change in instrumental contingency.

A short extinction session was not sufficient to destabilise instrumental memory. In fact, MK-801-treated rats in this experiment responded significantly more than controls at test. This increase may be explained as impaired extinction rather than reconsolidation. NMDAR antagonists have previously been shown to impair instrumental extinction (Lissek & Güntürkün, 2003; Kelamangalath *et al.*, 2007) leading to elevated pressing at test. While brief extinction sessions are conventionally used to destabilise pavlovian memories, the fact that saline-treated animals given the 2-minute reactivation showed both low responding and very little extinction over the course of the test strongly suggests they had extinguished already during the reactivation procedure, despite its brevity. Given that extinction and reconsolidation may be competing processes (Eisenberg *et al.*, 2003), which are both impaired by MK-801 in pavlovian settings (Lee *et al.*, 2006a; Flavell & Lee, 2013), it remains possible

that a shorter extinction session that does not result in behavioural extinction could destabilise the instrumental memory.

The brief FR1 session also did not destabilise the instrumental trace. This is important as the destabilisation caused by the VR5 reactivation cannot simply be attributed to the presence of the reinforcer. A training trial has been shown to destabilise fear (Duvarci & Nader, 2004; Lee, 2008), appetitive pavlovian (Milekic *et al.*, 2006; Valjent *et al.*, 2006) and object recognition memories (Kelly *et al.*, 2003; Akirav & Maroun, 2006). However, full-length (Hernandez & Kelley, 2004) and brief (Mierzejewski *et al.*, 2009) training sessions have been ineffective in instrumental settings, consistent with our findings in this study. The salient difference between the VR5 and FR1 reactivation sessions is that the latter involved no alteration in the instrumental contingency. Recent studies have demonstrated that a change reinforcer contingency can destabilise pavlovian fear memory (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013). Since new (contingency) information was present during the VR5 session it may have been more effective at engaging memory updating, the hypothesised function of reconsolidation (Lee, 2009).

The disruptive effect of systemic MK-801 was replicated by the co-infusion of AP-5/SCH23390 into the NAc. The NAc is known to play a key role in the acquisition of instrumental learning; blockade of NMDARs (Kelley *et al.*, 1997), D1Rs (Smith-Roe & Kelley, 2000), protein synthesis (Hernandez *et al.*, 2002) or dysregulation of PKA (Baldwin, Sadeghian, Holahan, *et al.*, 2002) in this region prevents the acquisition of instrumental memory. It is important to note that while protein synthesis or PKA inhibition appears to impair memory consolidation (Baldwin, Sadeghian, Holahan, *et al.*, 2002; Hernandez *et al.*, 2002), the disruptive effect of AP-5 and co-infusion of AP-5/SCH23390 into the NAc appears to focus on acquisition as there is no effect of these infusions if given after training sessions (Hernandez *et al.*, 2005). Taken with the results presented here, it seems reasonable to suggest that the NAc functions as a key locus of re-organisation and re-encoding during the updating of memory content. While our present data do not preclude the possibility the NAc acts as a site of memory storage this seems unlikely given that post-learning lesions of the NAc do not impair retrieval of

instrumental memory when there is no delay between response and reward delivery (Cardinal & Cheung, 2005).

While intra-NAc AP-5/SCH23390 impaired instrumental reconsolidation, this does not demonstrate that the NAc was a primary central locus of action of systemic MK-801. Infusion of MK-801 directly into the NAc did not have the same effect as AP-5/SCH23390, nor as systemically-applied MK-801. Moreover, infusion of AP-5 was also without effect on lever pressing, further supporting the interpretation that blockade of NMDARs alone in the NAc is insufficient to prevent instrumental memory reconsolidation. Thus, systemic injections of MK-801 likely have functional effects in brain areas other than the NAc. One interesting possibility is that systemic MK-801 mimicked the effect of AP-5/SCH23390 by a combined effect on both the NAc and ventral tegmental area (VTA); the VTA supplies dopaminergic input to the NAc. Systemic MK-801 evokes large increases in accumbal dopamine via an effect on the VTA (Mathé *et al.*, 1999). It may be an MK-801-mediated increase in accumbal dopamine caused internalisation of dopamine receptors, known to occur following increases in dopamine, or dopamine receptor agonism (Dumartin *et al.*, 1998; Hara & Pickel, 2007; Schroeder *et al.*, 2009). In conjunction with NMDAR blockade of the NAc the functional effect of systemic MK-801 may be a combined reduction in D1R and NMDAR activity, similar to that of AP-5/SCH23390 infusion; or perhaps the process of D1R internalisation impairs the interaction between D1Rs and NMDARs (Zhang *et al.*, 2007, 2009). A simpler explanation for the difference between systemic and intra-accumbal MK-801 may be that reconsolidation of instrumental memory also depends on NMDAR activity in other brain regions. The medial prefrontal cortex (Baldwin *et al.*, 2000; Baldwin, Sadeghian, & Kelley, 2002), anterior cingulate cortex (McKee *et al.*, 2010), dorsal striatum (Yin *et al.*, 2004, 2005; Shiflett *et al.*, 2010), hippocampus (Corbit & Balleine, 2000), amygdala (Baldwin *et al.*, 2000; Andrzejewski *et al.*, 2005) and VTA (Sharf *et al.*, 2006; Zellner *et al.*, 2009) have all been implicated in operant learning, and it remains unclear whether NMDAR activity in these areas is required for instrumental reconsolidation.

The long-term impairment of combined AP-5/SCH23390 was not due to just AP-5 or SCH23390, as these drugs did not impair instrumental reconsolidation when infused alone. Therefore, our results suggest that co-activation of D1Rs and NMDARs in the NAc is required for the reconsolidation of a goal-directed instrumental memory. A growing body of evidence suggests D1Rs and NMDARs directly interact (Salter, 2003), reciprocally modulating receptor function through direct protein-protein interactions (Lee *et al.*, 2002; Pei *et al.*, 2004); however whether direct interaction between D1Rs and NMDARs, or simply co-activation, is required for the reconsolidation of instrumental memory remains unclear. It is well established that local NMDAR activity is required for reconsolidation of appetitive (Milton, Lee, Butler, *et al.*, 2008; Wu, Li, Gao, *et al.*, 2012) and aversive (Lee & Hynds, 2012; Milton *et al.*, 2013) pavlovian memories. In contrast, there is less evidence that D1R antagonism impairs reconsolidation (Sherry *et al.*, 2005; Diergaarde *et al.*, 2008; Maroun & Akirav, 2009). Existing literature has demonstrated a role for D1Rs in consolidation of long-term memory (Smith-Roe & Kelley, 2000; Nagai *et al.*, 2007; Maroun & Akirav, 2009), and perhaps future studies need to consider the co-activation hypothesis before excluding a role for D1Rs in reconsolidation. While it remains possible that higher doses of each drug alone could have impaired reconsolidation, this seems unlikely as lower doses of both AP-5 (De Leonibus *et al.*, 2005) and SCH23390 (Dalley *et al.*, 2005) have been shown to be effective in preventing memory consolidation when infused into the NAc.

Notably, systemic MK-801 and intra-NAc AP-5/SCH23390 also resulted in acute effects at reactivation. Systemic MK-801 increased lever pressing during the extinction reactivation, whereas AP-5/SCH23390 caused a profound decrease during VR5. The acute effect of systemic MK-801 can be explained by the hyperactivity caused at doses used in our experiment (Hargreaves & Cain, 1995). Elevated responding was not observed in either reinforced reactivation procedure, most likely due to the limit on pellets that could be acquired in these sessions. The acute effect of MK-801 is short-lived and cannot account for the long-term performance effects; either the increase in the extinction condition or the decrease in the VR5 condition. The absence of any significant elevation in lever

pressing at pr-STM, or in the non-reactivated drug-injected group, provides additional confirmation that the hyperactivity is transient.

The acute effect of AP-5/SCH23390 co-infusion was attributable to both substances, as AP-5 and SCH23390 infused alone had no acute effect on performance at reactivation. Although one would expect SCH23390 alone to acutely impair responding (Smith-Roe & Kelley, 2000; Hernandez *et al.*, 2005), it is possible that experimental differences between our work and past research account for the apparent discrepancy. For example our subjects were weakly trained with no reward-paired signals. The restriction of responding during reactivation may also have prevented a performance deficit being observed. The exact reason AP-5 and SCH23390 cause an acute impairment when administered together, but not alone remains unclear; however it may be related to the necessity for co-activation of D1Rs and NMDARs in reconsolidation. Regardless of its underlying cause, the acute effect of AP-5/SCH23390 is temporary, as demonstrated by the lack of group differences in the non-reactivated condition.

In summary, these results demonstrate that instrumental A–O memories do undergo reconsolidation which can be disrupted by systemic MK-801 or intra-NAc AP-5/SCH23390. However, instrumental responding after longer periods of training is supported by S–R as well as A–O associations. Thus it remains to be seen if the reconsolidation of habitual S–R memories can also be disrupted to reduce reward-seeking behaviour.

CHAPTER 5

RECONSOLIDATION OF HABITUAL INSTRUMENTAL MEMORIES

Introduction

Once learned, memories become stabilised by a process of consolidation (McGaugh, 2000) but can then be destabilised (Nader, 2003) in order to update memory content (Lee, 2009). The process of reconsolidation has been demonstrated in a variety of appetitive (Wang *et al.*, 2005; Brown *et al.*, 2008; Milton, Lee, & Everitt, 2008) and aversive settings (Nader *et al.*, 2000; Lee *et al.*, 2004; Milekic *et al.*, 2007); however current literature suggests that instrumental memories do not undergo reconsolidation (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). We have previously shown (Chapter 4) that goal-directed Action–Outcome (A–O) memories can be destabilised by a change in reward contingency, and their reconsolidation disrupted by systemic antagonism of N-methyl-D-aspartate receptors (NMDARs) using MK-801. We have proposed that a change in instrumental contingency is sufficient to destabilise instrumental memory. This chapter tests this hypothesis in a well-trained setting.

With brief amounts of training (such as in Chapter 4) behaviour is mediated by A–O memory; however responses come under habitual Stimulus–Response (S–R) control following extended training (Dickinson, 1985). Previous research into the reconsolidation of instrumental memories have studied well-trained, and therefore likely habitual, animals (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009); however these studies also used training trials to reactivate the memory trace. A brief training session was ineffective at destabilising a goal-directed memory (Chapter 4), thus a likely explanation for the lack of any reconsolidation effect in past literature is the reactivation parameters were inappropriate reactivation. Alternatively, S–R memories may not undergo reconsolidation.

In order to establish a habitual memory, a similar protocol was employed as previously (Chapter 4) but extended to 10 days of training in line past research on instrumental reconsolidation (Hernandez

& Kelley, 2004). Behaviour has been demonstrated to be habitual after 500 rewarded responses (Adams, 1982) under similar experimental parameters and this was used as a general guide when designing the present experiments. The ability of the training protocol to produce habitual behaviour was tested by examining the sensitivity of responding to reward devaluation. While goal-directed memories are affected by changes in reward value, the defining feature of a habit is its insensitivity to reward devaluation (Dickinson, 1985). In order to confirm rats behaved habitually following training the reward pellets were devalued by pairing to LiCl-induced gastric malaise.

We first attempted to disrupt the reconsolidation of a lever pressing habit using the VR5 reactivation session from Chapter 4 combined with administration of the NMDAR antagonist, MK-801. This was followed up with a more conventional brief extinction session and a reactivation involving a larger change in contingency. Habitual animals are less sensitive to contingency degradation (Yin & Knowlton, 2006; Ostlund & Balleine, 2008b) and it was hypothesised that a greater change in contingency may be required in order to produce a sufficient error signal to destabilise the S–R trace. Brief extinction sessions are typically used to destabilise memories in other settings (Nader *et al.*, 2000; Milton, Lee, & Everitt, 2008; Robinson, Ross, *et al.*, 2011); however as well-trained memories may require longer reactivation exposure in order to be destabilised (Suzuki *et al.*, 2004; Reichelt & Lee, 2012), a 5-minute extinction session was used.

Materials and Methods

Subjects

Subjects were 88 male Lister-Hooded rats (Charles River), weighing 200-350g at the start of the experiment. Rats were housed in cages of 4 at 21^{o}C on a 12-hour light-dark cycle (lights on at 0700) and fed a restricted diet of 15g per day. Water available *ad libitum*. All procedures were carried out in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act (PPL 40/3205).

Firstly, 8 rats were used in the devaluation study in order to confirm behaviour was habitual. Next, 16 rats were used in order to test the VR5 reactivation.

24 rats were used in order to test the brief extinction reactivation, divided into two drug treatment groups (n=12), then into reward-devalued (LiCl-paired), and reward-valued (non-LiCl-paired), groups (final n=6).

A further 24 rats were used in the VR20, split into MK-801 and saline treated groups (n=12 each), which were then further sub-divided into LiCl-paired and non-devalued groups (final n=6 per group). Finally, 16 rats were used for a non-reactivated control group, again divided into drug-treatment and LiCl-paired groups (final n=4 per group) following the initial post-reactivation test. No rats were excluded from any experimental group.

Drugs

LiCl (Sigma-Aldrich) was dissolved in deionised water to a concentration of 0.12M. During devaluation rats were administered a dose of 10ml/kg paired with the reward pellets.

MK-801 (AbCam) was dissolved in sterile saline to a concentration of 0.1mg/ml. 30 minutes prior to memory reactivation, rats were administered intraperitoneally with 0.1mg/kg of MK-801 or equivalent volume of saline vehicle.

Instrumental Training

Training, memory reactivation and testing sessions took place in 8 operant boxes (MedAssociates) as described in Chapter 4. Rats were initially pre-trained to collect 45mg sucrose pellets (TestDiet) from the magazine, delivered at random intervals (mean 60 seconds) for 15 minutes. Instrumental training began immediately after the pre-training session. During training sessions the left lever was extended into the box and delivered a sucrose pellet into the magazine when pressed, on a fixed-

ratio (FR1) schedule; the lever did not retract and no reward-paired stimuli were presented during training. Training sessions lasted 30 minutes or until a maximum of 60 pellets had been obtained. Rats received a total of 10 training sessions.

Reward Devaluation

The ability of the training procedure to produce S–R responding was confirmed by devaluation of the sucrose pellets by LiCl-pairing. Devaluation was conducted as described in Chapter 4.

Reactivation Procedures

Following the 10th day of training, rats were semi-randomly divided into two groups, matched for lever pressing performance during training. The day after the end of the training phase rats were administered drug followed by a reactivation session.

VR5 reactivation: Rats were exposed to the VR5 reactivation as described in Chapter 4.

Brief extinction session: Rats were placed in the operant boxes for 5 minutes with the lever extended. No rewards were delivered during the session.

VR20 reactivation: This reactivation was similar to the VR5, except the reinforcement schedule was extended to VR20 (mean: 20, range: 10-30). The session ended after 20-minutes, or after a maximum of 20 rewards was obtained.

No reactivation: Additional rats were trained and received drug-injection but no behavioural session.

Behavioural Testing

24 hours after reactivation rats were returned to the operant boxes and tested in a 30-minute extinction session. The lever was extended, however no pellets were delivered. The day after this

first test, rats which received brief extinction, VR20 or no reactivation received LiCl-reward pairings as above in order to assess whether responding was under A-O or S-R control following the experimental manipulation. The day following reward devaluation, performance was tested again in a second 30-minute extinction test (6 days after reactivation).

Statistical Analysis

Training data was analysed using repeated measures analysis of variance (ANOVA) in order to assess whether the task was learned, and whether groups were equally performing, with Training and Treatment as factors. Reactivation was analysed using one-way ANOVA with drug Treatment as a factor. Test data was divided into 5-minute bins and analysed with Extinction and Treatment as factors. In the case of the VR20 and 5-minute extinction reactivations, data were compared directly with non-reactivated controls using three-way ANOVA with Extinction, Treatment and Reactivation as factors. Results with $p<0.05$ were deemed significant. A Greenhouse-Geisser correction was used to correct for non-spherical data where appropriate.

In the case of the 5-minute extinction, VR20 or no reactivation conditions, rats received reward devaluation and were tested again in order to gauge whether responding remained habitual. As we were interested in whether individual experimental groups were susceptible to reward devaluation the effect of Devaluation was tested using bonferroni-corrected planned-comparisons (effective $p<0.0125$ for the VR20 reactivation, $p<0.025$ for the 5-minute extinction).

Analysis of overall nosepoking behaviour was performed in order to assess response vigour during experimental sessions.

Results

Reward Devaluation

The ability of the training parameters to produce habitual behaviour was first tested by devaluing one group of rats after the 10th day of training. Over training (Figure 5.1A) rats significantly increased their lever pressing ($F_{(9,54)}$=11.36, p<0.001) indicating the task was learned, with no group differences (Devaluation: $F_{(1,6)}$=0.79, p=0.409; Training x Devaluation: $F_{(2.24,13.46)}$=1.31, p=0.306). Following training the reward pellets were devalued. At test (Figure 5.1B) ANOVA did not reveal any significant effect of Devaluation ($F_{(1,6)}$=0.02, p=0.898), confirming responding was driven by S–R learning.

ANOVA revealed nosepokes significantly reduced (Figure 5.2A) over the course of Training ($F_{(2.24,13.45)}$=4.28, p=0.033), but equally between groups (Devaluation: $F_{(1,6)}$=0.21, p=0.661; Training x Devaluation: $F_{(2.24,13.45)}$=0.47, p=0.657). During the test session both groups nosepoked (Figure 5.2B) at similar levels (Devaluation: $F_{(1,6)}$=0.75, p=0.421).

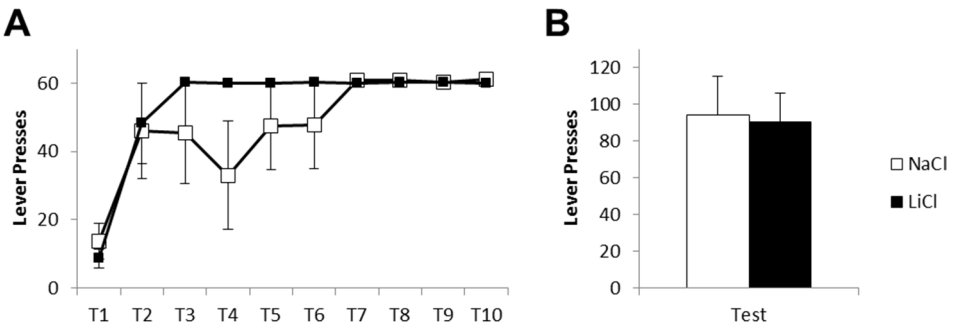

Figure 5.1: Rats behaved habitually, demonstrated by the insensitivity of behaviour to reward devaluation following training. **A**, both LiCl (black squares) and NaCl control (white squares) groups acquired the lever pressing response to equivalent levels during training. A maximum of 60 pellets could be acquired on any one training session. T1-T10 marks each day of training. **B**, after training rats received injection of LiCl (n=4) or saline control (n=4) and were tested in a 30-minute extinction test. LiCl-treated (black bar) rats did not reduce their lever pressing performance compared to NaCl

controls (white bar). The insensitivity of behaviour to reward devaluation indicated responding was under S–R control. Data expressed as mean ± SEM.

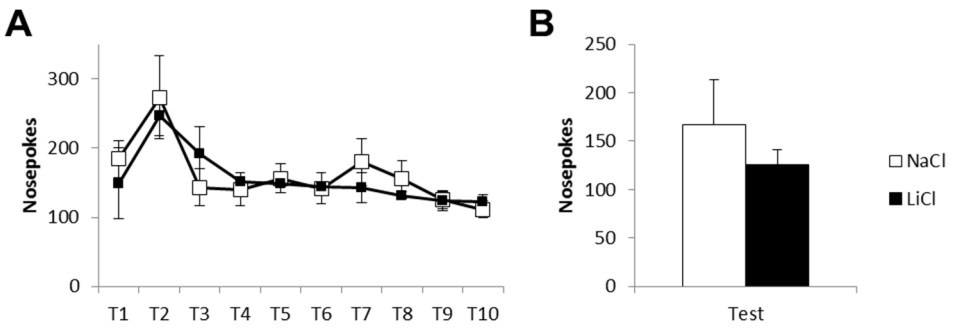

Figure 5.2: Pairing the reinforcer with LiCl did not devalue nosepoke responding. **A**, both LiCl (black squares) and NaCl (white squares) make equivalent numbers of nosepoke responses during each training session. Nosepokes initially increase over the first (T1) and second (T2) training days, decreasing from days three through ten (T3-T10). **B**, following training rats were re-exposed to the reward pellets and given an injection of either LiCl or saline vehicle. When tested in extinction, both LiCl-injected (black bar) and NaCl control (white bar) groups made equivalent nosepoke responses showing no devaluation effect. Data expressed as mean ± SEM.

VR5 reactivation

We first tested whether the VR5 reactivation which successfully destabilised goal-directed memories in the previous chapter would be equally successful in a habit setting. Rats significantly increased their responding during Training ($F_{(9,126)}$=26.60, p<0.001) with no difference between Treatment groups (Figure 5.3A; Treatment: $F_{(1,14)}$=0.63, p=0.439; Training x Treatment: $F_{(1.97,27.63)}$=0.71, p=0.500). At reactivation (Figure 5.3B) both Treatment groups responded equally ($F_{(1,14)}$=0.01, p=0.947). At test, (Figure 5.3C) ANOVA revealed a significant effect of Extinction ($F_{(5,70)}$=42.70, p<0.001), however there was no main effect of Treatment ($F_{(1,14)}$=0.36, p=0.559) or Extinction x Treatment interaction ($F_{(2.26,31.60)}$=0.70, p=0.521).

Analysis of nosepokes during training (Figure 5.4A) revealed a significant reduction over Training ($F_{(9,126)}$=14.16, p<0.001) with no group differences (Treatment: $F_{(1,14)}$=0.004,p=0.949; Training x Treatment: $F_{(2.40,33.46)}$=0.68, p=0.538). There was no significant difference between treatment groups at either Reactivation ($F_{(1,14)}$=0.04, p=0.853; Figure 5.4B) or Test ($F_{(1,14)}$=2.15, p=0.165; Figure 5.4C).

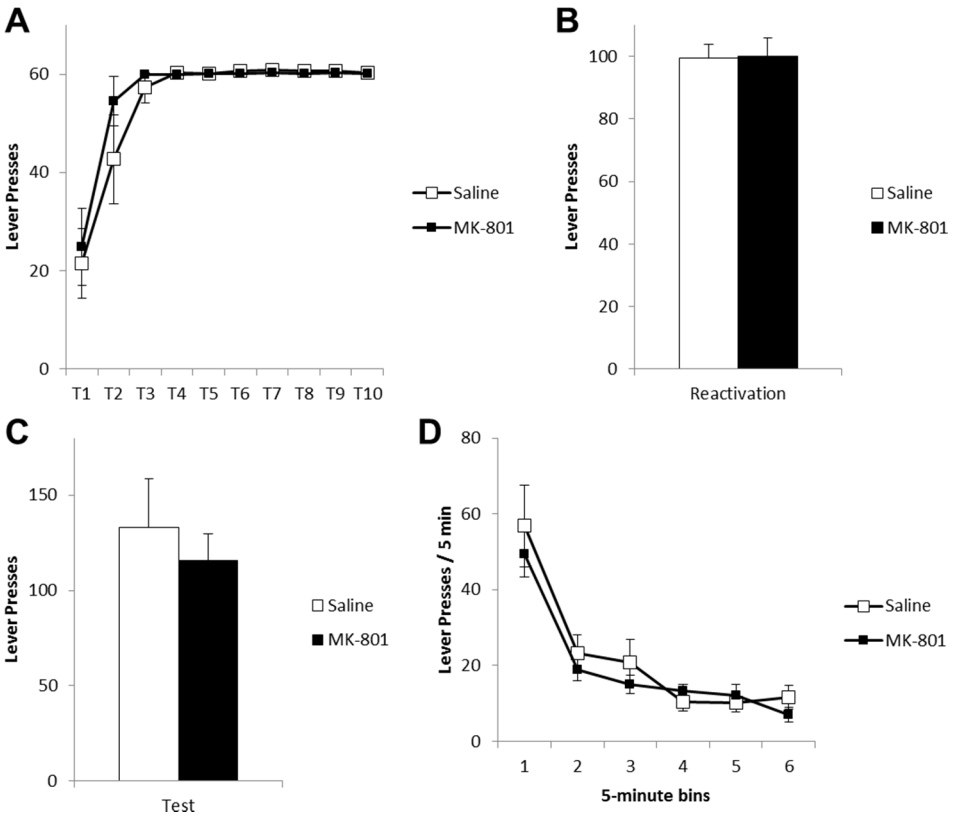

Figure 5.3: The VR5 reactivation was ineffective in destabilising instrumental memory when animals are habitual. **A**, during training both saline (white squares) and MK-801 (black squares) groups learned the lever press responses to equivalent levels of performance. Rats increased their responding over training days one to three (T1-T3), reaching plateau performance on training days four to ten (T4-T10). A maximum of 60 reinforcements could be obtained on any one training session. **B**, during the VR5 reactivation MK-801 treated rats (black bar, n=8) did not differ in their lever pressing performance compared to saline-injected controls (white bar, n=8). A maximum of 20 pellets could be obtained during this reactivation session. **C**, when tested 24 hours after reactivation, there was no overall difference in lever pressing performance between saline (white bar) and MK-

801 (black bar) treated groups. **D**, examination of within-session extinction bins during the test session showed no significant difference in the rate of lever pressing throughout the test session between saline (white squares) and MK-801-injected (black squares) rats. Data expressed as mean ± SEM.

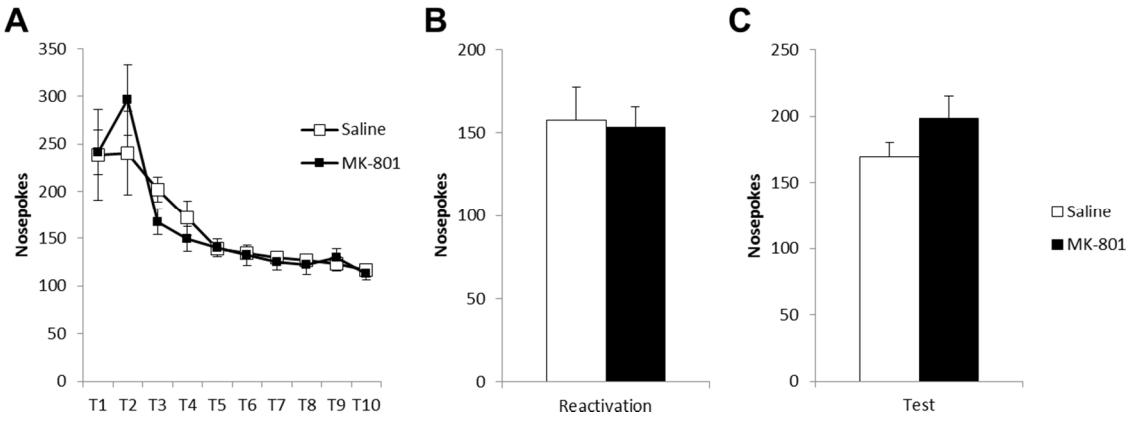

Figure 5.4: Treatment groups made equivalent numbers of nosepokes during the VR5 reactivation experiment. **A**, during the training phase, nosepoke responses decreased as training progress. Nosepoking was initially high during the first (T1) and second (T2) training days, falling sharply on the third (T3). Nosepokes continued to decrease over sessions four to ten (T4-T10), appearing to plateau. Both saline (white squares) and MK-801 (black squares) groups made showed similar nosepoking performance on each training day. **B**, both saline (white bar, n=8) and MK-801 (black bar, n=8) experimental groups made similar nosepoke responses during the VR5 reactivation session. **C**, when tested 24 hours after reactivation, saline (white bar) and MK-801-injected (black bar) rats showed no significant difference in nosepoke responses, suggesting rats were similarly motivated during the test. Data expressed as mean ± SEM.

Brief extinction reactivation

Rats acquired the lever pressing response (Figure 5.5A) during Training ($F_{(2.98,107.44)}$=44.34, p<0.001) with all groups learning at equal rates (Treatment: $F_{(1,36)}$=0.01, p=0.940; Reactivation: $F_{(1,36)}$=0.71,

p=0.406; Treatment x Reactivation: $F_{(1,36)}$=0.07, p=0.789; Training x Treatment: $F_{(2.98,107.44)}$=0.37, p=0.772; Training x Reactivation: $F_{(2.98,107.44)}$=0.92, p=0.433; Training x Treatment x Reactivation: $F_{(2.98,107.44)}$=0.42, p=0.737). During the reactivation session (Figure 5.5B) there was a significant acute increase in lever pressing with MK-801 Treatment ($F_{(1,22)}$=10.52, p=0.004).

At test (Figure 5.5C), ANOVA revealed a significant main effect of Extinction ($F_{(2.58,92.81)}$=82.23, p<0.001) with significant Extinction x Treatment ($F_{(2.58,92.81)}$=4.41, p=0.009) and Extinction x Reactivation ($F_{(2.58,92.81)}$=7.80, p<0.001) interactions, with no main effects of Treatment ($F_{(1,36)}$=0.15, p=0.698) or Reactivation ($F_{(1,36)}$=0.79, p=0.379). There was no overall Treatment x Reactivation interaction ($F_{(1,36)}$=1.98, p=0.168), nor any Extinction x Treatment x Reactivation ($F_{(2.58,92.81)}$=1.38, p=0.255) effect over the course of the session. Analysis of simple main effects did not reveal any significant Treatment effect in any one extinction bin (all F<4, all p>0.05); however rats given the 5-minute reactivation responded significantly lower than non-reactivated controls in the first extinction bin ($F_{(1,38)}$=9.75, p=0.003). There was no effect of Reactivation in any other extinction bin (all F<2, all p>0.05). Therefore the Extinction x Reactivation interaction was driven by higher responding in non-reactivated controls at the beginning of the test session, while the Extinction x Treatment interaction was driven by a general greater decline in response rate in MK-801-treated animals over the session.

In order to assess whether responding was goal-directed or habitual following experimental intervention, groups were divided into equally performing halves; half received pellet-LiCl pairings, the others received saline control solution. Performance was tested again the following day. During this devaluation test (Figure 5.6) there were no main effects of Treatment ($F_{(1,32)}$=0.13, p=0.725), Reactivation ($F_{(1,32)}$=1.80, p=0.189) or Devaluation ($F_{(1,32)}$=0.59, p=0.448) and no interactions (Treatment x Reactivation: $F_{(1,32)}$=2.59, p=0.118; Treatment x Devaluation: $F_{(1,32)}$=0.06, p=0.815; Treatment x Reactivation x Devaluation: $F_{(1,32)}$=1.09, p=0.304). Planned group comparisons (effective p<0.025) did not show any effect of LiCl devaluation in 5-minute reactivated rats treated with MK-801 ($F_{(1,10)}$=0.47, p=0.509) or Saline ($F_{(1,10)}$=0.88, p=0.371), nor in non-reactivated MK-801 ($F_{(1,6)}$=0.65,

p=0.451) and Saline ($F_{(1,6)}$=4.69, p=0.074) treated animals, compared to their non-devalued controls; this demonstrated that lever pressing was under S–R control.

Analysis of nosepoking during training (Figure 5.7A) revealed a significant effect of Training ($F_{(3.55,127.94)}$=25.78, p<0.001) with no difference between experimental groups (Treatment: $F_{(1,36)}$=0.01, p=0.909; Reactivation: $F_{(1,36)}$=2.70, p=0.109; Treatment x Reactivation: $F_{(1,36)}$=0.002, p=0.963; Training x Treatment: $F_{(3.55,127.94)}$=0.84, p=0.492; Training x Reactivation: $F_{(3.55,127.94)}$=1.89, p=0.123; Training x Treatment x Reactivation: $F_{(3.55,127.94)}$=0.29, p=0.865). There was also no significant effect of Treatment on nosepoking on the reactivation (Figure 5.7B) session ($F_{(1,22)}$=0.45, p=0.510), nor did the experimental groups differ in their nosepoke responses at test (Figure 5.7C; Treatment: $F_{(1,36)}$=0.52, p=0.474; Reactivation: $F_{(1,36)}$=2.72, p=0.108; Treatment x Reactivation: $F_{(1,36)}$=0.28, p=0.599). During the devaluation test (Figure 5.7D) ANOVA revealed significant main effects of Reactivation ($F_{(1,32)}$=5.40, p=0.027) and Devaluation ($F_{(1,32)}$=8.32, p=0.007), with no interaction between the two ($F_{(1,32)}$=0.61, p=0.442). There was no main effect, or interaction with, prior drug treatment at test (Treatment ($F_{(1,32)}$=0.14, p=0.706; Treatment x Reactivation: $F_{(1,32)}$=0.75, p=0.393; Treatment x Devaluation: $F_{(1,32)}$=0.42, p=0.522; Treatment x Reactivation x Devaluation: $F_{(1,32)}$=0.13, p=0.720).

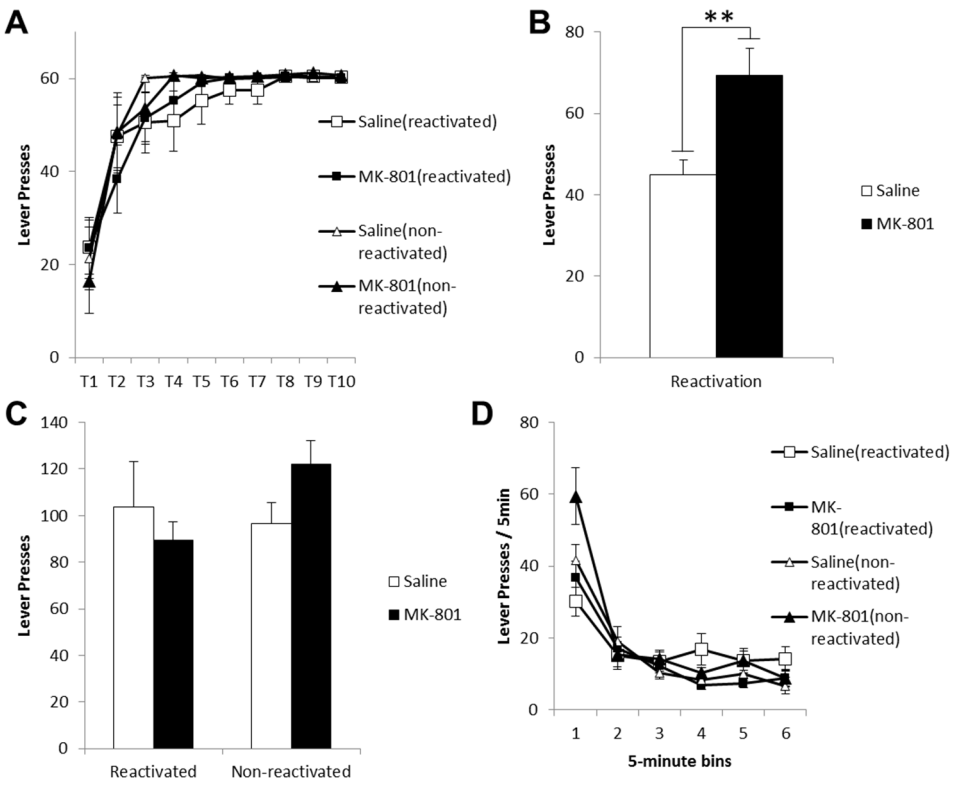

Figure 5.5: Rats injected with MK-801 prior to a 5-minute extinction, or no reactivation extinguish significantly faster at test. **A**, during training, rats from brief extinction reactivation groups to be given saline (white squares) or MK-801 (black squares) acquired the lever pressing response to similar performance as non-reactivated saline (white triangles) and MK-801 (black triangles) controls. Performance increased rapidly over training days one (T1), two (T2) and three (T3), levelling off during training sessions four to seven (T4-T7). A maximum of 60 pellets could be obtained on any training session; this plateau was reached by all animals on training days eight to ten (T8-T10). **B**, during the brief 5-minute extinction reactivation, MK-801-treated rats (black bar, n=12) acutely increased lever press responses compared to saline controls (white bar, n=12). **C**, when tested 24 hours after reactivation there were no overall differences in responding between MK-801 (black bars) and saline (white bars) injected rats regardless of whether they received the reactivation (left) or not (right). **D**, analysis of within-session extinction bins of the test session show rats given the 5-minute reactivation with either saline (white squares) or MK-801 (black squares) lever pressed less than non-reactivated saline (white triangles, n=8) or MK-801 (black triangles, n=8) controls at the beginning of the test session. Additional rats that were previously injected with MK-801 (regardless

of whether they were reactivated or not) did extinguish to a significantly lower baseline level during the test session. Data expressed as mean ± SEM. (**) indicates significant differences, p<0.01.

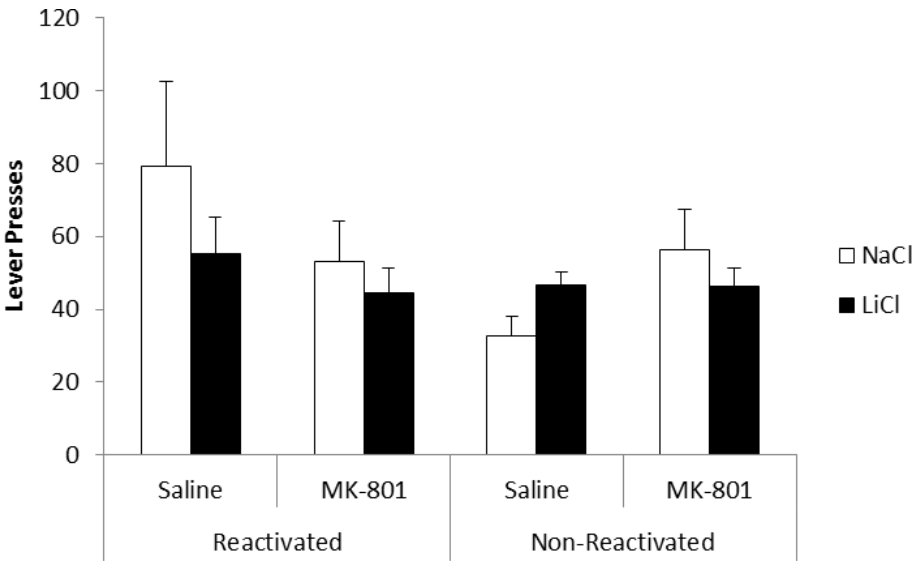

Figure 5.6: Lever pressing was not sensitive to reward devaluation following the 5 minute extinction session, regardless of drug treatment or reactivation session. Following earlier testing each experimental group was given free access to the reward pellets, which were then paired with either LiCl-induced malaise or saline control. When re-tested (6 days after reactivation) rats treated with LiCl (black bars; reactivated saline, n=6; reactivated MK-801, n=6; non-reactivated saline, n=4; non-reactivated MK-801, n=4) did not show any significant reduction in performance compared to their NaCl-treated controls (white bars; reactivated saline, n=6; reactivated MK-801, n=6; non-reactivated saline, n=4; non-reactivated MK-801, n=4) for any experimental group. Data expressed as mean + SEM.

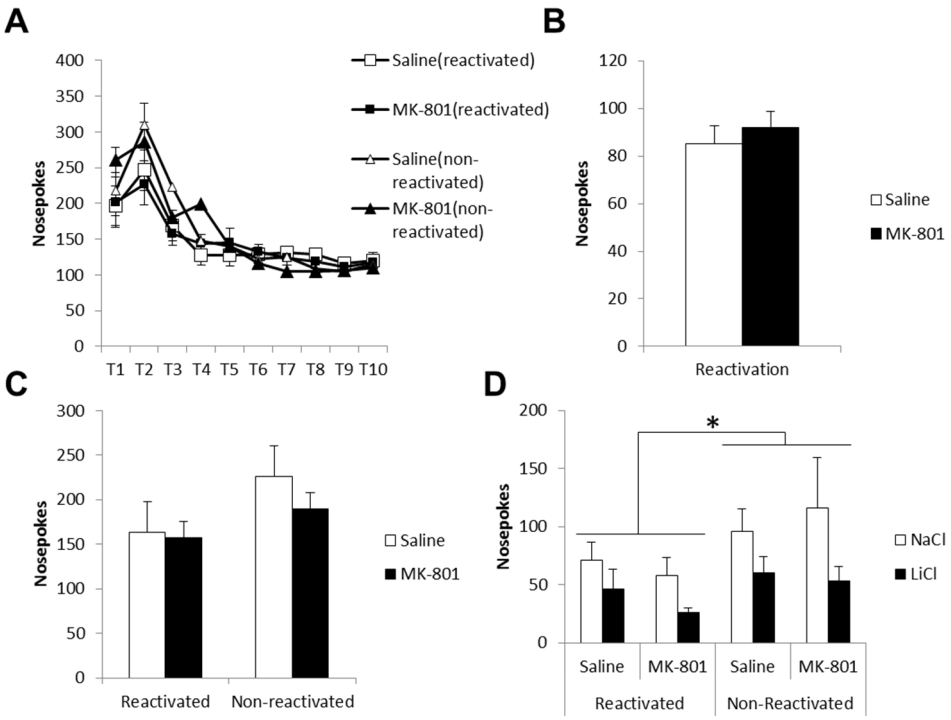

Figure 5.7: There were no differences in nosepoking behaviour between groups on any session. **A**, nosepokes reduced sharply decreased during training session one (T1), two (T2) and three (T3), plateauing over training sessions four to ten (T4-T10). Reactivated saline (white squares, n=12) and MK-801 (black squares, n=12) showed similar levels of nosepoke performance to each other and to non-reactivated saline (white triangles, n=8) and MK-801 (black triangles, n=8) controls. **B**, during the brief 5-minute extinction reactivation, MK-801 (black bar) did not produce any acute effect on nosepoke performance compared to reactivated saline controls (white bar). **C**, when tested 24 hours after reactivation, reactivated (left) and non-reactivated (right) groups displayed similar nosepoke performance regardless of whether they were saline (white bars) or MK-801-treated (black bars). **D**, at the devaluation test, non-reactivated rats nosepoked significantly more than those given the 5-minute reactivation. Furthermore, LiCl-treated animals (black bars; reactivated saline, n=6; reactivated MK-801, n=6; non-reactivated saline, n=4; non-reactivated MK-801, n=4) significantly reduced their nosepoke performance compared to NaCl-injected controls (white bars; reactivated saline, n=6; reactivated MK-801, n=6; non-reactivated saline, n=4; non-reactivated MK-801, n=4). There was no effect of drug treatment at this test. Data expressed as mean ± SEM. (*) indicates significant differences, $p<0.05$.

VR20 reactivation

All rats acquired the lever press response (Figure 5.8A) during Training ($F_{(9,324)}$=48.67, p<0.001) with no group differences (Treatment: $F_{(1,36)}$=0.01,p=0.930; Reactivation: $F_{(1,36)}$=0.36, p=0.554; Treatment x Reactivation: $F_{(1,36)}$=0.24, p=0.627; Training x Treatment: $F_{(2.82,101.51)}$=0.09, p=0.960; Training x Reactivation: $F_{(2.82,101.51)}$=0.33, p=0.789; Training x Treatment x Reactivation: $F_{(2.82,101.51)}$=0.35, p=0.778). At reactivation (Figure 5.8B) MK-801-injection acutely increased lever pressing ($F_{(1,22)}$=8.16, p=0.009).

At test (Figure 5.8C) ANOVA revealed a significant Treatment x Reactivation interaction ($F_{(1,36)}$=4.43, p=0.042) with no main effect of Treatment ($F_{(1,36)}$=0.10, p=0.748) or Reactivation ($F_{(1,36)}$=0.01, p=0.921). There was also a significant overall effect of Extinction ($F_{(5,180)}$=93.75, p<0.001) with no interactions including the Extinction factor (Extinction x Treatment: $F_{(2.37,85.26)}$=1.46, p=0.236; Extinction x Reactivation: $F_{(2.37,85.26)}$=1.86, p=0.154; Extinction x Treatment x Reactivation: $F_{(2.37,85.26)}$=1.87, p=0.154). Analysis of simple main effects did not reveal any effect of Treatment in either the reactivated ($F_{(1,22)}$=2.66, p=0.117) or non-reactivated ($F_{(1,14)}$=3.50, p=0.083) groups. Orthogonal main effects revealed an effect of Reactivation in MK-801-treated rats ($F_{(1,18)}$=4.46, p=0.049) but not in saline-injected controls ($F_{(1,18)}$=1.58, p=0.224). Thus the overall interaction was driven by the reduction in responding of reactivated MK-801-treated rats compared to their non-reactivated counterparts.

Following the test session rats were devalued and then retested (Figure 5.9) in order to determine whether habitual responding was intact. Analysis of this test session revealed a significant Treatment x Reactivation interaction ($F_{(1,32)}$=12.61, p=0.001) with a main effect of Reactivation ($F_{(1,32)}$=6.27, p=0.018), but no significant effect of Treatment ($F_{(1,32)}$=0.51, p=0.480). There was no overall significant effect of devaluation ($F_{(1,32)}$=2.04, p=0.16) or other interactions (Treatment x Devaluation: $F_{(1,32)}$=0.97, p=0.332; Reactivation x Devaluation: $F_{(1,32)}$=3.65, p=0.065; Treatment x Reactivation x Devaluation: $F_{(1,32)}$=3.65, p=0.065); however planned-comparisons (effective p<0.0125) showed a significant devaluation effect in rats which received MK-801 prior to VR20 reactivation ($F_{(1,10)}$=10.50,

p=0.009). Planned-comparisons of other groups did not reveal any significant devaluation effect (Saline(reactivated): $F_{(1,10)}$=3.56, p=0.089; Saline(non-reactivated): $F_{(1,6)}$=4.69, p=0.074; MK-801(non-reactivated): $F_{(1,6)}$=0.65, p=0.451). Analysis of simple main effects revealed a significant effect of Treatment in reactivated ($F_{(1,22)}$=9.92, p=0.005) but not non-reactivated ($F_{(1,14)}$=2.63, p=0.128) rats, indicating that the interaction was primarily driven by the overall reduction in responding of reactivated, MK-801-treated rats compared to reactivated saline controls.

Analysis of nosepoke behaviour during training (Figure 5.10A) revealed a significant reduction in nosepoking over the course of Training ($F_{(9,324)}$=31.44, p<0.001), with no significant main effects of Treatment ($F_{(1,36)}$=0.03, p=0.856), Reactivation ($F_{(1,36)}$=0.35, p=0.561) or any interactions (Treatment x Reactivation: $F_{(1,36)}$=0.02, p=0.903; Training x Treatment: $F_{(3.30, 118.75)}$=1.13, p=0.341; Training x Reactivation: $F_{(3.30, 118.75)}$=0.27, p=0.865; Training x Treatment x Reactivation: $F_{(3.30, 118.75)}$=0.61, p=0.623). Injection of MK-801 had a significant acute effect on nosepokes (Figure 5.10B) at Reactivation ($F_{(1,22)}$=4.59, p=0.044). At test (Figure 5.10C) ANOVA revealed a significant increase in nosepoking in non-reactivated controls, compared to reactivated test groups ($F_{(1,36)}$=10.87, p=0.002); however there was no overall effect of Treatment ($F_{(1,36)}$=0.13, p=0.722) or Treatment x Reactivation interaction ($F_{(1,36)}$=1.78, p=0.190). During the devaluation test (Figure 5.10D) ANOVA revealed a significant overall effect of Devaluation ($F_{(1,32)}$=4.70, p=0.038) with no other significant main effects or interactions (Treatment: $F_{(1,32)}$=1.93, p=0.175; Reactivation: $F_{(1,32)}$=1.31, p=0.261; Treatment x Reactivation: $F_{(1,32)}$=1.00, p=0.326; Treatment x Devaluation: $F_{(1,32)}$=1.70, p=0.202; Reactivation x Devaluation: $F_{(1,32)}$=0.60, p=0.445; Treatment x Reactivation x Devaluation: $F_{(1,32)}$=0.24, p=0.626). Planned-comparisons did not reveal any significant effect of LiCl devaluation in any one group compared to non-devalued controls (Saline(reactivated): F(1,10)=0.24, p=0.638; MK-801(reactivated): F(1,10)=1.75, p=0.215; Saline(non-reactivated): F(1,6)=2.25, p=0.184; MK-801(non-reactivated): F(1,6)=1.92, p=0.215).

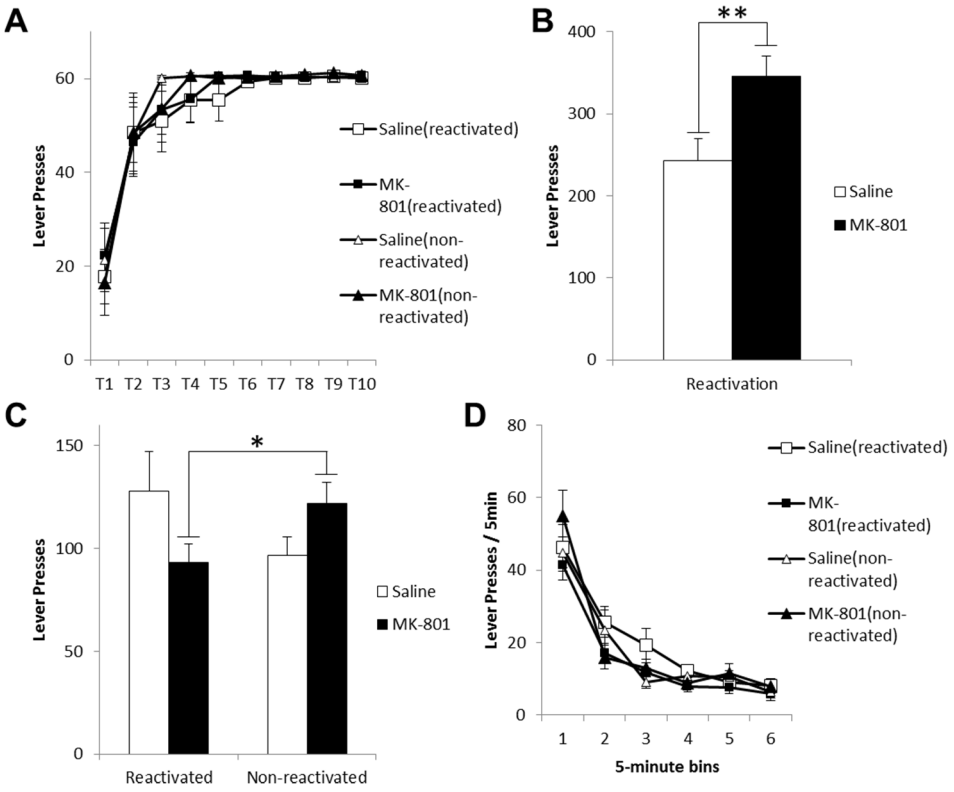

Figure 5.8: MK-801 significantly impairs lever pressing when given prior to a VR20 reactivation. **A**, rats learn to press the lever for food reward over ten days. Performance of the lever press response improves rapidly over training days one (T1), two (T2) and three (T3), plateauing over days four to ten (T4-T10). A maximum of 60 reinforcements could be obtained on any one training session. Lever pressing performance did not significantly differ on any session between reactivated saline (white squares, n=12) and MK-801 (black squares, n=12), and non-reactivated saline (white triangles, n=8) or MK-801 (black triangles, n=8). **B**, during the VR20 reactivation MK-801 (black bar) acutely increased lever pressing performance compared to saline-injected controls (white bar). **C**, when tested 24 hours after reactivation, there is a reactivation-dependant effect of MK-801 on lever pressing performance. This effect was driven by reduced responding of reactivated MK-801 (left, black bar) rats compared to their non-reactivated MK-801-injected counterparts (right, black bar). Non-reactivated saline controls (right, white bar) did not significantly differ in their responding compared to their reactivated counterparts (left, white bar). **D**, analysis of within-session extinction bins during the test session showed that all groups extinguished at similar rates throughout the test (reactivated saline = white squares; reactivated MK-801 = black squares; non-reactivated saline =

white triangles; non-reactivated MK-801 = black triangles). Data expressed as mean ± SEM. (*) indicates significant differences, p<0.05. (**) indicates significant differences, p<0.01.

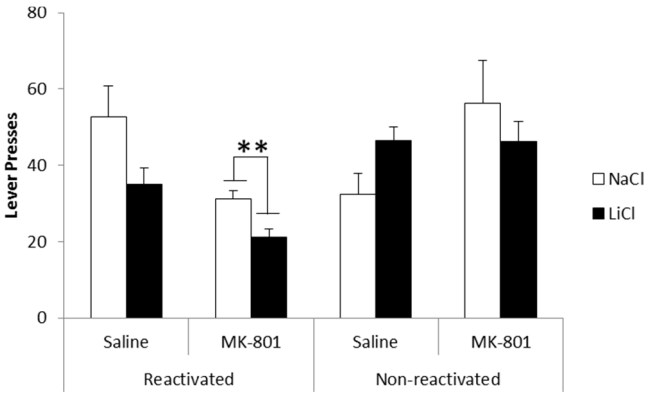

Figure 5.9: Reinforcer pairing to LiCl significantly impairs lever pressing only in rats which previous received MK-801 in combination with memory reactivation session. After initial testing each experimental group was exposed to the reward pellets, which was subsequently paired with either LiCl or saline control. When re-tested (6 days after reactivation), rats previously treated with MK-801 in conjunction with the VR20 reactivation showed a significant reduction in performance when treated with LiCl (n=6) compared to reactivated, MK-801-injected rats given NaCl control-pairings (n=6). Other groups did not shown any significant reduction in performance following treatment with LiCl (black bars; reactivated saline, n=6; non-reactivated saline, n=4; non-reactivated MK-801, n=4) compared to their NaCl-treated controls (white bars; reactivated saline, n=6; non-reactivated saline, n=4; non-reactivated MK-801, n=4). Data expressed as mean + SEM. (**) indicates significant differences in planned comparisons between LiCl and NaCl-treated rats (effective p<0.0125).

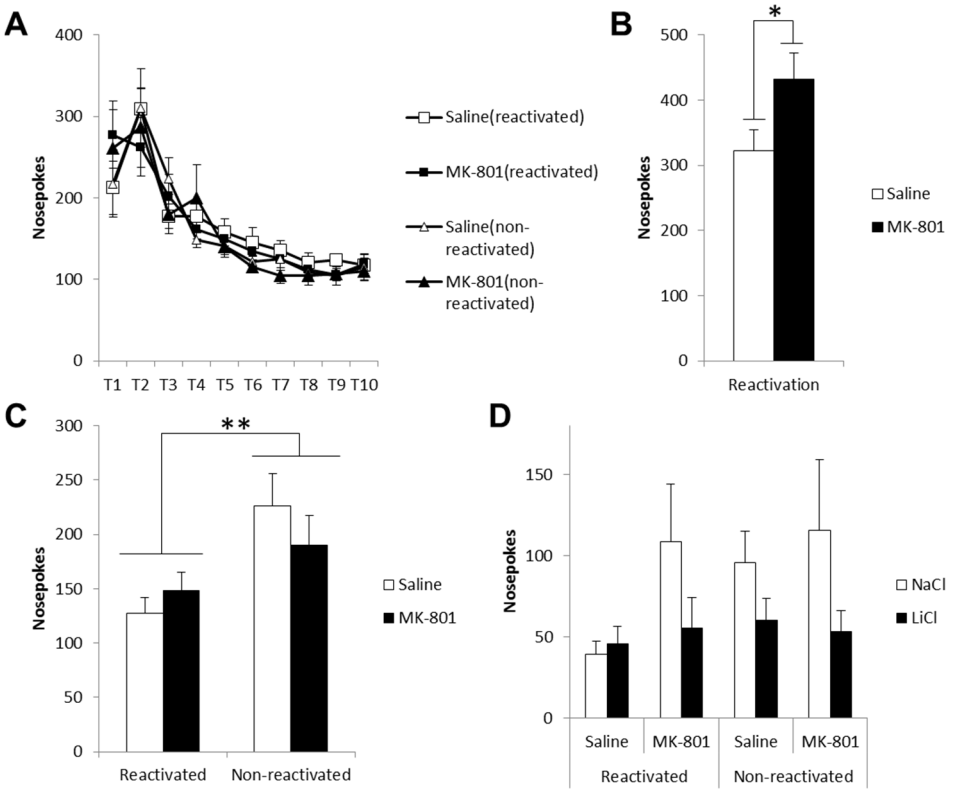

Figure 5.10: MK-801 causes an acute temporary increase in nosepoking at reactivation. Nosepokes are also elevated at test in non-reactivated groups at test. **A**, rats reduce their nosepokes rapidly during over the first (T1), second (T2) and third (T3) days of training. Nosepoking plateaus over the seventh (T7) to tenth (T10) training sessions. Rats in reactivated saline (white squares, n=12) and MK-801 (black squares, n=12) showed similar levels of nosepoke performance as non-reactivated saline (white triangles, n=8) and MK-801 (black triangles, n=8) controls throughout the training phase. **B**, MK-801 injected rats (black bar) experience an acute increase in nosepoking compared to saline controls (white bar) during the VR20 reactivation. **C**, when tested 24 hours after reactivation, non-reactivated rats (right) make significantly more nosepokes than reactivated rats (left) at test, regardless of previous treatment with saline (white bars) or MK-801 (black bars). **D**, there is an overall effect of devaluation following treatment with LiCl (black bars; reactivated saline, n=6; reactivated MK-801, n=6; non-reactivated saline, n=4; non-reactivated MK-801, n=4), however this is not driven by a significant difference in any one group. While visually, the non-devalued reactivated saline group (n=6) appear lower than their non-devalued, NaCl paired counterparts in other groups (reactivated MK-801, n=6; non-reactivated saline, n=4; non-reactivated MK-801, n=4), this difference

was not significant. Data expressed as mean ± SEM. (*) indicates significant differences, $p<0.05$. (**) indicates significant differences, $p<0.01$.

Discussion

The present data show habitual lever pressing can be impaired by administration of MK-801 prior to either a brief extinction or VR20 reactivation session; however MK-801 injected prior to a VR5 session did not produce any long-term behavioural effect. The effect with VR20 was reactivation-dependant, demonstrating the intervention impaired reconsolidation; however the impairment with the 5-minute extinction was not reactivation-dependant. In addition the reconsolidation disruption observed with the VR20 reactivation also caused sensitivity to reward devaluation following treatment, implying the habitual S–R memory was indeed disrupted and behaviour was mediated by an intact A–O trace. These data demonstrate that habitual instrumental memories do destabilise and undergo reconsolidation when exposed to a suitable change in operant contingency.

MK-801 administered prior to the VR20 reactivation appeared to successfully disrupt the reconsolidation of a habitual lever pressing memory. Administration of MK-801 produced a reactivation-dependant effect on the first test session, however this was driven by both a reduction in responding of reactivated-MK-801 and non-reactivated saline groups. This reduced performance in non-reactivated saline animals does not appear to indicate new learning in the reactivated-saline group, as these latter rats performed at similar levels to non-reactivated MK-801 controls. While there was no significant difference between drug and vehicle-injected reactivated rats during the first test, this is consistent with past studies which have disrupted habit memory; typically performance is maintained by the remaining A–O association, and behaviour becomes sensitive to reward devaluation (Yin *et al.*, 2004) or changes in contingency (Packard & McGaugh, 1996; Yin *et al.*, 2006). Importantly, the VR20 session caused rats treated with MK-801 to once again become sensitive to reward devaluation. Since behaviour was shown to be insensitive to reward value (thus habitual) following training, it is reasonable to believe reactivated MK-801-treated rats became

amnesic for the S–R memory, owing to a disruption of the reconsolidation process. Furthermore, the reactivation-dependent effect of MK-801 was still present on the devaluation test (6 days after reactivation); persistence of amnesia has been suggested to be an important criterion for the assessment of a reconsolidation impairment (Dudai, 2006). Moreover, the reactivation-dependant effect on the devaluation test was driven by a significant reduction in the responding of reactivated, MK-801-treated rats compared to their saline controls, perhaps highlighting that this group was more sensitive to the devaluation treatment. Neither the saline vehicle nor non-reactivated controls showed any significant devaluation effect post-treatment, implying the behaviour of these groups remained habitual; however as there was no devaluation interaction in the overall ANOVA it may be that the devaluation analysis was underpowered. The reactivated saline-injected rats did show a trend towards a devaluation effect, but sensitivity to reward devaluation has been demonstrated previously with similar group sizes (Chapter 4); thus it is unlikely this trend would be realised with increased rat numbers.

Nosepokes have been used previously to measure pavlovian motivational memory (Diergaarde *et al.*, 2006). Reductions in nosepoke responding may represent either a lack of motivation, or reduced expression of pavlovian memory. For example, the training data show a consistent initial rise in nosepoking, followed by a reduction with extended training. Since prolonged training leads to habitual responding (Adams, 1982) it may be the sharp reduction in nosepoking on the 3rd day of training, and the moderate decline as training continues, represents a behavioural shift to S–R driven responding. The reducing levels of nosepokes over the course of training are unlikely to represent reductions in vigour, however, given that lever pressing increases in parallel to maximal levels. This pattern of data, in which nosepokes initially rise then reduce with time has been observed in other instrumental lever experiments with prolonged training (Hernandez *et al.*, 2005), although it may be the case that it is related to the use of a pre-training session (Smith-Roe & Kelley, 2000). During testing there was no significant reduction of nosepokes in MK-801-injected rats compared to their respective saline controls, regardless of reactivation condition. This would strongly suggest drug and vehicle groups were equally motivated; however nosepokes were significantly reduced in VR20 and

5-minute reactivation groups compared to the non-reactivated controls. In the case of the 5-minute session this may be because of extinction during reactivation, consistent with the evidence from the lever pressing data (discussed below). In the case of the VR20 condition the reduction in nosepokes may also represent extinction learning or alternatively degradation of the pavlovian stimulus–outcome contingency, as nosepokes will have been less rewarded than in training. Notably this reduction in nosepokes with the VR20 session affected both saline and MK-801-treated rats equally. While MK-801-injected made fewer lever presses, they made increased numbers of nosepokes. Therefore, the reduction in lever pressing cannot be attributed to a loss of motivation. This would strongly indicate that the pavlovian-incentive memory was intact, and thus that the lever pressing impairment was due to a loss of instrumental habit memory.

The finding that the reconsolidation of instrumental habit memory was disrupted contradicts past literature on instrumental reconsolidation which has suggested instrumental memories do not undergo reconsolidation (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009). A possible explanation for this discrepancy is that previous literature has used training trials to destabilise the memory, with no change in the contingency of reward delivery. The present study found no effect of the VR5 reactivation on reconsolidation and given the dependency of destabilisation on contingency change in a goal-directed setting (Chapter 4) and the likely function of reconsolidation as an updating mechanism (Lee, 2009, 2010) it seems unlikely an FR1 schedule would have produced any effect. Additionally, we have previously shown a brief training trial to be ineffective in destabilising a goal-directed instrumental memory (Chapter 4), and training trials do not destabilise pavlovian fear memory when the contingency is well learned (Díaz-Mataix *et al.*, 2013). Importantly, the effect of VR20 was reactivation-dependant and sharply contrasts with previous studies. Thus, the lack of an instrumental effect in past literature appears to be the use of inappropriate reactivation parameters.

MK-801 also appeared to cause a greater decline in responding over the course of the test session when given in conjunction with a 5-minute extinction reactivation; however this effect of MK-801 was not reactivation-dependant and therefore unlikely to represent an impairment of

reconsolidation. Consistent with this interpretation, rats given the 5-minute reactivation in the present study did not subsequently show sensitivity to reward devaluation, suggesting their behaviour remained habitual; the implication being that reconsolidation of the S–R memory was not disrupted. A possible explanation for the effect of MK-801 may be that the 5-minute reactivation caused extinction, a plausible theory given a brief 2-minute session triggers new extinction learning in a goal-directed setting (Chapter 4) and supported by the significantly lower responding at the beginning of the test session in rats given the 5-minute reactivation compared to non-reactivated controls. Interestingly MK-801 did not impair this new extinction, and this may have been due the specific parameters of the reactivation session as under certain conditions MK-801 impairs neither reconsolidation or extinction (Flavell & Lee, 2013). The specific reactivation parameters seem the most likely explanation for the lack of an MK-801 effect on extinction, as it has previously been shown to impair instrumental extinction learning (Lissek & Güntürkün, 2003; Kelamangalath *et al.*, 2007). The precise cause of the effect of MK-801 with the 5-minute reactivation remains unclear. The MK-801 appeared to cause a general change in extinction pattern throughout the test session; a visual inspection of the data appears to show MK-801-treated rats started the extinction session at a slightly higher rate of lever pressing, and then reach their baseline responding sooner than saline controls. The higher initial responding may be due to the hyperactivity caused by MK-801 at the doses used here (Hargreaves & Cain, 1995), however the acute effect of MK-801 wears off after a few hours and thus should not have influenced test responding. Regardless of the precise cause of the effect, the 5-minute reactivation did not destabilise S–R memory.

The VR5 reactivation condition produced no significant effect on lever pressing, or nosepoking. While it cannot be determined whether an effect on S–R memory was masked by an intact A–O association, as no post-reactivation devaluation was carried out in this experiment, this seems unlikely since MK-801 administered prior to the VR20 did produce a modest decrease in lever pressing. Also, a visual inspection of the data shows MK-801 injected rats respond at a slightly higher level following VR5 compared to VR20 reactivation. The lack of effect with VR5 would suggest that destabilisation of memory did not occur with this reactivation session. This demonstrates that the differing effects of

5-minute extinction exposure and the VR20 reactivation was not simply due to reinforcer exposure in the latter condition. The ineffectiveness of the VR5 reactivation to destabilise habitual memories is surprising given we have previously shown the VR5 condition to be successful in a goal-directed setting (Chapter 4). Destabilisation of memories is believed to occur following a prediction-error signal (Lee, 2009; Sevenster *et al.*, 2013); however the 5-minute extinction session, VR5 and VR20 schedules all represent a change from the training conditions and should all trigger an error signal. Learning theory proposes that prediction-error signals drive learning and are proportional to the salient difference between the observed and expected outcome (Rescorla & Wagner, 1972). The lack of effect with the 5-minute extinction may be due to the lack of pellets, and thus does not cause a salient learning signal. In the case of the rewarded reactivations, as the VR20 schedule represents a larger deviation from the training parameters, thus involves greater uncertainty, it likely produced a larger error signal than the VR5; a given magnitude of error signal may be required to reach a threshold necessary to trigger destabilisation. Alternatively it has been suggested reconsolidation deficits may be proportional to the prediction-error signal generated (Reichelt & Lee, 2013b), and it maybe the larger putative error signal generated by the VR20 reactivation produced a larger deficit. While VR20 did produce a larger magnitude of reduction in lever pressing at test, neither the VR20 or 5-minute extinction session led to any overall significant reduction in responding during the first test; but only MK-801 given prior to the VR20 reactivation caused behaviour to become sensitive to reward devaluation. Thus the data presented here seem to support the interpretation that a certain threshold of prediction-error is required in order to memory to be destabilised.

An alternative explanation for the lack of effect with VR5 may be that rats which are behaving habitually cannot detect the VR5 contingency change. Habitual rats are less sensitive to contingency changes (Yin & Knowlton, 2006; Ostlund & Balleine, 2008b) and it may be the more extreme VR20 was required for the rats to detect a salient change (and thus produce an error signal). The VR20 reactivation also had a greater variability in reward delivery than the VR5 session and it is unclear whether the change in contingency or unpredictability of reward delivery provided the salient difference between the VR5 and VR20 sessions. A possible solution is to test both fixed-ratio and

variable/random-ratio schedules of reinforcement. If changes in contingency trigger reconsolidation then fixed and random-ratio schedules should be equally effective in destabilising instrumental memory. If unpredictability is required then only variable/random-ratios should be capable of inducing reconsolidation. Finally, the lack of effect with VR5 may be related to memory age or strength; the VR5 reactivation has previously been shown to be effective in a weakly-trained goal-directed setting (Chapter 4). This would be consistent with previous studies, which have shown that older (Suzuki *et al.*, 2004) or stronger (Reichelt & Lee, 2012) memories typically require longer or more frequent stimulus exposure at reactivation in order to destabilise. Further study is required to determine to contribution of memory age and strength to the reactivation parameters required for destabilisation.

One final interesting finding is the nosepoke behaviour following reward devaluation. Nosepoke responses did show a significant devaluation effect in rats given either 5-minutes extinction or no reactivation. While there was only an effect in the overall ANOVA, this is consistent with past literature showing devaluation of nosepokes in habitual animals (Killcross & Coutureau, 2003). Following the VR20 reactivation the MK-801-treated rats showed a similar trend towards devaluation of nosepokes as rats given other reactivation sessions, however saline controls show no such effect. In fact, devalued-saline-treated animals showed almost equal nosepokes non-devalued controls, and both groups nosepoked at a low level. This pattern of data in VR20-reactivated saline controls may represent a successful reconsolidation process during reactivation, leading to updating of the contingency and/or strengthening of memory. As rewards now require more lever presses to obtain, nosepokes will be more infrequently rewarded and thus one would predict frequency of nosepoking should reduce if the contingency was updated. This does not appear to have affected lever pressing vigour, as VR20-saline-injected animals lever press at similar levels to MK-801-injected non-reactivated controls. Furthermore, since nosepoke performance was not impaired in VR20-MK-801-treated rats (at least compared to non-reactivated controls) the lever pressing impairment of this group cannot be attributed to any loss of vigour or motivation. As such it appears habit memory was indeed impaired in VR20-reactivated, MK-801-injected rats.

The VR20 reactivation successfully destabilised an instrumental S–R association, the reconsolidation of which was disrupted by systemic MK-801. These data demonstrate the reconsolidation of habit memories appears to be dependent upon NMDAR transmission within the brain. Possible loci of action include the nucleus accumbens, known to been important for the acquisition (Kelley *et al.*, 1997; Hernandez *et al.*, 2002) of instrumental behaviour and reconsolidation of A–O memory (Chapter 4), or the dorsolateral striatum (DLS). The DLS a likely site of memory storage as lesions (Yin *et al.*, 2004) or inactivation (Packard & McGaugh, 1996; Yin *et al.*, 2006) of this region impair habit expression. Alternatively the reconsolidation-impairing action of MK-801 may have been driven by its dysregulation of modulatory neurotransmitters in the brain, such as dopamine, serotonin and noradrenaline (Yan *et al.*, 1997; López-Gil *et al.*, 2009). Impairments in the function of these modulatory transmitters may have impaired reconsolidation alone, or acted in concert with systemic NMDAR antagonism; for example NMDAR and dopamine-1 receptor co-activation have previously been shown to be important for the reconsolidation of goal-directed instrumental memories (Chapter 4). The β-blocker, propranolol has previously been shown to disrupt reconsolidation in appetitive (Diergaarde *et al.*, 2006; Milton, Lee, & Everitt, 2008) and aversive (Muravieva & Alberini, 2010; Soeter & Kindt, 2011) pavlovian memories, suggesting a role for noradrenaline in some forms of pavlovian memory reconsolidation. Further investigation is required to elucidate the potential role of monoamine transmitters in the reconsolidation of instrumental memories.

There were also acute effects of MK-801 during the VR20 and 5-minute reactivations. In both conditions MK-801 increased lever pressing, likely due to the hyperactivity effect caused by the doses used here (Hargreaves & Cain, 1995). The lack of any acute effect during the VR5 reactivation is likely due to rats all reaching the reward-cap during the session, preventing an effect from being observed. Interestingly MK-801 only had a significant acute effect on nosepoking during the VR20 reactivation session. Again this is likely due to hyperactivity caused by the drug. The absence of any acute effect on nosepokes with the VR5 session is again likely to be caused by all rats obtaining the maximum number of pellets. The cause of the inability of MK-801 to acutely increase nosepokes, but still increase lever presses, in the 5-minute extinction session is unclear; it is possible that the absence of

the pellets during this session resulted in this difference, possibly due to faster extinction of nosepokes than lever presses.

In summary, the data presented here suggest habitual S–R memories do undergo reconsolidation, which can be disrupted by systemic MK-801. The destabilisation of the memory trace requires a larger change in contingency than that required for goal-directed associations, possibly due to memory strength or age, or the reduced sensitivity of habits to contingency change. After disruption of the habit, behaviour became sensitive to reward devaluation, suggesting it was under A–O control.

CHAPTER 6

GENERAL DISCUSSION

This thesis set out to demonstrate the reconsolidation phenomenon in the memories underlying instrumental behaviour, a major class of memories which existing literature has not yet shown to undergo reconsolidation. The initial experiments were without success, however this was not due to MK-801 being ineffective at disrupting reconsolidation as MK-801 appeared to disrupt memory reconsolidation in two novel aversive paradigms. While these two aversive experiments were perhaps inconclusive, the potency of MK-801 to disrupt memory was confirmed. Later appetitive findings demonstrated reactivation-dependant amnesia in both goal-directed and habitual instrumental memories. Both types of memory were found to destabilise following a change in contingency, with habits requiring a more dramatic VR20 schedule, rather than the VR5 used to successfully to destabilise goal-directed lever pressing memory. Reconsolidation of both types of instrumental memory was disrupted by systemic administration of the N-methyl-D-aspartate receptor (NMDAR) antagonist, MK801, suggesting instrumental memory reconsolidation is dependent upon NMDAR transmission. Furthermore, the reconsolidation of goal-directed memories was shown to require co-activation of dopamine-1 receptors (D1Rs) and NMDARs in the nucleus accumbens (NAc), suggesting a possible role for dopaminergic signalling in the reconsolidation process. These results have implications for our understanding of memory as they fill a key gap in our knowledge, and suggest that reconsolidation is a universal process of memory maintenance. The requirement for a change in contingency in order for the reconsolidation process to be initiated is particularly interesting as habits are traditionally thought to be insensitive to outcome contingency. These results are interpreted as suggesting a role for motivational feedback signals and value updating in the destabilisation of memory traces. The ability to disrupt habit memories may also be useful in developing novel treatments for maladaptive memory disorders such as compulsive food seeking, drug addictions and obsessive compulsive disorder.

Reconsolidation as a universal mechanism

The process of reconsolidation has been demonstrated in a variety of both appetitive and aversive memories (see general introduction). Among these are contextual fear (Lee *et al.*, 2004; Lee, 2008), auditory fear (Nader *et al.*, 2000), inhibitory avoidance (Milekic & Alberini, 2002; Boccia *et al.*, 2004; Inda *et al.*, 2011), pure contextual memory (Lee, 2010), conditioned place preference (Milekic *et al.*, 2006; Valjent *et al.*, 2006; Brown *et al.*, 2008; Robinson & Franklin, 2010; Robinson, Armson, *et al.*, 2011), pavlovian appetitive context memory (Fuchs *et al.*, 2009; Wells *et al.*, 2011), autoshaping (Lee & Everitt, 2008c), incentive memory (Wang *et al.*, 2005) and conditioned reinforcement (Lee *et al.*, 2005; Milton, Lee, & Everitt, 2008; Théberge *et al.*, 2010). Furthermore, data in this thesis also support that conditioned place aversion (CPA) memories undergo reconsolidation (Chapter 3), consistent with past literature (Wu, Li, Yang, *et al.*, 2012). The key finding of this thesis to current knowledge of reconsolidation, is the addition both goal-directed (Chapter 4) and habitual (Chapter 5) instrumental memories to the list of memories which are believed to undergo reconsolidation. This list is not exhaustive and reconsolidation has not been tested for the memories underlying certain behaviours, for example latent inhibition; however reconsolidation seems to occur in all types of memories that have so far been tested, provided the appropriate reactivation parameters are used.

Furthermore, the reconsolidation process has been shown to occur in many different species, including humans (Hupbach *et al.*, 2007, 2009) and other vertebrates (Nader *et al.*, 2000; Cestari *et al.*, 2006; Perrin *et al.*, 2007; Coureaud *et al.*, 2009), as well as several invertebrates (Pedreira *et al.*, 2002; Stollhoff *et al.*, 2008; Carbó Tano *et al.*, 2009; Cai *et al.*, 2012). While typically studies have used adults, reconsolidation has also been demonstrated in neonatal rabbits (Coureaud *et al.*, 2009) and adolescent rats (Achterberg *et al.*, 2012; Flint *et al.*, 2013). Thus reconsolidation has been demonstrated in a variety of species, and a variety of memory settings. While there may be an as yet untested species or memory type which does not undergo reconsolidation, reconsolidation appears to be conserved across the animal kingdom and across experimental memory paradigms. As such reconsolidation is likely an evolutionarily conserved mechanism of memory maintenance.

Dependence of reconsolidation on NMDARs

Reconsolidation of both appetitive goal-directed and habitual instrumental memories (Chapters 4 and 5), aversive CPA, and potentially also active avoidance (Chapter 3) were found to be disrupted by systemic administration of MK-801. This adds to a body of research suggesting NMDARs are required for the reconsolidation process (Lee *et al.*, 2006a; Brown *et al.*, 2008; Milton, Lee, Butler, *et al.*, 2008; Winters *et al.*, 2009). This finding is consistent with literature which lays out a key role for NMDARs in learning and memory, both in reconsolidation and memory consolidation (McGaugh, 2000; Tronson & Taylor, 2007; Milton, 2013). There are still, however, certain paradigms in which the requirement for NMDARs has not been tested, such as inhibitory avoidance. While the initial acquisition of inhibitory avoidance requires NMDAR activity (Liang *et al.*, 1994), and inhibitory avoidance memories do undergo reconsolidation (Milekic *et al.*, 2007; Baratti *et al.*, 2008), it may be reconsolidation is NMDAR-independent in certain paradigms; although NMDAR activity does appear to be a universal requirement given our current knowledge.

Notably, protein synthesis inhibition is considered the canonical demonstration of reconsolidation (Tronson & Taylor, 2007); however this thesis made use of the NMDAR antagonist MK-801. Use of protein synthesis inhibition in appetitive settings suffers from the problem that it appears to be capable of conditioning to the reinforcer, forming a conditioned taste aversion (Hernandez & Kelley, 2004). This effect has not always been observed (Wang *et al.*, 2005; Mierzejewski *et al.*, 2009), but has been noted using intra-cerebral, as well as systemic, inhibition of protein synthesis (Jonkman & Everitt, 2009, 2011). This presents a particular confound for appetitive studies as they must dissociate between loss of reward-incentive, and impairment of memory. The present thesis used nosepoke responses to provide a measure of the incentive motivation to acquire the pellets, and loss of A–O and S–R memory was observed without any reduction in nosepoking (and thus presumably without any effect on US-incentive). In the case of the habit study (Chapter 5), loss of S–R memory was also confirmed by a sensitivity to reward devaluation. The present thesis can provide a framework for future studies to investigate the requirement for protein synthesis in instrumental

memory reconsolidation, however care must be taken to dissociate any putative memory deficit from a loss of motivation or incentive.

Involvement of dopamine in instrumental reconsolidation

This thesis made extensive use of the NMDAR antagonist, MK-801. However, there may also be other effects of MK-801 on monoamine transmission (Yan *et al.*, 1997; López-Gil *et al.*, 2009) which may make a contribution to the amnestic effect of the drug. For example, β-adrenergic receptors are believed to play a role in pavlovian memory reconsolidation (Milton, Lee, & Everitt, 2008; Kindt *et al.*, 2009; Muravieva & Alberini, 2010) and the reconsolidation of a goal-directed instrumental memory was shown to require co-activation of D1Rs and NMDARs in the NAc (Chapter 4). Thus there may be a requirement for dopamine in instrumental memory reconsolidation. As MK-801 is known to affect dopamine transmission, this may have been inadvertently disrupted by the MK-801 in the present experiments. Certainly, there appears to be a role for accumbens dopamine in pavlovian memory consolidation (Dalley *et al.*, 2005).

Alternatively, the finding of a dopaminergic contribution to instrumental reconsolidation may be related to the involvement of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) in instrumental behaviour. Dopamine has been strongly linked to the acquisition of instrumental behaviour. Antagonism of D1Rs in the NAc impairs acquisition of lever pressing (Smith-Roe & Kelley, 2000; Hernandez *et al.*, 2005) and electrical intra-cranial self-stimulation of the VTA, the primary source of accumbal dopamine input (Westerink *et al.*, 1996; Hnasko *et al.*, 2012), is sufficient to support acquisition of a lever pressing response (Cheer *et al.*, 2007). Interestingly, optogenetic activation of dopaminergic VTA neurons is not sufficient to support instrumental lever pressing (Adamantidis *et al.*, 2011); however lever pressing can be supported by optogenetic activation of the SNc (Rossi *et al.*, 2013), suggesting dopamine from the VTA and SNc serves different behavioural functions. While this finding may imply VTA dopamine is not involved in instrumental acquisition, NMDAR activity in the VTA is required for acquisition of reward behaviours (Zellner *et al.*,

2009; Ranaldi *et al.*, 2011), and acquisition of habit behaviours appears to require NMDARs expressed in dopaminergic neurons (Wang *et al.*, 2011), possibly involving activity in both the VTA and SNc (Faure *et al.*, 2006).

An interesting finding by Adamantidis *et al.* (2011) was that, while optogenetic stimulation of the VTA was not sufficient to support instrumental learning, when two levers were reinforced with food it was sufficient to bias responding towards a specific lever. This effect appears to have been driven by enhancing the motivation value of pavlovian cues associated with food-delivery. Consistent with this finding, inactivation of the VTA using baclofen and muscimol impairs pavlovian-instrumental transfer (PIT) effects (Murschall & Hauber, 2006); additionally a similar VTA-inactivation also impairs destabilisation of pavlovian goal-tracking memory (Reichelt *et al.*, 2013). This may imply additional roles for the VTA, or a role of motivation in destabilisation. The latter of these possibilities seems more likely, given that PIT is a motivation based process; it seems likely that the VTA is involved in signalling incentive salience (Berridge, 2012). This may be used to signal shifts in attention and "surprise", which may be a key component of prediction error signalling.

Production of a prediction error signal is believed to be required for reconsolidation to occur (Lee, 2009; Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013), and is likely dopaminergic in nature (Schultz, 2007). Given the finding here that co-activation of NMDARs and D1Rs in the NAc is required for goal-directed memory reconsolidation (Chapter 4), and given the VTA is the primary source of dopamine to the NAc, the VTA appears to be involved in both memory acquisition, destabilisation and reconsolidation. Additional research is required to elucidate these different functions in learning and memory, however it may be there are specific sub-populations of neurons in the VTA performing different functions. Certainly different cell populations appear to signal reward and punishment (Cohen *et al.*, 2012; Lammel *et al.*, 2012) and different sub-populations of neurons appear to project to different brain regions (Ikemoto, 2007). Furthermore more medial VTA dopamine neurons appear to co-release dopamine and glutamate in the NAc and are less sensitive to dopamine-2 receptor (D2R) mediated inhibition (Hnasko *et al.*, 2012). As it was possibly these co-releasing

dopamine/glutamate neurons that were involved in goal-directed memory reconsolidation, a different subpopulation than that targeted by Reichelt *et al.* (2013) using D2R antagonism, it seems these two studies likely disrupted different neural circuits. As such different dopaminergic circuits may be involved in destabilisation and reconsolidation, and/or may have differential involvement in pavlovian and instrumental tasks. Furthermore, central amygdala (CeN) and SNc neurons are believed to signal prediction errors in pavlovian tasks (Lee *et al.*, 2010). Given that the CeN interacts with the dorsolateral striatum (DLS) in the expression of habits (Lingawi & Balleine, 2012), there may be a role for SNc (which projects to the DLS) prediction errors in habit destabilisation and/or reconsolidation.

Notably, dopamine neurons in both the VTA and SNc also receive input via acetylcholine (Dani & Bertrand, 2007), although expression of receptor subtypes differs between the regions (Wooltorton *et al.*, 2003). Acetylcholine signalling has previously been shown to play a role in operant memory expression (Bradfield *et al.*, 2013), and also appears to play a role in inhibitory avoidance memory reconsolidation (Boccia *et al.*, 2004; Blake *et al.*, 2012). However, it is currently unknown whether cholinergic mechanisms also play a role in the reconsolidation of operant, or other, types of memory.

Receptor co-activation in reconsolidation

One interesting finding was the involvement of D1-NMDAR co-activation in the reconsolidation of a goal-directed instrumental memory (Chapter 4). Interestingly, co-release of neurotransmitters appears to part of the normal functioning of neurons (Hnasko & Edwards, 2012); for example GABAergic neurons can co-release GABA and glycine (Wojcik *et al.*, 2006). Notably certain neurons in the VTA co-release glutamate and dopamine from their synaptic terminals (Joyce & Rayport, 2000; Descarries *et al.*, 2008), and these neurons specifically co-release glutamate and dopamine in the shell of the NAc, but not the dorsal striatum (Stuber *et al.*, 2010). It may be that co-infusion of SCH23390 and AP-5 specifically disrupted function in a certain subset of accumbens neurons, independent of dopamine's putative error-signalling role in destabilisation. This does seem plausible

as dopamine signals to the accumbens appear to stem from several VTA populations (see above). Furthermore, different regions of the NAc appear to show different sensitivity to glutamate antagonism during cocaine seeking (Xie *et al.*, 2012), and different subpopulations appear to signal reward to cocaine versus natural (i.e. food) reinforcers (Carelli *et al.*, 2000); also, only a small specific sub-region appears to form a reward-signalling "hedonic hotspot" (Peciña, 2008). Given different functional circuits appear to exist within the accumbens it is reasonable to assume there may also be distinct prediction error and reconsolidation mediating neurons. Whether some of the functionality of these circuits overlaps is currently unknown. While this would strongly imply a role for a VTA-NAc shell circuit in the reconsolidation of instrumental memory, it may be that the accumbens shell region has a broader role in appetitive conditioning, as disconnection of the shell from the hippocampus can impair appetitive spatial learning (Ito *et al.*, 2008b). There may also be different VTA-accumbens circuitry mediating destabilisation and reconsolidation (Reichelt *et al.*, 2013). Furthermore certain populations of cholinergic (Manns *et al.*, 2001), serotonergic (Nicholas *et al.*, 1992) and noradrenergic neurons (Fung *et al.*, 1994) may all co-release glutamate with their monoamine transmitters (Ottersen & Storm-Mathisen, 1984; El Mestikawy *et al.*, 2011). Given that β-adrenergic receptors have been implicated in the reconsolidation of certain types of memory (Milton, Lee, & Everitt, 2008; Muravieva & Alberini, 2010; Robinson, Armson, *et al.*, 2011; Wu, Li, Yang, *et al.*, 2012) it may be interesting for future studies to investigate the requirement for co-activation of β-adrenergic and glutamatergic receptors in other brain regions, such as the amygdala, in the reconsolidation of certain memories.

Boundary conditions on the reconsolidation of instrumental memories

Memories will not always destabilise and undergo reconsolidation following reactivation. Several factors influence whether destabilisation occurs including the age (Milekic & Alberini, 2002; Suzuki *et al.*, 2004; Robinson & Franklin, 2010) and strength of the memory (Suzuki *et al.*, 2004; Robinson & Franklin, 2010; Reichelt & Lee, 2012; Flavell & Lee, 2013), in addition to the precise parameters of the

reactivation session (Suzuki *et al.*, 2004; Lee & Everitt, 2008b; Reichelt & Lee, 2012). As reconsolidation is not engaged under certain circumstances, there must exist several boundary conditions on the reconsolidation process which determine whether or not a memory will destabilise in any given circumstance. In the case of instrumental memories, goal-directed Action–Outcome (A–O) associations were found to be destabilised by exposure to a new VR5 contingency (Chapter 4). This is consistent with recent pavlovian reconsolidation literature which suggests that changes in contingency can destabilise pavlovian memory (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013). In the same vein, habitual Stimulus–Response (S–R) behaviour was destabilised by switching to a VR20 schedule of reinforcement (Chapter 5); however the VR5 reactivation condition produced no significant effect on habit memory. There were other notable failures to destabilise instrumental memories. Neither A–O nor S–R associations were destabilised by a brief extinction session (Chapter 2, 4 and 5), in fact the brief extinction session appeared to cause extinction in the 2-minute condition used in the goal-directed study (Chapter 4). While it may be unexpected that brief extinction session do not trigger destabilisation of instrumental traces given they are typically used to induce reconsolidation (Lee *et al.*, 2006a; Milton, Lee, & Everitt, 2008), past studies that have used extinction reactivations in instrumental settings have not destabilised the instrumental components of memory (Lee & Everitt, 2008b). The longer 5-minute extinction reactivations used in Chapters 2 and 5 did not appear to elicit extinction, however this may have been due to the extended duration of training in the habit study (or alternatively extinction learning did occur but was not blocked by MK-801) and the presence of an overt pavlovian cue (the lever retraction) in the early Chapter 2 experiments. This may have led to the 5-minute extinction being intermediate between the conditions required to elicit either destabilisation or reconsolidation; under such conditions MK-801 has been shown previously to have no long-term effect on expression of pavlovian food memory (Flavell & Lee, 2013). It remains to be determined whether even briefer extinction sessions can destabilise instrumental memories. While based on the present thesis this seems unlikely, there is some evidence that *zif268* expression is increased in the posterior dorsolateral striatum (pDLS) following a single retrieval trial of a spatial navigation habit (Milton & Everitt, 2012); however

whether this increase represents a reconsolidation process is unclear. Expression of *zif268* does appear to play a role in instrumental learning and memory (Hernandez *et al.*, 2006), however its contribution to the reconsolidation of instrumental memories remains unknown.

Goal-directed memory was also not destabilised by a brief training session with no change in contingency; given the ineffectiveness of the VR5 condition in the habitual setting and the requirement for a more dramatic change in contingency, it seems unlikely that an FR1 reactivation session would have been effective. These findings are consistent with past literature which has found instrumental memories do not destabilise following brief training trials (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009), or brief extinction reactivations (Lee & Everitt, 2008b). Since both actions and habits have been shown to undergo reconsolidation in the present thesis, the interpretation is that past literature did not observe a reconsolidation effect owing to lack of appropriate reactivation parameters.

Requirement for reinforcer presentation

Past literature has shown memory destabilisation to be sensitive to precise reactivation conditions (Milekic *et al.*, 2006; Valjent *et al.*, 2006; Lee & Everitt, 2008b; Reichelt & Lee, 2012; Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013). Among these, Lee and Everitt (2008) showed that in an instrumental setting a sucrose-paired pavlovian conditioned stimulus (CS) must be contingent upon a lever press in order for the CS–sucrose memory to destabilise. Interestingly the requirement for lever–CS contingency in destabilising CS–reward memory was not present in a similar experiment using intra-venous cocaine as a reinforcer (Lee *et al.*, 2006b); this may have been due to the different reinforcers or the mode of delivery, as sucrose pellets must be collected and eaten. Since sucrose pellets were used in the present thesis, it may be the lack of effect with the brief extinction reactivations in Chapters 4 and 5 was due to a lack of a reward paired CS that was present during training. While every effort was made to minimise pavlovian influences on memory in these studies, there may still have been some unavoidable conditioning, such as to the sound of the pellets

dropping into the magazine. This sound would not have been present at reactivation as pellets were not delivered, and this may have prevented destabilisation.

It seems highly unlikely that the presence of the pellets in the VR5 and VR20 reactivations was the cause of their effectiveness. In Chapter 2 reactivation involved the retraction of the lever, likely to be a CS (Davey *et al.*, 1981). This should have aided destabilisation were pellet associated cues required during reactivation; this was not the case. Furthermore the rewarded reactivations, which would have had all stimuli present as during training (including the act of collecting an eating the reward pellets), did not reliably trigger destabilisation. In the case of a goal-directed memory, MK-801 prior to an FR1 session was without significant effect, for habit memories VR5 was ineffectual. Thus it seems that the presence of the reinforcer during reactivation sessions is not sufficient for destabilising instrumental memories, although it may be necessary in certain circumstances.

Additionally, the presence of the unconditioned stimulus (US) did not appear to be required in order to destabilise CPA memory (Chapter 3). This may have been caused by exposure to the non-confined context triggering updating of that context (see Chapter 3 for discussion). In contrast the confined reactivation was ineffective at destabilising CPA memory. Confined reactivations are frequently used to destabilise conditioned place preference (CPP) memory (Milekic *et al.*, 2006; Valjent *et al.*, 2006; Wu, Li, Gao, *et al.*, 2012), however these are only effective when combined with US exposure (Milekic *et al.*, 2006; Valjent *et al.*, 2006). Furthermore, non-confined reactivations are also effective in triggering destabilisation of CPP (Kelley *et al.*, 2007; Itzhak, 2008; Robinson & Franklin, 2010; Robinson, Ross, *et al.*, 2011). Thus, US exposure is not always necessary, nor always sufficient for the destabilisation of memory traces.

Changes in contingency

Both A–O and S–R memories were destabilised by changes in reward contingency. However, a greater change in contingency was required to destabilise an S–R association. The VR5 reactivation

was effective in a goal-directed but not habitual setting, where VR20 was successful. The lack of effect with VR5 in the habitual setting may be due to a boundary condition on memory destabilisation. Older (Milekic & Alberini, 2002; Suzuki *et al.*, 2004), or more well-trained memories (Suzuki *et al.*, 2004; Reichelt & Lee, 2012), are typically more resistant to memory destabilisation, and often require "stronger" reactivation sessions compared to younger ones in order to destabilise; this typically involves longer, or more frequent, CS exposure (Suzuki *et al.*, 2004; Reichelt *et al.*, 2013). Thus, the better-trained habit memory may have required a more extreme ("stronger") contingency change during reactivation in order for the memory to destabilise. Alternative explanations for the requirement of a larger VR20 contingency change in order for destabilisation to occur may be that the session preferentially engaged habitual or goal-directed processes, or even engaged both simultaneously (discussed below). It may be that certain neural systems must be actively engaged in behaviour for reconsolidation of instrumental memory to occur.

One key outstanding question from Chapters 4 and 5 is precisely what features of the variable-ratio sessions was required for memory destabilisation to occur. The session involved lever pressing, US presentation, and also a reduction in the frequency of reward delivery (establishing a change in contingency between the response and outcome). Since brief extinction sessions and an FR1 (for actions, VR5 for habits) contingency were not sufficient to destabilise instrumental memory (see above) it is highly unlikely that simple exposure to the lever and/or pellets during the reactivation were the salient features which elicited destabilisation. Thus the salient reconsolidation-trigger of the reactivation must have been the change in contingency of US delivery; however it is not entirely clear what aspect of this was important to reconsolidation initiation. The contingency change included both an increase in the mean number of responses required to obtain a pellet (the ratio aspect), and an unpredictability of reward delivery (the variability part). The knock on effect of this contingency change was also a reduction in the temporal density of reward delivery. Anecdotally, on the 10^{th} day of training (Chapter 5) habitual rats obtained the maximum number of rewards very quickly, with a mean delay of approximately 5 ± 1 (range: 3 – 20) seconds for each rat. This is far quicker than the rats can eat the pellets, and rewards typically accumulated in the magazine for

many of the rats during the 7th-10th days of training. During the VR5 reactivation session this increases to approximately 20 ± 2 (range: 10 – 50), and 90 ± 10 seconds (range: 50 – 250) in the VR20 condition; this mirrors findings that more unpredictable schedules increase the delay between reward deliveries (Derusso *et al.*, 2010). Both VR5 and VR20 lead to increases in variability (therefore unpredictability) and decreases in reinforcer density; however only VR20 was sufficient to destabilise a habit memory in well-trained animals. A possible reason that only the VR20 destabilised habit memory is that a larger difference is needed in order to produce an error signal of sufficient magnitude to elicit destabilisation. This may be because habits are less sensitive to these contingency changes (Yin & Knowlton, 2006; Ostlund & Balleine, 2008b) and as such a larger change is necessary for a salient change to be detected. An even simpler explanation is that older (Milekic & Alberini, 2002) or stronger memories (Suzuki *et al.*, 2004; Reichelt & Lee, 2012) are resistant to destabilisation, requiring a "stronger" reactivation session (i.e. longer session duration, more frequent CS exposure). One final alternative is that only the VR20 session lead to a reduction in hedonic feedback as the delay between pellet deliveries was longer. It may have been that the pellet deliveries in the VR5 session were still too close together (at least for habitual animals) such that the pellets could be accumulated faster than they were eaten, resulting in no change in hedonic sucrose response during the reactivation as compared to training. In the weakly-trained goal-directed group (Chapter 4) it may be that the VR5 was enough to reduce US frequency to a sufficient degree (such that pellet were eaten faster than they were obtained) that a salient change could be detected, producing an error signal. It may be a change in the hedonic feedback is required to produce a prediction error, believed to initiate reconsolidation (see later for discussion).

A further explanation for the requirement for the VR20 session in order to destabilise habit memory is that the session may have been more effective at engaging habit mechanisms; or even more bizarrely goal-directed processing, which may have been required for habit destabilisation. Actions and habits may be competing processes (Daw *et al.*, 2005; Balleine & O'Doherty, 2010), with evidence for this coming from findings of dissociable neural systems in the brain. Recall that lesions or inactivation of the dorsolateral striatum cause goal-directed behaviour to resurface in well-trained

animals (Packard & McGaugh, 1996; Yin *et al.*, 2004, 2006), and lesions or inactivation of the dorsomedial striatum appear to force behaviour under habitual control (Yin *et al.*, 2005), with a similar dissociation within the medial prefrontal cortex (Coutureau & Killcross, 2003; Killcross & Coutureau, 2003). Furthermore certain training schedules of reinforcement appear to favour one process over the other. Interval schedules are known to favour habit responding; while ratio schedules typically favour goal-directed behaviour (Dickinson *et al.*, 1983; Yin & Knowlton, 2006; Derusso *et al.*, 2010; Wiltgen *et al.*, 2012). It has been suggested that the salient habit-favouring feature of interval schedules is the unpredictable relationship between responding and outcome delivery (Derusso *et al.*, 2010). It is possible that the VR20 may have functionally mimicked an interval schedule by diminishing the predictability between the enacting of a lever press and delivery of the reward. Furthermore more unpredictable schedules appear to increase the delay between reward deliveries (Derusso *et al.*, 2010), and this certainly appears to be the case in the VR20 condition (see above). If the VR20 did mimic an interval schedule it may have been more effective at engaging habit processing, and by extension, more effective at engaging reconsolidation of the habit memory (over the putatively competing A–O memory). If this is the case then, conversely, one might have expected the VR5 reactivation to have been more effective at engaging goal-directed processes. If so we might have expected the VR5 session to have triggered reconsolidation of the goal-directed component of instrumental memory in the habit study (Chapter 5); however this could not have been detected in the habit study as behaviour would have remained habitual and therefore resistant to reward devaluation (just as it was following training). Furthermore impairments in goal-directed, but not habitual, behaviour do not appear to impinge on overall responding when the task is well learned (Killcross & Coutureau, 2003; Yin *et al.*, 2005).

An alternative account of instrumental behaviour suggests that actions and habits can sometimes interact in an integrated fashion, particularly in action selection (Balleine & Ostlund, 2007; Balleine *et al.*, 2009; Balleine & O'Doherty, 2010). One demonstration of this is reinforcer devaluation. While devaluation of the reinforcer does not impair habitual lever pressing in extinction, re-training with the now devalued reward leads to a rapid reduction in performance (Dickinson *et al.*, 1983).

Interestingly inactivation of the dorsomedial striatum appears to diminish this effect (Yin *et al.*, 2005), implying a role for goal-directed processes in the modulation of (putatively) habitual responding. It has been suggested the reason for this effect is that the dorsomedial striatum may suppress habit responding when appropriate (Balleine *et al.*, 2009). Furthermore, it has been argued that since inactivation of the dorsolateral striatum can cause an immediate shift to goal-directed behaviour (and equally dorsomedial inactivation to habits), that both action and habit processes must have been active at the time of inactivation, and indeed may always be simultaneously active (Balleine & O'Doherty, 2010). There is also some evidence of simultaneous processing from lesions of the medial prefrontal cortex (mPFC). While lesions of the infralimbic mPFC cause habitually responding rats to return to goal-directed performance, interestingly lesions of the prelimbic mPFC can cause goal-directed responding to become habitual, even with brief periods of training (Killcross & Coutureau, 2003). This goes against conventional wisdom that suggests behaviour is goal-directed following brief periods of training, becoming habitual with extended training (Dickinson, 1985). The results of Killcross & Coutureau (2003) would imply that A–O and S–R memories are acquired in parallel, and may even interact, during training. Another example of integrated behaviour is outcome-specific pavlovian-instrumental transfer (PIT). Here presentation of an outcome-associated cue biases responding to the response associated with the signalled outcome (Rescorla & Colwill, 1989; Rescorla, 1994; de Wit *et al.*, 2006). Interestingly outcomes of responses can act as predictive stimuli, as well as reinforcing outcomes (Ostlund & Balleine, 2007). Rescorla & Colwill (1989) demonstrated that responses become biased towards the anticipated (rather than presented) reinforcer, indicating that PIT likely engaged use of an A–O association as rats appeared to understand the consequences of their actions, rather than being driven by presentation of an obtainable outcome acting as a stimulus. In another demonstration de Wit *et al.* (2006) showed that muscimol (GABA receptor agonist) inactivation of the mPFC in rats impaired conflict resolution. In this task one outcome signalled the ability to perform a response to obtain a second different outcome, however the signalling-outcome was itself a reinforcing outcome for a different response. Rats were normally capable of solving this task, however inactivation of the mPFC impaired the

ability to select the appropriate response. One interpretation of this finding is that the signalling-outcome cued both responses (as it was itself one obtainable outcome, and a signal for the other), however rats solved the task by using A–O associations to understand the consequences of each action and select the appropriate one. Since an outcome can act as a predictive stimulus (much as in conventional PIT experiments) this has led to the suggestion that the sensory properties of the outcome can cue possible responses via a form of S→R process, with goal-directed processing putatively acting to select appropriate actions from this list of cued possible responses (Balleine *et al.*, 2009; Balleine & O'Doherty, 2010). It may be that the reason habitual processes dominate behaviour following extended training is a strengthening in the ability of sensory cues to solely drive behaviour (Balleine *et al.*, 2009; Balleine & O'Doherty, 2010), rather than a shift in processing regions (i.e. from dorsomedial to dorsolateral, see above). This idea of co-operative goal-directed and habit processes in the selection of actions has a parallel with "actor/critic" models of instrumental behaviour (Daw *et al.*, 2005). In this model the "actor" selects actions based on a set of stored predictions, and these predictions are evaluated by the "critic". This model is particularly relevant here as engagement of the evaluative (and possibly goal-directed) "critic" processing is important for the generation of prediction error signals, which update the stored predictions of the "actor" (Daw *et al.*, 2005; Balleine & O'Doherty, 2010). Thus, it may be a requirement to engage goal-directed processing during the reactivation session in order to generate a prediction error signal that will destabilise instrumental memory.

Since the VR20 session was a ratio-schedule, which are believed to favour goal-directed responding (Dickinson *et al.*, 1983; Wiltgen *et al.*, 2012), in principle it could have engaged these evaluative goal-directed behavioural processes. However, if the VR20 had engaged and destabilised solely goal-directed memory, then we would predict that the session would have had no observable effect, as rats would remain habitual following treatment with MK-801; however, MK-801 injection prior to the VR20 did cause behaviour to become sensitive to reward devaluation, suggesting a return to goal-directed control of behaviour. Therefore, it is very likely habit memory was destabilised by the reactivation; this would imply that, at the very least, habit memory was active during reactivation.

One possibility is that the VR20 session engaged *both* action and habit processes simultaneously. It may even be that activation of both A–O and S–R traces is required in order to the habit to destabilise, as this may be needed to activate "actor/critic" evaluative feedback an generate a prediction error signal. This has parallels with theories of action control and decision making which suggest that both goal-directed and habit processes normally function together and in parallel (see above). As such the ability of the VR20 condition to destabilise habitual instrumental memory may stem from its ability to induce co-operative processing between goal-directed ("critic") and habit ("actor") systems. If both goal-directed and habit functions (and presumably also memory traces) were active during the VR20 reactivation, this raises an interesting question to whether treatment with MK-801 disrupted the A–O, or S–R traces, or even both. A similar question could also be asked of the goal-directed study using a VR5 reactivation schedule (Chapter 4), since habits also appear to be learned during brief periods of training (Killcross & Coutureau, 2003). It is possible that treatment in conjunction with an appropriate reactivation disrupted the reconsolidation of both goal-directed and habitual memories in both studies. If it was the case that both A–O and S–R associations were disrupted, then remaining responding must have been enabled by purely pavlovian mechanisms. This is hypothetically possible as the lever could act as a conditioned stimulus, which rats could then conditioned approach to and then press incidentally in the process of autoshaping to the lever (Davey *et al.*, 1981; Cleland & Davey, 1983). While this is a theoretically possible account of behaviour (Bindra, 1978), as A–O and pavlovian stimulus–outcome memory cannot be behaviourally distinguished by sensitivity to reward devaluation (Dickinson & Balleine, 1994), this question cannot be answered at this time at least in the case of the goal-directed study using VR5 reactivation (Chapter 4). In the case of the habit memory study (Chapter 5) it seems highly likely that goal-directed memory was intact following the reactivation treatment as responding was maintained at a high level, consistent with other studies which have selectively targeted habit processes (Killcross & Coutureau, 2003; Yin *et al.*, 2004). In the goal-directed study (Chapter 4) habit memory could theoretically have been disrupted, and if habits do function to select possible actions (as in the "actor" part of the "actor/critic" model) then impairment of habit memory in the goal-directed study

would have theoretically impaired the ability of rats to select and initiate lever pressing (indeed this appears to be what was observed). While habit memory may have been disrupted in the VR5 condition of the goal-directed study (Chapter 4), this does not seem likely as impairment of habit processes with limited training does not appear to impair performance (Killcross & Coutureau, 2003), as was observed in Chapter 4. As such the most likely explanation for the findings of Chapter 4 and 5 is that VR5 destabilised goal-directed memory with brief amounts of training, while the VR20 destabilised habit memory when training was prolonged. However, destabilisation of habit memory may have required activity in goal-directed circuits in order for there to be evaluative feedback with which to generate a prediction error signal.

Changes in outcome contingency have previously been shown to destabilise pavlovian fear memories (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013), thus it seems highly likely that the contingency change during the variable-ratio sessions was the necessary feature of the reactivation to destabilise instrumental memory. A possible explanation for the ability of the VR20 condition to destabilise habitual, but seemingly not goal-directed, memory may be that it mimicked an interval schedule; interval schedules are believed to favour habit processes (Dickinson *et al.*, 1983; Derusso *et al.*, 2010; Wiltgen *et al.*, 2012). One outstanding question is whether the destabilisation of memory, or even the detection of this contingency change required engagement of goal-directed processing structures. Activation of an evaluative (possibly goal-directed) "critic" may have been necessary in order to produce a destabilisation-inducing error signal. The integrated co-operation of action and habit behaviours is believed to be important in action selection (Balleine *et al.*, 2009; Balleine & O'Doherty, 2010) and it may be that brain regions that have been implicated in decision making and action selection, such as the prefrontal cortex (de Wit *et al.*, 2006; O'Doherty, 2011; Kovach *et al.*, 2012; Duque *et al.*, 2013), may make a contribution to destabilisation and/or reconsolidation of instrumental memories, perhaps through the generation of prediction error signals. In fact by targeting the prelimbic and infralimbic mPFC it may even be possible to artificially induce a prediction error signal to destabilise either A–O action or S–R habit memory, or at the very least enhance the likelihood of destabilisation occurring.

Implications for the treatment of maladaptive habits

Several maladaptive memory disorders are believed to be underpinned by the presence of a persistence maladaptive habit memory; notably, drug addiction (Everitt *et al.*, 2008; Milton & Everitt, 2012), compulsive food seeking (Balleine, 2005; Johnson & Kenny, 2010) and obsessive compulsive disorder (Boulougouris *et al.*, 2009; Gillan *et al.*, 2011). Several reconsolidation-based treatments have recently been developed for maladaptive memory disorders based around the behavioural retrieval-extinction paradigm (Kindt *et al.*, 2009; Monfils *et al.*, 2009; Quirk *et al.*, 2010), similar to that used in Chapter 2. In this setup memory is retrieved, and putatively destabilised, prior to a long extinction session, and has been used successfully to diminish fear responses in human volunteers (Kindt *et al.*, 2009). Whether this procedure does truly destabilise and weaken memory has recently been questioned, and the effect may be due to enhancement of extinction (Chan *et al.*, 2010; Baker *et al.*, 2013). Regardless of the nature of the impairment, this does not diminish its value as a clinical treatment.

In the case of drug addiction, one recent study reduced subjective craving for heroin-associated cues in abstinent addicts using retrieval prior to a long extinction session (Xue *et al.*, 2012), as compared to control subjects which received extinction alone or a 6 hour delay between retrieval and extinction. This reduction persisted for 6 months, however craving ratings did not reduce to the level of a neutral control cue. This is perhaps because the treatment only diminished (some of) the pavlovian components of the drug seeking behaviour; however drug seeking is underpinned by both pavlovian and instrumental processes, and both of these can contribute to relapse (Milton & Everitt, 2012; Milton, 2013). In particular, it is believed that the development of a persistent, compulsive habit is the key memory underpinning drug-seeking behaviour.

While traditionally drug addiction is believed to be caused by maladaptive habit processing (Everitt & Robbins, 2005; Milton & Everitt, 2012), there may also be an important contribution of goal-directed dysfunction to this condition. One of the hallmarks of drug addiction is the persistence of the behaviour in the presence of negative consequences (Milton & Everitt, 2012). Normally presentation

of a negative consequence biases behavioural responding away from actions that have negative outcomes. For example, while reward devaluation does not impinge on habitual responding in extinction (Adams, 1982; Dickinson, 1985), presentation of a devalued reinforcer causes responding to shift towards alternative actions (Dickinson *et al.*, 1983; Yin *et al.*, 2005) and this may involve engagement of goal-directed processing (Yin *et al.*, 2005; Balleine *et al.*, 2009). Thus the weakening of goal-directed memory (as well as habit strengthening) may also play a role in the development of drug addiction. Addictive drugs are known for increasing dopamine transmission, particularly in the NAc (Di Chiara, 2002). Given the NAc may play a role in reconsolidation of A–O memory (Chapter 4), it may be that addictive drugs enhance destabilisation of goal-directed memories and disrupt their reconsolidation. In theory this would weaken the outcome-encoding goal-directed memory, and allow the habit to dominate behaviour without any evaluative consideration for the consequences of drug-seeking behaviour. The weakening of evaluative (putatively goal-directed) processing may explain the apparent loss of executive control that occurs in drug addiction (Balleine *et al.*, 2009; Milton & Everitt, 2012).

The data from this thesis demonstrate, in principle, that goal-directed and habit memories undergo reconsolidation (Chapters 4 and 5). Therefore it may be possible to destabilise and weaken habit memory in order to treat persistent drug addiction. Disruption of the habit should return behaviour to goal-directed control (Chapter 5), which while not necessarily preventing drug craving as these are likely mediated by a pavlovian mechanisms (Milton & Everitt, 2010), it should remove the loss of control that is a key feature of drug addiction. Targeting the maladaptive habit is perhaps the most obvious means of treatment, however it may also be possible to target the goal-directed memory in order to diminish drug-seeking behaviour. Experimental manipulations typically disrupt reconsolidation with a view to weakening memory, but it is also possible to artificially strengthen memory by enhancing reconsolidation in some cases (Tronson *et al.*, 2006; Chen *et al.*, 2011; Coccoz *et al.*, 2013; Rodríguez *et al.*, 2013). Indeed strengthening of memories may be one of the normal functions of the reconsolidation process (Lee, 2008, 2009). If goal-directed A–O memory could be strengthened then this could potentially suppress the habit by exerting top-down control of

behaviour; in fact goal-directed memory may act normally to suppress inappropriate habitual responses (de Wit *et al.*, 2006; Balleine *et al.*, 2009; Balleine & O'Doherty, 2010). As such the focus of an A–O targeted treatment would be on returning the action system to a healthy balance between A–O and S–R processing, thus restoring executive control of behaviour, rather than on weakening behavioural responding.

Targeting, and attempting to strengthen, goal-directed memory may even prove a more effective treatment than disrupting the habitual component of behaviour. While targeting and weakening habit memory would reduce drug-seeking in theory, this would not prevent a former addict from becoming re-addicted by re-learning the habit. On the other hand, a suppressive A–O memory should continue to inhibit drug-seeking behaviour for the rest of an addict's life, at least in theory. In principle this would make A–O based treatments more persistent and long-lasting than those targeting habit memory. One problem may be that during the course of drug addiction the A–O memory may be weakened, diminished or even erased making it difficult to target and strengthen this memory; however a solution could be to artificially create a false A–O memory which could suppress responding.

A similar strategy of implanting a false memory has been used to diminish alcohol preference (Clifasefi *et al.*, 2013). In this study Clifasefi *et al.* (2013) asked participants to answer questions about life events they experienced before the age of 16. Among them were question asking whether they had felt sick after drinking too much vodka, and another question about feelings sick after drinking too much rum (participants were asked how likely it was these events would have occurred). Participants were then fed an "individualised food and drink" profile, containing some of their supposed food preferences (the list was of course completely fictional and most preferences were the same for every participant), however the experimental group were also told in the profile that they had got sick from drinking alcohol (either vodka or rum). Subjects were then asked to recount (or imagine) the event where they had felt sick. A few weeks later the belief of participants that the false event had occurred was tested, with those in the experimental group showing higher rates of

false belief (compared to their responses in the initial questionnaire); however interestingly these subjects also reported a lower preference for alcohol. This experiment has many parallels with reward devaluation (see Chapters 4 and 5, and above) as the reduction in alcohol preference was also specific to the "devalued" spirit. It may be that a similar paradigm could be employed to create a false reward devaluation memory, and in doing so diminish drug seeking. One problem with this is that typically reward devaluation requires re-exposure to the reinforcer in order to update incentive memory (Dickinson & Balleine, 2002; Balleine, 2011). It is unknown whether a false memory can provide the encoding for this, although in principle it should be capable of devaluing the outcome the next time it is encountered. Another problem with using false memory as a treatment is the relatively low rates of false belief in experimental groups (Bernstein *et al.*, 2005; Clifasefi *et al.*, 2013). In the case of Clifasefi *et al.* (2013) this was just under 20%. It is unknown whether these rates could be improved through pharmacological enhancement of memory. As such false memory paradigms may provide a means of implanting suppressive memories by using suggestion, but key problem currently is the low rate of uptake for the false suggestion. Perhaps reconsolidation-mediated strengthening mechanisms could be used (or manipulated) to strengthen false memories which suppress drug-seeking. In fact combining a false memory strategy with reconsolidation-disruption of a maladaptive habit may lead to a more effective treatment plan. Weakening the habit by disrupting its reconsolidation should restore goal-directed control of behaviour (Chapter 5). Combined with false memory devaluation of the drug, this should leave behaviour controlled by a goal-directed memory for a devalued reinforcer. The presence of a suppressive memory may assist in preventing further relapse as it would inhibit patients from becoming re-addicted and re-learning the maladaptive behaviour.

Using reconsolidation mechanisms to manipulate both goal-directed and habit memory may also be useful in the treatment of obsessive compulsive disorder, as this is also believed to result from dysfunction of goal-directed processes, with a maladaptive over-dependence upon S–R habit-mediated control of behaviour (Gillan *et al.*, 2011). Furthermore, reconsolidation-based treatments

for drug addiction may extend to compulsive food seeking as the neurobiological mechanisms of these two processes may be similar (Johnson & Kenny, 2010).

Hypothetical role of motivational incentive in reconsolidation

If contingency change was the salient feature of the VR reactivations this raises a key issue over the sensitivity of instrumental behaviour to contingency changes. While A–O memories are well accepted to be sensitive to contingency degradation or omission schedules (Hammond, 1980; Balleine & Dickinson, 1998a), habits are believed to be less sensitive (Balleine & Dickinson, 1998a; Yin *et al.*, 2006; Derusso *et al.*, 2010); however this is not to suggest they are insensitive (Yin *et al.*, 2006; Derusso *et al.*, 2010). This may represent differing sensitivities of actions and habits to the specific sensory outcome. The US in conditioning is generally considered to be neurally represented by at least two representations: a sensory representation (US_S) encoding the specific features of the US, and a motivational representation (US_M) encoding its motivational or incentive value (Blundell *et al.*, 2001; Delamater, 2012). As noted above the contingency change in the VR reactivations consisted of both a change in sensory contingency (the number of physical pellet deliveries) and an increase in the inter-reward delay (a decrease in value density). It may be that instrumental response (R) memories are encoded as both R–US_S and R–US_M memories, and it was the change in R–US_M contingency (i.e. the reduction in incentive reinforcement) that was important for triggering reconsolidation; a similar model of instrumental memory has been proposed previously in order to explain how certain training conditions can favour either goal-directed or habitual responding (Dickinson *et al.*, 1983).

It may even be that the instrumental response also possesses multiple neural representations, and motivational value of its own; not unreasonable given that CSs can take on their own incentive value to become conditioned reinforcers (Lee *et al.*, 2005; Milton, Lee, & Everitt, 2008). In the case of the lever pressing paradigms used in the current studies the response lever is both a sensory object (R_S), and an interact-able object by means of an associated lever pressing motor pattern (R_R). Pressing of

the lever then leads to a motivationally relevant incentive outcome (R_M). Given that USs appear to possess multiple representations, and it is likely CSs do to, this is not an unreasonable suggestion to make for response memories. Indeed there is some evidence for the existence of distinct motor associations in certain patient populations. Visual agnosia refers to a condition in which patients cannot identify visual objects, however there appears to be a dissociation between perception and interaction representations (Milner & Goodale, 2008). One patient, DF, failed to orient a card into the same orientation as a letterbox slot, but when asked to "post" the letter showed no impairment, successfully orientating the card immediately and posting it without hindrance (Goodale *et al.*, 1991). Another patient could not recognise objects by sight, but could successfully identify objects if the experimenter "pantomimed" their use (Ferreira *et al.*, 1998). These patients strongly imply that there exists a dissociable R_R component of object memory which encodes the appropriate use of that object. Thus, it is entirely plausible that memories have multiple representations in different neural units (sensory, incentive, motor). As such the instrumental memory may exist as part of a multiple component representations in a $R_S \rightarrow R_M \rightarrow R_R$ association (Figure 6.1A), with parallel circuits for instrumental and pavlovian memory; although if the functional units contain an inbuilt sensory stimulus representation and an appropriate motor response there may be no need to have separate instrumental and pavlovian circuits.

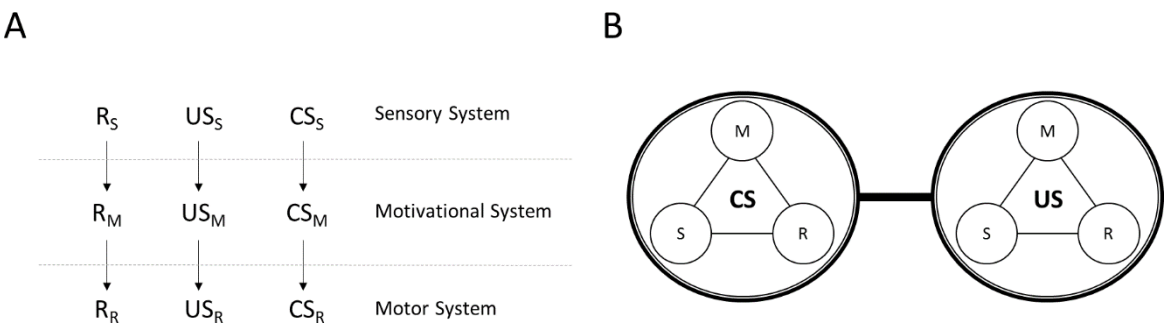

Figure 6.1: Theoretical associative structure of memory. A, memories may be represented in different neural systems consisting of dissociable sensory (subscript S), motivational incentive (subscript M) and motor (subscript R) representations. There may be separate pavlovian (CS), outcome (US) and instrumental (R) memories which operate in parallel to influence behaviour. B, individual memories may be represented as self-contained units each containing a stimulus, assigned incentive value, and an appropriate response for that stimulus. For example, lever is good, levers are pressed, and pressing the lever is equally good. These self-contained units may then become associated to other units to allow flexible outcome-sensitive processing (i.e. lever pressing produces US in the case of goal-directed memories). Retrieval can occur via any of the individual sensory, motivational or motor components within a unit, or by activation of an associated representation (i.e. the CS unit can retrieve the US unit).

One question in the above model (Figure 6.1) is how memories are retrieved. Traditionally in behavioural literature it has been believed that exposure to a sensory stimulus triggers the retrieval process, as in the classic Thorndikian S–R association (Thorndike, 1911; Hull, 1943); however experimental evidence would suggest that memories can also be retrieved by exposure to the reinforcer. For example exposure to ethanol can increase both craving and ethanol-seeking (Chutuape *et al.*, 1994). It has been suggested that this occurs through activation of an outcome–response association (de Wit & Dickinson, 2009), mirroring older philosophical theories of goal-directed action in which thoughts of the goal precede action (James, 1890; Stock & Stock, 2004). Thus memories can be retrieved via a sensory cue or a paired association (as perhaps might be

intuitively obvious), but studies of visual agnosia patients (see above) would also suggest retrieval can occur via a motor representation (Ferreira *et al.*, 1998). Furthermore, the motivational value of a memory may also play a role in its retrieval (Bousfield, 1950; Lewis & Critchley, 2003). An example of this is mood-congruent retrieval of memory (Mayer *et al.*, 1995; Lewis & Critchley, 2003) in which being in a happy mood promotes retrieval of "happy" memories, while being sad favours retrieval of negative memories. Thus memory may be better modelled by a unit consisting of multiple representations (Figure 6.1B) in which the memory unit can be retrieved by activation of any of the sensory, motivational or motor sub-nodes within it, or by a paired unit (such as a CS).

If R_R possesses its own incentive value, R_M, independent of the US_M this could model habit behaviour. If habits possessed a representation of the incentive value of the response (i.e. how much that response should be performed in future), this would make them sensitive to changes in the contingency of incentive reinforcement (i.e. reinforcer density), but not devaluation of the US as the response would not be directly paired with any US representation. In the hypothetical memory model above (Figure 6.1B) enacting of a habit would only require activation of a single unit (which could contain sufficient information to allow retrieval and performance of a response); however goal-directed behaviour would involve activation of the US unit which would encode information about the current value of the reinforcer. If incorporating an incentive component into instrumental memory models in order to explain reconsolidation of habit memories, this raises a question of whether an incentive component is required for other forms of memory reconsolidation. The incentive component of memory is of particular interest as it involves a form of evaluative feedback in order to be learned (Balleine, 2011); in the case of reconsolidation feedback is believed to produce a prediction error signal which leads to destabilisation (Lee, 2009; Díaz-Mataix *et al.*, 2013; Reichelt *et al.*, 2013; Sevenster *et al.*, 2013).

The feedback involved in incentive learning appears to require experiencing hedonic responses to stimuli (Dickinson & Balleine, 2002). Using reward devaluation as an example, pairing the reward pellets with LiCl causes goal-directed responding to be reduced by inducing gastric-malaise (see

Chapters 4 and 5). Notably, if the LiCl is injected immediately after reinforcer exposure, rats must be re-exposed to the reinforcer in order to update their incentive memory (Balleine & Dickinson, 1991; Balleine, 2011). This implies that rats must experience the reinforcer in the presence of a negative hedonic state in order for the incentive value of the pellets to be devalued. Given that the incentive value is being updated to a new, lower value, one might hypotheses that this process involves prediction-error and reconsolidation mechanisms (Figure 6.2); while this has not been tested, incentive memories have been shown to undergo reconsolidation (Wang *et al.*, 2005). In the case of habitual lever pressing, it may have been the mismatch in hedonic reward (reinforcer densitiy) and expected outcome (incentive) that produced the error signal to trigger reconsolidation.

Incentive prediction error signals

Reconsolidation is believed to be engaged in response to a prediction error signal (Lee, 2009; Díaz-Mataix *et al.*, 2013; Reichelt *et al.*, 2013; Sevenster *et al.*, 2013). In the case of reward devaluation pairing the sensory reinforcer representation with LiCl does not by itself reduce incentive value, the reinforcer must be re-experienced in a negative hedonic state in order for incentive value to be updated. This effect leans naturally towards a prediction error interpretation as the hedonic experience provides a measure of *current* incentive value, while the pellets have a high *predicted* incentive (Figure 6.2A) as demonstrated by the lack of a devaluation effect prior to reinforcer re-exposure (Balleine & Dickinson, 1991). Since the sensory US_S reinforcer representation has previously been paired with prediction of sickness, the sensory presentation of the pellets now elicits a negative hedonic reaction of disgust. It is then conceivable that the mismatch between the incentive and hedonic values produces a prediction error signal, updating memory via reconsolidation (Figure 6.2B).

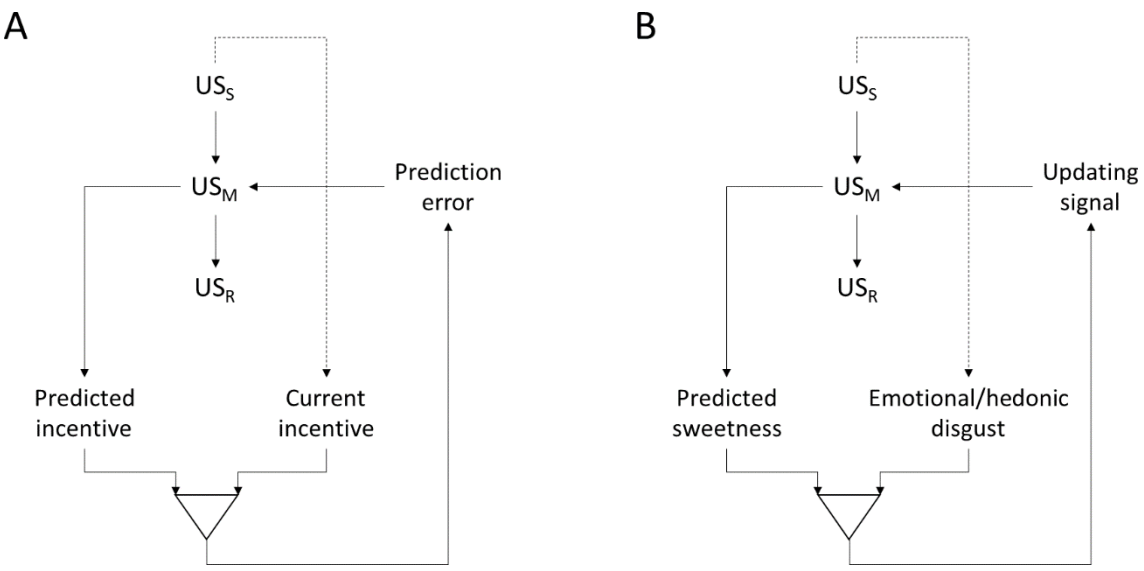

Figure 6.2: Conceptual diagram showing how the difference between predicted incentive and experienced hedonic value could produce a prediction error updating signal in evaluative conditioning (A), using LiCl reward devaluation (B) as an illustrative example.

Hedonic-incentive feedback in memory destabilisation

If changes in incentive value are required in order for memories to be updated then, behaviourally, all reactivation sessions should involve some form of hedonic change, producing a prediction-error between the predicted incentive value and experienced hedonic response. In the case of this thesis reactivation was successful in triggering reconsolidation in the case of brief non-confined context exposure (Chapter 3), VR5 contingency (Chapter 4) and VR20 contingency (VR20). Past literature has typically used either brief extinction sessions or reinforced training trials in order to destabilise memories. The capacity of these different reactivation parameters to elicit deviations in predicted hedonic incentive and memory destabilisation will be considered in turn.

Reinforced reactivations

Reinforced reactivations are perhaps the simplest set of reactivation parameters to fit into and incentive prediction error framework as the reactivations include an overt hedonic value (by the

presence of the US). One might predict that early in training while memory is not well learned, training trials will induce reconsolidation due to being unable to accurately predict the motivational outcome, leading to memory updating/strengthening; however once memory is well-established and able to accurately predict the incentive outcome, training trials should be incapable of destabilising memory.

In the case of the VR5 reactivation (Chapters 4 and 5), it successfully destabilised goal-directed memory, but not habit memory where a VR20 session was sufficient for destabilisation to occur. As discussed above these contingency changes also involved a reduction in temporal reinforcer density. For habitual rats (Chapter 5) if they were able to obtain pellets in the VR5 session faster than they could eat them (as seems likely) then there would be no reduction in physical hedonic reaction compared to training; however presumably the large increase in reinforcer delay in the VR20 reactivation facilitated a reduction in hedonic experience to the sucrose, producing an error signal. Hypothetically if the delay between reinforcer deliveries is important in determining the effectiveness of the contingency change to destabilise instrumental memory, then there may well exist a boundary condition of inter-reinforcer delay on instrumental memory reconsolidation. This could potentially be investigated using video-tracking, although this was not available in these studies.

The hypothesis that a change in hedonic reinforcement is required to trigger memory destabilisation may also explain the lack of effect in Hernandez and Kelley's (2004) study on instrumental reconsolidation. They found that anisomycin, used in their study, devalued the reinforcer rather than impaired reconsolidation. In order to bypass this confound, they used a reactivation procedure in which the reinforcer was changed from sucrose to chocolate pellets (Hernandez & Kelley, 2004). From the traditional memory-updating perspective, one might expect this session to trigger destabilisation as the instrumental outcome-contingency has changed; however the hedonic reinforcement hypothesis would predict that there would be no prediction-error signal, provided the chocolate pellets had a similar hedonic and incentive value to the sucrose pellets. This prediction

would suggest that destabilisation can be prevented by substituting the US for another US or conditioned reinforcer of equivalent incentive value. This may also explain the lack of any effect in Chapter 2 in which the lever retracted during a brief 5-minute reactivation session, as the retraction may have acted as a conditioned reinforcer, taking on the reinforcing value of the pellets (Chapter 2). If the lever-retraction would possess the same incentive value as the predicted value of the response; thus, there would have been no prediction-error signal. An extension of this interpretation is the prediction that conditioned reinforcers should block destabilisation of instrumental memory, provided the conditioned reinforcer and the instrumental outcome had equivalent incentive values.

Does this interpretation extend to other memory settings? Training trials have successfully destabilised emotional fear memory (Eisenberg & Dudai, 2004; Duvarci *et al.*, 2006; Lee, 2008), and appetitive conditioned place preference (CPP; Milekic *et al.*, 2006; Valjent *et al.*, 2006; Wu, Li, Yang, *et al.*, 2012). In the case of fear memory, the amount of training used in these studies was very limited. Two of these studies (Duvarci *et al.*, 2006; Lee, 2008) used only single training trials, while Eisenberg & Dudai (2004) gave only two-days of training (although with multiple trials). It may be that the updating of incentive motivational properties of a CS requires re-exposure to the CS so it can be experienced in a relevant hedonic state, much as devaluation of sucrose pellets requires re-exposure to the pellets following LiCl injection. In fact since updating of sucrose incentive during reward devaluation only requires re-exposure to the pellets, and not the LiCl (Balleine & Dickinson, 1991), this interpretation would predict that a one-trial-learned memory will always undergo reconsolidation-mediated updating and strengthening following a second training trial, or CS re-exposure alone; indeed this appears to be the case (Przybyslawski *et al.*, 1999; Boccia *et al.*, 2004; Duvarci *et al.*, 2006; Baratti *et al.*, 2008) and CS re-exposure (memory retrieval) has been suggested to strengthen newly acquired memories (Inda *et al.*, 2011), which may be occurring via reconsolidation. In appetitive settings one-trial learning is typically not carried out, however training trials have been shown to be sufficient to destabilise CPP memory and these studies typically only use two drug conditioning pairings (Milekic *et al.*, 2006; Valjent *et al.*, 2006; Wu, Li, Gao, *et al.*, 2012). Given that only two conditioning sessions were used in the conditioned place aversion (CPA) study

(Chapter 3), one might predict that a training trial would have been sufficient to engage reconsolidation in that experiment. Thus, provided memory is weakly learned the CS should not be a completely accurate predictor of the hedonic outcome and thus training trials should destabilise memory via a prediction error.

Another variation on a reinforced reactivation trial is a US-only reactivation (Schneider & Sherman, 1968; Debiec *et al.*, 2010). In this setup a non-contingent US (without context or CS presentation) appears to reactivate and destabilise the memory trace, allowing amnestic treatment to be effective. One interpretation of these results is that the representation of the US activated the CS representation and thereby destabilised the CS–US memory. This is perhaps at odds with the hedonic-error hypothesis, as there should have been no predicted value from the CS with which to produce a prediction error signal and initiate reconsolidation. However, amnestic treatment in combination with this type of reactivation appears produce an amnesia that is specific to the US and also impairs memory for all US-paired associations (Debiec *et al.*, 2010), consequently an alternative interpretation is that it was not the CS–US memory that was impaired but rather the US–incentive memory. This could then have a knock-on effect on US-paired associations by removing the incentive motivation for rats to respond to the US (or the predicted presence of the US). This interpretation is also consistent with an appetitive study which used US exposure to destabilise US–incentive memory without any effect on instrumental A–O associations (Wang *et al.*, 2005). How then can reconsolidation of incentive memory be explained by the hedonic-error hypothesis? In the case of Wang *et al.* (2005) there was a change in motivational state (rats were no longer food deprived) and so rats would need to learn the new incentive value of the US in the new motivational state (Dickinson & Balleine, 2002). In this respect the reward (US) exposure sessions were, in essence, training sessions for US–incentive memory. Since there was only one prior incentive training session we might predict the new incentive memory was weakly learned, and therefore should undergo reconsolidation in order to strengthen (as above) in a second "training" (exposure) session. A similar argument applies to the fear memory paradigms used by Debiec *et al.* (2010). Rats only received three US exposures (all paired with a CS) in a single session. During the training sessions rats

presumably learn the predictive relationship between the CS and US, but are also learning the incentive value of the US (i.e. footshock = bad). As there were only 3 US exposures rats would have had limited opportunity to learn about the incentive value of the aversive USs used in this study. Thus a US-exposure reactivation session was likely a training session for US–incentive memory. Given it was relatively weakly learned, we might expect this memory to destabilise in order to be strengthened via reconsolidation. Thus, the hedonic-error hypothesis would therefore interpret US reactivations as training trials for US–incentive memory.

A further prediction of the hedonic-error interpretation is that as the number of training trials increases, the ability of a training trial to destabilise memory will diminish. Eventually the incentive prediction of the CS should become a near-perfect predictor of incentive outcome, thus a training trial would produce no error signal. This is a similar prediction to the "updating consolidation" interpretation of reconsolidation (Rodriguez-Ortiz & Bermúdez-Rattoni, 2007). This is certainly consistent with the lack of effect of an FR1 schedule on goal-directed memory (Chapter 4). While there were only two-days of training, rats did receive up to 90 reinforcements so the relationship between lever pressing and pellet delivery was likely well learned. There have also been several other demonstrations in reconsolidation-literature that memories become resistant to training trial-induced reconsolidation when memory is well learned, in both appetitive and aversive settings (Hernandez & Kelley, 2004; Mierzejewski *et al.*, 2009; Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013).

Extinction reactivations

Results from studies using training trials to destabilise memories are consistent with an incentive prediction error interpretation, however the vast majority of reconsolidation studies have used non-reinforced extinction sessions in order to trigger memory destabilisation. These sessions should naturally produce an error signal owing to the lack of reinforcement; however this does not always trigger reconsolidation. Brief sessions typically trigger reconsolidation, while longer sessions suppress

responding by new learning (Pedreira & Maldonado, 2003; Suzuki *et al.*, 2004; Robinson & Franklin, 2010; Reichelt & Lee, 2012; Flavell & Lee, 2013). How might these results be interpreted in an incentive-error signal framework? Using CS-fear memory as an illustrative example, during conditioning the rats learns the CS predicts mild electric footshock. During the reactivation session the CS should elicit an expectation of a negative hedonic reaction, however it is conceivable that the unexpected end of a brief reactivation session elicits a positive hedonic reaction such as "relief". This is quite possibly similar to the putative process of rebound-excitation by which stimuli that predict reinforcer omission taken on an opposite incentive value (Dickinson & Dearing, 1979; Dickinson & Balleine, 2002). In the case of fear memory reconsolidation a CS should activate the aversive motivational system, inhibiting the appetitive system; from there, unexpected removal from the experimental context should cause a rebound excitation of the appetitive system, producing a prediction error signal (current appetitive hedonia with aversive CS incentive).

In the case of longer sessions this putative rebound-error signal presumably does not occur. The reason for this may lie in the gradual decrease in motivation throughout a longer session. While this might be predicted to cause an error signal, owing to the difference between the predicted incentive of the CS and the current motivational state, this is not necessarily the case as the incentive value of the CS, and any hedonic feedback, should theoretically be proportional to the current motivational state (Dickinson & Balleine, 2002). For example when hungry, food has both a higher incentive value (higher "wanting") and increased palatability (more "liking"). If you miss breakfast and lunch, dinner will have a higher incentive and hedonic value as you are food-deprived, however the process of eating dinner will gradually decrease hunger and both the incentive and hedonic reaction to food such that by the end of the meal you are no longer hungry, nor do you have any desire to eat more food; in fact overeating can induce a negative hedonic state (being bloated) actively dis-incentivise further overeating. Importantly the modulation of incentive by motivational states must be learned through experiencing the hedonic reactions to outcomes in the motivational state (Dickinson & Balleine, 2002), and this may explain why training trials trigger destabilisation when memory is weakly learned (i.e. not much experience with the outcome). If prediction errors are generated from

the difference between predicted incentive and the actual hedonic outcome then the eating of a meal should not produce any prediction error, as these two factors remain in proportion as you become full. Likewise during extinction of cued-fear, the absence of the US should diminish motivation, reducing incentive to escape the shock and emotional fear of the shock in proportion to each other, producing no error signal. This interpretation would suggest that extinction learning is a conditioned loss of motivation, which it does appear to be given behaviour can renew and reinstate from extinction (Bouton, 2004), rather than unlearning or a sensory [CS_S]–[No US_S] memory.

Notably there also appear to be a point between reactivation and extinction where neither process occurs (Flavell & Lee, 2013). These results can also be incorporated into a motivational-error signal framework. As was suggested above, a deviation in expected hedonic feedback may be required to produce an error signal. In the case of extinction reactivations this may be mediated by rebound excitation of an opposing motivational system (Dickinson & Balleine, 2002). One of the assumptions inherent in a cross-inhibitory network is that it can exist in a steady state. Hypothetically, at the beginning of a fear extinction session the aversive system becomes increasingly active, suppressing a putative appetitive system (Figure 6.3). This initial over activity in the aversive system allows rebound-excitation (and therefore prediction-error induced reconsolidation) to occur. As the session continues activation of the aversive system plateaus and the system normalises to a steady state in which all units are equally active, preventing any rebound error signals. The lack of a US should then gradually diminish activity in the aversive system allowing previously inhibited systems to reassert themselves. In the case of extinction reactivations, this incentive-prediction error interpretation is fundamentally very similar to behavioural dominance theory (Eisenberg *et al.*, 2003), in which reconsolidation and extinction are believed to be opposing systems with only the "behaviourally dominant" memory undergoing reconsolidation. In the case of our exemplar fear memory, the incentive-error interpretation suggests the aversive system initially "dominates" behaviour but is eventually suppressed by opposing systems in a long extinction session (and fear extinction learning may be learned suppression of the aversive system). In this respect the incentive-error interpretation is simply an expansion of behavioural dominance theory to include a steady-state condition under

which neither reconsolidation nor extinction can occur. While these competing motivational systems have, for simplicity, been presented here as appetitive versus aversive, there likely exist multiple competing systems. For example, the need to urinate suppressed salivation in Pavlov's dogs (Pavlov, 1927) and the presence of an oestrus female can suppress seeking of a liquid reinforcer in male rats (Hughes *et al.*, 1987).

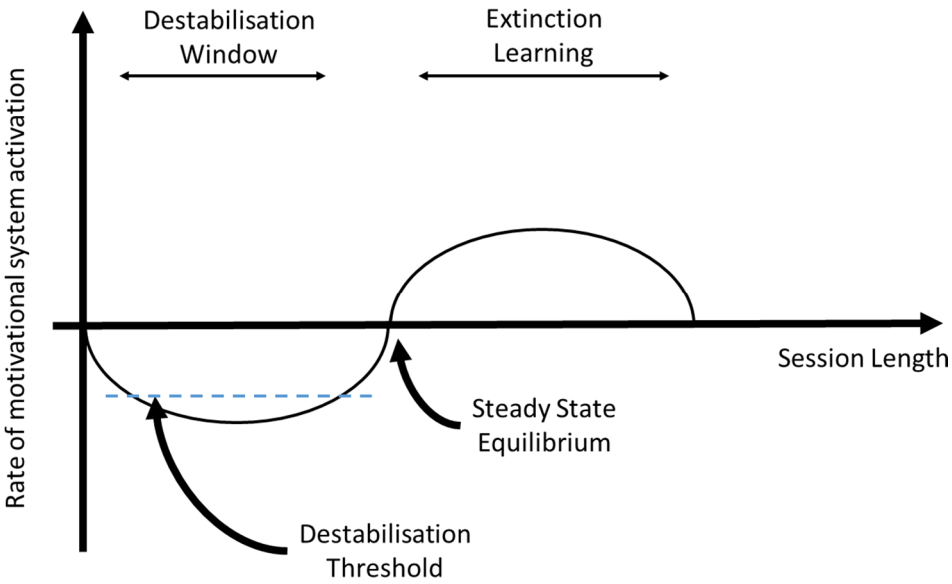

Figure 6.3: Conceptual representation of the aversive motivational system activation during the extinction of a CS–fear memory. Initially the aversive system becomes increasingly active, driven by activation from the CS. At the peak rate of change removal from the context, thereby signalling absence of the US and removing the activation from the CS, should trigger rebound excitation of opposing (appetitive) motivational systems (eliciting "relief") and signalling a prediction error which enables destabilisation and reconsolidation. At the beginning of the session the capacity for rebound excitation is minimal as the rate of activation is initially slower, but at the maximal rate of change the capacity to trigger reconsolidation should be optimal. As the session continues activity of the aversive system should reduce, eventually reaching a steady state in which no new learning can occur (counter-intuitively coinciding with the point of maximal motivational system activation). With extended exposure activation of the aversive system is suppressed, giving way to activation of opposing (appetitive) systems.

In order to be a satisfactory interpretation of reconsolidation boundary conditions, the incentive-error interpretation must also be able to explain why memories seem to require longer reactivation sessions, or greater CS exposure, following extended periods of training (Suzuki *et al.*, 2004; Reichelt & Lee, 2012). An interpretation of these findings is that a minimum amount of CS extinction exposure is required for memory to destabilise. One possibility to explain this is that as training progresses the CS begins to take on the incentive properties of the US. For example a sucrose-paired CS will act to predict the US but in addition will gain appetitive value, eliciting sign-tracking behaviour (Robinson & Flagel, 2009) and the ability to act as a conditioned reinforcer (Milton, Lee, & Everitt, 2008). It may be that the conditioned reinforcing properties of the CS are able to substitute for the reinforcer to a degree preventing production of an error signal; however this is not an entirely satisfactory explanation as this would predict that either memory would never undergo reconsolidation (does not appear to be the case) or that it would only if the incentive value of the CS was completely or partially extinguished (contrary to evidence suggesting reconsolidation to be "behaviourally dominant" prior to extinction, see above). An alternative explanation may lie in the putative requirement for hedonic feedback in the production of destabilisation-inducing error signals. Responses to a CS may be both preparatory and consummatory and the hypothesis presented here predicts that the difference between predicted incentive and experience hedonic outcome elicits an error signal; however experiencing the hedonic reaction may require a consummatory response (or at least consummatory attempt). As the amount of training increases it may be that consummatory CS and US responses compete. This appears to be pronounced during the habitual lever pressing study (Chapter 5) as nosepoking (presumed pavlovian approach) decreases markedly after 3 days of training and continues to decrease as training progresses, in fact the nosepokes may become indirectly punished (as time spent nosepoking is time not spent lever pressing) leading to domination of the conditioned lever press response over behaviour; this has also been observed in other studies (Hernandez *et al.*, 2005), although this may be related or exaggerated by the use of a pre-training session (Smith-Roe & Kelley, 2000). Likewise in pavlovian goal-tracking, the goal-tracking response

may compete with CS-sign-tracking. Thus in the case of extinction reactivation sessions it may be longer sessions are required before there is a failed US-specific consummatory attempt. In briefer sessions consummatory responses to the CS (sign-tracking) may compete more effectively with US-consummatory responses, inhibiting production of a prediction error as the incentive outcome is the CS, which will always have the same incentive value as the CS predicted (in essence the CS becomes its own reward for a CS-consummatory response). As such there may be a minimum amount of CS exposure required (Figure 6.3) before a failed consummatory attempt will occur, and this may explain such findings in the literature (Suzuki *et al.*, 2004; Reichelt & Lee, 2012). Ultimately this argument reduces to saying that destabilisation and reconsolidation require reactivation of the memory. Recall our hypothetical structure of memory (Figure 6.4A). Behaviour can be mediated purely by the left-hand CS circle, however in order to produce an error signal the CS–US association must be reactivated such that the hedonic outcome to a US-response can influence the incentive value of the CS (Figure 6.4B). In the absence of reactivation a CS-mediated conditioned response can mediate behaviour, but ultimately the only outcome will be the CS which has the same value as the CS predicted. This interpretation would also imply that destabilisation of an instrumental trace requires activation of both goal-directed and habitual process (as was suggested may be necessary earlier) and may suggest an associative structure in which goal-directed and habit process are integrated, rather than parallel processes. Furthermore this differentiation between behaviour and reactivation is important, as behavioural expression (or memory retrieval) is not a requirement for destabilisation to occur (Milton *et al.*, 2013).

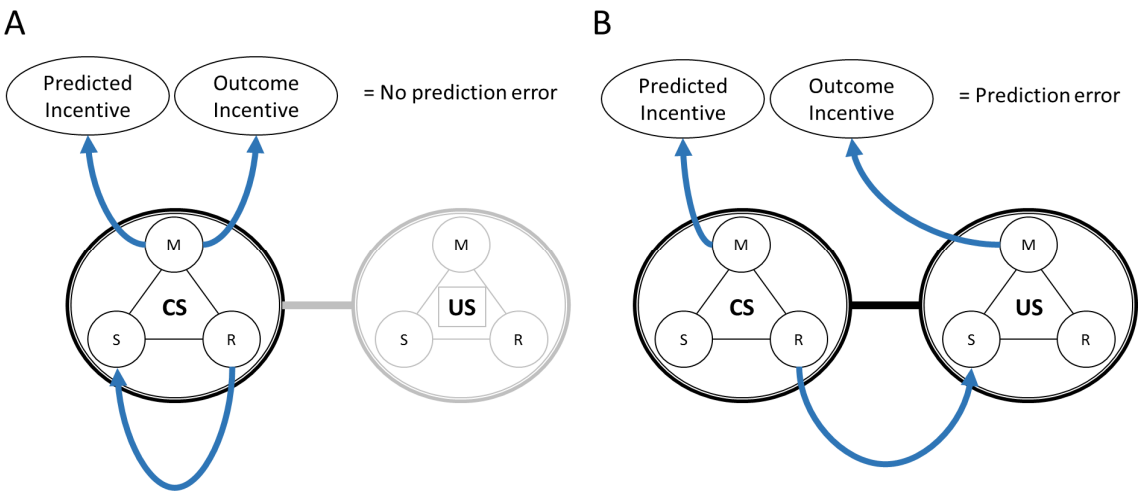

Figure 6.4: Reactivation of both the CS and US representations should be necessary for memory reconsolidation to occur. A, Behavioural expression can be mediated solely by the left-hand CS representation and its associated conditioned response. B, engagement of both CS and US associative structures should be necessary for reconsolidation in order to there to be an experienced difference between the hedonic value of the US and the predicted value of the CS.

The hedonic prediction error interpretation proposed here requires there to be hedonic feedback in order for a prediction error to be generated, and this feedback may require a US-consummatory experience (or at least a consummatory attempt). There is some evidence for this hypothesis in the conditions under which putative seeking behaviour diminishes. The simplest example is that used earlier of reward devaluation. In order for the devaluation treatment to diminish instrumental responding rats must be re-exposed to a consummatory experience of the reinforcer (Balleine & Dickinson, 1991). Notably, devaluation can occur in the absence of re-exposure, however this appears to be related to the use of hypotonic LiCl which induces an immediate negative hedonic state (Balleine & Dickinson, 1992) which may directly affect the consummatory experience of the reinforcer. Similar devaluation results have also been found for sexual behaviours (Everitt & Stacey, 1987). Everitt & Stacey (1987) showed that castration impaired sexual motivation; however instrumental responding of males for an oestrus female was not impaired on the first test following castration (i.e. prior to any copulation attempt, and thus prior to any consummatory feedback).

Performance only diminished following a failed copulation attempt. Taken together these results might suggest that experience of the hedonic value (or lack of hedonic value) of the US consummatory response is required in order to update incentive value. This may offer an explanation for the lack of any reconsolidation effect on instrumental lever pressing using extinction sessions in this thesis, as it may be these sessions lacked a consummatory experience of the pellet. While it may be inferred that nosepoking was the consummatory response in these experiments (Chapters 2, 4 and 5) this is not necessarily the case, in fact the consummatory response may have been that act of picking up and eating/chewing the pellet (the absence of the pellets in the extinction reactivations of course meant this response could not be enacted or even attempted). Interestingly, Everitt & Stacey (1987) also lesioned the medial preoptic area (POA) of the hypothalamus which appears to diminish the ability to engage in consummatory sexual intercourse, but not sexual arousal or interest (Everitt & Stacey, 1987; Hughes *et al.*, 1987). In contrast to castrated males (see above), rats with POA lesions did not reduce their instrumental responses in pursuit of a female immediately following failed copulation, and required 4 test sessions (a thus 3 prior failed intercourse attempts) before the instrumental responding started to diminish. One interpretation of this is that since the POA lesion impaired enacting of consummatory sexual behaviour, there was no consummatory feedback, or at least diminished consummatory feedback, requiring multiple sessions (and perhaps partial recovery from the lesion with time) in order for instrumental incentive to be updated. There is potentially a parallel to be made here with certain, typically appetitive, reconsolidation studies which have required multiple reactivation sessions in order for reconsolidation mechanisms to be engaged (Brown *et al.*, 2007, 2008; Sadler *et al.*, 2007; Fricks-Gleason & Marshall, 2008; Robinson & Franklin, 2010; Reichelt & Lee, 2013b). Four of these studies demonstrate that amnestic treatment was ineffective after a single reactivation session, however a memory deficit is observed following at least four reactivation treatments (Sadler *et al.*, 2007; Fricks-Gleason & Marshall, 2008; Robinson & Franklin, 2010; Reichelt & Lee, 2013b). These studies used either the β-adrenergic receptor antagonist, propranolol (Fricks-Gleason & Marshall, 2008; Robinson & Franklin, 2010), or the NMDAR antagonist, MK-801 (Sadler *et al.*, 2007; Reichelt & Lee, 2013b). Notably both these agents have

effects on motivation, with repeated propranolol treatments able to induce place aversion (Robinson, Armson, *et al.*, 2011) particularly in rats with morphine addiction history, and MK-801 place preference (Layer *et al.*, 1993). One possible interpretation is that these studies impaired the reconsolidation of incentive memory (thus impairing performance), which does undergo reconsolidation (Wang *et al.*, 2005) and appears to require re-exposure (Dickinson & Balleine, 2002). An alternative interpretation for these results is that repeated treatment with the drugs may allow the drugs to induce a motivational or hedonic state, which can then act to induce a prediction error signal. MK-801 appears to induce food craving, although interestingly may impair motivation for morphine conditioned place preference (Yonghui *et al.*, 2006). Thus prolonged treatment with these drugs may induce a change in motivational or hedonic state which has a facilitatory effect on hedonic prediction error (by enhancing, or reducing, the hedonic value of a US-specific consummatory response). Finally, multiple sessions may have been required in order to engage the incentive systems necessary to produce a prediction error signal (much as multiple sessions were required before sexual incentive was reduced in Everitt & Stacey's experiment).

One final finding from studies using extinction reactivations is that longer reactivation sessions are sometimes required following the passage of time, without any additional training sessions (Milekic & Alberini, 2002; Suzuki *et al.*, 2004). This could reflect "systems consolidation", or a lingering consolidation process (Alberini, 2005, 2011), in which the memory strengthens over time or it may be that the memory spontaneously reactivates during the temporal delay period, undergoing memory strengthening. In opposition to this finding there has been a report suggesting that delays between training and reactivation can enhance memory destabilisation using an extinction reactivation session (Robinson & Franklin, 2010). Notably this was a morphine-induced place preference study, and it may have been the delay between training and reactivation allowed rats to enter a morphine-deprived motivational state, such that when returned to the training context the activity of an appetitive motivational system would have been increased, enhancing the putative rebound-excitation error signal. As such the finding that delays between training and reactivation can enhance destabilisation can be explained within a hedonic-feedback framework, however the hypothesis must

make some appeal to a form of lingering consolidation interpretation of the finding that the passage of time can strengthen memories. It may be that this strengthening represents spontaneous memory reconsolidation, cued perhaps by the time of day. The spontaneous retrieval of memory may explain the peculiar finding in the non-reactivated control groups used in Chapters 4 and 5. These studies showed that the performance of non-reactivated vehicle animals decreased (although not significantly) compared to MK-801-injected rats. Typically this finding would be interpreted as an impairment of extinction learning, however the rats received no behavioural session. It is possible that rats "imagined" the behavioural task, and then extinguished their memory following the lack of reinforcement. While this hypothesis is not directly testable, if it were true one might expect increases in retrieval-associated gene expression following a cue for the task (such as the presence of the experimenter or even the time of day). Since rats were food restricted during these experiments (maximally so just prior to the experimental sessions) it may be the level of food-deprivation also acted as a cue for memory retrieval.

Changes in contingency

Changes in contingency also appear to be sufficient to trigger memory destabilisation in both aversive fear (Díaz-Mataix *et al.*, 2013; Sevenster *et al.*, 2013) and appetitive instrumental (Chapters 4 and 5) settings. The case of the instrumental studies presented here is may be, as was suggested earlier, the reduction in temporal reinforcer density that drove destabilisation due to a reduction in hedonic feedback producing an error signal. In the case of aversive changes in contingency it may be that as the temporal contingency becomes well-learned the timing of the CS comes to predict both shock and safety (if one knows exactly when the shock will occur, then the periods of safety before and after the shock can also be predicted). As such the temporal contingency may act as a safety signal, which would then allow for both direct activation and rebound-excitation (see above) of an aversive motivational system to drive a prediction error signal to induce reconsolidation, as the shock would have a more negative hedonic value than predicted.

Non-emotional memories

One potential problem for an incentive error signal interpretation of reconsolidation is that non-emotional memories would be predicted not to undergo reconsolidation; however reconsolidation has been demonstrated for pure contextual (Lee, 2010), object recognition (Akirav & Maroun, 2006; Winters *et al.*, 2009) and episodic memories (Hupbach *et al.*, 2007, 2009). A key question is what motivational value would a non-emotional memory have? The answer may lie in that novel objects appear to have a "default" positive motivational value. When exposed to a novel context rats show greater motor activity (and by implication greater motivational activation) which diminishes as rats become habituated to the context (Hooks & Kalivas, 1994, 1995). Furthermore, access to novelty can elicit conditioned place preference (Bevins & Bardo, 1999; Bevins *et al.*, 2002) strongly suggesting that novelty has a positive intrinsic value. If a non-emotional context or object is initially assigned a "novelty" motivational value which is then updated to "neutral" following repeated exposure (i.e. training), then we might predict that these memories should function in a similar manner to conventional memories in that they should initially be vulnerable to destabilisation and become increasingly resistant with extended training. That is to say that CS (or context) exposure, fundamentally equivalent to a training trial, should be insufficient to destabilise a non-emotional memory once it is well learned. This does appear to be the case, with a weakly-learned pure context memory undergoing reconsolidation following re-exposure (Lee, 2010) but a well-learned memory being resistant (Biedenkapp & Rudy, 2004; Lee, 2010). If new information is presented during the session then this should trigger reconsolidation mechanisms via a hedonic-feedback error signal; again this appears to be the case as presentation of a mild electric footshock in the previously non-emotional context engages reconsolidation mechanisms (Lee, 2010). Similar results have been found for non-emotional object recognition memory (Winters *et al.*, 2009). The incentive-prediction error hypothesis would suggest that it is not the presence of new information per say that is responsible for induction of reconsolidation in these tasks, but rather the motivational salience of novel objects.

Predictions of the hedonic-prediction error interpretation

The incentive prediction error hypothesis proposed here suggests that memories only undergo reconsolidation following a difference between predicted and experienced incentive value, mediated via hedonic feedback. This has many parallels with the theories of incentive salience (Berridge, 2009, 2012) and the somatic marker hypothesis (Damasio, 1996), however is distinct in that it proposes the difference between these two processes produces a prediction error signal which triggers destabilisation and updating via reconsolidation. The hypothesis asserts that reconsolidation is an updating mechanism, as has been proposed previously by others (Lee, 2009), however it differs from previous theories in that it purports that it is not new information per se that triggers destabilisation of the memory trace, but rather a change in hedonic feedback (see above).

A possible means of testing this hypothesis is to keep the information content of the memory constant, while changing the motivational or hedonic value of that information at reactivation. One way to do this would be changing the motivational state. This theory would predict that training trials should have a diminished capacity to trigger reconsolidation as the memory becomes well learned (see above), in much the same way as alternative accounts such as "lingering consolidation" (Alberini, 2005, 2011) or "updating consolidation" (Rodriguez-Ortiz & Bermúdez-Rattoni, 2007); however changing the motivational state should allow updating by incorporating new incentive information into the memory trace. There are two problems with testing the hypothesis in this way. Firstly, the changing of the motivational state may affect the value of the reinforcer (e.g. hunger would increase the value of food) and thus may lead to reduced performance in a vehicle control group, and thus mask a reconsolidation effect. One solution may be to use an irrelevant incentive. For example conditioning a CS–fear memory under food restriction and then changing the motivational state to thirst. A problem with this setup may be that the new motivational state is interpreted as a new context, and thus engages consolidation rather than reconsolidation. Although given the double dissociation between hippocampal Brain-derived Neurotrophic Factor (BDNF) and *zif268* in context memory consolidation and reconsolidation (Lee *et al.*, 2004; Lee, 2010), one might

predict that if the new motivational state was interpreted as a new context this would engage consolidation and BDNF expression, while *zif268* expression should increase if the change in motivational state triggered reconsolidation of the context memory. Previous studies have demonstrated latent inhibition to be lifted by changes in motivational state, however interestingly latent inhibition appears to be specific to reinforcers that are relevant to the motivational state (Killcross & Balleine, 1996; Dickinson & Balleine, 2002). Thus whether changes in irrelevant motivation would be effective in enabling reconsolidation is unclear. It may also be possible to enhance destabilisation by influencing incentive prediction or hedonic feedback more directly, for example by pharmacologically manipulating μ-opioid receptors in the NAc (Peciña, 2008) and ventral pallidum (Berridge, 2009), which appear to be mediating incentive and hedonic reaction respectively.

An alternative, and opposite, means of testing this hypothesis is to provide novel information in the reactivation session, but keep the motivational value equivalent. An example might be training rats to lever press for sucrose pellets, and then changing the reinforcer during the reactivation session to a new reward with either a higher, lower or equivalent hedonic value. The incentive-hedonic feedback hypothesis would predict that equivalent value reinforcer would not elicit reconsolidation as it did not change the incentive outcome. Indeed Hernandez and Kelley (2004) found that substituting sucrose for chocolate pellets did not engage reconsolidation, presumably because the sucrose and chocolate has similar hedonic values.

A more interesting way to test the requirement for novel information verses hedonic prediction error to trigger destabilisation might be using object recognition to target the reconsolidation of the training context. If a novel object is provided in a context every day then that context should maintain a constant incentive value of “novel”. Since new objects are presented every day the memory should eventually become well learned, producing no error signal and thus no reconsolidation. This is at odds with the updating hypothesis which would predict reconsolidation to occur as the context contained new information. So long as training was minimal each day (so as not to allow habituation) one should predict the context to maintain its novelty-value. Then either

omission of a novel object, or re-exposure to a non-novel object, should trigger reconsolidation due to the change in incentive outcome. Memory for the context should then destabilise and be updated to learn the context has no incentive value. Under this set of behavioural circumstances the presentation of novel information should block reconsolidation, and thus would provide a means to dissociate between updating with new sensory information and updating changes in incentive outcome (Figure 6.5). Furthermore, one might predict that consolidation of the context memory to require BDNF in the hippocampus on the first day, with a requirement for *zif268* expression during reconsolidation (Lee *et al.*, 2004; Lee, 2010). An additional prediction is that only the memory for the novel object should undergo consolidation each day, leaving context memory unperturbed. Thus giving MK-801 or another amnestic agent on the day prior to reactivation would diminish the ability of the object used that session to block reconsolidation, as the blocking of object recognition memory would mean it were still a novel object during reactivation (thus not producing any error signal). Context memory could then be measured by using an immediate-shock fear conditioning paradigm similar to Lee (2010); only those rats with intact context memory should learn the contextual fear memory.

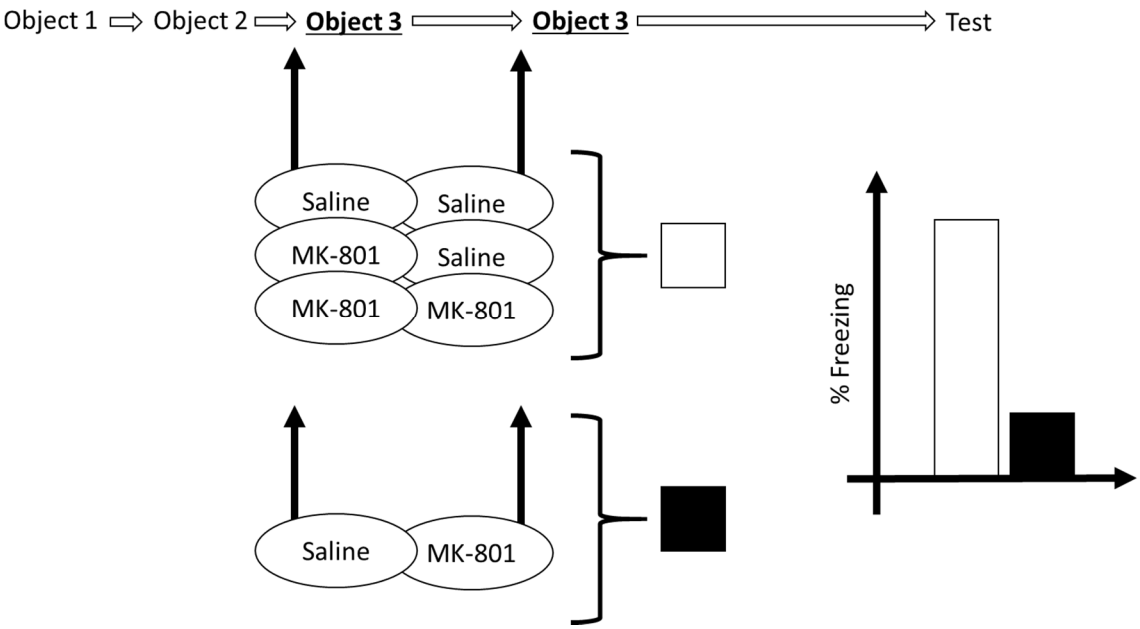

Figure 6.5: Hypothetical experimental design to test whether new sensory or new incentive information is required in order to destabilise memory. Exposure to a novel object (objects 1, 2 and 3) in the experimental context each day should produce a well-learned memory for the novelty-value of the context (top). Re-exposure to a previously recognised object (e.g. object 3) should then trigger reconsolidation and memory updating of the context memory as the object is no longer novel (and the value of the context has also changed). This reconsolidation could then be blocked by MK-801. One additional prediction is that MK-801 should also block consolidation of object recognition memory when a new object is presented. Re-presentation of an object previously presented in the presence of MK-801 should not the trigger reconsolidation as it would still be considered novel on the test day (as the consolidation of the original novelty memory would have been prevented by MK-801). Rats which were re-exposed to a non-novel object should undergo reconsolidation, which should be blocked by MK-801. These rats should then be impaired in the learning of a contextual fear memory using an immediate-shock design (right), demonstrating loss of the contextual memory.

Given the central role of the incentive-motivational system in the hedonic-feedback model, one might expect pathologies of this system to cause alterations in the conditions under which reconsolidation will occur, leading to memory dysfunction. One such disorder is schizophrenia, which

is associated with both anhedonia and memory dysfunction. In particular the lack of hedonic reaction appears to be related to "wanting" and incentive deficits, rather than immediate "liking" reactions (Horan *et al.*, 2006; Strauss & Gold, 2012). Interestingly schizophrenics appear particularly vulnerable to false memory effects (Lee *et al.*, 2007; Paz-Alonso *et al.*, 2013). While the precise cause of schizophrenia is unclear, patients do have larger NAc volume (Lauer *et al.*, 2001) (but see (Mamah *et al.*, 2007)) and dopaminergic dysfunction has been linked to the pathology (Davis *et al.*, 1991). Depression can also lead to changes in hedonic processing and appears to lead to changes to the NAc and VTA circuitry (Nestler & Carlezon, 2006). Interestingly depressed patients appear to over-generalise their memory, forming a general-"gist" or schema more easily but being impaired in the formation of specific autobiographical memory, particularly for memories with a positive emotional valence (Williams & Scott, 1988; Kuyken & Dalgleish, 1995). In fact this bias towards overgeneralisation may not be caused by depression, but in fact be an endophenotype trait of those predisposed to depression (Hasler *et al.*, 2004). Subjects with negative self-image (but not full blown depression) also appear to possess this bias towards overgeneralisation (Sperduti *et al.*, 2013), and overgeneralisation of memory appears to predict development and progression of depression (Riutort *et al.*, 2003; Sumner *et al.*, 2010). It is unclear exactly the cause of overgeneralisation, however it may be related to a tendency to engage reconsolidation processes and updating existing schemas, as opposed to engagement of consolidation in the formation of a new memory. Further investigation of the incentive system in learning and memory may also prove a fruitful avenue for understanding the pathologies of disorders associated with anhedonia.

Summary

This thesis primarily set out to demonstrate the phenomenon of reconsolidation in the memories underlying instrumental memories. Both goal-directed and habitual instrumental memories were shown to undergo reconsolidation following a suitable change in contingency. While this finding expands our understanding of reconsolidation, equally important are the conditions under which

reconsolidation was found not to occur. In the case of instrumental memory, no change (or an insufficient change) in contingency did not destabilise memory, and nor did extinction reactivations of varying length. In fact a very brief 2-minute extinction session initiated new extinction learning in a weakly-learned goal-directed memory, contrary to conventional beliefs that brief extinctions session should initiate reconsolidation processes. Furthermore, a novel conditioned place aversion paradigm demonstrated that reconsolidation does not occur following a brief confined reactivation, but does following an equivalent non-confined session, replicating similar past findings in conditioned place preference studies. In order to interpret these finding a new hypothesis was proposed in which the difference between predicted incentive salience and experience hedonic value produces a prediction error signal that initiates the reconsolidation-mediated updating process. This is subtly different from the traditional view that reconsolidation mediates updating of memories with new information, and means of experimentally distinguishing these two hypotheses were proposed. This hypothesis has implications for the precise boundary conditions of the initiation of reconsolidation updating mechanisms. Investigation of the precise parameters under which memories become unstable may be important in the development of novel treatments for maladaptive memory disorders, such and drug addiction, compulsive food seeking and post-traumatic stress disorder. In particular, the knowledge that habit memories can be made labile and disrupted may lead give new hope to the development of reconsolidation-based addition therapies.

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