INVESTIGATION OF THE BOUNDARY CONDITIONS AND THE FUNCTION OF THE VENTRAL HIPPOCAMPUS FOR WELL-LEARNED INSTRUMENTAL MEMORY RECONSOLIDATION

BY

CHAORAN CHENG

A thesis submitted to the University of Birmingham for the degree of
DOCTOR OF PHILOSOPHY

Disclaimer

This thesis was mostly completed on my own, except that part of the data in Experiment 1 of Chapter 2 was acquired by Marc T. J. Exton-McGuinness and Kalina Boytcheva. In particular, the work with the context extinction group（12 rats), the delayed reactivation group (12 rats) and the delayed non-reactivation group (8 rats) were carried out by Marc T. J. Exton-McGuinness, and part of the 6 hours delay group (8 out of 24 rats) was carried out by Kalina Boytcheva. Agreement has already been made to use those data. Part of the data (chapter 1 and 2) in this thesis was published by Cheng el al. in the 2022 thesis "Procedures between training and reactivation influence affecting the destabilization of instrumental sucrose memory".

Index

Abstract

Well-learned instrumental memory was thought both to be robust and unable to undergo reconsolidation. However, the current study showed that a certain contingency change was required to overcome the prediction error signal threshold in order to trigger a well-learned memory reconsolidation. In this study, we investigated different experimental models to study well-learned memory reconsolidation, which neural circuit may be involved and the boundary conditions of well-learned instrumental memory reconsolidation. We found with a mild contingency change (VR5) the two-lever model is the most suitable model to study well-learned memory reconsolidation and the delayed reactivation method which reactivated 48 hours (a gap day) after training to be the most consistent reactivation parameter to destabilise the memory. This may be ascribed to the gap day facilitating memory destabilization. But any procedures except memantine injection, even handling, during the gap day may prevent the facilitation effect and animals do not show signs of instrumental memory destabilization in the following test session. Furthermore, our data also suggest the ventral hippocampus may be required to trigger instrumental memory destabilization. Despite a lack of statistical power, our immunohistochemistry data of zif268 expression suggests that the infralimbic region and prelimbic region may be involved in instrumental memory reconsolidation. However, further studies are required for confirmation. Overall, this study demonstrates a new protocol that can consistently destabilise the well-learned instrumental memory which can be used for further instrumental memory studies and showed the ventral hippocampus is required for well-learned instrumental memory destabilisation.

General Introduction

History of consolidation hypothesis

This study focussed on the memory reconsolidation process. Before the emergent hypothesis of memory reconsolidation, consolidation had already been suggested as a memory processing hypothesis for more than a century (Müller & Pilzecker, 1960). Memory consolidation was suggested as a process that consolidates short-term memory to long-term memory which is more resistant to modification (Müller & Pilzecker, 1900; Hebb, 1949; McGaugh, 1966).

The beginning of the consolidation hypothesis

Müller and Pilzecker observed in 1900 that acquiring different information shortly after the learning trial would interrupt that learning, causing impairment of the retention performance, but interference information acquired after a long delay would not have such an effect. This suggested that short-term memory could be interrupted or modified, whereas, after consolidation, long-term memory was resistant to such effect. Further study of active avoidance showed that electroconvulsive shock treatment (ECS) 20 seconds after the training would cause rats to have significantly more freezing behaviour than the other groups. This impairment of behaviour was suggested as being retroactive amnesia (RA) (Duncan, 1949). However, such behaviour was arguably the fear of punishment rather than RA due to the disruption of the consolidation (Coons & Miller, 1960). Further study using passive inhibitory avoidance (IA) showed that 5 seconds after brief training, the rats who had ECS stepped on the charged platform a significantly higher number of times than the control group (Madsen & McGaugh, 1961). If the performance in the active aversive inhibitory avoidance was due to fear of punishment, the results in the passive aversive inhibitory avoidance should have yielded a contrasting result. Further studies using hypothermia, hypercapnia, cortical spreading depression, anaesthesia and convulsive drugs also induced RA in the newly learned memory (Gisquet-Verrier & Riccio, 2018). This leads to the idea that memory consolidation, as a newly learned memory, is labile and able to be disturbed in a short period by interference information or amnesic treatment.

The amnesic treatment-induced RA has a temporal-dependent effect. The ECS-induced amnesia gradient decreases as the interval between the training and treatment increases. One

hour after the training, the experimental group showed a similar performance to the control group (Duncan, 1949). This was also found in appetitive instrumental memory consolidation. Amnesic treatment at three minutes after training showed the greatest impairment in instrumental behaviour, whereas treatment with an interval of 30 minutes and above did not show any impact on memory (Ungerer, 1973). These findings suggest the memory consolidation process only takes a short period to transfer this labile memory into long-term memory, which is resistant to further change, but it does not imply that the consolidation process is finished in this short period. Human positron emission tomography (PET) study showed that brain activity shifted in different regions for several hours after learning a motor skill (Shadmehr & Holcomb, 1997). In guinea pigs, the learning-induced neuronal receptive field (RF) in the auditory cortex showed that neural activity continued for several days (Bjordahl, Dimyan, & Weinberger, 1998; Galván, Chen, & Weinberger, 2001).

After training, neuronal activity may persist to underpin the formation of LTP – long-term potentiation - (Hebb, 1949). This reverberating neural activity corresponds with the later long-term potentiation findings. LTP is suggested as triggering synaptic plasticity (Bruel-Jungerman, Davis, & Laroche, 2007; Nicoll, 2017; Takeuchi, Duszkiewicz, & Morris, 2014). Synaptic plasticity involved new protein synthesis and was hypothetically important for new memory formation (Martin, Grimwood, & Morris, 2000). This was supported by a study showing that short-term memory did not require protein synthesis, but long-term memory formation could be blocked by the protein synthesis inhibitor, puromycin (Agranoff, Davis, & Brink, 1966). Injecting a protein synthesis inhibitor in the dentate gyrus also blocked LTP (Otani & Abraham, 1989). This is not the only similarity between LTP and the consolidation process. They both induced depolarisation of synapses and reactivation of receptors; this, in turn, activated kinase and immediate early genes (IEG) which trigger the protein synthesis (Laroche, 2010). Thus, synaptic plasticity is likely to be why there is a small labile window for disruption. The new neuron connection is thought to be important for long-term memory formation (Müller and Pilzecker, 1900). Disrupting such a process is thought of as an impairment to new memory storage.

The RA shows a delayed onset. In the well-trained passive inhibitory avoidance experiment, a test at 15 minutes after ECS did not show the impairment of latency, but the tests at 30 minutes, 60 minutes, and 120 minutes all showed the impairment of retention performance. Furthermore, the 15-minutes group retested after 24 hours also showed impairment (Miller, Ralph R. & Springer, 1971). The same results were found in hypothermia and cycloheximide

treatment (Geller, Robustelli, & Jarvik, 1970; Hinderliter, Webster, & Riccio, 1975). This was explained by dual-trace theory, in other words that short-term memory, which lasts a few seconds to hours, is independent of long-term memories (Gerard, 1949). This is supported by the fact that the drug can only block either short-term memory or long-term memory in goldfish (Agranoff et al., 1966). As such, it suggests that the long-term encoding processing may be disrupted by amnesic treatment (Landfield, Mcgaugh, & Tusa, 1972; McGaugh, 2000; Miller& Matzel, 2000). Therefore, the original hypothesis of RA was memory encoding and storage impairment, and RA was thought irreversible (Duncan, 1949; McGaugh, 1966).

Problems with the early consolidation hypothesis

However, a few factors cannot be explained by this memory encoding and storage impairment theory. If it causes encoding and storage impairment, RA should completely impair the retention performance. However, the RA does not completely impair retention performance except in weak learning (Duncan, 1949; Madsen & McGaugh, 1961; Rodriguez-Ortiz, Carlos, Garcia-DeLaTorre, Benavidez, Ballesteros, & Bermudez-Rattoni, 2008). For example, in the appetitive instrumental setting, the rats with the impairment showed a reduction in, but still a noticeable degree of lever pressing in the non-reward test session (Ungerer, 1973). This partial deficit in test performance makes it challenging to determine whether the RA is in fact an impairment in encoding and storage.

The non-reversible part is most challenged by the fact that the amnesia can be reversed by the passage of time (Cooper & Koppenaal, 1964; Murchison et al., 2004; Squire & Barondes, 1972) or by a reminder (Land, Bunsey, & Riccio, 2000; Miller, Ralph, & Springer, 1972, 1972; Miller, Ralph, Ott, Berk, & Springer, 1974). For example, in the automated visual discrimination task the number of correct trials in the drug group was significantly lower than the saline controls in the first two days after treatment. However, there was no difference from the third day onward (Miller, Ralph, & Springer, 1972). This may be attributed to the fact that there was a slowly developing memory storage process that was unaffected by the amnesic treatment (Miller, Ralph, & Springer, 1972), or simply, that RA is reversible.

Further studies, about a reminder being able to reverse the RA, suggest that RA is reversible

and rejected the slow development memory process theory. The ECS-induced amnesia could be reversed by the reminder session in both appetitive memory and aversive memory (Miller, Ralph, & Springer, 1972; Miller et al., 1974). The reminder session started to have effect from two hours after the treatment to up to two weeks (Miller, Ralph, & Springer, 1972). Due to those experiments using a certain degree of reinforcement, for example, mild shock or reward, the reversal performance was argued as a generalisation to the test situation rather than recovering the original memory (Gold, Paul, & King, 1974). Later studies used the extinction session, which does not include the reinforcement-as-reactivation session, against the previous argument (Gordon, William & Mowrer, 1980; Wittman & DeVietti, 1980). These factors suggest that RA is a reversible retrieval failure rather than a non-reversible encoding and storage impairment (Lewis, 1979; Miller, Ralph, & Springer, 1973; Spear, 1973). Moreover, this result showed that the consolidated memory does not always stay in a consolidated form; it can be reactivated. Further study showed that if the rats were trained in a fear conditioning experiment, the ECS could not impair the fear memory 24 hours after training unless there was a brief exposure to a conditional stimulus (CS) before ECS (Misanin, Miller, & Lewis, 1968). The reminder session's function is suggested as reactivating the memory back to the labile stage, so it can be disturbed by the amnesic treatment. After that, the memory re-consolidates back to a persistent state which is not affected by the amnesic treatment (Lewis, 1979).

Current view of memory consolidation

Current thinking about consolidation is more dynamic than the original hypothesis, but the basic structure is similar. The new memory needs a process to stabilise and become fixed. This process has a very short window for amnesic treatment but takes much longer to complete. The protein synthesis (Rudy, Biedenkapp, Moineau, & Bolding, 2006), protein synthesis-induced LTP (Bruel-Jungerman et al., 2007; Nicoll, 2017; Takeuchi et al., 2014), and synaptic plasticity (Müller and Pilzecker, 1900) are thought important in the consolidation mechanism. The amnesic treatment, such as N-Methyl-D-aspartic acid (NMDA) receptor antagonist or protein synthesis inhibitor, will prevent synaptic plasticity thus causing impairment of memory representation within the structure (Elsey & Kindt, 2017). So, amnesia is a memory retrieval impairment and the amnesia is reversible (Lewis, 1979; Miller, Ralph, & Springer, 1973; Spear, 1973). The changing part of the current

consolidation view is that the consolidated memory is not thought to be a fixed state; it can be reactivated by the reminder and undergoes re-consolidation. The amnesic treatment during the re-consolidation process also will cause amnesia (Mactutus, Riccio, & Ferek, 1979; Mactutus, Charles, Concannon, & Riccio, 1982).

Age of reconsolidation

In the 1990s, there was an increased interest in reconsolidation studies. Amnesic treatment after the reminder session will also induce RA (Misanin et al., 1968), which suggested the memory was re-consolidated after the reactivation (Lewis, 1979). Further study showed that old fear memories, up to 14 days old, can still be reactivated (Nader, Schafe, & Le Doux, 2000). Other studies showed three conditioned fear training sessions were able to form a memory that still could be reactivated 45 days after training (Debiec, LeDoux, & Nader, 2002). Therefore, as the original concept of memory consolidation likened it to raw iron shaped into a specific form, current thinking about memory is more akin to water and ice. The consolidation changes the water into ice, but this ice can be melted by the reminder, integrating new ingredients, then changed back to the stable ice form.

The similarities and differences between consolidation and reconsolidation

The reconsolidation process shares similarities with memory consolidation. Reconsolidation and consolidation start when the memory is in the labile state and through a series of processes, which include protein synthesis, LTP, and synaptic plasticity, transfer the memory into a persistent state which has resistance to the disruption (Besnard et al., 2012; Lee et al., 2017). The difference is that consolidation is about the newly learned memory transferred into long-term memory, and reconsolidation is the long-term memory reactivated by a reminder, which then integrates new information and re-consolidates back to the persistent form (McGaugh, 2000; Reichelt & Lee, 2013a).

Though the process of reconsolidation is similar to consolidation, the two processes are doubly dissociable. The antisense oligodeoxynucleotides (ASO) of brain-derived neurotrophic factor (BDNF) infused into the dorsal hippocampus 90 minutes before context fear conditioning training, but not testing, impaired the long-term memory (LTM) but not short-term memory (STM). The ASO of transcription factor zif268, infused at the same time points, had no effect. For reconsolidation, after the training, the rats received a non-reinforced session (extinction session) as their reactivation session. The ASO of zif268, but not the ASO

of BDNF, infused 90 minutes before reactivation, impaired the LTM. This study showed that consolidation which induces synaptic plasticity to form a new LTM and reconsolidation which induces synaptic plasticity to maintain access and restabilise the existing memory were different in molecular mechanisms (Lee, Everitt, & Thomas, 2004). Therefore, the reconsolidation process is not only re-consolidation.

Reconsolidation is a generalisation mechanism across memory types and species

All known memory undergoes reconsolidation (Lee et al., 2017). For aversive memory, fear conditioning was the most used task in the studies. The fear is normally caused by electric footshock (as an unconditional stimulus, US) and paired with a specific cue (conditional stimulus, CS) such as tone or light, or context. To reactivate the memory, normally an extinction session was used as the reactivation session. This means the session would only have CS or novel/trained context without US (Reichelt & Lee, 2013a). Systematic injection of MK-801 could successfully impair these memory reconsolidations (Ben Mamou et al., 2006; Charlier & Tirelli, 2011; Lee et al., 2006b; Nader et al., 2000). The conditioned taste aversion (CTA) task paired an unfamiliar flavoured fluid (as CS) with a malaise-inducing agent (as US). The rats were normally trained to drink water from two pipettes. Water in one of the pipettes was changed to CS on the reactivation. US was applied after the reactivation. The test had the same setting as the reactivation. The unconditioned rats showed a preference for CS rather than water and the conditioned rats showed a contrasting preference (Merhav & Rosenblum, 2008; Rosenblum, Meiri, & Dudai, 1993; Stern, Chinnakkaruppan, David, Sonenberg, & Rosenblum, 2013). Only a few studies were about this memory. The systematic injection of a protein synthesis inhibitor could successfully impair this memory reconsolidation (Flint & Marino, 2007). The IA memory was also shown to undergo reconsolidation. The systematic injection of MK-801 and β-adrenergic receptor antagonists could successfully impair this memory reconsolidation (Flint, Noble, & Ulmen, 2013; Przybyslawski, Roullet, & Sara, 1999). These aversive memory reconsolidation processes also commonly relied on protein synthesis in the hippocampus and amygdala (Debiec et al., 2002; Lee. et al., 2004; Milekic, Pollonini, & Alberini, 2007; Nader et al., 2000).

As some of aversive settings corresponded to post-traumatic stress disorder (PTSD) in humans, some of the appetitive settings mainly tried to seek the mechanism for drug

addiction memories. The drug influences the instrumental behaviour through the conditioned approach which helped to make the instrumental response, conditioned motivation which enhanced motivation for the instrumental response (increased the outcome value), and conditional reinforcement which supported the response for delayed reinforcement. With the passage of time, the instrumental response would shift from a goal-directed response (associated with a drug high) to a habitual response (Milton, 2013).

In the animal model, the conditional approach was studied as a goal-tracking experiment. Goal tracking as a CS (CS+) was paired with the reinforcement and a CS (CS-) was not. Through the training, the rats should learn to approach the food magazine at CS+ rather than CS- (Costa & Boakes, 2009). The post-reactivation protein synthesis inhibitor injection did not impair the goal-tracking memory (Blaiss & Janak, 2007). However, a different training schedule, using a short session and fixed intervals rather than random intervals, showed that a pre-reactivation injection of MK-801 could impair the 6-day trained goal-tracking memory. However, it could not impair the stronger 12-day trained memory (Reichelt & Lee, 2012). The other setting for the conditioned approach was sign-tracking and Pavlovian-instrumental transfer. The rats needed to learn to press the lever with a specific CS+ but not a CS-. Compared to goal-tracking, this setting lacked both flexibility and the goal-directed nature of instrumental behaviour (Everitt, Barry, Dickinson, & Robbins, 2001). This memory was also able to be disrupted by systematic injections of MK-801 (Lee, & Everitt, 2008c; Milton et al., 2012).

The most widely used appetitive setting is conditioned place preference (CPP). Animals were paired with the drug and a context i.e., by injecting the drug before putting it into context A. Other animals were paired with saline and context B in the same way. The animals were reactivated and tested by freely exploring the three contexts – A, B, and one for control, without a drug or saline injection. The time spent on the drug-associated context was reduced by a systematic MK-801 injection which reflected the fact that the memory had undergone reconsolidation (Alaghband & Marshall, 2013; Brown, Travis, Lee, & Sorg, 2008; Sadler, Herzig, & Schmidt, 2007). Due to the context-related nature, this memory reconsolidation was also amygdala- and hippocampus-dependent (Milekic, Brown, Castellini, & Alberini, 2006; Sakurai, Yu, & Tan, 2007).

The conditioned reinforcement was the instrumental response (lever pressing for reward) paired with a CS (light or tone). The CS acted as a direct predictor for the reward (sucrose pellets or drug infusion) and reinforced the instrumental response. The extinction session was

normally used as a reactivation and test session, where animals received saline infusion (for drug) or no reward (for pellets) for the lever pressing (still paired with CS). This memory could be disrupted by a systematic MK-801 injection (Flavell & Lee, 2013; Lee, & Everitt, 2008a) and relied on BLA (Lee, Di Ciano, Thomas, & Everitt, 2005; Milton et al., 2008). Recently, an alternative reactivation parameter was used for cocaine-seeking memory. The reactivation used a contingency change of reward (randomly altering the number of lever pressings per sucrose pellet) rather than a non-reinforcing extinction parameter. The pre-reactivation of an MK-801 injection reduced the drug-seeking behaviour in the test. This showed that, rather than just extinction parameters, certain exposure to reward can also reactivate the drug-seeking memory (Exton-McGuinness & Lee, 2015; Exton-McGuinness, Drame, Flavell, & Lee, 2019).

There are only a few studies about instrumental behaviour, which is where a specific action is paired with an outcome (Thorndike & Woodworth, 1901). Dickinson (1985) suggested that instrumental behaviour may be classified as two types. The first is the response to a stimulus. This Stimulus-Response (S-R) model reflected a passive non-intelligential performance for a stimulus. But not all the behaviour was like that; the second process was based on knowledge of the consequence of performance. The behaviours were performed with a purpose or goal, so the active behaviour was different from the "response". These were referred to as "actions". When the action was performed, an outcome was expected, so this association was referred to as Action-Outcome (A-O).

To observe whether the memory was goal-directed, a common method was to devalue the reward. For appetitive memory, the common method was through the injection of lithium chloride which would cause gastric malaise in the rat after the free eating reward. If the behaviour was goal-directed, the rats would learn the reward caused gastric malaise, so they would reduce the reward-associated behaviour. Otherwise, it showed the behaviour was habit dominated, which is insensitive to the reward value (Dickinson, 1985; Exton-McGuinness et al., 2014; Holland, 2004).

Later, Dickinson's study (1995) showed that instrumental behaviour was a dual process. The rats with four training sessions (120 reinforcements) showed a different lever-pression pattern according to their deprivation state, whereas the rats with 12 trained sessions (360 reinforcements) showed no difference. If the behaviour was goal-directed, the low-deprivation state rats were less eager to obtain the food (low reward value), so the rats were less likely to press more levers in the extinction session. For the high-deprivation state, the

rats were eager to get food (high reward value), so they would press significantly more than in the low-deprivation state but quickly reduced the lever-pressing, so the behaviour in the less-training group appeared goal-directed. On the other hand, the well-trained groups had similar lever-pression rates regardless of the deprivation state. This reflected that habitual behaviour was insensitive to reward value. Interestingly, rats that received eight sessions (240 reinforcements) which delivered food randomly with no lever in the chamber, and four normal training sessions (120 reinforcements) showed a similar habitual behaviour as the 12 training sessions group. This showed the training did not reflect how many training trials the subjects took, but just how many reinforcements the subjects obtained. Dickinson suggested the S-R process contributed more to the manifest behaviour as the training proceeded. So, the behaviour shifted from goal-directed to habitual. It is worth noting that the training schedule in Dickinson's study (1995) used a random interval (RI) schedule in which the reward would be delivered following a random interval after pressing the lever. The RI schedule proved less sensitive to reward value and had a slower performing rate than the variable ratio (VR) schedule (a different number of lever presses for each reward) with matched outcome probabilities (Dickinson et al., 1983). The study by Mackintosh (1974) suggested the long inter-response times (IRTs) hypothesis, in other words, in a RI schedule, the longer the interval between each response, the more likely the reward is to be delivered. So, the longer IRTs correspond to the high possibility of reward delivery for the next response, whereas reward delivery in the VR schedule was based on the number of lever presses. So, animals using the VR schedule often had a higher response rate than using the VI schedule.

The well-learned instrumental memory was considered unable to undergo memory reconsolidation until 2014. This was due to the normal reactivation parameter which was that an extinction session was unable to trigger memory destabilisation. Therefore, a protein synthesis inhibitor could not impair memory (Hernandez & Kelley, 2004; Mierzejewski et al., 2009). But Exton-McGuinness et al. (2014) showed that with certain reinforcement exposure rather than complete non-exposure, the well-learned instrumental memory could be impaired by MK-801. By showing that the animal behaviour in the test session was goal-directed, it was suggested that the habitual S-R memory was impaired after reactivation. This confirmed that instrumental habitual memory was destabilised. Exton-McGuinness et al. (2015) further demonstrated the goal-directed A-O memory was able to reconsolidate and required a lower contingency change than the well-learned memory. The inhibition of the dopamine-1 receptor (D1R) or NMDA receptor separately in the nucleus accumbens (NAc) would not affect

memory reconsolidation, but the inhibition of both receptors showed a disruption of instrumental memory. This suggested coactivation of D1R and NMDA receptors was important for instrumental memory destabilisation and reconsolidation. Therefore, it showed that instrumental memory was able to reconsolidate.

Reconsolidation is a general memory process across species. Most studies used rodent models. Most aversive memories, such as conditioned fear memory and contextual fear memory; appetitive memories, such as drug-seeking memory and Pavlovian conditioned instrumental memory, and spatial memory, were observed to undergo memory reconsolidation (Morris et al., 2006; Reichelt & Lee, 2013a). But other species also showed signs of memory reconsolidation. The medaka fish has a simpler brain than the rodent, but its fear memory also undergoes reconsolidation. This was observed by pairing light with an electric shock. After two days of training, amnesic treatment before the reactivation session impaired the freezing behaviour of the fish. This was a sign of reactivation-dependent amnesia (Eisenberg, Kobilo, Berman, & Dudai, 2003; Eisenberg & Dudai, 2004). In the case of Chasmagnathus crabs, a visual danger stimulus paired with the training context could reactivate the fear memory in a novel context. The impairment of response was observed when treated with cycloheximide from one hour before reactivation to two to six hours after reactivation (Pedreira & Maldonado, 2003).

Human memory also was able to undergo reconsolidation. Most amnesic reagents, such as NMDA receptor antagonist MK-801 or protein synthesis inhibitor anisomycin, have severe side effects with systematic injection. This means that most studies used propranolol treatment, stress/cortisol, Ketamine, glucose, or behavioural means (memory interference) to prevent the formation of long-term memory (Agren, 2014). Most of the human experiment settings were similar to those of the animal models. For example, most experiments follow the acquisition, extinction and test procedure (Lonsdorf et al., 2017). In the fear conditioning experiment, human participants had to remember some fear-relevant stimulus (such as a picture of spiders), and one of the stimuli was paired with an electric shock (Kindt, Soeter, & Vervliet, 2009). In the animal model fear-conditioning experiments, however, the electric shock was also paired with a tone or light (Reichelt & Lee, 2013a). Both models used the extinction session as a reactivation parameter and took place 24 hours after training. In the test, the animal model quantified the fear reaction as a percentage of freezing time (Lee, Dickinson, & Everitt, 2005), whereas the human model used a questionnaire (Kindt et al., 2009). However, unlike most animal experiments using single populations, human

experiments often have subpopulation differences, even individual differences, often perceived as noise. For this reason, group analysis may not provide meaningful information (Lonsdorf & Merz, 2017).

Universal results across various species are supported by the memory reconsolidation hypothesis. Though it shares several similar features with the consolidation process, reconsolidation also has its distinctive features in addition to re-consolidation. Furthermore, there are a few boundary conditions that may affect the reconsolidation process.

Boundary conditions of memory reconsolidation

The most significant difference between the consolidation studies and reconsolidation studies is that a reminder/reactivation session takes place after the training and normally 24 hours before the test. The reminder session needs to provide new information to activate the memory, so it needs to contain certain factors of the training session but cannot be the same as the training (Lewis, 1979). The instrumental memory reconsolidation studies showed that using the training session as a reactivation session could not trigger memory reconsolidation (Exton-McGuinness et al., 2014; Exton-McGuinness & Lee, 2015). Therefore, this reactivation session suggested generating a prediction error signal which is the key to triggering memory destabilisation (Lee, 2009). However, for some memories, the training session can also be used as the reactivation session. For example, in the context of fear memory, the second training session could reactivate the memory of the first session. This was confirmed as a reconsolidation process but not a consolidation process due to the ASO of zif268, but not BDNF ASO, impairing memory (Lee, 2008). This may be due to the weak learned memory being easier to reactivate. This was supported by the results from the rats with one-day spatial training which could be reactivated by the extinction session, while the six-day training could not (Morris et al., 2006). This shows there are several boundary conditions affecting memory reconsolidation. Those boundary conditions are summarised in Figure 1.

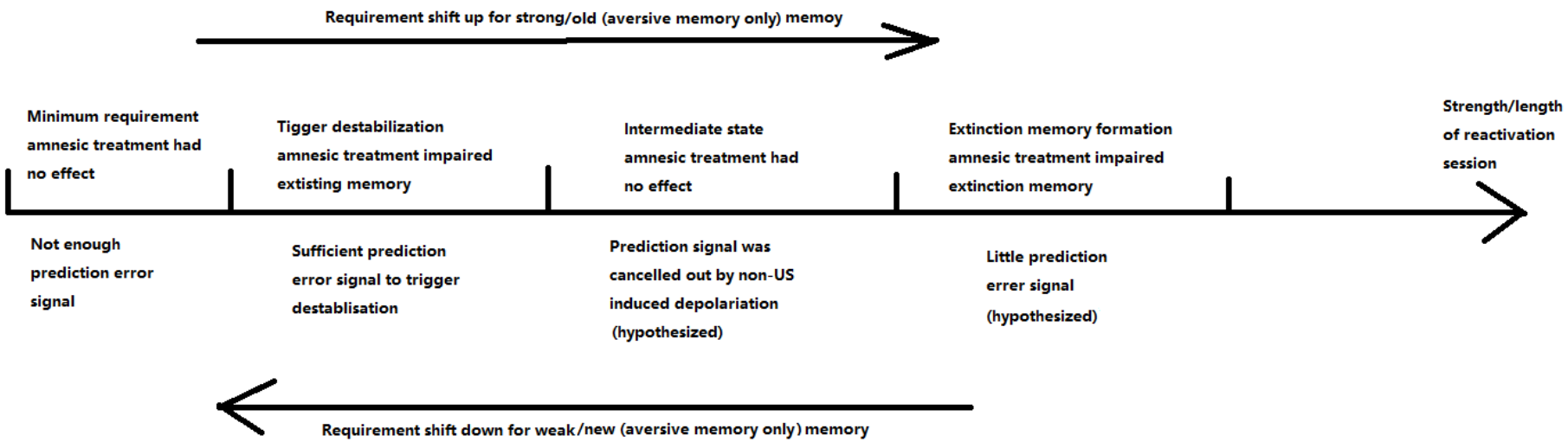


Figure 1. The effect of reactivation strength/length, memory age and memory strength for memory and prediction error signal.

Memory strength

One of the commonly considered boundary conditions is memory strength. Several studies suggested that the different memories or the extinction memory could compete. The notable hypothesis is the "trace dominant" framework (Eisenberg et al., 2003). Eisenberg et al. (2003) showed the rats had reconsolidation if there were two training trials before reactivation conditioned taste aversion (CTA). But if there was only one training session, the reactivation would result in forming CS-no US trace (extinction trace) rather than reconsolidation. The hypothesis this result suggested is that the extinction trace would compete with the learned aversive CS -US trace. Thus, if the aversive memory was stronger, it would dominate, and the amnesic treatment would impair the original aversive memory. However, if the CS-US memory was weaker than the newly learned CS-no US memory (extinction memory) (reactivation session as an extinction session), the extinction trace would dominate. Thus, the amnesic treatment would impair the newly learned extinction memory. This competition theory is also supported by a few appetitive studies. The goal-tracking studies showed the rats with six-day training could be reactivated by an extinction session with three CS+ (CS with reinforcement in training) and three CS- (CS without reinforcement in training). However, saline controls rats with only three days of training would show extinction behaviour which should equal response to CS+ and CS- rather than the normal response (where CS+ responds more than CS-). This suggested that the weak memory was not dominant, therefore the extinction memory was formed rather than the goal-tracking memory being reconsolidated (Reichelt & Lee, 2012).

Though few studies showed that instrumental memory reconsolidation followed the "trace dominant" framework, several instrumental studies showed such memory competition occurred in the instrumental setting. First, the weak-learned instrumental memory had similar results to the weak-learned goal-tracking memory. When reactivated with a brief extinction session, it showed amnesic treatment-impaired extinction behaviour in the test rather than instrumental memory impairment (Exton-McGuinness & Lee, 2015). Also, through instrumental training, the manifest behaviour would shift from the reward value sensitive A-O behaviour to the reward value insensitive S-R behaviour. The manifest behaviour was determined by the strength of the memory, so the behavioural result also reflected the change of memory strength (Dickinson, Balleine, Watt, Gonzalez, & Boakes, 1995). Such results showed that the trace dominant hypothesis also works between appetitive instrumental memories. Also, this trace dominant hypothesis works between different types of memory.

Reed (1990) showed that context extinction sessions before the test would promote instrumental behaviour. The context extinction sessions were thought to reduce the context-reward association, so the elevated instrumental responses corresponded to the reduction of the strength of the opponent's memory.

In addition to the competitive memory strength affecting the reconsolidation, the strength of the training memory also affects reconsolidation. In simple terms, the stronger memory is harder to reactivate. In context fear conditioning, mice trained with one footshock could reactivate the training memory by the extinction session (no footshock, same context). But the same reactivation session could not reactivate the training memory with three footshocks in the training. This was suggested to be due to the three footshocks having formed a stronger fear memory than one footshock (Suzuki et al., 2004). The factors of this were related to dorsal hippocampus function and the NMDA receptor GluN2B subunit was down-regulated in the LBA (Wang, Szu-Han et al., 2009). In auditory fear conditioning, the rats trained with 10 CS-US were insensitive to the amnesic treatment, but the trained 1 CS-US pair were sensitive. The dorsal hippocampus lesion did not affect auditory fear memory reconsolidation but caused the strong fear memory to be sensitive to the amnesic treatment. The western blot after training showed the GluN2B receptor was only down-regulated in the ten CS-US paired rats, but not the rats with only one CS-US pair or the rats with ten CS-US paired with the dorsal hippocampus lesion. The GluN2B subunits in BLA were reported as important for memory destabilisation but not reconsolidation (Milton et al., 2013). The dorsal hippocampus was suggested as having the role of recording spatial-contextual information and memory. Reactivation required hippocampus representation to guide to the easily inferenced neocortical representation which recorded content information (Nadel & Hardt, 2011). Therefore, the mechanism of the boundary condition was suggested as destabilisation failure (Wang, Szu-Han et al., 2009) and may be related to the enhanced spatial-contextual representation in the dorsal hippocampus.

Strong memory being hard to reactivate may also apply to some appetitive memories. A weak contingency session can reactivate a weak-trained, goal-directed appetitive instrumental memory (Exton-McGuinness, M. T. & Lee, 2015), but did not work on over-trained habitual memory (Cheng, Exton-McGuinness, unpublished data). This was suggested as a prediction error signal insufficient to trigger memory destabilisation. The same was found in the goal-tracking study. The rats trained for six days were able to be reactivated by three sets of CSs (CS+ and CS-), but the rats with 12 days of training were not (Reichelt & Lee, 2012).

However, some studies showed that memory strength was unrelated to reconsolidation. For the cue-induced drug-seeking memory, a well-learned drug-seeking memory (ten days of training, around 500 CS paired reinforcements) could be reactivated by an extinction session (Lee, Milton, & Everitt, 2006a). Similar results were found in the well-learned conditioned reinforcement reconsolidation (Lee & Everitt, 2008b), but not in the well-learned non-cued instrumental memory (Exton-McGuinness et al., 2014).

Memory age

The other factor affecting memory reconsolidation is the age of the memory. The suggestion is that memory stability was increased with the passage of time. Therefore, older memories are hard to reactivate (Reichelt & Lee, 2013a). This was observed in several studies. With the same reactivation session, the eight-week-old context fears memory could not be reactivated, but both a one-week-old memory and a three-week-old memory can. However, if a longer extinction session is used, the eight-week-old memory could still be reactivated (Suzuki et al., 2004). The other inhibitory avoidance experiment showed that reactivation-dependent amnesia was only observed two and seven days after training but not 14 and 28 days after training (Milekic, Maria, & Alberini, 2002). But these results were not consistent: a context fears study showed that memory could undergo reconsolidation 3 days, 15 days, and even 45 days after training (Debiec et al., 2002). The 45-day-old memory was suggested as being hippocampus-independent (Anagnostaras, Gale, & Fanselow, 2001; Debiec et al., 2002; Kim & Fanselow, 1992). Such results suggested that the older memory being harder to reactivate may not be due to the old memory being more stable but may be due to the decay of hippocampus representation (Hardt, Nader, & Nadel, 2013).

As Hardt et al. (2013) suggested, the context and spatial information encoded in hippocampus representation acted as the guide to the neocortical encoded content information. The hippocampus representation only decayed within days to weeks, but the neocortical representation was more persistent. Therefore, the older memory being harder to reactivate indicates the loss of hippocampus representation. It follows that it needs a stronger/longer reactivation parameter to reactivate. Moreover, the hippocampus representation may also "teach" the neocortical representation to integrate new information over time. The neocortical representation would become sparser and more efficient (McClelland, McNaughton, & O'Reilly, 1995). The new neocortical representation was hypothesised no longer to need

hippocampus representation for retrieval (Hardt et al., 2013). The results showed the hippocampus-dependent results for the 45-day-old memory only occurred after the reactivation session. The lesion impaired the memory within 24 hours after reactivation but had no effect after 48 hours which showed the process was related to reconsolidation (Debiec et al., 2002). Thus, the results may suggest that memory destabilisation may be triggered by the neocortical representation and re-established the hippocampus representation linkage to the neocortical representation. As the neocortical representation encoding mechanisms are easily interfered with by other neocortical representations (McClelland et al., 1995; McCloskey & Cohen, 1989), this may suggest the interfering memory may affect memory retrieval (Hardt et al., 2013).

An auditory fear memory study result may support that the old memory was harder to reactivate due to memory forgetting. A strong fear memory (ten CS-US paired) could not be reactivated by a five CS extinction session, but could be reactivated by the one CS extinction session if the memory was 30 days or 60 days old (Wang, Szu-Han et al., 2009).

In an appetitive setting, the age of memory seems unaffected by memory reconsolidation. The typical appetitive memory unaffected by ageing is drug-seeking memory. The relapse (reinstatement) of the drug-seeking memory can be over a few weeks and the memory has high resistance to forming an extinction memory (Bossert, Marchant, Calu, & Shaham, 2013). The other conditional reinforcement study showed that well-trained conditioned reinforcement could be reactivated 21 days after training (Diergaarde, Schoffelmeer, & De Vries, 2006). However, it is worth noting that most long-interval studies had more training sessions in appetitive settings (around ten sessions) than the aversive setting (usually one or two sessions). It is, therefore, possible that the results are due to the well-learned appetitive memory taking longer to forget.

Reactivation session length/strength

The third factor affecting memory reactivation is the length/strength of the reactivation session. In both aversive settings and appetitive settings, memory appeared to require a minimum number of exposures to CS or US to trigger the reconsolidation (Exton-McGuinness et al., 2014; Exton-McGuinness & Lee, 2015; Suzuki et al., 2004). However, over-exposure would cause extinction memory formation rather than training memory reconsolidation (Flavell & Lee, 2013; Suzuki et al., 2004). This first demonstrated, in the

context of fear memory, that no exposure (zero minutes) or short exposure (one minute) to the context would not trigger memory reconsolidation. 30-minute exposure to the extinction session would result in extinction memory formation. Only intermediate exposure length (three minutes) triggered the fear memory reconsolidation (Suzuki et al., 2004).

A similar result was also found in the auditory fear experiment. The exposure of ten CS without the US caused the formation of extinction memory, whereas only one CS caused fear memory reconsolidation (Lee et al., 2006b; Wang, Szu-Han et al., 2009). Similar results were also found in Chasmagnathus crabs (context fear conditioning) (Pedreira & Maldonado, 2003) and in medaka fish (cued fear conditioning) (Eisenberg et al., 2003). Flavell & Lee (2013) showed that the conditioned reinforcement memory followed the same principle. The extinction session with ten CS reactivated the appetitive memory, whereas the 50 CS session (same session length) caused the formation of extinction memory. The intermediate 30 CS session neither reactivated the memory nor caused extinction memory formation. However, it is worth noting that the effect of the reactivation strength/length is related to the memory strength, and the requirement of the exposure increases by the memory strength. For example, a 3-CS-paired extinction session would reactivate a well-learned (six-day training) goal-tracking memory but cause extinction memory formation for the three-day training memory and could not trigger the reconsolidation for the 12 days of training (Reichelt & Lee, 2013a).

In this study, we investigate well-learned instrumental memory. As stated above, this memory is relatively strong and therefore requires a stronger reactivation parameter (VR20) to reactivate (Exton-McGuinness et al., 2014). However, in this study, we used a relatively weak reactivation parameter (VR5) and also reactivated the well-learned instrumental memory. This could suggest that other boundary conditions may affect the instrumental memory reconsolidation.

Prediction error in memory reconsolidation

One of the explanations for contingency change other than an extinction session that could reactivate the instrumental memory is that the contingency change induced a suitable prediction error signal which is required to destabilise the memory (Exton-McGuinness et al., 2014). It is suggested that this prediction error signal is delivered from the ventral tegmental area (VTA) to the Nucleus accumbent (Nac) and further suggested to be required for memory destabilisation in many studies (Exton-McGuinness et al., 2014; Reichelt et al., 2013).

Neuroanatomy results for prediction error

The destabilisation was suggested to have had a different molecular pathway with restabilisation/reconsolidation. It was shown that destabilisation/strengthening of the cued fear memory required BDNF but not zif268 expression in the hippocampus (Lee, 2008). Further study showed the double dissociation requirement of NMDA receptor components for destabilisation and restabilisation. The destabilisation selectively required the GluN2B subunit and the restabilisation selectively required the GluN2A subunit (Milton et al., 2013). The prediction error signal is suggested to be important for destabilisation (Exton-McGuinness, Lee, & Reichelt, 2015). This is supported by studies about the substantia nigra/ventral tegmental area complex (SNc/VTA). In monkeys' midbrains, the dopaminergic neurons (DA) in SNc/VTA increased activity when exposed to reward paired CS (CS+). If the reward was delivered, the neuron maintained the firing rate, otherwise the neurons would be hyperpolarised, which reflected the negative prediction error signals. This signal reflects as an unexpected omission of reward or a received lesser reward (Hollerman & Schultz, 1998; Takahashi et al., 2009). On the other hand, the non-reward CS (CS-) also triggered a smaller depolarisation of the dopaminergic neurons but was followed by a hyperpolarisation. The no-reward scenario did not change the neuron firing rate, but the reward delivery after the CS-caused neuron activity elevation reflected positive prediction error signals (Waelti, Pascale, Dickinson, & Schultz, 2001). The function of the VTA was the same in rodents. The inactivation of VTA would prevent error-driven learning in rats (Rodriguez-Ortiz, Carlos, Garcia-DeLaTorre, Benavidez, Ballesteros, & Bermudez-Rattoni, 2008). However, over-expectation training for goal-tracking training could reactivate the goal-tracking memory

which was previously thought unable to undergo the reconsolidation process (Reichelt & Lee, 2013b). Dysregulation of VTA by B/M or sulpiride prevented Pavlovian reinforcement memory reconsolidation but infusing the MK-801 or AP-5 did not impair the existing memory. This suggested the prediction error signal from VTA was required to destabilise the existing memory, but the VTA was not required for memory reconsolidation (Reichelt, Exton-McGuinness, & Lee, 2013).

Prediction error and destabilisation

The weak learned appetitive memory is easy to reactivate even through a training session (Rodriguez-Ortiz, Carlos et al., 2008). This is suggested as the association is not well established, thus the subjects cannot predict the outcome, so even a training trial can generate enough prediction error signals to trigger destabilisation (Lee, 2008). Through the training, the amnesic effect of protein inhibitors decreased (Rodriguez-Ortiz, De la Cruz, Gutierrez, & Bermudez-Rattoni, 2005). This was hypothesised as proportionate to the prediction error signal (Reichelt & Lee, 2013b). Therefore, a well-learned memory would have a better prediction for the training session, so the training session generated fewer prediction error signals which were unable to trigger the destabilisation.

A more recent study supported the role of prediction error signals in destabilisation. Exton-McGuinness et al. (2014) showed the well-trained instrumental memory (training for one lever press for one sucrose pellet) could be reactivated by a VR20 protocol (12–28 lever presses for a sucrose pellet, average rate 20 lever presses per pellet) but could not be reactivated by the FR20 (fixed ratio 20 lever presses per pellet). On average the contingency change is the same, the putative prediction error signal should be similar between FR20 and VR20, but the results in behaviour were different. This suggested that not only was the amplitude of the prediction error signal important to trigger the destabilisation, but there were also other factors.

The hypothesis of temporal difference affecting memory destabilisation

Two hypotheses arose from the later review by Exton-McGuinness et al. (2015). The first was that the unpredictability of the VR20 procedure generated greater attention from the rats than

the fixed FR20. The attention to the CS was suggested as facilitating learning and memory reconsolidation (Hall & Pearce, 1982; Pearce & Hall, 1980; Wilson, Boumphrey, & Pearce, 1992). The other hypothesis was about the temporal difference (TD) model of prediction error signals. In the classical Bush and Mosteller model, the results had the same weighted average of past rewards, so there was no difference (Bush & Mosteller, 1951a; Bush & Mosteller, 1951b). But in the TD model, none of the sessions were considered as a whole, but rather as multiple discrete moments. In each discrete moment, the prediction was not only for the reward at this moment but also the prediction of the reward in subsequent moments (Glimcher, 2011; Sutton & Barto, 1998). So, for the FR20, if the session was divided by each reward, the pattern of each discrete moment should have the same number of lever presses. On the other hand, the pattern of VR20 should be more randomised, so each moment could be treated as a training session. The rats with FR20 generally learned that 20 lever presses were needed for a pellet. As the animal learns the protocol and gets the expected reward, the session, as shown by Rodriguez-Ortiz, Carlos et al. (2008), should generate fewer prediction signals. By contrast, for the VR20, the outcome was delivered more randomly, so the rats' predictions had a high error rate, which should generate a continuous prediction error signal (increased neuron activity for CS-) and thus be a potential trigger for destabilisation.

This may also explain why long exposure to an extinction session would cause extinction memory formation rather than memory reconsolidation. With a short exposure, subjects were exposed to the CS without the US. The subject would wrongly predict that the CS was associated with US, and this would generate the prediction error signal, so it may trigger destabilisation. The more unpaired CS to which the subjects were exposed, the more the CS-no US pairing would be learned. This non-reward caused the hyperpolarisation of the dopaminergic neurons, and this may shift the CS+ to CS-. So the intermediate exposure did not trigger memory destabilisation. Finally, the rats would eventually learn the CS- no US trace and cause the extinction memory formation, so the process shifted from destabilising the existing memory to forming the new extinction memory. Such reconsolidation-intermediate-extinction shift with CS was found in the appetitive conditional reinforcement study described earlier (Flavell & Lee, 2013). A conditional fear study showed more detail about such process. The reconsolidation group showed significant high NMDA receptor activity and low calcineurin (CaN) expression in the basal lateral amygdala (BLA), and the extinction group showed the opposite. The experiment results are most strongly expressed in the three stage model: the reconsolidation stage which was presented with 1 CS required for

NMDA receptor activity in BLA but was not required for CaN expression; the intermediate or "limbo" state which was presented with 4 CS was NMDA receptor activity independent; and the extinction stage which was presented with 7 CS onward required NMDA receptor activity and CaN expression. Furthermore, the NMDA receptor activity in this stage was positively related to CaN expression (Merlo et al., 2014). But it is worth noting that without an independent measure of prediction error, the necessity for prediction error in destabilisation cannot be substantiated.

Neural circuits may be involved in instrumental memory reconsolidation

There was no study about which neural circuit may be involved in instrumental memory reconsolidation, but with instrumental learning studies and studies of other memory reconsolidations, it is suggested that several circuits may be important for instrumental memory reconsolidation (figure 2). The ventral medial prefrontal cortex (vmPFC) - nucleus accumbent (Nac) circuit was suggested to be involved in several instrumental behavioural circuits (Bouton et al., 2021; Cooper et al., 2017; Everitt & Robbins, 2005). In vmPFC, different subregions project to different Nac subregions, the IL major projected to Nac shell (AchSh) and PL was preferentially projected to Nac core (Nacc) (Sesack & Grace, 2010). Those two projects are important for instrumental learning, as a lesion of vmPFC would prevent the acquisition of goal-directed learning but did not prevent expression (Kalivas et al., 2005). Apart from the ventral striatum, the dorsal striatum was a suggested response for habitual behaviour* (Everitt & Robbins, 2005). Lesions of the dorsal striatum prevent habitual formation but did not affect reward expectancy (Yin et al., 2004). The input of the dorsal striatum was suggested from vmPFC and Nacc via substantia nigra (Graybiel & Grafton, 2015). Though there is no evidence of BLA and dorsal hippocampus (dHPC) involvement in instrumental reconsolidation, those regions were suggested as a response to contextual information in instrumental learning and involvement in multiple aversive and appetitive memory reconsolidations (Reichelt & Lee, 2013). The current view of BLA was that it mainly concerns processing discrete CS, whereas the glutamatergic input from dHPC to Nac especially AchSh was processing contextual or spatial stimuli (Janak & Tye, 2015). It was suggested the dHPC -Nac circuit may compete with the BLA-Nacc circuit over goal-direct behaviour (Everitt & Robbins, 2005; Peak et al., 2020; White & McDonald, 2002). Lesion of the hippocampus impaired the contextual conditioning but enhanced discrete CS conditioning, whereas lesions of BLA had the opposite effect (Everitt & Robbins, 2005). In memory reconsolidation studies, blocking BLA or the hippocampus impaired memory reconsolidation in fear conditioning, inhibitory avoidance, and CPP (Reichelt & Lee, 2013). Apart from memory reconsolidation, it is suggested that BLA and dHPC direct and indirect projection to Nac are important in instrumental learning (Bouton et al., 2021; Everitt & Robbins, 2005). Glutamatergic input from BLA to Nacc facilitated reward-seeking and

positive reward learning, and lesions of BLA or Nacc prevented the acquisition of cocaine seeking (Millan & McNally, 2011; Millan et al., 2015). The circuit of BLA to AchSh was thought important in reducing or inhibiting the instrumental response during an extinction session (Bouton et al., 2021). Those different projections and functions suggest that BLA may have a complex role in instrumental memory learning. There was evidence to suggest the function of dHPC was dependent on BLA activation in cocaine paired renewal; asymmetrical blocked dHPC and BLA attenuated drug seeking behaviour in the context used in extinction sessions (extinction context), whereas ipsilateral or unilateral inactivation had no effect (Fuchs et al., 2007). However, direct projection to Nac, dHPC and BLA may also have indirect control of Nac through the ventral hippocampus (vHPC). The vHPC was suggested as the site which integrates spatial/contextual information from the dHPC and emotional information from the BLA (French et al., 2003; French & Totterdell, 2003; Oleskevich et al., 1989). The linkage of those circuits is as in figure 2.

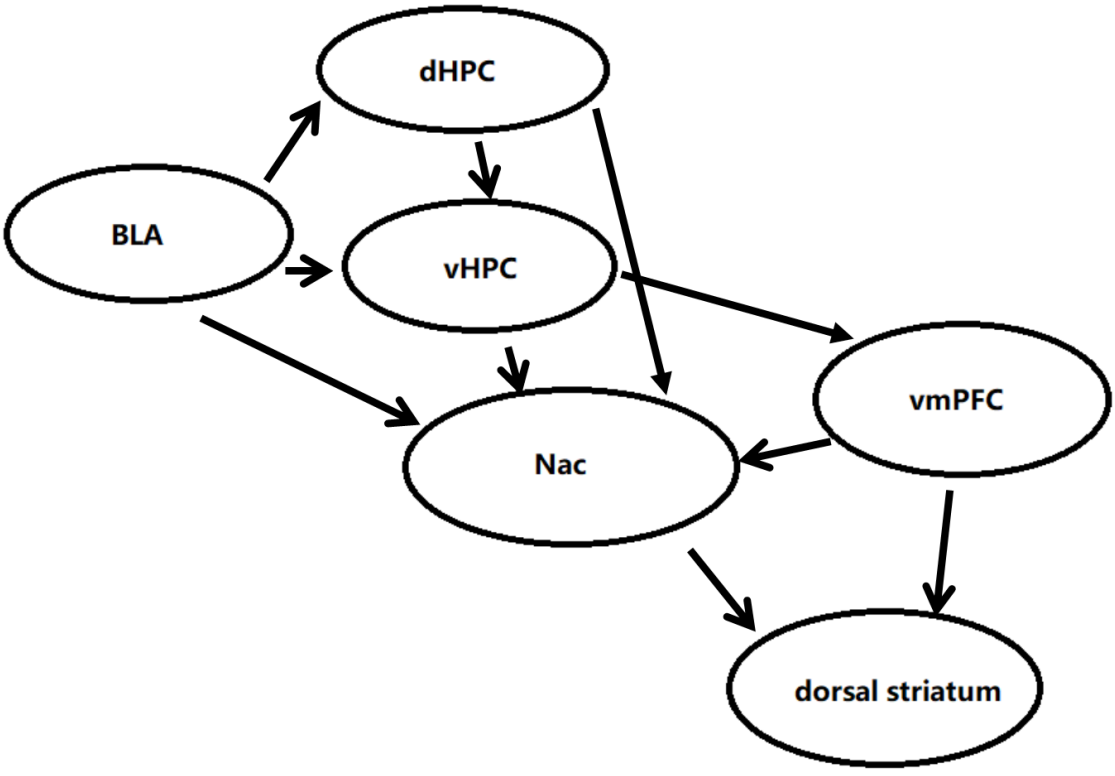


Figure 2. The neural circuits which may be involved in well-learned instrumental memory reconsolidation.

Ventral mediate prefrontal cortex

There is no evidence for infralimbic (IL) and prelimbic (PL) involvement in instrumental memory reconsolidation. However, the expression of zif268 in PL and IL was upregulated after cocaine-induced CPP memory reconsolidation (Li et al., 2016). Furthermore, the dichotomous roles of IL and PL in different types of instrumental learning were suggested by several studies (Kalivas & Volkow, 2005; McLaughlin & See, 2003; Peters et al., 2009). IL and PL receive the DA signal from VTA and regulate Nac activation by glutamate signal (Everitt & Robbins, 2005; Sesack & Grace, 2010). This VTA-vmPFC-Nac circuit was suggested as important to the regulation of prediction error signals (Cooper et al., 2017). Inhibition of PL by $GABA_{A/B}$ receptor agonist baclofen/muscimol (B/M) or dopamine receptor antagonist fluphenazine prevented the reinstatement of cocaine-seeking behaviour (James et al., 2018; McFarland & Kalivas, 2001). Thus PL was suggested to have a role in promoting instrumental behaviour after reinstatement (Bouton et al., 2021). PL was also suggested as having the same function in sucrose instrumental learning, as deactivation of IL reduced the instrumental action when re-exposed to the same context after the extinction session but no effect for re-exposure to a different context (Bossert et al., 2011; Shipman et al., 2018; Warren et al., 2016). On the other hand, IL was suggested as being important for extinction learning and inhibition of instrumental behaviour after extinction sessions (Bouton et al., 2021). The IL inactivation within a short window (up to 20s) after an extinction session increased response during extinction training and cue-induced reinstatement of cocaine seeking (Gutman et al., 2017). For sucrose instrumental learning, reversible inactivation of IL increased the response in the extinction context after an extinction session but also reduced the response in the training context (Eddy et al., 2016). The impairment of IL may impair the expression of inhibition extinction context-response association and prevent the expression of appetitive context-reward association (Bouton et al., 2021). Though IL and PL were well studied in instrumental learning, those two regions were rarely targeted by memory reconsolidation studies. Given their role in the renewal circuit and the extinction circuit, they may also be involved in instrumental memory reconsolidation.

Hippocampus and basal lateral amygdala

Dorsal Hippocampus and BLA were focused on both appetitive and aversive memory reconsolidation studies (Reichelt & Lee, 2013). Those two regions were suggested as important for contextual information processing and required for several types of memory reconsolidation (Cooper et al., 2017; Reichelt & Lee, 2013). For example, the selectively required zif268 in the hippocampus is involved only in contextual fear memory reconsolidation but not in consolidation (Lee et al., 2004). In an appetitive setting, CCP memory reconsolidation can be impaired by infusion of NMDA antagonist AP5 in the hippocampus (Sakurai et al., 2007). The zif268 level is increased in CA1, CA2, and CA3 after cocaine-induced CPP memory reconsolidation (Li et al., 2016). The role of BLA in memory reconsolidation was studied in different models (Reichelt & Lee, 2013). In aversive memory reconsolidation, the BLA was required for cue fear conditioning (Ben Mamou et al., 2006; Lin et al., 2006), inhibitory avoidance (Milekic et al., 2007; Pedroso et al., 2013), and conditioned taste aversion (Koh & Bernstein, 2003). In an appetitive setting, BLA inhibited by protein synthesis inhibitor anisomycin, zif268 antisense oligodeoxynucleotides (Lee et al., 2005), or NMDA antagonist AP5 caused a reactivation-dependent impairment in conditioned reinforcement (Milton et al., 2008). In BLA, zif268 expression was found after cocaine-induced CPP memory reconsolidation, and BLA was also required for drug-associated memory learning (Hamlin et al., 2008; Li et al., 2016).

The ventral hippocampus is another potential region that might be recruited in instrumental memory reconsolidation. While there are no studies directly implicating the VH, its projection from ventral CA1 to the IL was activated after context-induced reinstatement of heroin seeking. This caused down-regulated synaptosomal Glu2 in IL and facilitated LTD in the vCA1-IL pathway (Wang et al., 2018). The ventral subiculum was important for cocaine-seeking renewal. Inactivation of the ventral subiculum (vSUB) prevents the reinstatement of cocaine- and heroin-seeking (Bossert & Stern, 2014; Lasseter et al., 2010). As the vHPC function suggested an integration of contextual and spatial information from DH and BLA to Nac, it may be - but is unlikely to be - involved in well-learned instrumental memory reconsolidation.

Striatum

There is no evidence for striatum involvement in instrumental memory reconsolidation, but Nacc involvement is suggested in drug-associated CPP memory reconsolidation as zif268 expression was upregulated after reconsolidation, and the reconsolidation process would be impaired if zif268 ASO or protein synthesis inhibitor was to be infused into Nacc (Li et al., 2016). Most instrumental learning studies focused on Nac in the ventral striatum. The dorsal striatum is also suggested to have importance in instrumental behaviour, especially habitual behaviour (Everitt & Robbins, 2005). Nac was suggested as important for the initial acquisition of food or liquid rewards (Everitt & Robbins, 2005; Haber et al., 2000; Kelley & Berridge, 2002), but the executive control would ultimately shift to the dorsal striatum through the training (Robbins & Everitt, 1999; Yin et al., 2004; Yin et al., 2005). This suggested the goal of direct behaviour involved a circuit of PFC to Nac and the dorsal striatum, then a shift to PFC and to the dorsal striatum through training (Everitt & Robbins, 2005). This is supported by microinfusion of antagonist α-flupenthixol to NAc but this did not affect well-learned cocaine-seeking behaviour under a CS paired second-order schedule but when infused to the dorsal striatum, greatly reduced such behaviour (Murray et al., 2012). As the Nac was more related to goal-directed behavioural or CS-paired behavioural learning, it is unlikely to be involved in well-learned instrumental memory reconsolidation. However, the dorsal striatum was suggested as more related to habitual learning. It is more likely to be involved in well-learned instrumental memory reconsolidation.

Molecular pathway in memory reconsolidation

The reconsolidation process was likely involved in new protein synthesis. Infusion of protein inhibitor systemically or in some key areas, like the hippocampus or amygdala, would impair the reconsolidation process in a number of appetitive and aversive settings. However, anisomycin's effect on reconsolidation was challenged by Rudy (2006) because anisomycin is a ribotoxin that induces apoptosis. So, the amnesic effect may be due to the death of critical neurons rather than the impairment of reconsolidation. However, protein kinase inhibition and transcription factor inhibition at the hippocampus or BLA would also impair memory reconsolidation in different settings. The NMDA receptor antagonist was normally used as an amnesic agent for memory reconsolidation. The mechanism was suggested as: the NMDA receptor was activated by glutamate, and the calcium influx activated small GTPase such as

Ras, Raf, and Rap which further activated the extracellular signal-regulated kinase pathway (ERK). The ERK pathway activated the transcription of proteins such ascAMP response element-binding protein (CREB) and zif268. PKA was also important for the memory reconsolidation process. An infusion of PKA inhibitor Rp-cAMPS in BLA impaired memory reconsolidation in auditory fear conditioning and conditioned taste aversion. An injection of PKA activator 6-BNZ-cAMP in BLA enhanced auditory fear memory reconsolidation. It was suggested that the PKA was activated by cAMP which is converted from ATP by the activation of β-adrenergic receptors. PKA directly or indirectly acted on the ERK pathway and activated transcription factor expression (figure 3).

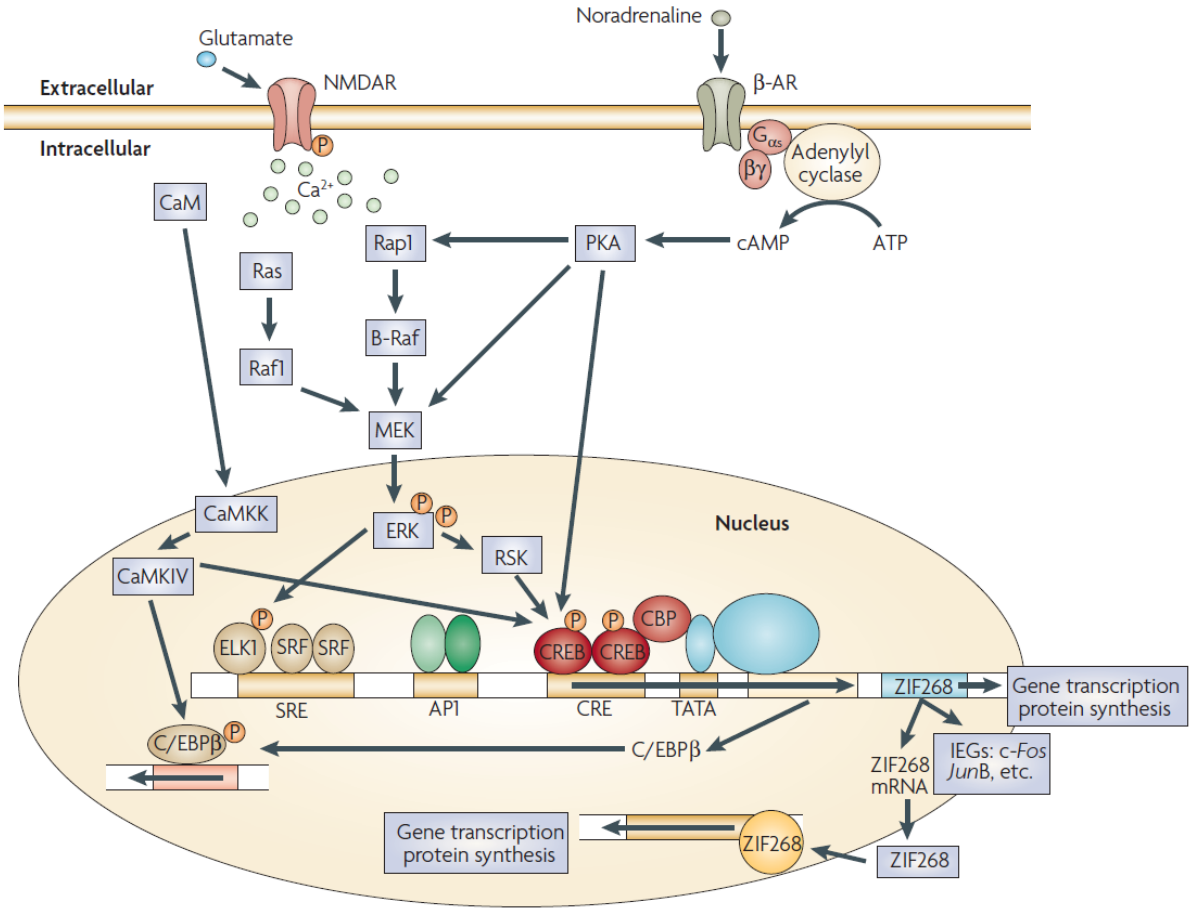


Figure 3. The signalling pathway suggested for memory reconsolidation (Tronson & Taylor, 2007).

Most memory reconsolidation could be impaired by the N-Methyl-D-aspartic acid (NMDA) receptor antagonist and enhanced by the agonist. This showed the importance of the NMDA receptor in memory reconsolidation. An NMDA receptor is an ionotropic glutamate receptor that normally consists of four subunits that have three types, GluN1–3, namely, an NMDA

receptor consisting of two GluN1 subunits (encoded by eight genes) and two GluN2 (encoded by four genes, GluN2A–D) and/or GluN3 subunits (encoded by two genes GluN3A and GluN3B) (Vyklicky et al., 2014).

The GluN2B and GluN2A subunits were selectively important for destabilisation and restabilisation in the basolateral amygdala (BLA). This was shown by an infusion of GluN2B specific NMDA antagonist ifenprodil (IFEN) before the reactivation session in the cued fear conditioning task. This prevented anisomycin-induced amnesia but not the retrieval of fear memory in reactivation (Milton et al., 2013). The post-reactivation of IFEN infusion showed no impact on memory reconsolidation (Ben Mamou, Gamache, & Nader, 2006). Thus, GluN2B was suggested to be selectively required for destabilisation but not restabilisation (Milton et al., 2013). Meanwhile, the GluN2A-specific NMDA receptor antagonist NVP-AAM077 (NVP) showed it impaired the freezing behaviour regardless of anisomycin treatment and had no impact on the fear memory retrieval in reactivation. This showed that the GluN2A was selectively required for restabilisation as opposed to destabilisation (Milton et al., 2013). Furthermore, the western blot showed that the well-learned memory had a lower expression of GluN2B in the lateral and basal amygdala (LBA). It was suggested that this was related to the strong memory being harder to destabilise (Wang, Szu-Han, de, & Nader, 2009).

Dizocilpine maleate (MK-801) is a widely used NMDA receptor inhibitor. The function is the ion channel blocker which means the inhibition is voltage- and use-dependent and requires the activation of the receptor. The inhibition increases with the probability of channel opening (Vyklicky et al., 2014). This inhibitor has a slow onset by intraperitoneal injection, so it is normally applied before the reactivation session. As an amnesic reagent, the MK-801 appeared able to impair most aversive and appetitive memory reconsolidation processes (Alaghband & Marshall, 2013; Charlier & Tirelli, 2011; Exton-McGuinness, Patton, Sacco, & Lee, 2014; Exton-McGuinness & Lee, 2015; Flavell & Lee, 2013; Lee Milton, & Everitt, 2006b; Lee & Everitt, 2008a).

Aim and hypothesis

This study investigates the mechanism of reactivation of well-learned instrumental memory by contingency change and possible neuron circuits that may be involved in the reconsolidation process. Well-learned instrumental memory could not be reactivated by an extinction session which was normally using as a reactivation session in other memory reconsolidation settings (Flavell & Lee, 2013; Hernandez & Kelley, 2004; Mierzejewski et al., 2009). However, it could be inducing a suitable contingency change of the reinforcement (Exton-McGuinness et al., 2014). The hypothesis of the mechanism of this was that the contingency change induced a series of prediction error signals which triggered memory destabilisation (Exton-McGuinness & Lee, 2015; Exton-McGuinness et al., 2014; Lee, 2009, 2010). Our hypothesis is that the requirement of prediction error is proportional to the memory strength, so a weak prediction error signal was able to trigger the destabilisation of weak instrumental memory but also able to destabilise the well-learned instrumental memory. However, a context extinction session after training, but not before, triggered well-learned instrumental memory reconsolidation with a lower contingency which was previously shown to be unable to trigger reconsolidation (Cheng et al., 2022). The hypothesis of this is that a context extinction session reduced the strength of context-reward association, which reduced the competition with an instrumental action-reward association, and so facilitated memory destabilisation. We would test this hypothesis in this study and try to identify the neural circuit that is involved in well-learned instrumental memory reconsolidation.

Chapter 1

Introduction

The mechanism that stabilises new memory into long-term memory is called consolidation, and such consolidated long-term memory is resistant to modification (McGaugh, 2000). However, this does not mean that the content of long-term memory is unchangeable. With a session containing certain training details but distinctive from the training session, the consolidated memory can be activated and destabilised (Exton-McGuinness et al., 2014; Exton-McGuinness & Lee, 2015). These memories are returned to a labile state and then integrate new information back into the consolidated stage (Lewis, Misanin, & Miller, 1968). The latter process is called memory reconsolidation. To observe such memory reconsolidation, memories must be consolidated and then reactivated into the labile state. Due to such lability, memories can be manipulated during the reactivation. This manipulation normally induces reactivation-dependent amnesia by amnesic agents (e.g., N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 or protein synthesis inhibitor anisomycin) (Reichelt & Lee, 2013a). If memories are reactivated in the reactivation session, they are in a labile state and, therefore, the injection of amnesic agents can disrupt the original memories and inflict reactivation-dependent amnesia (Mactutus et al., 1979; Thompson & Grossman, 1972). The difference in behavioural performances during the latter test session can then be observed. This is viewed as evidence of memory reconsolidation occurring during the reactivation session.

Few studies have investigated instrumental memory reconsolidation. Instrumental learning associates a specific action, such as a lever press, with a specific outcome, such as a sucrose reward. The well-learned habitual stimulus-response (S-R) memory, which is formed by overtraining, showed high memory strength and resistance to the value of the outcome (Adams, 1982; Dickinson, 1985) or consequent actions (Balleine & Dickinson, 1998). In memory reconsolidation, several boundary conditions have been observed that influence

whether memories undergo reconsolidation. Two of these are memory strength (Reichelt & Lee, 2012; Suzuki et al., 2004) and memory age (Suzuki et al., 2004). Stronger or older memories are harder to manipulate, and therefore are unable to trigger reconsolidation (Lee, 2009). Consequently, it may be unsurprising that the well-learned instrumental memory is not able to be reactivated by the normal reactivation parameters of an extinction session (Lee & Everitt, 2008b), reinforcer exposure (Wang, Ostlund, Nader, & Balleine, 2005) or a training trial (Hernandez & Kelley, 2004). Moreover, the inhibition of protein synthesis, which disrupts the reconsolidation of other aversive and appetitive memories (Debiec et al., 2002; Lee et al., 2005; Milekic et al., 2006; Milekic, M. H. et al., 2007; Nader et al., 2000) did not affect the S-R response (Hernandez, Sadeghian, & Kelley, 2002). Therefore, over a long period, the well-learned instrumental memory was believed not to undergo memory reconsolidation.

The current view of memory reconsolidation is of a memory-updating process and the memory reconsolidation is hypothesised to be triggered by a prediction error signal (Lee, 2009; Lee, 2010). Reactivation parameters used by previous studies, such as extinction session or training trials, did not induce enough prediction error, and this may be the reason why those studies failed to trigger memory reconsolidation, so a minimum number of stimulus presentations was required to trigger reconsolidation (Suzuki et al., 2004). Moreover, an extinction session that involved a great number of CS- no US pairings was more likely to generate new extinction memory rather than a destabilisation of the old memory (Flavell & Lee, 2013). Interestingly, an intermediate number of stimulus exposures did not trigger memory reconsolidation, nor new learning of extinction memory (Flavell & Lee, 2013). Given this parametric complexity of triggering reconsolidation using extinction training, a potentially advantageous approach is to change reward contingency (Exton-McGuinness et al., 2014; Exton-McGuinness & Lee, 2015) or timing (Diaz-Mataix, Ruiz

Martinez, Schafe, LeDoux, & Doyere, 2013; Tallot et al., 2017). In instrumental memory, a reduction in absolute reward contingency successfully triggered memory reconsolidation after extensive training (Exton-McGuinness et al., 2014). However, it appeared that there was an additional requirement for uncertainty, as only a variable-ratio (VR) and not fixed-ratio (FR) contingency change was effective (Exton-McGuinness. et al., 2014).While it appeared that a VR20 reactivation session (12–28 lever presses for a sucrose pellet) was able to destabilise a well-learned instrumental memory (Exton-McGuinness. et al., 2014), we previously demonstrated that placing a context extinction session before a VR5 reactivation (1–9 lever presses for a sucrose pellet), which could not previously trigger the memory reconsolidation, was shown as able to trigger reconsolidation (Cheng et al., 2022). This effect could be explained by behavioural competition theories in which the manifesting behaviour was dependent on the comparison of the values of the two associations (Reed & Reilly, 1990). During training, rats did not only learn the instrumental association, but also the context-reward association; those two associations are independent of one another. A weaker context-reward association in reward delayed instrumental learning meant the manifest response shifted to instrumental behaviour (Reed & Reilly, 1990). The 'trace dominance' hypothesis suggested that the CS-US association competed with the extinction association (CS- no US association) for domination, and that the amnesic reagent would disrupt the dominant trace (Eisenberg et al., 2003). Based on these findings, our hypothesis was that the context extinction session reduced the context-reward association; therefore, instrumental association overpowered the context-reward association. The instrumental association dominated the reactivation session, so the S-R memories were disrupted by the MK-801.

To characterise this trace dominance hypothesis of instrumental memory destabilisation, we aimed to analyse the zif268 level in several brain regions that were shown to be important in context and instrumental memory, Zif268 being an immediate early gene, normally

expressed after NMDA receptor activation and protein expressed 60-90 minutes after training (see general introduction figure 3 for detail, Veyrac et al., 2014). The hippocampus is well-known for its crucial role in contextual memory storage and reconsolidation (Hardt et al., 2013; Lee, 2010); for example, the inhibition of protein synthesis – especially zif268 – in the hippocampus would impair context fear memory reconsolidation (Debiec et al., 2002; Lee, 2010). The basolateral amygdala (BLA) is another brain region often studied with regard to aversive memory reconsolidation. The BLA mediates outcome encoding (Balleine, Killcross, & Dickinson, 2003) and is reported as crucial in multiple memory reconsolidation, including cued fear conditioning (Ben Mamou et al., 2006), inhibitory avoidance (Pedroso et al., 2013), conditioned taste aversion (Koh & Bernstein, 2003), conditioned reinforcement (Lee et al., 2005) and conditioned place preference (Milekic et al., 2006). On the other side, the striatum and ventral medial prefrontal cortex (vmPFC) were found to be important in instrumental learning. The striatum, especially the dorsal striatum, is also crucial for S-R association learning and instrumental conditioning (Everitt. & Robbins, 2005; Packard & Knowlton, 2002; Yin, Ostlund, Knowlton, & Balleine, 2005; Yin, Knowlton, & Balleine, 2005). Inhibition of this region prevents well-learned S-R behaviour performance such as drug-seeking (Belin & Everitt, 2008; Zapata, Minney, & Shippenberg, 2010). The vmPFC, which includes the infralimbic (IL) cortices and prelimbic (PL) cortices, is involved in the instrumental learning process. The PL is responsible for goal-directed learning (Corbit & Balleine, 2003; Coutureau & Killcross, 2003; Coutureau, Esclassan, Di Scala, & Marchand, 2012; Naneix, Marchand, Di Scala, Pape, & Coutureau, 2009; Ostlund & Balleine, 2005; Tran-Tu-Yen, Marchand, Pape, Di Scala, & Coutureau, 2009), whereas the IL is required for the learning of S-R association (Coutureau & Killcross, 2003; Killcross & Coutureau, 2003).

To examine the activity in those brain regions, it was suggested that the zif268 expression

level was correlated with memory reconsolidation (table 1). Zif268 expression was showed as a marker for double dissociation of reconsolidation and consolidation of context fear memory, as zif2688 expression in the hippocampus was found to be required only for reconsolidation, and BDNF which was required only for consolidation in the hippocampus (Lee et al., 2004). The inhibition of the protein synthesis – especially zif268 in the hippocampus – would impair context fear memory reconsolidation (Debiec et al., 2002; Lee, 2010). Zif268 expression was also found to upregulate in the hippocampus during cocaine memory reconsolidation (Li et al., 2016), and it is important for memory reconsolidation in other brain regions. The Zif268 mRNA level was also found to be elevated in the amygdala and Nacc core after both contextual fear conditioning retrieval sessions and the auditory conditioned fear retrieval session (Hall, Thomas, & Everitt, 2001a; Thomas, Hall, & Everitt, 2002). An upregulation of zif268 was also found in the prelimbic cortex for context fear memory reconsolidation (Stern, Gazarini, Vanvossen, Hames, & Bertoglio, 2013), and zif268 was also expressed in the medial prefrontal cortex and IL in cocaine memory reconsolidation (Li et al., 2016). Overall, zif268 expression was found to be important for memory reconsolidation in the tested brain regions. Therefore, the zif268 expression level was a good indicator for the involvement of those regions in instrumental memory reconsolidation.

Table 1 Summary of memory reconsolidation studies targeting zif268

Behavioural paradigm	Target region	Manipulation	Result	Reference
Contextual fear conditioning	Hippocampus	In situ *hybridization*	*Zif268* mRNA expression	(Hall et al, 2001b)
	Hippocampus	immunohistochemistry	*Zif268* mRNA expression	(Besnard et al., 2014)
	Hippocampus	anti-sense Oligodeoxynucleotide	impaired long-term memory retrieval	(Lee et al., 2004)
	Amygdala	In situ *hybridization.*	*Zif268* mRNA expression	(Hall et al., 2001)
	Amygdala	immunohistochemistry	*Zif268* mRNA expression	(Besnard et al., 2014)
	Nucleus accumbens	In situ *hybridization.*	*Zif268* mRNA expression	(Thomas et al., 2002)
	Prefrontal cortex	In situ *hybridization.*	*Zif268* mRNA expression	(Thomas et al., 2002)
Cued fear conditioning	Amygdala	In situ *hybridization.*	*Zif268* mRNA expression	(Hall et al, 2001b)
	Amygdala	western plot and immunohistochemistry	*Zif268* mRNA expression	(Maddox et al., 2011)
	Amygdala	anti-sense Oligodeoxynucleotide	impaired long-term memory retrieval	(Maddox et al., 2011)
Inhibitory Avoidance Task	Hippocampus	western plot and immunohistochemistry	*Zif268* mRNA expression	(Cheval et al., 2012)
novel-object recognition	Hippocampus	Knock out zif268 gene	impaired long-term memory retrieval	(Bozon et al., 2003)
Conditioned reinforcement	Hippocampus	anti-sense Oligodeoxynucleotide	impaired long-term memory retrieval	(Lee et al., 2005)
	Infralimbic cortex	immunohistochemistry	Zif268 expression	(Li et al., 2016)
	Medial prefrontal cortex	immunohistochemistry	Zif268 expression	(Li et al., 2016)
	Nucleus accumbens core	immunohistochemistry	Zif268 expression	(Li et al., 2016)
	Nucleus accumbens shell	immunohistochemistry	Zif268 expression	(Li et al., 2016)
	Amygdala	immunohistochemistry	Zif268 expression	(Li et al., 2016)
	Ventral tegmental area	immunohistochemistry	Zif268 expression	(Li et al., 2016)
	Hippocampus	immunohistochemistry	Zif268 expression	(Li et al., 2016)
	Supramammillary nucleus	immunohistochemistry	Zif268 expression	(Li et al., 2016)

In this study, we would examine the zif268 expression in IL, PL, dorsal striatum, dorsal hippocampus, and BLA. A delayed reactivation group that received nothing on the context extinction day was used as the negative control, to match the interval between the end of instrumental training and VR5 reactivation. If the trace dominance hypothesis is correct, the reactivation session of the gap day should be dominated by contextual memory which may have a higher zif268 expression in contextual memory-related brain regions like the hippocampus and BLA, whereas the context extinction group should have a higher zif268 expression in the IL, PL and dorsal striatum. A non-reactivation group that did not receive a reactivation session served as a baseline for the zif268 expression. However, the delayed reactivation group showed a highly similar zif268 expression level to the context extinction group in most brain regions, especially those brain regions that were important for instrumental learning. This led to further studies of whether or not the delayed reactivation group was a negative control. Further studies showed context extinction, delayed reactivation, and direct reactivation as reactivation parameters had contradictive results over three studies, so they were not a reliable reactivation method for habitual memory reactivation in the one-lever model. Meta-analysis suggested that among those three studies, the delayed reactivation group had the most consistent results. This research revealed that instrumental memory had a complex boundary condition, i.e. one lever may not be a suitable model for habitual memory reconsolidation studies.

Method

Subjects

Subjects were 60 male experimental-naive Lister-Hooded rats from Charles River, weighing 200-350g at the beginning of the experiment. The rats were housed four per cage in a room with a constant 21°C temperature and a 12-hour light/dark cycle (lights on at 07:00). Each rat was provided with 20g of rat chow per day upon acclimation in the holding room (48-72 hours). Water was available ad libitum throughout except during the behavioural sessions. All procedures followed the United Kingdom Animals (Scientific Procedures) Act 1986, Amendment Regulations 2012, and the act under project licence PPL P3B19B9D2.

Behavioural Apparatus

Training sessions, the context extinction session, and the memory retrieval session all took place in eight operant boxes, each of which was in a sound-attenuating chamber (MedAssociates, VT). All boxes were the same size: 25 x 32 x 25.5cm. The boxes consisted of two Perspex walls (a door and a rear wall), a Perspex ceiling, two steel walls (the right featured a food magazine and two levers, the left featured a light), as well as a grid floor with 19 stainless steel rods (4.8mm diameter, positioned 1.6cm from centre to centre). Each food magazine had an infrared detector to measure nosepokes. All rats received the same training.

Behavioural Procedure

Experiment 1

Training

The rats were pre-trained to collect 45mg sucrose pellets delivered at random intervals (30-90 seconds; mean = 60 seconds) in a food magazine for 15 minutes before training on day one only. The training session immediately took place after the pre-training session. The rats were trained for ten days. One lever was presented in the chamber. The training session started with an illumination of the house light and the presentation of levers. When the active lever was depressed, a 45mg sucrose pellet was delivered to the food magazine (FR1 schedule). The lever did not retract and no stimulus was paired with the reward. The training session lasted for 30 minutes or when 60 sucrose pellets were obtained - whichever occurred first. Only one training session was given to each rat per day for a total of ten days.

Reactivation

The delayed reactivation group and contextual extinction group received a reactivation session. The reactivation session was similar to the training session, except reinforcement occurred under a VR5 schedule. VR5 required a random number of active lever presses to gain a sucrose reward (mean: 5, range: 1–9). Reactivation lasted 20 minutes, or until the maximum of 20 pellets was obtained.

- In the delayed reactivation condition, the VR5 reactivation session took place 48 hours after the last training session.
- In the context extinction condition, a 30-min exposure to the training context alone (houselight illuminated and levers retracted through the session) took place 24 hours after the last training session. The VR5 reactivation session took place a further 24 hours later (48

hours after the last training session).

• In the non-reactivation condition, the rats were perfused 48 hours after training.

• See Figure 1 for a comparative overview of the different procedures.

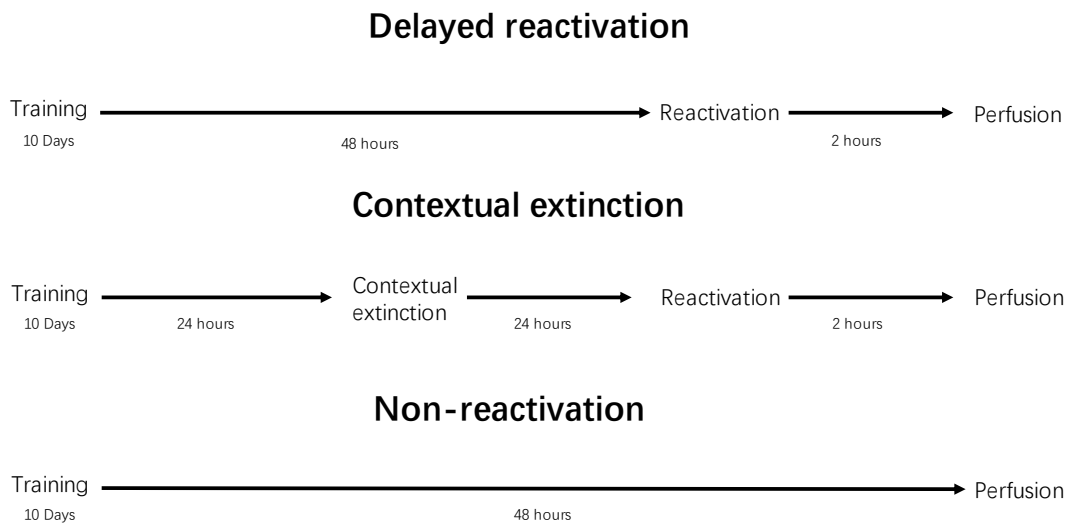


Figure 1 experiment procedure for experiment 1.

Experiments 2 & 3

Training

The training was the same as in experiment 1; new groups of rats were used for each experiment.

Reactivation

All experimental groups received a reactivation session. The reactivation session was the same as in experiment 1, except rats were injected i.p. with MK-801 or saline 30 min before the reactivation session, unless otherwise indicated:

• In the direct reactivation condition, the VR5 reactivation session took place 24 hours

after the last training session.

- In the delayed reactivation condition, the VR5 reactivation session took place 48 hours after the last training session.
- In the context extinction condition, a 30-min exposure to the training context alone (houselight illuminated and levers retracted through the session) took place 24 hours after the last training session. The VR5 reactivation session took place a further 24 hours later (48 hours after the last training session).
- See Figure 2 for a comparative overview of the different procedures.

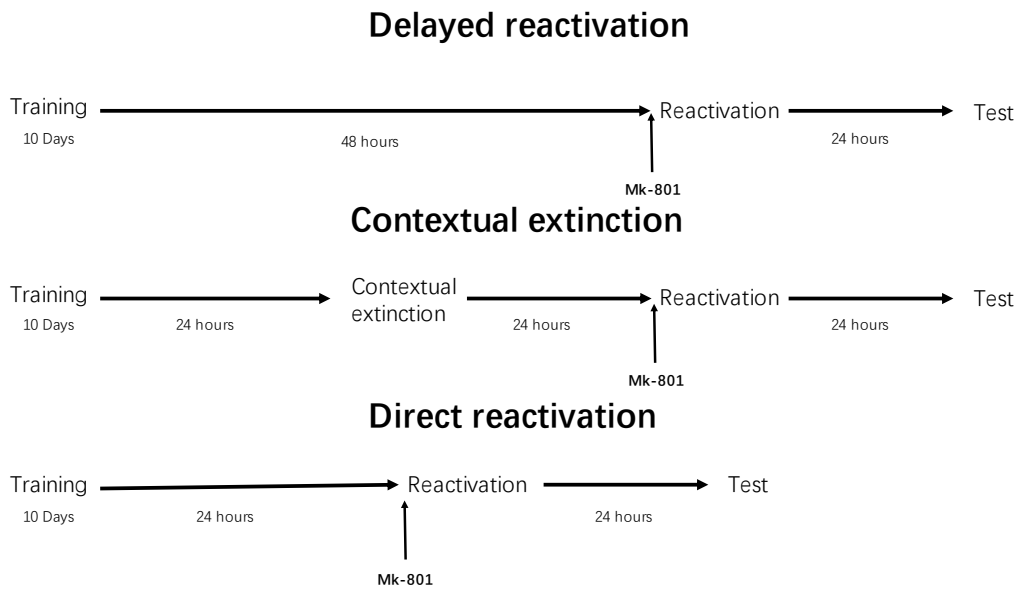


Figure 2. Experiment procedure for Experiments 2 &3.

Extinction Testing

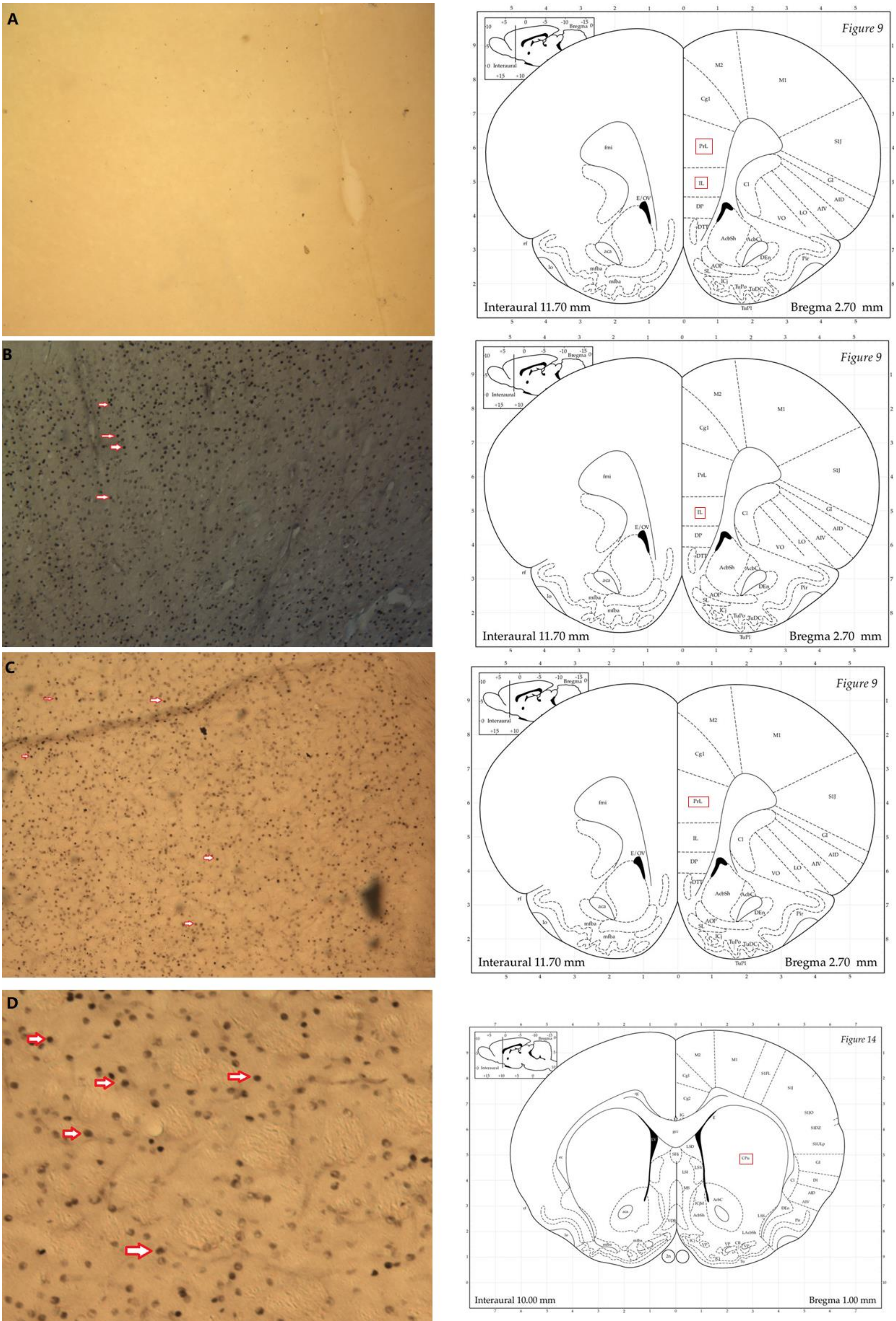
Behavioural groups were placed into the chamber 24 hours following reactivation for a 30-minute session. The levers were extended and the house lights were illuminated during the session. However, no sucrose pellet was delivered when the lever was pressed. No limitation was imposed upon the lever pressing; CO2 was used to cull the rats following the experiment.

Tissue Preparation and Immunohistochemistry

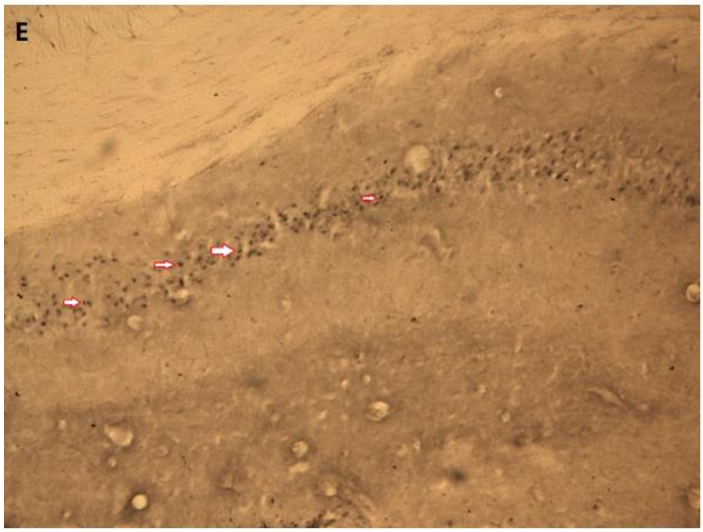
The rats were killed using an overdose of an anaesthetic drug (200mg/ml Pentobarbital) two hours following the end of the reactivation session. The rats were transiently perfused with 4% paraformaldehyde (PFA) in PBS. Following the perfusion, the brains were excised and stored in a PFA fixation solution and transferred into a 30% sucrose solution within a 24-hour period. The brains were cut into 40 μm slices at -20°C. Every third slice was processed for zif-like immunoreactivity. Slices were incubated in 0.3% hydrogen peroxide for 30 minutes to remove endogenous peroxidases. After being washed in PBS on three occasions, the slices were placed into a blocking solution (3% goat serum from a Vectastain® ABC kit [Vector Laboratories LTD] in a 0.1% triton X-100 PBS solution) to block non-specific binding sites for 30 minutes. The slices were then left overnight in a 4°C primary antibody solution (1:2000 for anti-zif268 antibody [Abcam Inc. RRID: AB_2097174, Lot no. K-1714] in a blocking solution (10% goat serum). The control slices were not added to the primary antibody, but the rest procedure was the same as the other slices. The slices were washed three times in PBS the following day, before being incubated in a secondary antibody (Vectastain® ABC kit) at room temperature for 2 hours. The ABC reagent was then applied as per the kit's instructions. The sections were stained with DAB (3-3, diaminobenzidine) peroxidase substrate kit (Vector Laboratories LTD).

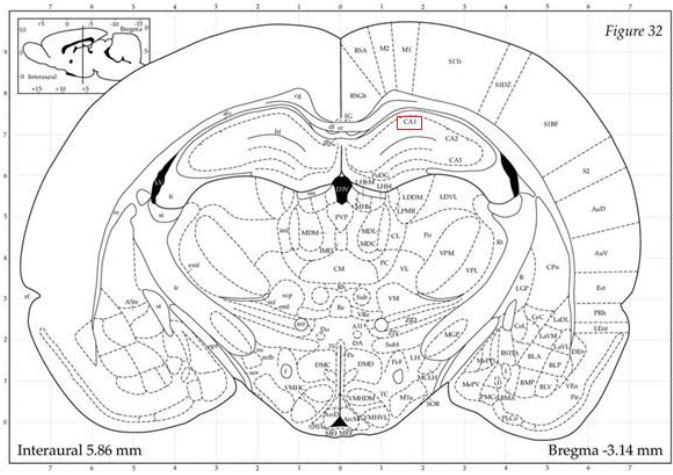
Brain slices were observed using an Olympus BX50 light microscope attached to a Leica DFC425 camera. Positive cells were marked using the Interactive Measurements module of the Leica Application Suite v3.7.

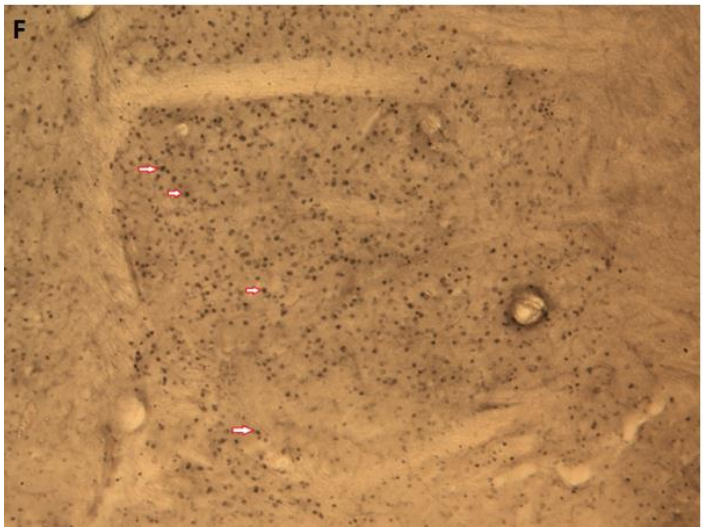
When counting positive cells, only fully stained cells were counted. The selected areas were in approximately the same place in each section (example as in figure 3). The zif268 positive cell number in each slice for the same brain was averaged for use in the statistical analysis.



A
B
C
D
Figure 9
Interaural 11.70 mm
Bregma 2.70 mm
M2
M1
Cg1
PrL
IL
DP
fmi
E/OV
Cl
SIJ
GI
AID
AIV
LO
VO
AcbSh
AcbC
DEn
Pir
aca
mfba
lo
rf
Figure 14
Interaural 10.00 mm
Bregma 1.00 mm
CPu







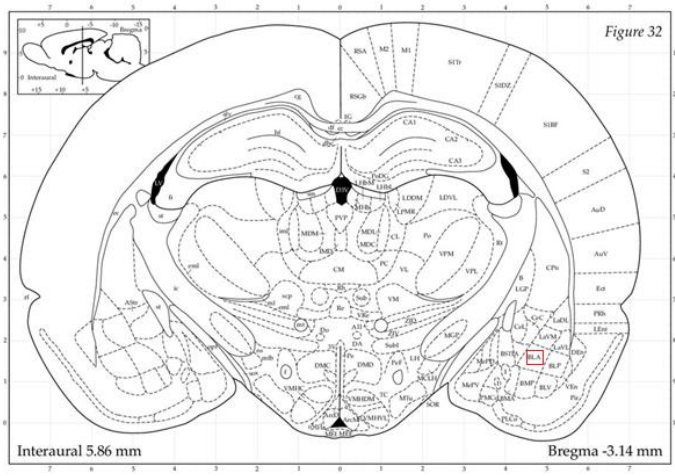


Figure 3. Example of zif268 positive cell in brain slice. A. control slice, bregma +2.70mm. B. Infralimbic, bregma +2.70mm. C. Prelimbic, bregma +2.70mm. D. Striatum, bregma +1.00mm. E. Hippocampus CA1, bregma -3.14. F. Basal lateral amygdala, bregma -3.14mm.

Statistical Analysis

Data were presented as mean + S.E.M. for the number of lever presses at each training session, retrieval session, and test session. Immunohistochemistry data was presented for several positive cells in each brain region. All data were checked for consistency, and any data lying more than two standard deviations from the mean were treated as outliers and excluded. All analysis was conducted using the IBM SPSS statistic 25 program. The training

data were analysed by repeated measures ANOVA to access whether the tasks were learned and whether all experimental groups performed similarly with the lever, using training days, reactivation method, and drug treatment (only for Experiments 2 and 3) as factors. Reactivation data and test data were analysed using a one-way ANOVA and post-hoc Tukey test with a group (context extinction (n=4) vs. delayed reactivation (n=4) vs. non-reactivation (n=4)) as a factor in Experiment 1. In Experiments 2 and 3, the data were analysed using a two-way ANOVA, with groups (delayed reactivation (saline n=7, MK-801 n=8) vs. context extinction (saline n=8, MK-801 n=8), delayed reactivation (saline n=8, MK-801 n=8) vs. direct reactivation (saline n=7, MK-801 n=7) and drug treatment (MK-801 vs. saline) being used as consideration factors. The reactivation and the test session's data were further analysed within each reactivation group, using drug treatment as the factor, by a one-way ANOVA. The data was further analysed within each group using a one-way ANOVA, with drug treatment as a factor.

Meta-analysis

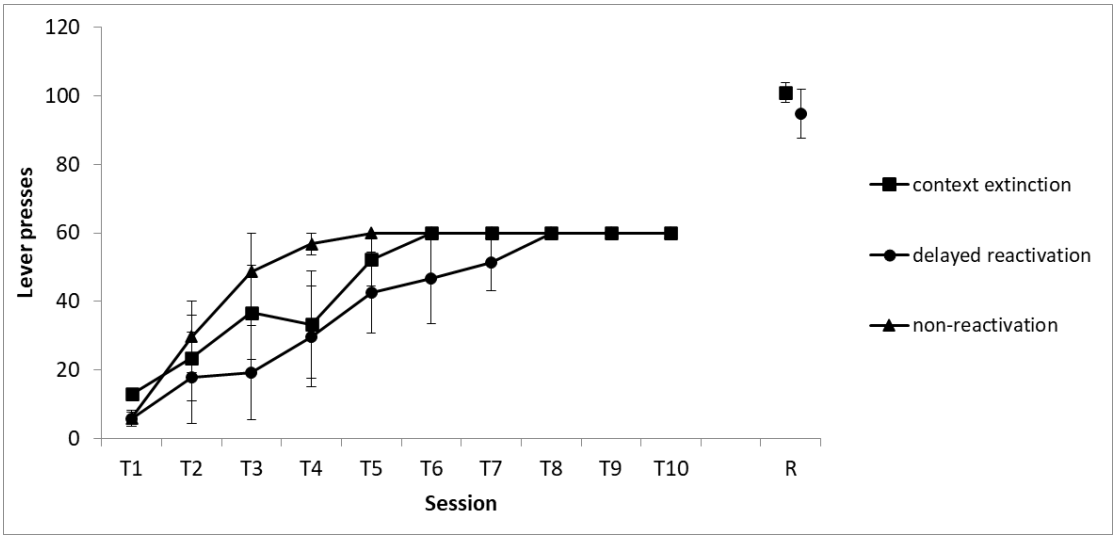
The workbook "Differences between independent groups - continuous data" was taken from *Meta-Essentials: Differences Between Independent Groups - Continuous Data 1.1* and the data input followed the user manual (Suurmond, van Rhee & Hak, 2017). One of the direct reactivation group data (saline n=12, MK-801 n=12) and one of context extinction group data (saline n=13, MK-801 n=12) were used from Cheng et al., 2022 study. Those data used the same experiment protocol of context extinction and direct reactivation as in this study.

Results

Experiment 1

Well-learned instrumental memory was reactivated by a direct VR20 contingency change but could not be reactivated by a direct VR5 contingency change (Cheng et al., 2022). However, a context extinction session after training, but not before, could reactivate the well-learned instrumental memory (Cheng et al., 2022). This was suggested as the context extinction session reducing the context-reward association and shifting the manifest behaviour toward instrumental. We hypothesized that the striatum, hippocampus, vmPFC, and BLA were involved in this reconsolidation progress (see general introduction figure 2 for detail). The reconsolidation process was suggested as related to new protein expression and had a relationship with the zif268 expression (Reichelt & Lee, 2013; Veyrac et al., 2014). So, comparison of zif268 expression in those brain regions between a reactivated group with context extinction and a reactivated group may show which region may relate to the reconsolidation process. A non-reactivation group would serve as a baseline control for zif268 level.

A



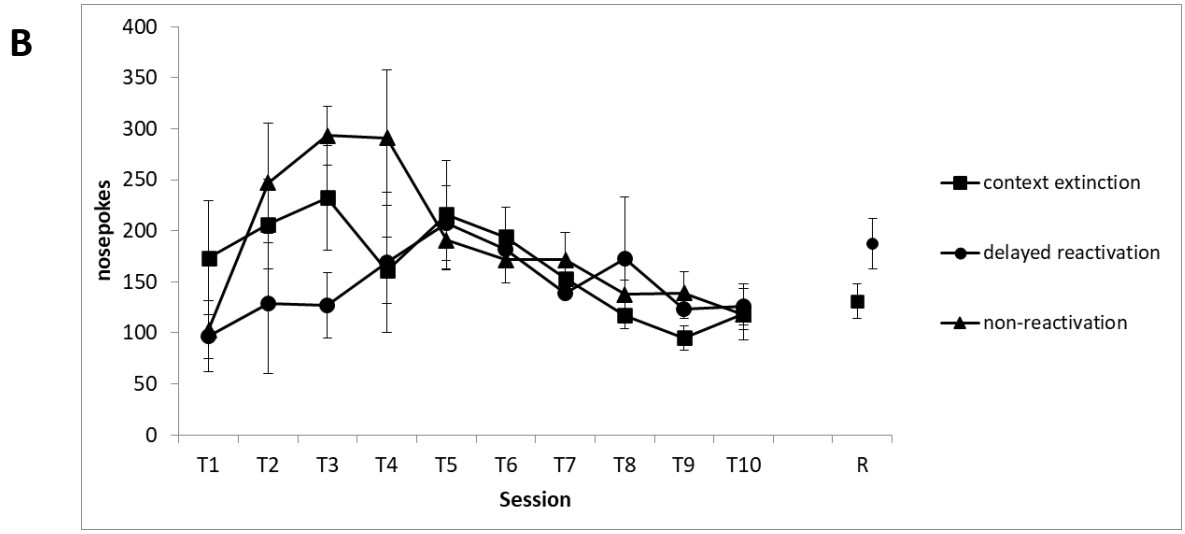


Figure 4. No behavioural difference in the training and reactivation session (a. Lever press; b. Nosepokes, n=4 for each group)

All groups exhibited a similar performance in the training session. The ANOVA showed there was no difference in overall lever pressing between each group (training x group, $F_{(5.5, 24.7)}$ =0.85, p=0.534) (see Fig.4a). As a control parameter for the motivation control, the number of nosepokes was also measured in the session. Data showed no difference in nosepokes between each group during the training (group x training, $F_{(6.9, 31.0)}$ =1.19, p=0.338) (see Fig. 4b).

Both the context extinction group and delayed reactivation group performed similarly in the reactivation session; all the rats that took part in the reactivation session acquired the maximum number of sucrose pellets before the allotted time elapsed. The ANOVA revealed no difference for total lever presses between the two groups ($F_{(1, 6)}$ =0.67, p=0.444) (see Fig. 4a). Therefore, the zif268 expression difference was unlikely to be due to the difference in reactivation. No difference was found in the number of nosepokes between the tested groups ($F_{(1, 6)}$ =3.53, p=0.109) (see Fig. 4b).

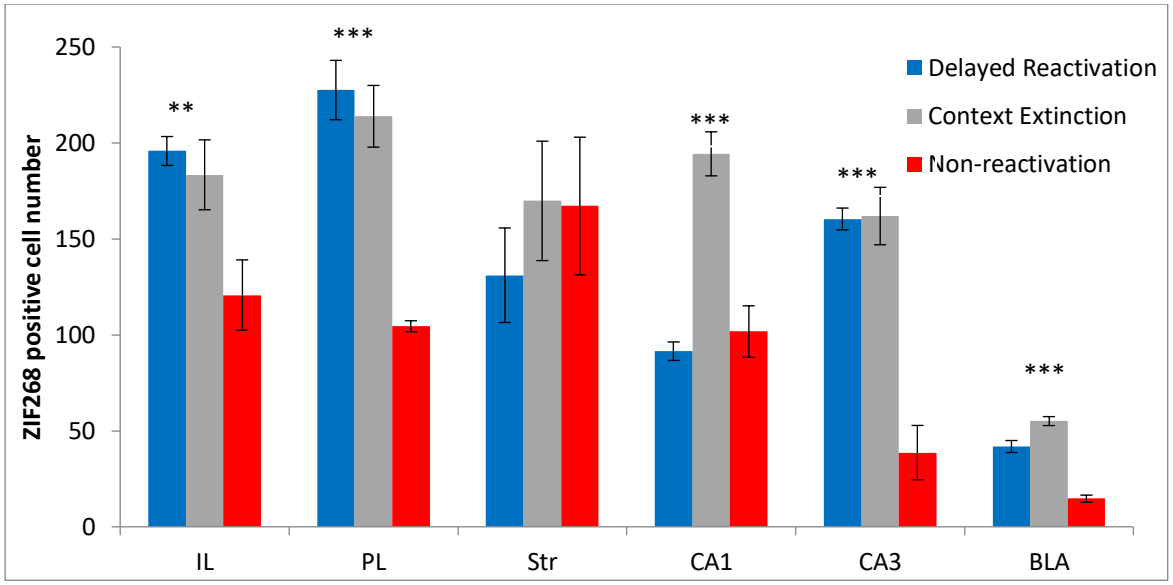


Figure 5. Zif268 positive cells in different brain regions in IL, PL, hippocampus CA1, CA3, and BLA. There were significant differences in zif268 levels between groups (n=4 for each group) There was no difference in the striatum (Str). Data expressed as mean + SEM.

Two hours after the end of the reactivation session, the rats were perfused and their brains were taken for immunohistochemistry. Using the non-primary antibody control slices as negative control (see figure 3), the fully stained, round cell bodies were counted as zif268 positive cells. In most of the tested brain regions (IL, PL, hippocampus CA1, CA3, and BLA, but not the striatum), the zif268 expression was significantly different between the groups (see Fig. 5; see Table 2). Further post-hoc tests showed that the test groups had a significantly higher zif268 level than the non-reactivation group in most tested areas; this demonstrated that zif268 expression in most of the brain regions we chose was likely due to the VR5 reactivation procedure. The delayed reactivation group and contextual extinction group had similar zif268 levels to the baseline in the striatum, which may indicate that the zif268 in striatum may be not activated by the VR5 procedure or there may be a change of neurone subtype activation, but the total number of activated neurones was the same. The contextual extinction group had a high zif268 level in the hippocampus CA1, but this was not the case in the delayed reactivation group or the non-reactivation group; this may indicate that some memory retrieval process occurred only in the contextual extinction group.

Table 2. Statistical data for zif268 immunohistochemistry.

Brain Regions	**ANOVA Statistics**	**Post-hoc Tukey HSD Test**	**Mean**	**Standard Error**	**Significant to Non-reactivation Group?**	**Significant to Context-extinction Group?**	**Significant to Delayed reactivation group?**
IL	$F_{(2, 9)}$ =6.71, **p=0.016**	Non-reactivation	120.75	18.31		Y, p=0.045	Y, p=0.019
		Context-extinction	183.42	36.44	Y, p=0.045		N, p=0.841
		Delayed reactivation	195.83	15.08	Y, p=0.019	N, p=0.841	
PL	$F_{(2, 9)}$ =27.02, **p<0.001**	Non-reactivation	104.5	5.73		Y, p=0.001	Y, p<0.001
		Context-extinction	213.92	32.13	Y, p=0.001		N, p=0.744
		Delayed reactivation	227.59	30.93	Y, p<0.001	N, p=0.744	
Str	$F_{(2, 9)}$ =0.49, p=0.627	Non-reactivation	167.17	35.87		N, p=0.998	N, p=0.697
		Context-extinction	169.83	31.12	N, p=0.998		N, p=0.661
		Delayed reactivation	131.08	24.63	N, p=0.697	N, p=0.661	
CA1	$F_{(2, 9)}$ =28.77, **p<0.001**	Non-reactivation	101.75	13.41		Y, p<0.001	N, p=0.777
		Context-extinction	194.33	22.95	Y, p<0.001		Y, p<0.001
		Delayed reactivation	91.5	9.62	N, p=0.777	Y, p<0.001	
CA3	$F_{(2, 9)}$ =32.86, **p<0.001**	Non-Reactivation	38.67	14.15		Y, p<0.001	Y, p<0.001
		Context-extinction	161.92	29.93	Y, p<0.001		N, p=0.996
		Delayed reactivation	160.42	11.39	Y, p<0.001	N, p=0.996	

BLA	$F_{(2, 9)}$ =68.10, **p<0.001**	Non-reactivation	14.67	1.86		Y, p<0.001	Y, p<0.001
		Context-extinction	55.08	4.67	Y, p<0.001		Y, p=0.011
		Delayed reactivation	41.83	6.26	Y, p<0.001	Y, p=0.011	

As the context extinction group and delayed reactivation group were not injected with an amnesic agent before reactivation and tested for behavioural impairment, we cannot draw any conclusion about whether the instrumental memory was destabilised by VR5 procedure in each group. As the delayed reactivation group shared similar zif268 expression patterns to the context extinction group in most of the tested brain regions, two contrasting conclusions can be drawn:

1. Zif268 expression in the regions of interest is not involved in instrumental memory reconsolidation.
2. There was no functional difference between the delayed reactivation and context extinction conditions in triggering instrumental memory destabilization.

Given the extensive evidence implicating zif268 expression in memory reconsolidation, and the remote likelihood that none of the analysed brain areas are necessary for instrumental memory reconsolidation, each group shared similar zif268 expression patterns to the context extinction group in most of the tested brain experiments but differed slightly from the direct reactivation condition that was previously contrasted with context extinction. Therefore, it was possible that the delayed reactivation group also triggered memory destabilisation. Therefore, further behavioural experiments were required to examine the possibility.

Experiment 2

To examine whether delayed reactivation could destabilise well-learned instrumental memory, we repeated the behavioural procedure in experiment 1 with new rats. Rats were injected with MK-801 or saline 30 minutes before memory reactivation. An extinction test session was added 24 hours after reactivation. If the delayed reactivation session destabilised the instrumental memory, MK-801 would be shown to impair the reactivated memory; therefore, the MK-801 group would show impaired performance at the test compared to saline controls.

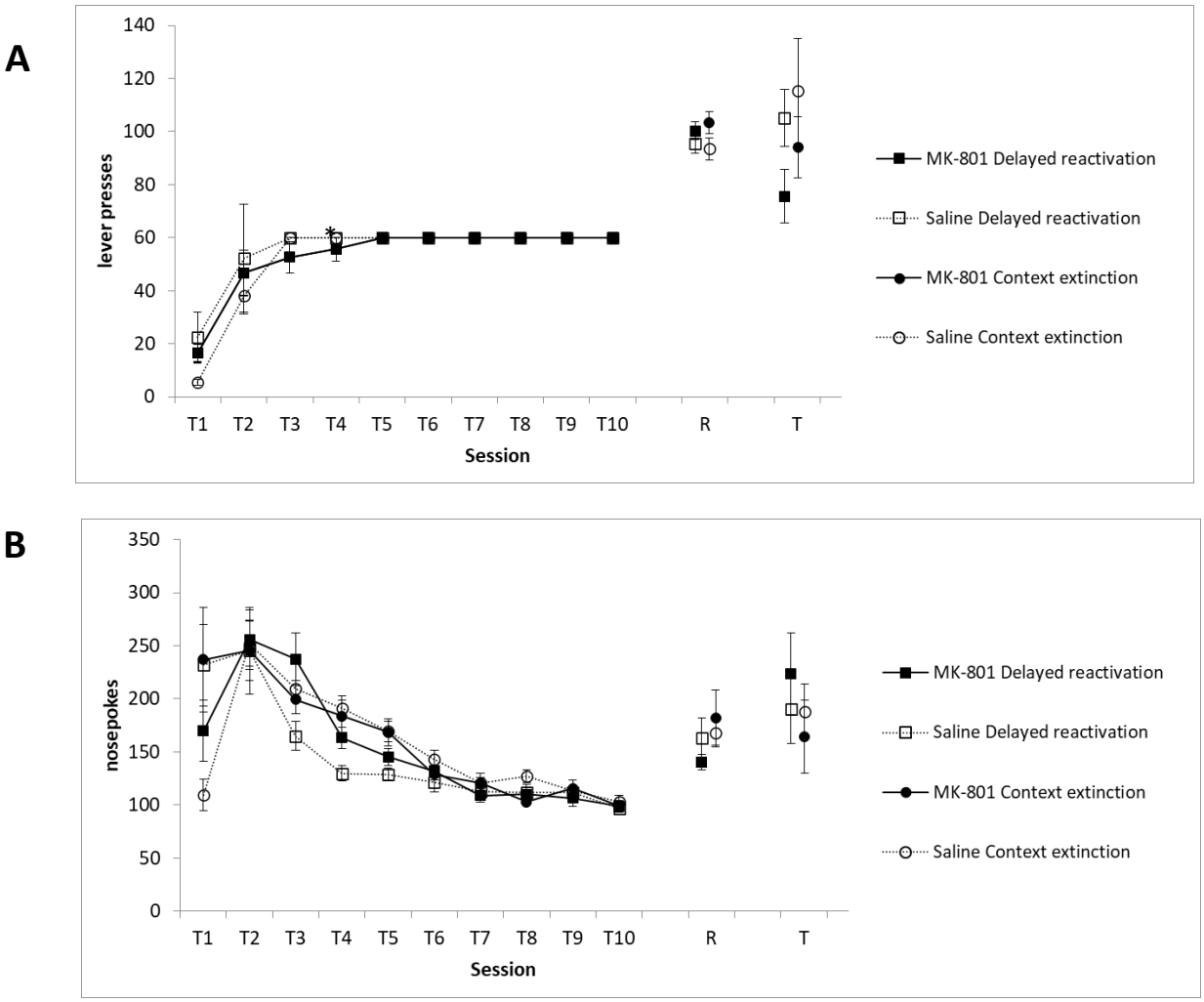


Figure 6. MK-801 group with delayed reactivation group but no other groups showed lever pressing impairment in the test: A. There was no difference in lever pressing except in the test whereas the MK-801 group of delayed reactivation showed fewer lever presses than the saline; (n=7 in the delayed reactivation saline group; n=8 in the other group; one outlier in the delayed reactivation-MK-801 group,). B. there was no difference in nosepokes. Data expressed as mean + SEM.

All groups showed similar behavioural performance in the training session. ANOVA revealed that there was no significant reactivation method x drug treatment effect ($F_{(1, 27)}$ =0.01, p=0.927), nor a simple main effect of drug treatment ($F_{(1, 27)}$=0.06, p=0.817). As a planned comparison of within-group effects, there was no drug effect within each group (delayed reactivation - drug treatment, $F_{(1, 11)}$ =0.01, p=0.935; contextual extinction - drug treatment, $F_{(1, 14)}$ =0.09, p=0.796) (see Fig. 6a) The number of nosepokes signified no group difference in the training session (drug treatment x reactivation method, $F_{(1, 21)}$ =0.01, p=0.921; drug treatment, $F_{(1, 21)}$ =0.24, p=0.629; delayed reactivation - drug treatment, $F_{(1, 11)}$ =0.12, p=0.734; context extinction - drug treatment, $F_{(1, 10)}$=0.12, p=0.735) (see Fig. 6b).

All groups showed similar behavioural performance in the reactivation session. All animals acquired all rewards during the reactivation session. Neither the 30-minute pre-reactivation drug treatment nor the difference in reactivation methods had any effect on lever presses during the reactivation (drug x reactivation method, $F_{(1, 27)}$ =0.40, p=0.533; drug treatment, $F_{(1, 27)}$ =3.45, p=0.073). There was no difference in lever pressings within each reactivation group (delayed reactivation, $F_{(1, 13)}$=0.91, p=0.358; contextual extinction, $F_{(1, 14)}$=2.75, p=0.119) (see Fig. 6a). No significant effect was found in the number of nosepokes in the reactivation session (drug treatment x reactivation method, $F_{(1, 27)}$=1.16, p=0.29; drug treatment, $F_{(1, 27)}$=0.06, p=0.831; delayed reactivation, $F_{(1, 13)}$=1.47, p=0.247; context extinction, $F_{(1, 14)}$=0.26, p=0.619) (see Fig. 6b).

In this test, the delayed reactivation group demonstrated reactivation-dependent amnesia, whereas the contextual extinction group did not. There was no overall effect on lever pressing (drug treatment x reactivation method, $F_{(1, 26)}$=0.10, p=0.760; drug treatment, $F_{(1, 26)}$=3.57, p=0.070). However, the planned comparisons of each reactivation group were subsequently

conducted. The ANOVA revealed that the MK-801 treatment significantly reduced lever presses in the delayed reactivation group ($F_{(1, 12)} = 6.22$, **p=0.028**), but not in the context extinction group ($F_{(1, 14)} = 0.86$, p=0.368) (see Fig. 6a). As a control parameter, the nosepoke data revealed no significant difference in each group (drug treatment x reactivation method, $F_{(1, 27)}=0.71$, p=0.407; drug treatment, $F_{(1, 27)}=0.02$, p=0.894; delayed reactivation, $F_{(1, 13)}=0.40$, p=0.537; context extinction, $F_{(1, 14)}=0.30$, p=0.593) (see Fig. 6b). Although the MK-801 in the delayed reactivation group had a significant effect, there was no overall effect on drug treatment or reactivation method. Therefore, the MK-801 effect may not be strongly concluded as reactivation-method dependent.

This experiment's results exhibited impairment in lever pressing at test in the delayed reactivation condition but not in the context extinction condition. This suggested the 48 hour gap between the end of the training and the reactivation session, rather than the context extinction session, may be important in triggering the reconsolidation. To test this, we would reactivate the animals directly (24 hours after training) with the VR5 schedule compared with the delay reactivation (48 hours after training).

Experiment 3

As the results of Experiment 2 suggested, the simple delay of the memory reactivation session may facilitate its effect in destabilising the instrumental memory. The present experiment tested this hypothesis by directly comparing direct reactivation and delayed reactivation conditions (the reactivation session being presented 1 and 2 days after the end of the training, respectively).

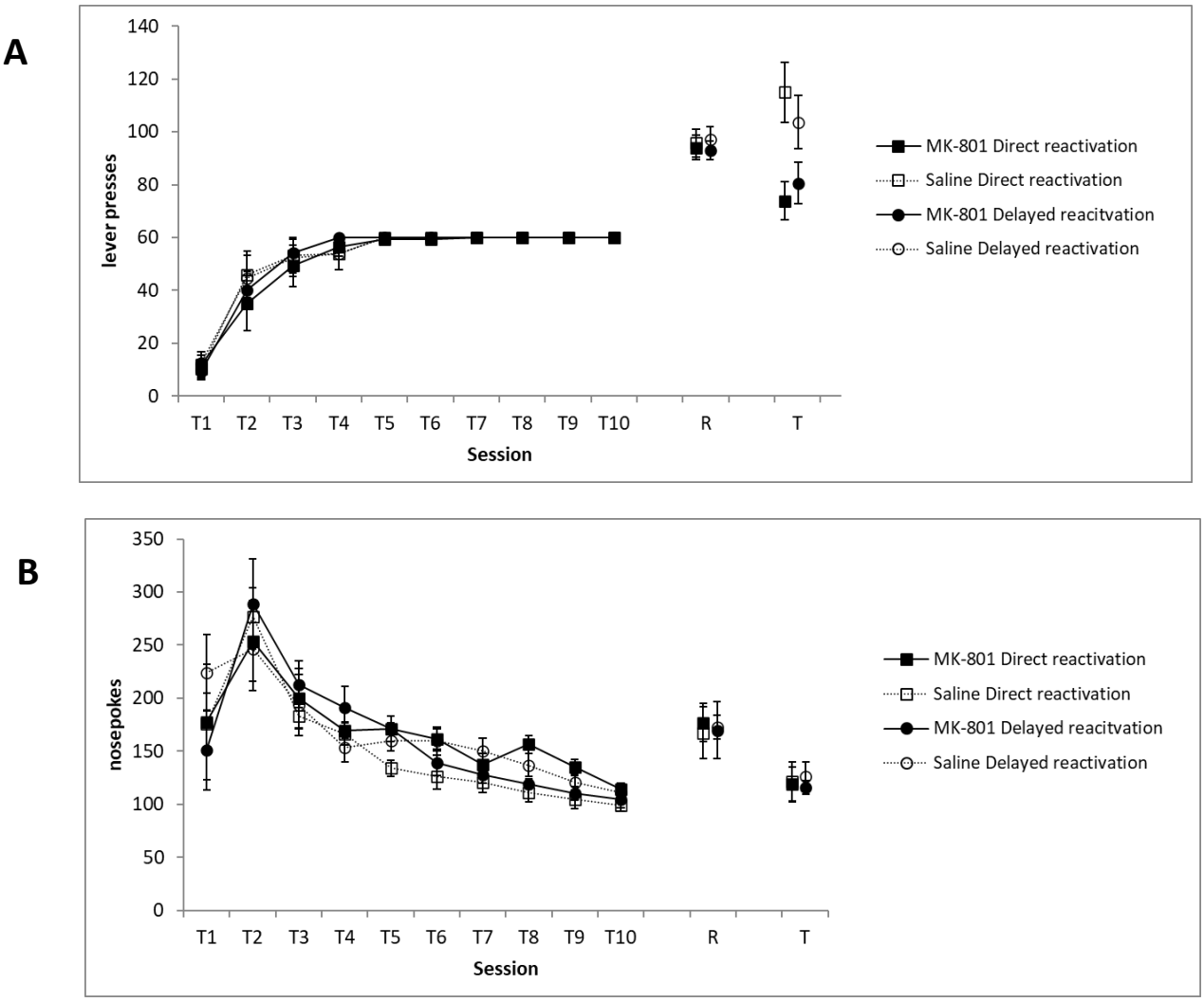


Figure 7. MK-801 group with direct reactivation group but no other groups showed lever pressing impairment in the test: A. There was no difference in lever pressing except in the test whereas the MK-801 group of direct reactivation showed fewer lever presses than the saline; (n=7 in the direct reactivation-MK-801 group; n=8 in the other group). B. There was no difference in nosepokes. Data expressed as mean + SEM.

All groups showed similar behavioural performance in the training session. ANOVA revealed there was no significant reactivation method x drug treatment effect ($F_{(1, 27)}$=0.09, p=0.768), nor a simple main effect of drug treatment ($F_{(1, 27)}$=0.08, p=0.777). As a planned comparison of the within-group effect, there was no drug effect within each group (delayed reactivation - drug treatment, $F_{(1, 14)}$=0.0001, p=0.992; direct reactivation - drug treatment, $F_{(1, 13)}$=0.14, p=0.716) (see Fig. 7a). Therefore, the reduction of lever pressing in the test was unlikely to be attributable to the differences in training performance and acquisition of the instrumental memory. The number of nosepokes did not differ between groups in the training session (drug treatment x reactivation method, $F_{(1, 27)}$ =2.34, p=0.138; drug treatment, $F_{(1, 27)}$=0.46, p=0.501; Delayed reactivation x drug treatment, $F_{(1, 14)}$=0.31, p=0.590; direct reactivation x drug treatment, $F_{(1, 13)}$=3.13, p=0.100) (see Fig.7b).

Despite the reactivation session not taking place on the same day, no statistical difference was found in lever pressing ($F_{(1, 27)}$=0.06, p=0.812) (see Fig. 7a). The MK-801 treatment did not cause any difference within each group (direct reactivation, $F_{(1, 13)}$=0.06, p=0.808; delayed reactivation, $F_{(1, 14)}$=0.42, p=0.528). Nosepokes showed no significant difference in reactivation ($F_{(1, 27)}$=0.08, p=0.775) (see Fig. 7b).

In this test, while there was not an overall effect on the lever pressing (drug treatment x reactivation method, $F_{(1, 27)}$=0.92, p=0.347), the drug treatment had a significant effect ($F_{(1,27)}$ =11.45, **p=0.002**). Planned comparisons of each reactivation group were subsequently conducted. The ANOVA revealed that the MK-801 treatment significantly reduced lever presses in the direct reactivation group ($F_{(1, 13)}$ =8.62, **p=0.012**), but not in the delayed reactivation group ($F_{(1, 14)}$=3.22, p=0.094) (see Fig. 7a). As a control parameter, the nosepoke data revealed no significant difference in each group (drug treatment x reactivation method, $F_{(1, 24)}$=0.07, p=0.789; delayed reactivation, $F_{(1, 12}$=0.42 p=0.527; direct reactivation, $F_{(1, 12)}$=0.01, p=0.915) (see Fig. 7b). Though there was no overall effect, the MK-801 treatment

was shown to have a significant effect on lever pressing. Furthermore, a within-group analysis showed that a significant effect was seen in the direct reactivation, not in the delayed reactivation. Therefore, there was stronger evidence for an impairment in the direct reactivation group than in the delayed reactivation group.

In Experiments 2 and 3, and our pilot study, the reactivation method, context extinction, delayed reactivation, and direct reactivation with MK-801 each showed statistically successful triggered lever-pression impairment once, as well as one failure. Therefore, it is difficult to conclude there was stronger evidence for an impairment in the direct reactivation group than in the delayed reactivation group. A mini meta-analysis could provide insight into which reactivation method was most effective.

Meta-Analysis

To identify which reactivation method was most consistent, a mini meta-analysis was conducted using six studies with a total of 109 subjects. The meta-analysis had a statistically significant effect (Z-value= -3.10, $p<0.001$). The combined effect size is Hedges' g -0.80 (95% CI -1.47 to -0.14). The analysis was moderate heterogeneity (I^2=48.51%, Q = 9.71) (see Table 3). Therefore, the entire data could not be treated as studies from the same population, and a subgroup analysis was conducted for further analysis.

Table 3. The result of Forest's plot showed the six studies' results were heterogeneous.

Combined Effect Size	
Hedges' g	-0.80
Standard error	0.26
CI Lower limit	-1.47
CI Upper limit	-0.14
PI Lower limit	-2.17
PI Upper limit	0.56
Z-value	-3.10
One-tailed p-value	0.00
Two-tailed p-value	0.00
Number of incl. subjects	109
Number of incl. studies	6
Heterogeneity	
Q	9.71
p_Q	0.08
I^2	48.51%
T^2	0.22
T	0.46

The delayed reactivation group was the only homologous population within all the reactivation methods (Q=0.05, I^2=0.00%); this is the most consistent method with an effect size of -1.16 (95% CI -1.16 to -1.33) (see Table 4).

Context extinction was the second-most effective method with moderate heterogeneity (Q=1.47, I^2=31.84%); so, it could not be meta-analysed as a single population. The effect size should focus on the prediction interval (PI), rather than the CI, with its effect size of -0.86

(95% PI 7.20 to 5.45).

The direct reactivation group was highly heterogeneous (Q=5.12, I^2=80.48%); its effect size was -0.52 (95% PI -16.12 to 15.09). This showed that direct reactivation was the least consistent reactivation method.

Table 4. Subgroup analysis results show that delayed reactivation is the most reliable effect in the one-lever model.

Study name / Subgroup name	Hedges' g	CI Lower limit	CI Upper limit	Weight	Q	p_Q	I^2	T^2	T	PI Lower limit	PI Upper limit
context extinction	-0.44	-1.46	0.58	46.47%							
context extinction	-1.22	-2.11	-0.33	53.53%							
context extincti	**-0.86**	**-1.65**	**-0.07**	**4.08%**	**1.47**	**0.23**	**31.84%**	**0.10**	**0.31**	**-7.20**	**5.48**
delayed reactivati	-1.25	-2.44	-0.05	45.97%							
delayed reactivati	-1.09	-2.17	0.00	54.03%							
delayed reactiva	**-1.16**	**-1.33**	**-0.99**	**94.85%**	**0.05**	**0.83**	**0.00%**	**0.00**	**0.00**	**-2.19**	**-0.13**
direct reactivatio	-1.32	-2.49	-0.16	46.99%							
direct reactivatio	0.20	-0.62	1.01	53.01%							
direct reactivat	**-0.52**	**-2.06**	**1.02**	**1.07%**	**5.12**	**0.02**	**80.48%**	**0.93**	**0.97**	**-16.12**	**15.09**
Combined Effect	**-1.14**	**-2.96**	**0.68**		**9.71**	**0.08**	**48.51%**	**0.22**	**0.46**	**-2.96**	**0.68**

Discussion

Our previous study showed that well-learned instrumental memory could be reactivated by the VR20 procedure 24 hours after training but could not be reactivated by a lower contingency change VR5. However, by inserting a context extinction session before reactivation, but not before training, the well-learned instrumental memory could be reactivated by the VR5 procedure (Cheng et al., 2022). To investigate the mechanism and neurocircuits that may be involved in instrumental memory reconsolidation, our hypothesis was that the context extinction session reduced the context-reward association, so the manifest behaviour may be dominated by instrumental behaviour, and the instrumental memory was reactivated. With this hypothesis, a group without context extinction session but reactivated at the same time (48 hours after training), called delayed reactivation, should not reactivate the memory. As the contextual-reward association was suggested as a competitive factor with an instrumental association, several neurocircuits involved in contextual memory reconsolidation and instrumental learning were hypothetically involved in instrumental memory reconsolidation (see general introduction figure 2). As zif268 expression was suggested as a requirement for memory reconsolidation (see table 1), we examined the zif268 lever in those brain regions 2 hours after the reactivation session. The highly overlapped result between the context extinction group and delayed reactivation suggested either zif268 expression may not be related to instrumental memory reconsolidation or the delayed reactivation and context extinction may have a similar function to trigger instrumental memory reconsolidation. As the delayed reactivation was not examined before, we examined the delayed reactivation procedure with context extinction or direct reactivation in two different experiments. The delayed reactivation showed a behavioural impairment by MK-801 injection in experiment 2 but not in experiment 3. Contradictive to the previous study (Cheng et al., 2022), the context extinction procedure was shown to be unable to trigger

instrumental memory reactivation in experiment 2, and delayed reactivation triggered instrumental memory in experiment 3. Overall, those behavioural results suggested the one-lever model maybe not be a rigid model for the investigation of well-learned instrumental memory reconsolidation, so the immunohistochemistry result may not be reliable data for investigating the neurocircuits required for instrumental memory reconsolidation.

Behavioural impairment due to MK-801 injection was likely due to instrumental memory impairment

Though we did not have any non-reactivation group behavioural test data in this study, we used the same training and test procedure and program as Exton-McGuinness et al.'s (2014) study. As the non-reactivation group did not show impairment with an MK-801 injection in Exton-McGuinness et al.'s (2014) study, it was unlikely to have a different result in this study. So, the reduction of lever pressing in the test was likely to be reactivation-dependent. Experiments 2 and 3 had one reactivation condition that showed impairment, in addition to another that did not; this showed that impairment was only observed in some, but not all, reactivation conditions. Therefore, this finding ruled out the possibility of MK-801 non-specifically reducing the lever pressings, since groups with the MK-801 treatment did not show any reduction in lever pressing. Based on the finding that the non-reactivation group in Exton-McGuinness et al.'s (2014) study did not show the impairment, and that not all the reactivation conditions observed such impairment, the impairment could be determined a reactivation-dependent reconsolidation impairment.

Another argument can be made as to whether this is a genuine reconsolidation effect or simply an impairment of new learning (consolidation) in the VR5 schedule. If this was a new learning effect and the MK-801 treatment prevented the rise in lever pressings, this caused an apparent impairment in lever pressing (Hernandez et al., 2002). The counter-argument could

be that, if this was a new learning of the VR5 schedule, it should be the same across all groups. However, the reduction of lever pressings was only observed in one group in each experiment. Therefore, it was unlikely that just one group in each experiment learned, while the other group did not, and the results of this study were unlikely to be obtained due to the new learning of the VR5 schedule.

As shown in the **Results** section, this lever-pression difference is unlikely to be due to the difference in training or reactivation sessions. All groups had statistically similar training profiles and reactivation profiles; the only differences were the reactivation method and drug treatment. Therefore, the impairment was likely to be due to the drug treatment given before reactivation under certain reactivation conditions.

Nosepokes are a commonly used control parameter in many studies (Exton-McGuinness et al., 2014; Exton-McGuinness & Lee, 2015; Klanker, Fellinger, Feenstra, Willuhn, & Denys, 2017; Weatherly, Arthur, & Nurnberger, 2006), though it is weak and indirect evidence for motivation and locomotor activity. This is not necessarily strong evidence for an absence of difference.

The impairment in the instrumental responding in the delayed reactivation condition was likely reflected as a disruption of S-R associations. Two types of instrumental memory are held up in the current view: goal-directed Action-Outcome (A-O) memory and habitual S-R memory.

The A-O memory was sensitive to the reward value, while the S-R memory was not. The A-O memory dominated at the beginning of the first few training sessions and the domination shifted to the S-R memory as the training continued (Dickinson et al., 1995). After ten days of training, the subjects showed insensitivity to devaluation of the reward, which offers the hypothesis that the S-R association was dominant (Adams, 1982; Exton-McGuinness et al., 2014). Exton-McGuinness and Lee (2015) demonstrated that the directive VR5 reactivation

could reactivate the weaker A-O memory, but it could not reactivate the stronger well-learned S-R memory (Cheng et al., 2022). The well-learned instrumental memory could be reactivated by a larger contingency change such as the VR20 procedure (Variant Ratio 20; 12–28 lever pressings per sucrose pellet; mean = 20 pressings per pellet). As this study used ten days of training, the dominant memory after the period of training was habitual S-R association (Dickinson et al., 1995). In Exton-McGuinness et al.'s (2014) study, the animals with behavioural impairment showed sensitivity to reward value whereas the saline group did not, so the impaired association was likely to be habitual S-R association (Exton-McGuinness et al., 2014). While this study used a variant VR5 procedure, rather than the VR20, the training time was identical to Exton-McGuinness et al.'s (2014) study. Thus, it was likely the impaired associate was same as in Exton-McGuinness et al.'s (2014) study. Therefore, it was more likely to be the case that the variant of the procedure facilitated the S-R memory reconsolidation process, rather than that the goal-directed memory was reactivated.

Delayed reactivation was the most consistent reactivation method.

Among the three reactivation methods, direct reactivation was found to be the least reliable. The direct reactivation in a previous study (Cheng et al., 2022) demonstrated that high possibility did not trigger memory reconsolidation, yet Experiment 3 showed significantly reduced lever pressings in the MK-801 group which suggested the instrumental memory was destabilised and impaired. The subgroup analysis showed as heterogeneity, so the two experiments could not be treated as the same population, therefore the confidence interval (CI) could not be used for this subgroup and its prediction interval (PI), which only predicted with some accuracy if no bias of population selection was involved, ranging from from 15.09 to -16.12. This means direct reactivation being predicted had an effect from significant positive affect lever pressings to significantly negative affect lever pressings. So, this was the

least reliable method among the three. The context extinction group also showed as heterogeneity, so the two experiments cannot be treated as a single population. Its prediction interval was from 5.48 to -7.20. Therefore, context extinction was not a reliable method for well-learned instrumental memory either. Delayed reactivation was the only method that showed homogeneity within the two experiments, meaning the two experiments can be treated as studies from the same population. This means within all three reactivation methods; only delayed reactivation had a measurable "true" effect. Thus, it showed that with a 95% confidence interval from -0.99 to -1.33, the delayed reactivation method had a general negative impact on lever pressings. Therefore, delayed reactivation had a more consistent result among the two experiments.

The hypothesis of the facilitation effect of delayed reactivation

Since delayed reactivation offered the most consistent effect, it is important to know why a gap day between training and reactivation can facilitate habitual memory reconsolidation. In the current view, the promnesic effect is diminished as the interval between the last training session, and the reactivation session increases (Gold & Van Buskirk, 1975). The gap day showed a completely opposite effect. Therefore, the mechanism of the promnesic effect of the gap day may differ from the other enhancers, such as increased arousal or the strength of the reinforcer (Gold & Van Buskirk, 1975; McGaugh, 2000). The gap day triggering habitual memory reconsolidation may be due to a weakening memory during the gap day.

Two theories exist surrounding long-term memory loss: memory decays over time (Ebbinghaus, 1885) or memory is interfered with by other memories (Anderson, Bjork, & Bjork, 1994). Although few studies supported the interference theory, fully consolidated memory is not vulnerable to interference unless it enters the labile state; for example, during memory reconsolidation (Cowan, Beschin, & Della Sala, 2004; Nader & Hardt, 2009). A

newly formed episodic memory may be consistent with two components: content-related representation is largely dependent on the neocortical network, and spatial-context representation is dependent on the hippocampus (Hardt et al., 2013). The hippocampus memory representation has little overlap due to the efficient patterning ability of the hippocampus (Leutgeb, Leutgeb, Moser, & Moser, 2007; McHugh et al., 2007; Nakashiba et al., 2012). However, the representation in the neocortical circuit is easily overlapped and hard to distinguish (McClelland et al., 1995; McCloskey & Cohen, 1989). During encoding, the neocortical representation is linked to the specific spatial-context representation; therefore, the hippocampus memory serves as an index for the neocortical memory (Danker & Anderson, 2010). The hippocampus (context)-neocortical circuit (reward) correlation is the context-reward association, whereas the S-R association may be purely based on the neocortical circuit. While the spatial-context trace can decay in days (Hardt et al., 2013; Oliver et al., 2016), the neocortical trace can persist for much longer (Hardt et al., 2013). Consequently, the forgetting of the context-reward memory may be more severe than the longer persistent instrumental memory.

The other possibility of reduction strength of contextual memory on the gap day may be due to similar memories competing during retrieval; and this decreased memory retention (Hardt et al., 2013) In this study, the rats were trained with sucrose reward on each weekday. On the gap day (also a weekday), the feeding only with food chow – instead of the training with sucrose reward – may generate a prediction error in the rats; this may induce the inference context-reward memory, which would decrease memory retention. Therefore, in the reactivation session, the S-R association was stronger than the context-reward association. Consequently, based on the trace dominance theory, instrumental memory was reactivated and showed sensitivity to MK-801. The statistical analysis showed MK-801 did not significantly reduce lever pressings in Experiment 3's gap day. However, the ANOVA also

indicated that the drug treatment had a significant overall effect, so the negative result was weak. Hence, this result may be due to the difference between the two groups of rats.

The other argument for the effect of the context extinction session can be based on Reed's experiment, which indicated that rats had elevated instrumental behaviour following context extinction when compared to delayed reactivation (Reed & Reilly, 1990). Accordingly, the contextual extinction group should have more elevated instrumental behaviour than the delayed reactivation group at reactivation, and context extinction should be more likely to trigger reconsolidation than in the delayed reactivation group. However, Reed and Reilly's experiment used a delayed reward, which resulted in an attenuation response (Grice, 1948; Schachtman, Reed, & Hall, 1987; Williams, 1976); our study used non-delay reinforcement that may have had a different effect. Furthermore, Reed and Reilly's experiment used five context extinction sessions (assuming one session per day, as per the authors' other papers), which corresponded with a five-day gap day in the delayed reactivation group; this further facilitated the instrumental behaviour in the extinction test. As this study had only one context extinction session, the facilitative effect may not be as significant as in Reed and Reilly's experiment, which may be why the context extinction was not as reliable a reactivation parameter as it was in the delayed reactivation.

Potential brain regions may be involved in instrumental memory reconsolidation.

We found a highly overlapped pattern of zif268 expression in context extinction and delayed reactivation groups. With the non-reactivation group's zif268 level as the baseline, both the context extinction group and delayed reactivation group showed higher zif268 expression in IL, PL, dorsal hippocampus CA3 (DH CA3), and basolateral amygdala (BLA). In the dorsal hippocampus CA1 (DH CA1), the context extinction group had higher zif268 expression, but

delayed reactivation had the same zif268 expression level as non-reactivation controls. There was no difference in zif268 expression in the striatum between test groups and non-reactivation controls. However, the power of this study is low due to the small sample numbers (n=4 in each group), which is likely to induce type II error, so the conclusion in this experiment had low reliability.

The first question about zif268 expression in those regions is whether this is due to motor activity. The non-reactivation group was in the cage during the reactivation session; therefore, some may argue those zif268 expressions may be due to motor activity. There may be some level of zif268 expression due to motor activity, but it is hard to explain if all the differences in zif268 expression were due to motor activity. Those two groups had a non-statistical difference in lever pressing and nosepokes during the reactivation session, so they likely had a similar level of motor activity. Therefore, if the expression was due to motor activity, they should have had the same expression pattern crossing every region. Though the zif268 expression highly overlapped in most areas for the two testing groups, there was a difference in DH CA1 and BLA in which the context extinction group had higher expression than the delayed reactivation group. Especially in DH CA1, the delayed reactivation group had the same zif268 expression as the non-reactivation group, but the context extinction group had a higher expression level than the other two. So at least in CA1 and BLA, those zif268 expressions were unlikely to be due simply to motor activity.

So at least to a certain degree, the zif268 expression in those regions may be due to memory reactivation. As the delayed reactivation is more reliable than the context extinction, it was more likely to trigger instrumental memory reconsolidation than the context extinction group in this experiment. So, the zif268 expression, especially in the context extinction group, is unlikely to be due to instrumental memory retrieval. As the expression was in CA1 and BLA, those regions were suggested to be highly involved in contextual and spatial information

processing (Chaaya et al., 2018), which may likely be due to the retrieval of context extinction memory. Apart from our hypothesis that context extinction may reduce the context-reward association, it was also suggested as forming the context-no reward association, i.e., context extinction memory. So, it is likely that during the reactivation session, this context-extinction memory was reactivated instead of instrumental memory. This may suggest that if the non-reactivation controls were to stay in the box without any lever present (i.e., context extinction session), they were likely to induce context extinction memory and may not be good controls for the experiment.

As the delayed reactivation likely reactivated instrumental memory during the reactivation session, the zif268 expressed in those regions may be related to the reconsolidation process. IL and PL were showed to be involved in several instrumental learning circuits, zif268 expression may also represent the involvement of instrumental memory reconsolidation. But it is worth noting that the context extinction group also had similar zif268 expression in those regions, so it may mean those regions had a general function in the memory retrieval process or the instrumental association was reactivated to a certain degree but did not dominate the manifested behaviour. CA3 and BLA are the other two regions with zif268 expression. The function of those regions was suggested as more contextual and Pavlovian (Chaaya et al., 2018; Reichelt & Lee, 2013). This may mean a certain degree of Pavlovian behaviour was involved in the instrumental behaviour or it may be, as in the suggestion above, that both instrumental association and context related association were likewise reactivated by VR5. If those associations were simultaneously reactivated and the domination was due to the strength of association, the BLA and CA1 data may suggest that the high zif268 expression in those regions prevents instrumental memory reconsolidation. If this is true, this may support our initial hypothesis which is that contextual memory was competing with instrumental memory for domination.

As the dorsal striatum was suggested to be involved in habitual learning (Everitt & Robbins, 2005) and Nac was suggested as integrating signals from the hippocampus, BLA, and vmPFC (Cooper et al., 2017), it is surprising that the zif268 level in the test groups was similar to non-reactivation controls. This zif269 expression pattern also was different from the Cahill et al. (2018) study. In the Cahill et al. (2018) study, the zif268 was elevated in the habitual group but not in the goal-directed group, and the zif268 knockdown altered the proportion of animals using habitual strategy 3 days after reactivation, but such alteration did not persist at the test session 7 days after reactivation. But the Cahill et al. (2018) study used the T-maze experiment rather than the lever-reward experiment we used in this study; the different results may be due to the difference of experiment parameter. The authors also mentioned that the western blot experiment was underpowered (habit group 2-hour n=2) in Cahill et al. (2018) study. This may affect the reliability of the western blot result.

It is worth noting that an absence of zif268 alteration does not mean those regions are not involved in the memory reconsolidation process. First, zif268 was expressed in lots of memory reconsolidation studies and instrumental learning studies (Li et al., 2016; Maroteaux et al., 2014; Piva et al., 2018; Veyrac et al., 2014), but is not necessarily involved in instrumental memory reconsolidation. This may mean the striatum was not involved in instrumental memory reconsolidation, but it is more likely to have some other explanation. For example, the level of zif268 did not represent the activity of that brain region, so maybe the activated signalling pathway in the striatum did not activate the zif268. The other possibility is that a different neuronal population was recruited. For example, the predominant cell type in Nac is medium spiny neurons (MSN). By expressing different DA receptors (D1-like or D2-like), those MSN can be classed as D1 or D2 (Gerfen et al., 1990; Gerfen & Surmeier, 2011). The activation of D1 and D2 neurons often yields contrasting results in drug addiction studies (Creed et al., 2016; Heinsbroek et al., 2017; James & Aston-

Jones, 2016; Smith et al., 2013; Wang et al., 2014). So, it is possible the active neuron was shifted to a different population in the striatum. This explanation may fit with the results in the study by Cahill et al. (2018), where the zif268 expression knockdown impacted on the proportion of animals using habitual strategy after reactivation in the T-maze experiment.

Overall, due to the immunohistochemistry experiment being underpowered and because no other protein was analysed, the data and conclusion are low in reliability. The brain regions that may be involved in instrumental memory reconsolidation need further studies.

Conclusion

Overall, the current study indicated that habitual instrumental memory had a complex boundary condition for reconsolidation. The conducted meta-analysis suggested that the gap day presented the most consistent results in this study; consequently, further studies should test this by comparing it with other reactivation methods using a two-lever model. A large contingency change - VR20 – was not found to reactivate the instrumental memory when using a two-lever model (Cheng, unpublished data); this may be due to the inactive lever pressing further diluting the reward contingency, so the VR20 protocol was more similar to the extinction session, rather than the reactivation session with unpredictable reward. Therefore, the VR5 protocol may be the correct contingency for the two-lever model.

Chapter 2

Introduction

Consolidated memory is not always the same: it can destabilise into a labile stage, integrate new information and return to a consolidated stage (Nader, 2003). To trigger memory retrieval, an extinction session is normally used as the reactivation method. Injection of an amnesic reagent, for example, the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801, resulted in long-term amnesia, which is normally used as the marker for the occurrence of memory reconsolidation (Tronson & Taylor, 2007). However, well-learned instrumental memory, which is an association of a specific action to an outcome, could not be destabilised in the extinction session (Hernandez & Kelley, 2004). Nevertheless, recent studies suggested that the reconsolidation process is a memory-updating process triggered by prediction error signals (Lee, 2009; Lee, 2010). Normal extinction session procedures cannot destabilise instrumental memory, possibly because reconsolidation may require a certain amount of prediction error signals to initiate (Debiec, Diaz-Mataix, Bush, Doyere, & LeDoux, 2013; Lee, 2009; Sevenster, Beckers, & Kindt, 2013). A sequential contingency change could generate putative prediction error signals and is triggered by instrumental memory reconsolidation (Exton-McGuinness et al., 2014).

A weak instrumental memory could be destabilised with a lower contingency change, but a well-learned instrumental memory could not (Exton-McGuinness & Lee, 2015). Inducing certain procedures, like context extinction or a gap day, between training and reactivation may reduce the threshold; this means well-learned instrumental memory could be destabilised with the contingency change that could not destabilise it before (Chapter One, Cheng, Exton-McGuinness, unpublished data). Within those reactivation methods, inducing a gap day before the reactivation was found to be the most consistent method by meta-analysis. Other methods had contradictive results between different experiments. Adding a second inactive lever is a common parameter for drug addiction studies (Fuchs, Bell, Ramirez, Eaddy, & Su,

2009; Lee et al., 2006a). So, it is important to establish whether the principles of instrumental memory destabilisation observed in the one-lever setting generalise to a two-lever paradigm, in which we could subsequently explore the mechanisms of destabilisation.

A particular mechanism that may trigger instrumental memory destabilisation arises from the trace dominance hypothesis, as it may explain the aforementioned effect of context extinction and a gap day to facilitate instrumental memory destabilisation. Eisenberg et al. (2003) showed that the extinction trace (CS-no US) competed with the original conditional stimulus (CS)- unconditional stimulus (US) trace in the rat-conditioned taste aversion trials and the medaka fish-fear conditioning trials. If the original trace was robust, the amnesic reagent would disrupt the original memory and leave the extinction trace untouched – and vice versa. Though Eisenberg et al.'s (2003) study was based on the same CS and comprised competition between the original excitatory memory and the inhibitory extinction memory, Reed and Reilly's (1990) study showed that a reduction of context-reward association would increase the instrumental behaviour, which may indicate that instrumental memory and context memory follow the trace dominance hypothesis. If the trace dominance theory is applied to instrumental memory reconsolidation, the manifesting behaviour might depend on the competition of two memories; strong memory dominates the behaviour and shows sensitivity to the blocker (Eisenberg et al., 2003). During training, a context-reward association is established alongside instrumental association (Reed & Reilly, 1990). Context extinction or a gap day may reduce the strength of context-reward memory by exposure to extinction conditions or by memory forgetting/interference. As a result, instrumental memory would be more likely to be destabilised.

In this study, we first investigated whether the reactivation methods in a one-lever model could carry through to the two-lever model. Then we investigated the mechanism of the successful reactivation method. In the previous chapter, the facilitation effect is suggested as

context memory forgetting/interference. To further investigate whether the facilitation effect is due to memory forgetting/ interference, we applied memantine to the rats 24 hours before reactivation. Memantine is an uncompetitive NMDA receptor antagonist (Plosker, 2015); it is used to sustain memories and prevent long-term memory loss (Sachser et al., 2016; Shinohara & Hata, 2014; Villarreal, Do, Haddad, & Derrick, 2002). If the facilitation effect is due to memory forgetting, memantine groups would be similar to the direct reactivation group, which should not show lever-pression impairment, and the saline controls should be the same as the delayed reactivation group, which showed lever-pression impairment. In a gap day based on the trace dominance hypothesis, only the dominant association is able to destabilise in the reactivation session (Eisenberg et al., 2003). To investigate whether instrumental memory followed the trace dominance theory as we expected, we would destabilise the subjects with two sequential reactivation sessions. Based on the trace dominance theory, the instrumental memory did not show that impairment in the test was due to other memory; for instance, contextual memory dominated the reactivation session. Therefore, instrumental memory did not destabilise. If this was the case, the MK-801 treatment should impair the dominated memory in the first reactivation session and the second reactivation session should destabilise the instrumental memory.

Method

Subjects

Subjects were 108 male experimental-naive Lister-Hooded rats from Charles River, weighing 200-350g at the beginning of the experiment. The rats were housed four per cage in a room with a constant 21°C temperature and a 12-hour light/dark cycle (lights on at 07:00). Each rat was provided with 20g of rat chow per day upon acclimation in the holding room (48-72 hours). Water was available ad libitum throughout except during the behavioural sessions. All procedures followed the United Kingdom Animals (Scientific Procedures) Act 1986, Amendment Regulations 2012, and the act under project licence PPL P3B19B9D2.

Behavioural Apparatus

Training sessions, the context extinction session, and the memory retrieval session took place in eight operant boxes, each of which is in a sound-attenuating chamber (MedAssociates, VT). All boxes were the same size: 25 x 32 x 25.5cm. The boxes consisted of two Perspex walls (a door and a rear wall), a Perspex ceiling, two steel walls (the right featured a food magazine and two levers, the left featured a light), as well as a grid floor with 19 stainless steel rods (4.8mm diameter, positioned 1.6cm centre to centre). Each food magazine had an infrared detector to measure nosepokes. All rats received the same training.

Behavioural Procedure

Training

The rats were trained for ten sessions. In each session, two levers were presented in the box. One lever was the active lever, while the other one was the inactive lever. The active lever

(left or right) was assigned pseudo-randomly and counterbalanced across groups. Once assigned, the active lever did not change place during the ten-day training period. The training session started with an illumination of the house light and the presentation of levers. When the active lever was depressed, a 45mg sucrose pellet was delivered to the food magazine (FR1 schedule). The lever did not retract and no stimulus was paired with the reward. No programmed consequences occurred if the inactive lever was depressed. The training session lasted for 30 minutes or when 60 sucrose pellets were obtained - whichever occurred first. Only one training session was given to each rat per day for a total of ten days.

Reactivation

Experiment 1

All experimental groups received a reactivation session. The reactivation session was the same as training, except rats were injected with MK-801 or saline 30 minutes before the reactivation session unless otherwise indicated:

- In the direct reactivation condition, the VR5 reactivation session (a random number of active lever presses to gain a sucrose reward, mean: 5, range: 1–9) took place 24 hours after the last training session.
- In the delayed reactivation condition, the VR5 reactivation session took place 48 hours after the last training session.
- In the context extinction condition, a 30-minute exposure to the training context alone (houselight illuminated and levers retracted through the session) took place 24 hours after the last training session. The VR5 reactivation session took place a further 24 hours later (48 hours after the last training session).
- In the 6 hour delayed condition, the rats took the VR5 reactivation session 48 hours

after the last training session but without MK-801 or saline injection. The MK-801 or saline injection took place 6 hours after the reactivation session.

- In the non-reactivation condition, the rats stayed in the home cage for 48 hours after the last training session and received an MK-801 or saline injection at the approximate same time as the direct reactivation group.
- In the delayed non-reactivation condition, the rats stayed in the home cage for 72 hours after the last training session and received an MK-801 or saline injection at approximately the same time as the delayed reactivation group.
- See Figure 1 for a comparative overview of the different procedures.

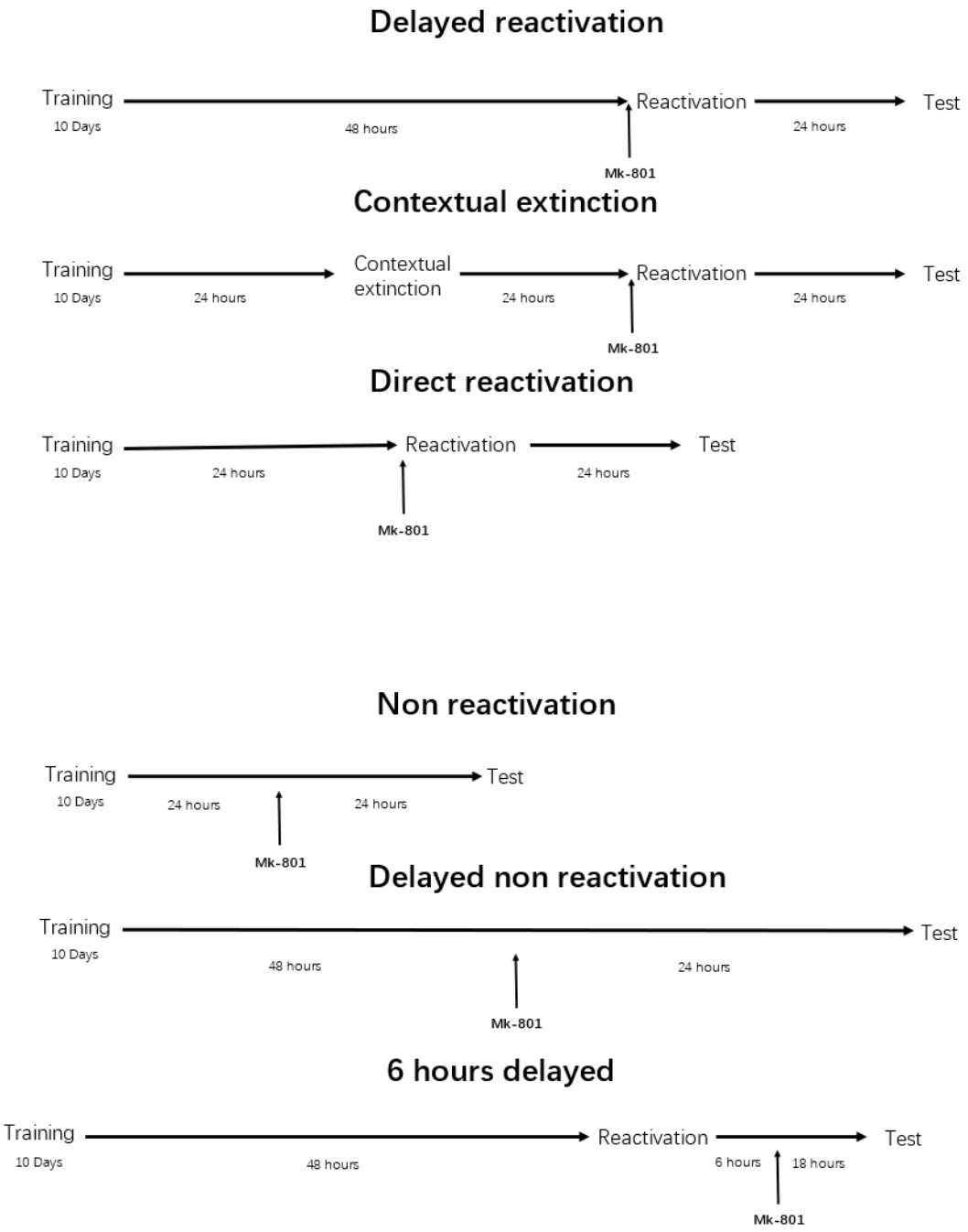


Figure 1. Schematic diagram of the experimental protocol in Experiment 1.

Experiment 2

Memantine injection

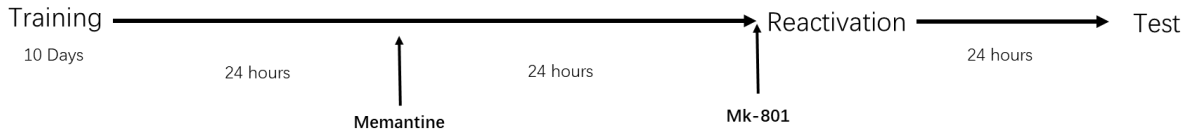


Figure 2. Schematic diagram of the experimental protocol in Experiment 2.

The procedure was the same as delayed reactivation in experiment 1 except the memantine group (14 rats) received a memantine (Tocris, 20mg/kg) injection and the saline group (15 rats) received a saline injection 24 hours after the last training session. See figure 2 for an overview.

Experiment 3

Double Reactivation

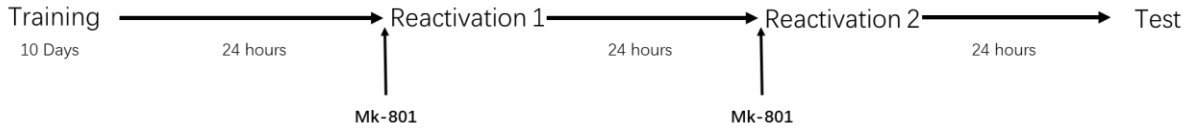


Figure 3. Schematic diagram of the experimental protocol in experiment 3.

The reactivation parameter was the same as the direct reactivation as in experiment 1 except the rats received a 2nd reactivation session 24 hours after the first one. See figure 3 for an overview.

Post-reactivation Testing

Behavioural groups were placed into the chamber 24 hours following the reactivation for a 30-minute session. The levers were extended and the house lights were illuminated during the session. However, no sucrose pellet was delivered when the lever was pressed. No limitation was imposed upon the lever pressing; CO_2 was used to cull the rats following the experiment.

Statistical Analysis

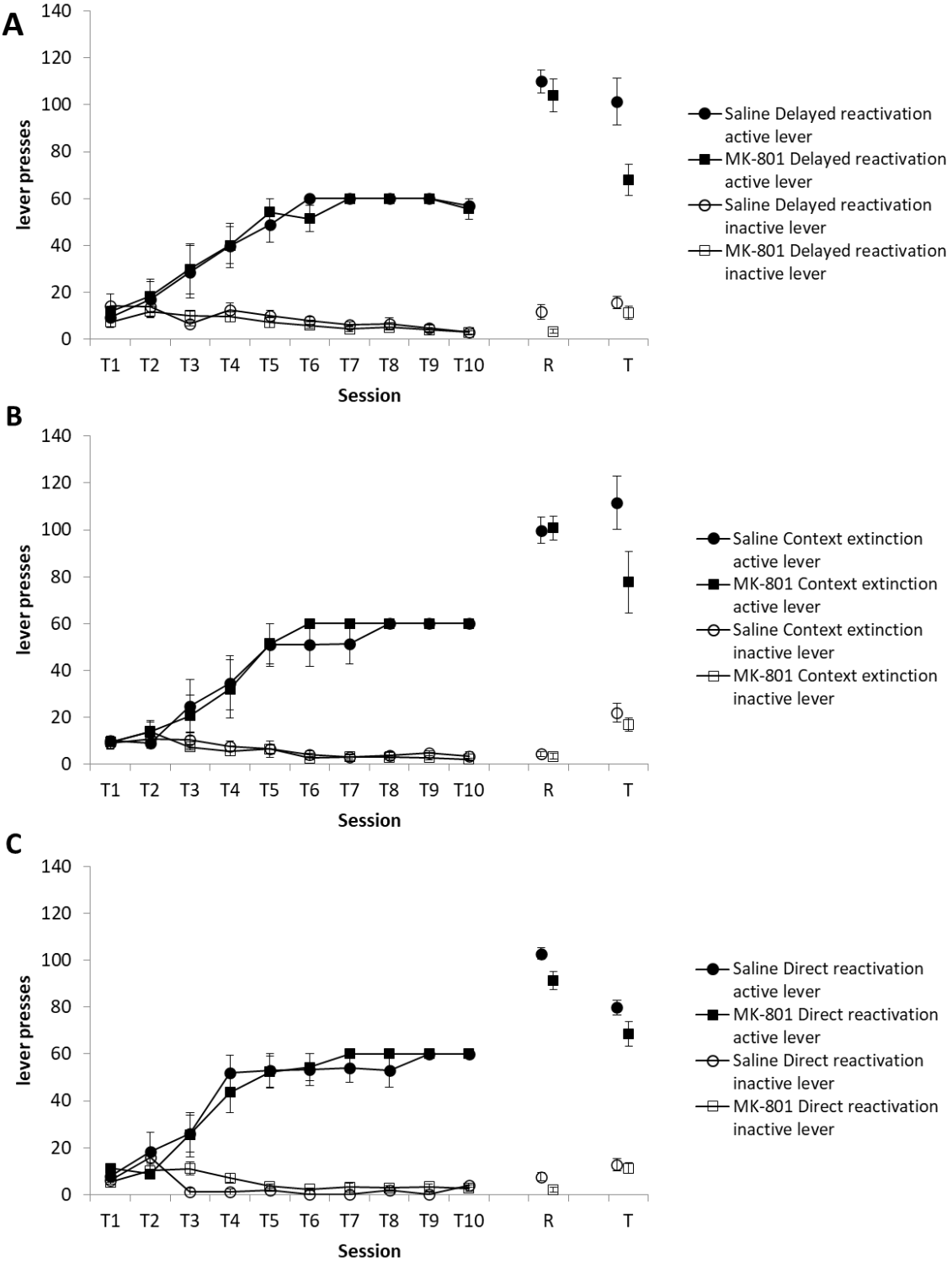
Data were presented as mean + S.E.M. for the number of lever presses at each training session, retrieval session, and test session. All data were checked for consistency, and any data lying more than two standard deviations from the mean were treated as outliers and excluded. All analysis was conducted using the IBM SPSS statistic 25 program. The training data were analysed by repeated ANOVA measures to access whether the tasks were learned and whether all experimental groups performed similarly; the lever, training days, reactivation method (Experiment 1 context extinction saline n=6, MK-801 n=6, delayed reactivation saline n=7, MK-801 n=7, direct reactivation saline n=8, MK-801 n=8, 6h delayed saline n=10, MK-801 n=10, non-reactivation saline n=7, MK-801 n=8, delayed non-reactivation saline n=3, MK-801 n=4), MK-801 treatment (injection 1 and injection 2 in Experiment 2, saline-saline: n=12, saline-MK-801: n=14, MK-801 saline: n= 14, MK-801-MK-801: n=14) and memantine treatment (Experiment 3 memantine MK-801: n=7, saline-MK-801: n=7, memantine saline: n=7, saline-saline: n=8) were used as factors. Reactivation data and test data were analysed using a three-way ANOVA, with lever (active vs. inactive), group (context extinction vs. delayed reactivation vs. direct reactivation vs. 6h delayed vs. non-reactivation vs. delayed non-reactivation in Experiment 1, memantine vs. saline in Experiment 2) and drug treatment (MK-801 vs. saline) as the factors. The reactivation and test session data were further analysed within each reactivation group using lever and drug

treatment as the factors in a two-way ANOVA.

Results

Experiment 1

All three reactivation methods were tested using a two-lever model to attempt to identify the most reliable method.



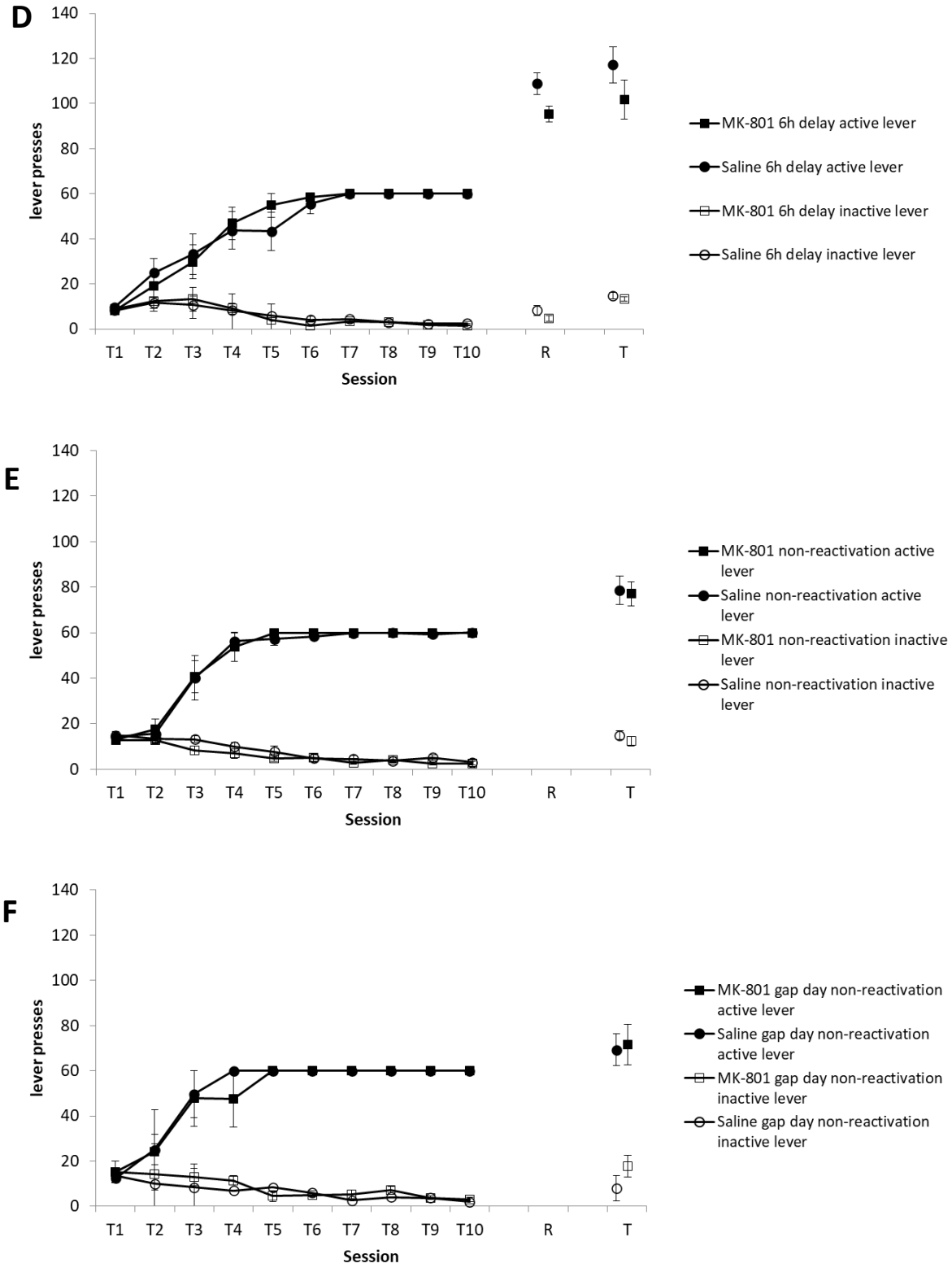


Figure 4. Delayed reactivation was the only reactivation method which had behavioural differences at the test (a) Delayed reactivation had a difference of lever presses in the test but not in training or in the reactivation session (MK-801, n=7 saline, n=7). (b) Context extinction had no difference in lever presses in training, reactivation session, or test (MK-801, n=6, saline, n=6). (c) Direct reactivation had no difference in lever presses in training, reactivation session, or test (MK-801, n=8, saline, n=8). (d) 6h delay had no difference in lever presses in training, reactivation session, or test (MK-801, n=10, saline, n=10). (e) non-reactivation had no difference in lever presses in training or test (MK-801, n=8, saline, n=7). (f) Delayed non-reactivation had no difference in lever presses in training or test (MK-801, n=4, saline, n=3).

Table 1. Planned comparison did not find any significant interaction between lever pressing and nosepokes during the training.

	Lever Pressings	Nosepokes
Delayed reactivation	$F_{(2.5, 30.3)}$=0.39, p=0.729	$F_{(3.2, 38.7)}$=0.41, p=0.758
Contextual extinction	$F_{(3.5, 34.6)}$=0.25, p=0.832	$F_{(2.6, 26.1)}$=0.44, p=0.756
Direct reactivation	$F_{(2.9, 31.5)}$=0.64, p=0.591	$F_{(2.9, 31.5)}$=0.84, p=0.478
6h Delay	$F_{(2.6, 46.0)}$=0.84, p=0.464	$F_{(3.8, 68.7)}$=1.86, p=0.130
Non-reactivation	$F_{(2.3, 29.4)}$=0.18, p=0.859	$F_{(3.2, 41.2)}$=1.47, p=0.237
Delayed non-reactivation	$F_{(2.1, 10.3)}$=0.46, p=0.658	$F_{(2.2, 11.0)}$=0.58, p=0.581

There was no difference in lever presses during training. ANOVA revealed there was no significant interaction of lever x training x reactivation method x drug treatment ($F_{(15.5, 214.3)}$ = 0.44 p=0.970); in the planned comparison, there were also no lever x drug treatment x training interactions found within each group (see Fig. 4; see Table 1).

The discriminated response in reactivation showed no difference between each group. All animals acquired all the rewards during the reactivation session. Neither the 30-minute pre-reactivation drug treatment nor a difference in reactivation methods had any effect on discriminated responses in the reactivation (lever x drug treatment x reactivation method, $F_{(3, 51)}$=0.72, p=0.544); lever x drug treatment, $F_{(1, 51)}$=0.59, p=0.448). As a planned comparison, no significance of lever x drug treatment interactions was found in any groups (direct reactivation group, $F_{(1, 14)}$=0.84, p=0.380; 6h delay group, $F_{(1, 18)}$=2.41, p=0.138; delayed reactivation, $F_{(1, 12)}$=0.05, p=0.820; contextual extinction, $F_{(1, 10)}$=0.07, p=0.801) (see Fig. 4).

The delayed reactivation group showed the effects of MK-801, whereas all other groups did not. A repeated measure ANOVA was conducted to examine the effect of lever, drug treatment, and reactivation methods on lever pressings. There was no interaction between whether the lever was active, the drug treatment, and the reactivation method on the lever pressing (lever x drug treatment x reactivation method, $F_{(5, 66)}$=1.26, p=0.291). However, MK-801 showed a significant effect on the discriminated lever pressings (lever x drug

treatment, $F_{(1, 66)}$ =8.17, p<0.01; drug treatment, $F_{(1, 66)}$ =7.73, p<0.01). A planned comparison of each reactivation group was subsequently conducted. The ANOVA revealed the MK-801 treatment had significantly impaired discriminated responding lever presses in the delayed reactivation group (lever x drug treatment, $F_{(1, 12)}$ =6.25, p=0.028), but not in direct reactivation (lever x drug treatment, $F_{(1, 11)}$ =0.46, p=0.511), context extinction (lever x drug treatment, $F_{(1, 10)}$ =3.40, p=0.095), 6h delay (lever x drug treatment, $F_{(1, 15)}$ =1.68, p=0.215), non-reactivation (lever x drug treatment, $F_{(1, 13)}$ =0.02, p=0.889) and delayed non-reactivation (lever x drug treatment, $F_{(1, 5)}$ =0.31, p=0.604) (see Fig. 4).

Among those reactivation methods, only the delayed reactivation group showed impaired discrimination in responding to the test. Consequently, the strongest evidence was for an effect in delayed reactivation. It is worth noting that the context extinction group also had a reduction of active lever pressing during observation, but such a difference was not statistically significant. This unreliability may be due to the memory competition of context extinction memory with instrumental memory (see Chapter One).

As a control parameter, nosepokes were measured throughout the experiment. Nosepokes showed no difference in the training (reactivation method x drug treatment x training, F (20.2, 278.5) = 1.00 p=0.459) and no drug treatment x training was found in the planned comparison (see Fig. 5).

No significant effect was found in nosepokes in the reactivation session, except the saline group in the delayed reactivation group had a higher number of nosepokes than the drug group (drug treatment x reactivation method, F (3, 51) =0.72, p=0.544; delayed reactivation, F (1, 12) =4.89, p=0.047; direct reactivation, F (1, 14) =0.002, p=0.964; context extinction, F (1, 10) =1.36, p=0.271; 6h delay, F (1, 18) =2.02, p=0.173) (see Fig. 5).

The nosepokes data revealed no significant difference in each group in the test (drug treatment x reactivation method, F (5, 69) =0.47 p=0.789; delayed reactivation, F (1, 12)

=1.39, p=0.261; direct reactivation, F (1, 11) =0.32, p=0.582; context extinction, F (1, 10) =0.12, p=0.739; 6h delay, F (1, 15) =0.03, p=0.861; non-reactivation, F (1, 13) =0.09, p=0.775; delayed non-reactivation, F (1, 5) =0.62, p=0.465) (see Fig. 5; see Table 1).

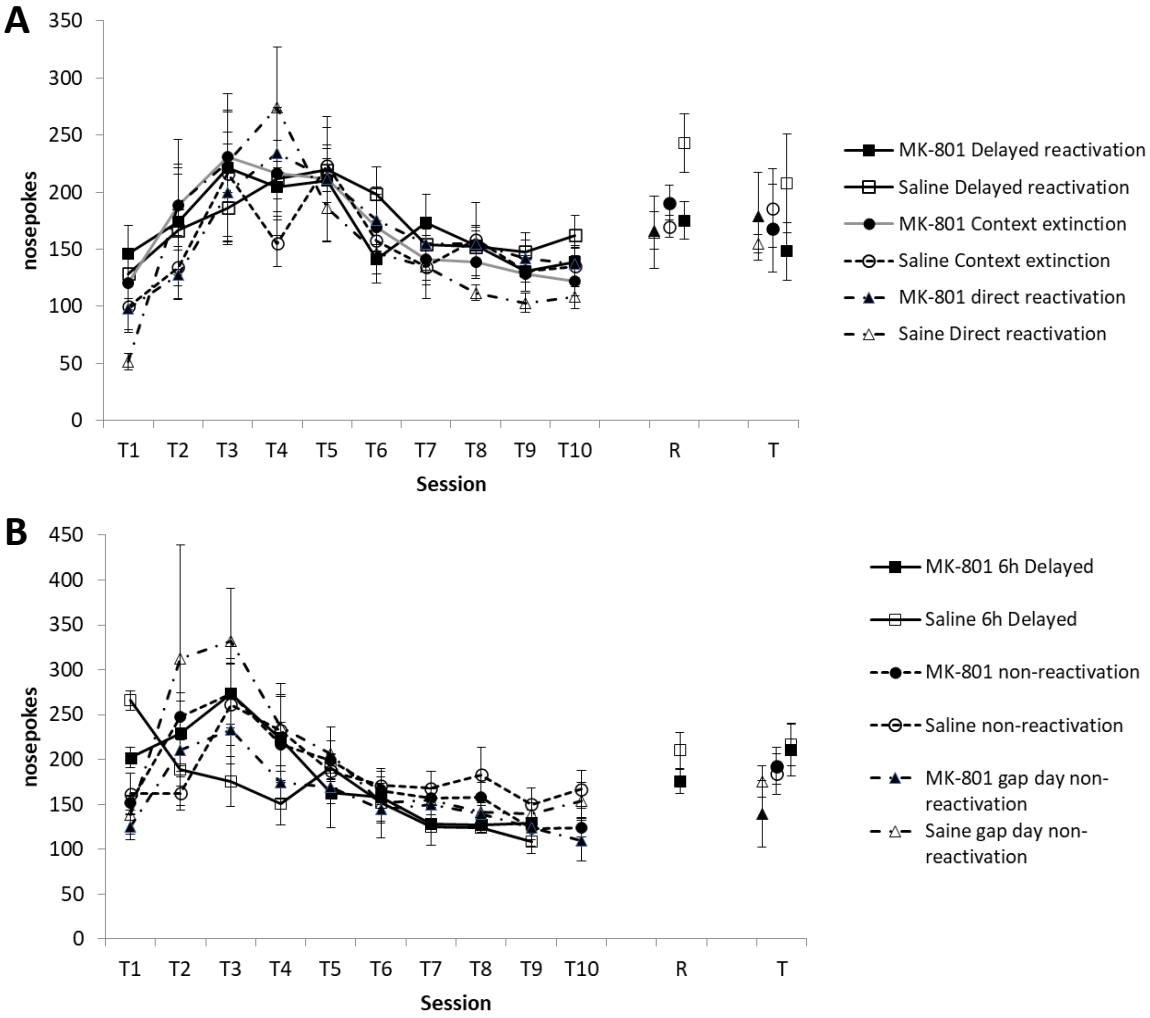


Figure 5. Nosepokes had no difference in test and training. In reactivation, only the delayed reactivation group had a difference in nosepokes. Test groups (A) Control groups (B)

The lower nosepoke responding at memory reactivation of MK-801-treated rats in the delayed reactivation group could result from a reduced motivation for sucrose. However, if this were the case, MK-801-treated rats should have been impaired regardless of the reactivation condition. It is unlikely that MK-801 would reduce motivation for sucrose only when the reactivation session is delayed. Moreover, it seems likely that the difference

between MK-801 and Saline treated rats is actually due to elevated nosepoke responding in the Saline group. This is because both groups in the 6h delayed condition (in which the reactivation session was delayed, but the injections took place after reactivation) nosepoked at a similar level to the MK-801 delayed reactivation group. As we do not have an immediate explanation for why the saline delayed reactivation group might have elevated nosepoke responding at reactivation, it may likely be due to chance and unlikely to affect the test results.

This experiment confirmed the meta-analysis result in the one-lever model, in which delayed reactivation was the most reliable reactivation method for well-learned instrumental memory.

Experiment 2

To test whether the instrumental memory reconsolidation followed trace dominance theory, we would destabilise the memory with two reactivation sessions. As shown in Experiment 1, the direct reactivation did not work in a two-lever model. If this was ascribed to other memory dominating the reactivation session, the MK-801 treatment in the first reactivation session may disrupt the dominated memory, and the second reactivation session may destabilise the instrumental memory.

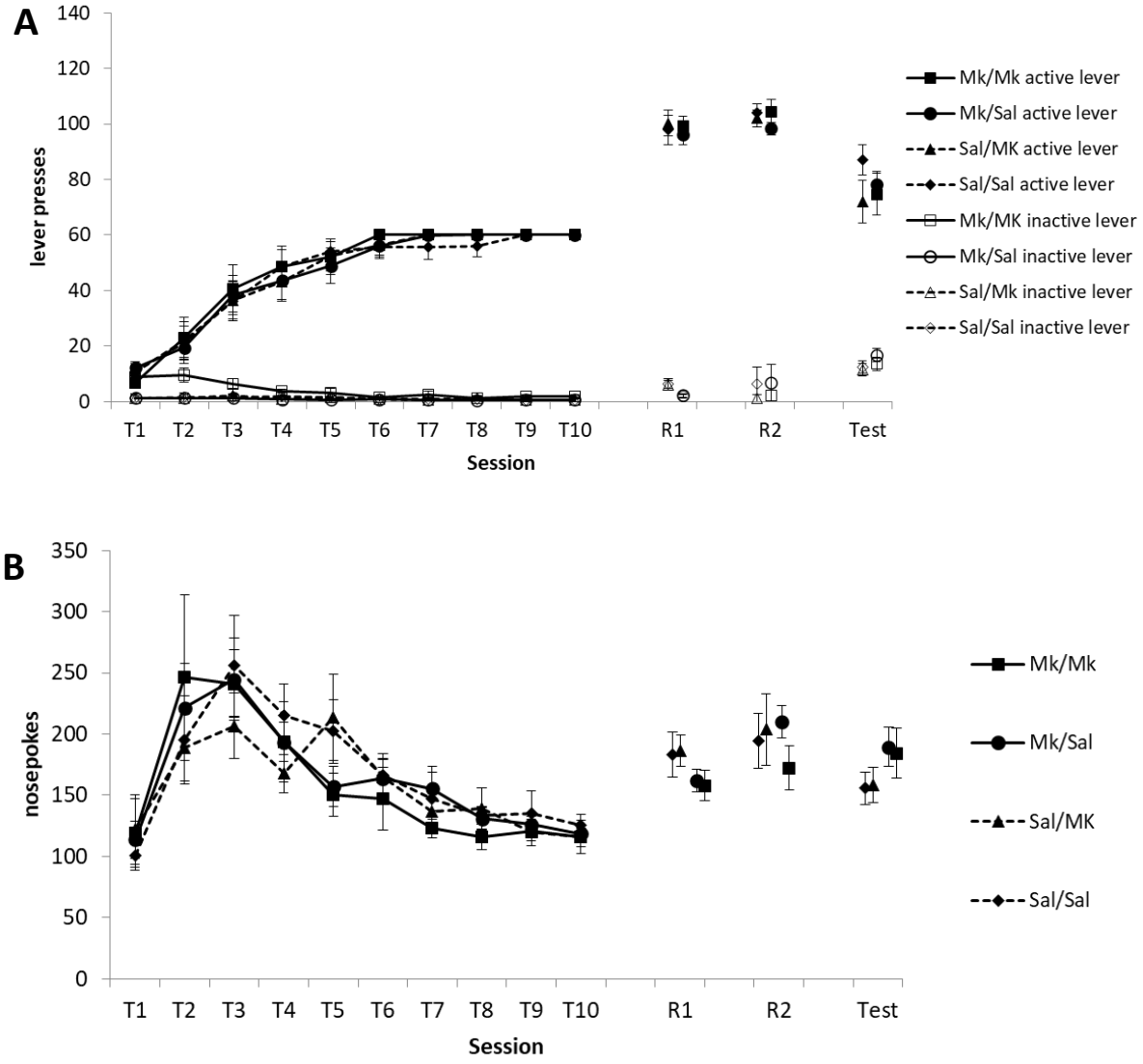


Figure 6. Rats showed similar performance on lever pressing and nosepokes during the training, reactivation sessions, and test. (a) Lever pressing (**Saline-saline**: n=12, **Saline-MK-801**: n=14, **MK-801 saline**: n= 14, **MK-801-MK-801**: n=14). (b) nosepokes

All groups exhibited a similar performance in the training. The ANOVA revealed there was no significant interaction of lever x training x Injection 1 x Injection 2 ($F_{(2.9, 142.7)} = 0.31$, $p = 0.812$). As a planned comparison, there was no lever x Injection 2 x training interaction found within each group (saline group in reactivation 1, $F_{(3.4, 80.8)} = 0.24$, $p = 0.891$; MK-801 group in reactivation 1, $F_{(2.2, 57.9)} = 0.28$, $p = 0.778$) (see Fig. 6a). Nosepokes showed no difference in the training (training x Injection 1 x Injection 2, $F_{(4.3, 213.4)} = 0.47$, $p = 0.766$) and no Injection 2 x training was found in the planned comparison (saline group in reactivation 1, $F_{(4.5, 108.1)} = 0.63$, $p = 0.660$; MK-801 group in reactivation 1, $F_{(2.6, 68.8)} = 0.38$, $p = 0.746$) (see Fig. 6b).

The discriminated response of the lever in two reactivation sessions showed no difference between each group. All animals acquired all the rewards during all the reactivation sessions. In reactivation 1, neither the 30-minute pre-reactivation MK-801 treatment in the first reactivation session nor the MK-801 treatment in the second reactivation session had any effect on discriminated responses in the reactivation (lever x Injection 1 x Injection 2, $F_{(1, 50)} = 1.06$, $p = 0.307$; lever x Injection 2, $F_{(1, 50)} = 0.36$, $p = 0.552$; Injection 2, $F_{(1, 50)} = 0.40$, $p = 0.533$). As a planned comparison, no lever x Injection 2 interaction was found in any group (saline group in reactivation 1, $F_{(1, 24)} = 0.16$, $p = 0.691$; MK-801 group in reactivation 1, $F_{(1, 26)} = 0.98$, $p = 0.331$) (see Fig. 6a). No significant effect was found in nosepokes in the first reactivation session (Injection 1 x Injection 2, $F_{(1, 48)} = 0.53$, $p = 0.470$; saline group in reactivation 1, $F_{(1, 22)} = 0.13$, $p = 0.722$; MK-801 group in reactivation 1, $F_{(1, 26)} = 0.46$, $p = 0.505$) (see Fig. 6b).

In reactivation 2, neither the 30-minute pre-reactivation MK-801 treatment in the first reactivation session nor the MK-801 treatment in the second reactivation session had any effect on the discriminated response of the lever in the reactivation (lever x Injection 1 x Injection 2, $F_{(1, 50)} = 0.07$, $p = 0.796$; lever x Injection 2, $F_{(1, 50)} = 1.09$, $p = 0.301$; Injection 2, F

$_{(1,\ 50)}$ =1.36, p=0.249). As a planned comparison, no significance was found in lever x Injection 2 interactions in any group (saline group in reactivation 1, $F_{(1,24)}$ =0.54, p=0.472; MK-801 group in reactivation 1, $F_{(1,26)}$ =0.63, p=0.435) (see Fig. 6a). No significant effect was found in nosepokes in the first reactivation session (Injection 1 x Injection 2, $F_{(1,\ 50)}$ =1.13, p=0.293; saline group in reactivation 1, $F_{(1,\ 24)}$ =0.05, p=0.827; MK-801 group in reactivation 1, $F_{(1,26)}$ =2.63, p=0.117) (see Fig. 6b).

No significant difference in lever pressing was found in the test. A repeated measure ANOVA was conducted that examined the effect of lever, MK-801 treatment in the first reactivation, and MK-801 treatment in the second reactivation on the discriminated response of the lever. There was no interaction between lever, MK-801 treatment in the first reactivation, and MK-801 treatment in the second reactivation on the discriminated response (lever x Injection 1 x Injection 2, $F_{(1,46)}$ =1.21, p=0.277). A planned comparison of each group using reactivation 2 as a factor was subsequently conducted. The ANOVA revealed no significant effects within each group (saline group in reactivation 1, lever x Injection 2, $F_{(1,22)}$ =1.86, p=0.186; MK-801 group in reactivation 1, lever x Injection 2, $F_{(1,24)}$ =0.003, p=0.955) (see Fig. 6a). As a control parameter, the nose-poking data revealed no significant difference in each group (Injection 1 x Injection 2, $F_{(1,46)}$ =0.02, p=0.892; saline group in reactivation 1, $F_{(1,24)}$ =0.09, p=0.769; MK-801 group in reactivation 1, $F_{(1,25)}$ =0.18, p=0.676) (see Fig. 6b).

None of the groups showed behavioural impairment in the test, which may indicate that the instrumental memory did not follow the trace dominance hypothesis. The first reactivation session may be disrupted by the facilitative effect of a gap day, therefore the reactivation which took place 48 hours after training did not destabilise the instrumental memory.

Experiment 3

One of the possibilities of the facilitation effect was due to memory forgetting on the gap day. Memantine is an NMDA receptor antagonist that is also often used to prevent memory loss (Sachser et al., 2016). If the facilitation effect was due to memory loss, treatment of memantine during the gap day should disrupt this effect. Therefore, the memantine MK-801 group should reverse the active lever-pression impairment.

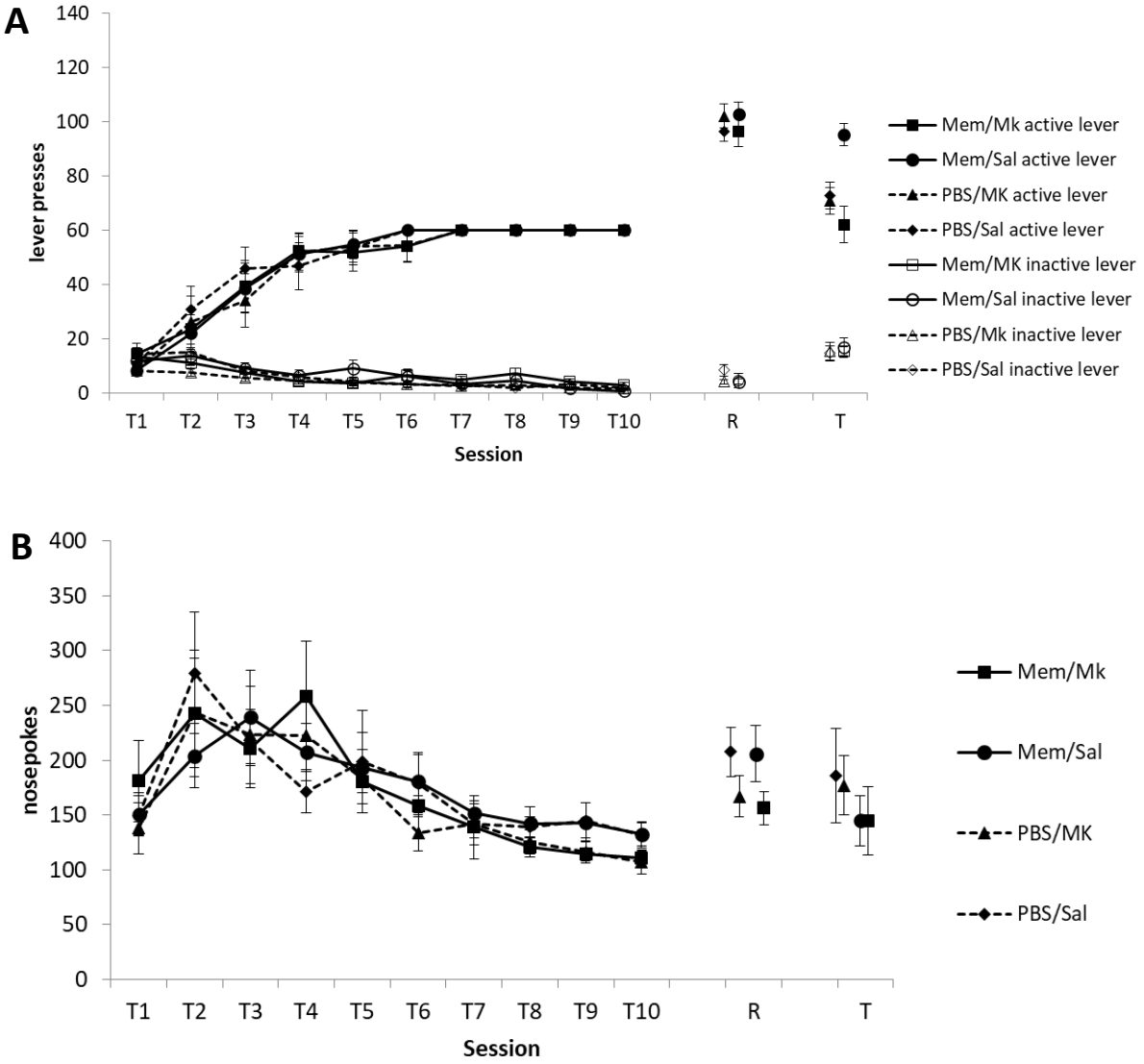


Figure 7. Memantine group showed a lever pressing difference in the test but PBS group did not. No behavioural difference in the training and reactivation sessions. (a) Lever pressing (**memantine MK-801:** n=7, **saline-MK-801**: n=7, **memantine saline**: n=7, **saline-saline**: n=8) (b) nosepokes

There was no difference in each group during the training. ANOVA revealed there was no significant interaction of lever x training x memantine treatment x MK-801 treatment ($F_{(3.0, 83.9)} = 0.21$ p=0.891). As a planned comparison, there was no lever x MK-801 treatment x training interaction found within each group (memantine treatment, $F_{(3.2, 45.4)} = 0.33$ p=0.822; control group, $F_{(2.5\ 35.6)} = 0.46$ p=0.682) (see Fig. 7a). Therefore, the reduction of discriminated response in the test was unlikely to be due to differences in training performance and the acquisition of the instrumental memory. Nosepokes showed no difference in training (training x memantine treatment x MK-801 treatment, $F_{(3.5, 86.8)} = 0.30$, p=0.855) and no MK-801 treatment x training was found in the planned comparison (memantine treatment, $F_{(3.4, 40.3)} = 0.54$ p=0.679; control group, $F_{(2.8\ 36.8)} = 0.50$ p=0.672) (see Fig. 7b).

The discriminated response in the reactivation showed no difference between each group. All animals acquired all the rewards during the reactivation session. Neither the 30-minute pre-reactivation MK-801 treatment nor the memantine treatment in the gap day had any effect on the discriminated response in the reactivation (lever x MK-801 treatment x memantine treatment, $F_{(1, 28)} = 2.62$ p=0.116; lever x MK-801 treatment, $F_{(1, 28)} = 0.08$ p=0.781; MK-801 treatment, $F_{(1, 28)} = 0.24$ p=0.628). As a planned comparison, no significance was found in the lever x MK-801 treatment interactions in either group (memantine group, $F_{(1, 14)} = 0.59$ p=0.455; control group, $F_{(1, 14)} = 3.77$ p=0.073) (see Fig. 7a). No significant effect was found in nosepokes in the reactivation session (memantine treatment x MK-801 treatment, $F_{(1, 25)} = 0.05$ p=0.832; memantine group, $F_{(1, 12)} = 2.77$ p=0.122; control group, $F_{(1, 13)} = 1.84$ p=0.198) (see Fig. 7b). The difference of discriminated responses in the test session was unlikely to be due to the difference in reactivation session.

In the test, the memantine group showed the effects of MK-801, whereas the control group did not. A repeated measure ANOVA was conducted to examine the effect of lever, MK-801

treatment, and memantine treatment on discriminated response. There was an interaction between lever, MK-801 treatment, and memantine treatment on the discriminated response (lever x MK-801 treatment x memantine treatment, $F_{(1, 26)} = 7.45$ **p=0.011**). A planned comparison of each group using MK-801 treatment as a factor was subsequently conducted. The ANOVA revealed the MK-801 treatment had a significantly reduced discriminated response in the memantine-treated group (lever x MK-801 treatment, $F_{(1, 13)} =14.59$, **p=0.002**), but not in the control group (lever x MK-801 treatment, $F_{(1, 13)} =0.58$, p=0.462) (see Fig. 7a). As a control parameter, the nose-poking data revealed no significant difference in each group (memantine treatment x MK-801 treatment, $F_{(1, 25)} = 0.02$ p=0.891; MK-801 treatment within each group: memantine group, $F_{(1, 12)} =0.00001$, p=0.997; control group, $F_{(1, 12)} =0.03$, p=0.867) (see Fig. 7b). The test results showed the MK-801 group with memantine treatment appeared to have behavioural impairment whereas the MK-801 group with PBS injection did not.

This experiment's results may suggest the injection during the gap day would disrupt the facilitative effect. Combined with the result in experiment 2, it is likely to be seen that any procedure act during the gap day may disrupt the facilitative effect. However the memantine was able to preserve such an effect or to have a similar function to the facilitative effect. For example, the memantine and MK-801 are both NMDA receptor antagonists, so the memantine injection on the gap day may affect the sensitivity of the rat to the MK-801 injection and shift the behaviour toward instrumental during the reactivation session.

Discussion

This study showed the two-lever model can also destabilise the well-learned instrumental memory. Additionally, it studied the boundary conditions of instrumental memory reconsolidation. In experiment 1, among the several reactivation parameters, the delayed reactivation with VR5 was the only one that can destabilise the well-learned instrumental memory. In experiment 2, none of the groups showed any sign of instrumental memory destabilisation; therefore, the reactivation inserted on gap day disrupted the facilitative effect rather than facilitating it. This may also suggest that the trace dominance hypothesis may not work in well-learned instrumental memory reconsolidation. In experiment 3, the memantine was used to discover the mechanism that may underlie the facilitative effect. The result shows that the PBS group did not show signs of instrumental memory destabilisation whereas the memantine group did. This may suggest that the injection and/or handling on the gap day disrupt the facilitative effect and the memantine injection may preserve the effect or have a similar function to the facilitative effect.

Reactivation Methods for the Two-lever Model

The current data have been used to influence the design of parallel instrumental cocaine-seeking studies, in which the delayed reactivation VR5 procedure has been found to successfully destabilise the instrumental cocaine memory in a two-lever setting (Exton-McGuinness et al., 2019). Here, we compared the delayed reactivation to the context extinction and direct reactivation also used in Chapter One. It was found that delayed reactivation was the only condition that showed impairment on active lever pressing. In the context extinction condition, the MK-801 group exhibited an observable reduction of active lever pressing, but such a difference was not statistically significant. Direct reactivation did not show any impairment on active lever pressing.

The impairment of active lever pressing in the delayed reactivation MK-801 group was likely due to an impairment of the reconsolidation process. The first stage of the entire process is memory destabilisation. Then, the labile memory integrates the new information and reconsolidates to a persistent state (Lee, 2009); this destabilisation is doubly dissociated from reconsolidation (Milton et al., 2013). If the destabilisation was impaired, the memory should be intact due to it being unable to trigger reconsolidation; this was not found in the delayed reactivation MK-801 group. If the reconsolidation was impaired by the MK-801 treatment, it would disrupt the reconsolidating memory and cause inaccessible memory (see the general introduction for detail), thus leaving a reactivation-dependent amnesia (Mactutus, Riccio, & Ferek, 1979; Thompson & Grossman, 1972). The amnesia was showed as behavioural impairment in the test. The delayed reactivation MK-801 group showed the behavioural impairment was likely to be the instrumental memory impairment, which is linked to active lever pressing resulting in a reward. This behavioural impairment is only found in the delayed reactivation group but not with the other reactivation methods nor in any non-reactivation controls, which means this behavioural impairment was not a general effect and was likely caused by the delayed reactivation method. The reactivated group with the six-hour delayed MK-801 treatment also showed no such impairment, which is a good control measure for the delayed reactivation group. It not only suggested that the reduction of active lever pressing was unlikely to be due to the non-specific effect of MK-801 but also indicated that the impairment was temporal-dependent, which was a characteristic found in the drug-seeking appetitive memory reconsolidations and also fits with the current view of reconsolidation (Fuchs et al., 2005; Gisquet-Verrier & Riccio, 2018; Milton & Everitt, 2012). Overall, this reactivation-dependent behavioural impairment in the MK-801 group was likely due to the impairment of instrumental memory.

Nosepokes as a control measure are often used in memory reconsolidation studies (Exton-

McGuinness et al., 2014; Exton-McGuinness & Lee, 2015; Klanker, Fellinger, Feenstra, Willuhn, & Denys, 2017; Weatherly, Arthur, & Nurnberger, 2006). While it is sometimes assumed to be indirectly reflective of general activity levels, it is also subject to influence from motivation to retrieve the sucrose reward. No statistical difference was found in most sessions of Experiment 1; this may suggest the behavioural impairment in the delayed reactivation group is likely due to the memory impairment because it is unlikely some factors only affected the delayed reactivation group but not the other groups. The only difference found was the nosepokes in the reactivation session: the delayed reactivation saline group exhibited higher nosepokes than the MK-801 group. As mentioned in the **Results** section, this difference was unlikely to affect the test results. Due to no injection being given before the reactivation session, the nosepokes in the 6h delayed group can be treated as a baseline. So, it is obvious the MK-801 group in delayed reactivation had a similar number of nosepokes to the 6h delayed group, whereas the nosepokes in the saline group were higher than the baseline. Though the reason for this elevation of nosepokes is unclear, it was likely due to chance and did not affect the test results.

Another explanation of the obtained results is that the reduction of lever pressing was due to an impairment of new learning in the VR5, rather than an impairment of memory reconsolidation. If this is due to rats learning the VR5 protocol in the reactivation session, rats will make a greater number of instrumental responses to achieve the same number of rewards. This will cause an elevation of lever pressing and this effect could be prevented by MK-801 treatments, which appears as lever-pression impairment (Hernandez et al., 2002); in this experiment, this possibility could not be ruled out conclusively. Here, it was observed that the delayed reactivation saline caused a higher lever pressing than the non-reactivation controls, which may be due to the new learning during the reactivation session. Moreover, the 6h delayed MK-801 group, in which the MK-801 treatment was unlikely to affect the test

result, also had a higher active lever pressing than the non-reactivation control. This finding may suggest that the MK-801 treatment before the reactivation was responsible for preventing the elevated lever pressings, rather than reducing the lever pressing. This finding led to the question as to whether the gap day could facilitate new learning, rather than reconsolidation; if this is true, the gap day could be said to weaken the old memory and induce new learning during reactivation. However, it is worth noting that Exton-McGuinness's (2014) VR20 (12–28 lever presses for a sucrose pellet) results suggested that S-R memory reconsolidation impairment also showed a similar pattern to the delayed reactivation groups. This pattern was not found in the one-lever model of Experiment 3 (delayed reactivation vs. direct reactivation), nor in the previous study (context extinction vs. direct reactivation). No difference was found in lever pressings in the context extinction group, so it is unlikely to explain the new learning only occurring in the delayed reactivation setting. Therefore, combining the results from this study with Exton-McGuinness's (2014) study, it follows that the reduction of active lever pressings in the delayed reactivation group remains most likely due to impaired instrumental memory reconsolidation.

Finally, it is notable that the impairment of the delayed reactivation group was not likely to be due to state dependency. One example of state dependency is the rats performing poorly in the T-maze test if they received an injection of sodium pentobarbital one day previously. However, if they were given a second injection of the drug, they exhibited perfect performance (Overton, 1964). While the MK -801 treatment was found to have state-dependent retrieval effects (Ceretta, Camera, Mello, & Rubin, 2008; Flint et al., 2013), this was hardly found to be the case in this study. Though the state-dependent retrieval effects are similar in performance to reconsolidation impairment, this should result in all the destabilised MK-801 group exhibiting similar results regardless (Gisquet-Verrier et al., 2015). However, such results were not found in this experiment; therefore, state dependency was unlikely to be

seen only in the delayed reactivation group.

Trace Dominance and Instrumental Memory

Based on trace dominance theory, associations compete for dominance of retrieval and reactivation in the reactivation session and only the strongest association can destabilise (Eisenberg et al., 2003). As later proposed in the discussion of the memantine data, the dominance of the context-reward memory might prevent instrumental memory destabilisation under certain conditions, principally the direct reactivation condition. Therefore, the context-reward association – but not the instrumental association – was destabilised in the reactivation session; if this was the case, the MK-801 treatment should impair the context-reward association. If there was a second reactivation session, there would no longer be a strong competing context-reward memory, allowing the reactivation of the instrumental memory, and the MK-801 group should show the impairment of lever pressing; however, this was not shown in the obtained results, as all groups did not show any impairment of lever pressing.

The failure to promote instrumental memory destabilisation in this double reactivation experiment was not likely due to an inability to disrupt the contextual memory. Many studies concerned with conditioned place preference (CPP) used systemic MK-801 injections with the same dose as this study to impair context-reward memory (Alaghband & Marshall, 2013; Sadler et al., 2007). Therefore, it was unlikely that the MK-801 did not work on the contextual memory in this study. Nevertheless, the context memory may not have been completely impaired by the first MK-801 treatment, so the second reactivation was still dominated by the context memory.

Nosepokes were measured as a control parameter, yet some studies use nosepokes as a measure to demonstrate that Pavlovian contextual associations remain intact (Exton-

McGuinness et al., 2014; Exton-McGuinness et al., 2019). Given that there was no reduction in nosepokes at the second reactivation session, this may indicate that the context memory remained intact and was not impaired by MK-801 at the first reactivation session. However, the sensitivity of nosepokes to context-reward memory may not be evident in our paradigm. It has previously been shown that context extinction sessions reduce the strength of the context-reward association and promote instrumental behaviour (Reed & Reilly, 1990). Therefore, if the nosepokes were related to the context-reward memory, the context extinction group in Experiment 1 should have shown a reduction of nosepokes when compared to other groups, but we did not observe such a pattern of results. Therefore, nosepokes might not reflect the strength of the context-reward memory.

There remains a speculative account of our data that fits within the trace dominance hypothesis and is consistent with the apparent failure of MK-801 at the first reactivation session to promote destabilisation at the second reactivation session. The original trace dominance hypothesis assumed that memories either undergo destabilisation or extinction. However, there is a minimum requirement of exposure to extinction which was required to destabilise the context memory (Suzuki et al., 2004). The reactivation session changed the value of the reward for lever pressing, but it may generate less prediction error for the context-reward association than the extinction session (context-no reward). Therefore, a strong context-reward memory may inhibit the competing instrumental memory from undergoing destabilisation at the first reactivation, while not itself being destabilised. Moreover, the context memory would neither be impaired by the MK-801 injection at the first reactivation session, nor would it be extinguished substantially, leaving it able again to be dominant at the second reactivation session. This might explain the lack of differences observed in any condition in the present experiment.

Perhaps the most obvious explanation for the results of Experiment 2 is actually that trace

dominance theory does not apply in our setting of instrumental memory reconsolidation. The traces in Eisenberg et al.'s (2003) experiment were the CS-US association and CS-no US association, which were all based on the same CS. Conversely, in this study, the context-reward association and the S-R association were based on different stimuli. Therefore, it could be that the failure of reactivation was irrelevant to the contextual association; it may just be an insufficient prediction error signal. However, it is worth noting that competing responses exist in instrumental behaviour (Dickinson, 1985). Reed and Reilly (1990) indicated that context extinction enhanced the instrumental response and that the context-reward association showed a competitive response for lever response and interfered with the instrumental behaviour (Reed & Reilly, 1990). Hence, it is possible that the VR5 did not have enough contingencies to change and generate enough prediction errors to trigger the reconsolidation, so direct VR5 reactivation for well-learned instrumental memory did not work regardless of the number of reactivation sessions.

Facilitative effect in delayed reactivation

As it was shown that the delayed reactivation was likely to destabilise the instrumental memory, we followed with an experiment to attempt to identify the possible mechanisms of this facilitative effect. One of the possibilities of this effect was due to memory forgetting during the gap day, the context memory being sufficiently weak and allowing instrumental memory to undergo reconsolidation (see Chapter One for detail). In this study, we used memantine to prevent contextual memory forgetting during the gap day; if this was successful, the facilitative effect should be reversed, which would make the results more akin to those seen in direct reactivation (i.e., no lever-pression impairment). However, the results obtained opposed the stated hypothesis: the memantine group showed memory impairment, but not the saline controls.

The first question asked is whether the memantine could prevent memory forgetting during the gap day, since memantine was also known as a neurogenesis enhancer that enhanced memory forgetting (Muller, Mutschler, & Riederer, 1995; Seif el Nasr, Peruche, Rossberg, Mennel, & Krieglstein, 1990; Volbracht, van Beek, Zhu, Blomgren, & Leist, 2006; Zajaczkowski, Frankiewicz, Parsons, & Danysz, 1997). For instance, mice receiving an intraperitoneal injection of memantine showed a reduction in freezing time in several contextual fear conditioning tests (Ishikawa, R., Fukushima, Frankland, & Kida, 2016). However, the memantine effect was dose-dependent and the dose used here had been proved to maintain the object recognition memory in Sachser et al.'s (2016) study: the neurogenesis effect was only shown in the higher memantine dose and the mice received memantine injections once a week for four weeks. Therefore, the memantine caused neurogenesis over a long period and when higher doses were given. Therefore, the memantine used in this study should prevent memory forgetting, rather than causing a memory forgetting by neurogenesis effect.

In our hypothesis, the memory forgetting during the gap day reduced the strength of contextual memory, and therefore facilitated instrumental memory reconsolidation. However, this experiment showed that memantine – which preserves memory against loss – did not diminish the effect, but rather maintained the facilitation effect; to identify this, we may need to look into the mechanisms of long-term memory forgetting.

In the current view, two theories of long-term memory forgetting exist: 1) memory decays over time (Ebbinghaus, 1885), or 2) memory is interfered with by other memories (Anderson, Bjork, & Bjork, 1994). The decay-over-time hypothesis is mainly focused on maintaining long-term potentiation (LTP) (Villarreal et al., 2002), as the function of memantine to prevent memory loss is also suggested to be maintaining LTP during the gap days (Ishikawa et al., 2016; Shinohara & Hata, 2014; Villarreal et al., 2002). However, if this is true, the question

that remains is as follows: why does delayed reactivation impair the reconsolidation, rather than create results akin to those achieved in direct reactivation? It may be argued that the memantine treatment may prevent instrumental memory loss, rather than context memory; however, this is highly unlikely to be the case, as there is no evidence that memantine can selectively prevent forgetting of one memory, but not others. It is worth noting, though there was no evidence that systematic injection of memantine may have greater effect on specific brain regions, memantine may, but is unlikely to, have different effects in different brain regions, such as the ventral hippocampus or nucleus accumbens core. This may affect instrumental memory destabilisation (see chapter 3 and Ramon unpublished data). Therefore, the argument may be that if the memantine prevents forgetting in both memories, the trace dominant hypothesis may not apply to instrumental memory reconsolidation, so the strength of the context memory is not relevant to instrumental memory reconsolidation. If the memantine has a general anti-forgetting effect, the gap day can be treated as not existing, and the results should be more like direct reactivation, rather than delayed reactivation. Therefore, such an explanation is inconsistent with the observation that the direct reactivation condition did not show any lever-pression impairment.

The alternative view that memory forgetting is due to retroactive interference of task or material (Müller & Pilzecker, 1900) may explain the unreliability of context extinction but is not easily applied to the present experiment. Memantine has consistently been shown to impair consolidation and reconsolidation (Alaghband & Marshall, 2013; Nandhra, Murphy, & Sule, 2013; Tucci et al., 2014), and so if memory over the gap day is due to the acquisition and consolidation of interfering information, memantine should impair, rather than preserve, the memory in gap day. Some may argue that consolidation and reconsolidation are temporal-dependent (Mactutus, Riccio, & Ferek, 1979; Thompson & Grossman, 1972); just as we showed in Experiment 1, the memantine injection may miss that labile window. However,

such an argument does not consider the data of saline control. If the memory had already entered the persistent state, why did the saline injection disrupt the effect? Therefore, adopting a memory interference framework is likewise unable to explain the pattern of data in this experiment.

The other possibility of lever-pression impairment was due to state-dependent effects (Ceretta et al., 2008; Flint et al., 2013). Because memantine and MK-801 were both NMDA receptor antagonists, the result may be due to state dependency. Again, this is highly unlikely. As the result was due to state-dependent retrieval effects, the memantine saline group which only had one drug treatment should show poor performance, yet a contrasting result was yielded. The memantine MK-801 group which had two drug treatments should have retention performance but showed a reduction of active lever pressing. So, the drug group had a completely different result to that predicted in the state-dependent retrieval. However, the memantine injection may cause rats to have sensitivity to an MK-801 injection and shift the behaviour toward instrumental in the reactivation session. It is worth noting that the rats in experiment 2 also received an MK-801 injection on the gap day (followed by a reactivation session) and did not show behavioural impairment in the test. This possibility may need further research to explain.

Though it is hard to explain the data by memory forgetting views and state dependency theory, the new view of reconsolidation may suggest a possible explanation for the data. The new view of reconsolidation treats the reconsolidation process as a memory integration process rather than reconsolidation (Gisquet-Verrier & Riccio, 2018; Lee, 2010; Lee, Jonathan, Nader, & Schiller, 2017). In this view, the reactivated memory integrates new information within hours. This is the malleable period for this memory, then the memory returns to an inactive state. If the information presented at reactivation is the same as the initial learning, this will enhance subsequent retention performance. If the information is

inconsistent with the original memory, the memory is updated resulting in disrupted test performance. If the context-food memory was destabilised during the gap day, which may be due to inducing the prediction error with no training that day, the information in the cage was different from the initial memory which was in the operant chamber. Thus, the context memory would be disrupted by endogenous memory updating during the gap day, with the effect that the instrumental memory is dominant at the reactivation session. If this is true, then memantine treatment may have no effect on this process at all (but see below), or it might impair the integrating context memory (or even produce additional memory updating), leaving only the instrumental association as being retrievable in the reactivation session. This would explain why the effect of MK-801 is seemingly more pronounced than in the absence of any treatment on the gap day (comparing visually against the data in Experiment 1).

While there may be several explanations for why memantine on the gap day did not affect the disruptive impact of MK-801, an important observation is that the saline controls did not show amnesia.

One possibility is that the act of injecting the rats, which necessitates their handling, is likely to form new independent memories. There are two major reasons why this handling/injection memory might be preferentially destabilised at the reactivation session. First, it is the most recent experience and so would benefit from recency effects (Delaney, Verkoeijen, & Spirgel, 2010). Second, the reactivation session is itself preceded by intraperitoneal injection (to administer saline or MK-801), the procedure of which might destabilise the handling/injection memory, and perhaps it is this memory that is impaired by MK-801. This would explain why the instrumental memory remains intact. In this view, administration of memantine would likely impair the acquisition/consolidation of the handling/injection memory in the gap day, leaving the reactivation session to destabilise the instrumental memory normally.

The second possibility is that the stress caused by injection may influence the facilitation effect. Stress can enhance consolidation through an elevation of glucocorticoid levels in the basolateral amygdala (BLA) (Roozendaal, 2002). Such elevation can also impair retention performance by blocking hippocampal-dependent influences on memory retrieval (Roozendaal, 2002). Therefore, the injection on the gap day may block the retrieval from the previous contextual memory so the gap day memory consolidation was enhanced. At the reactivation session, the enhanced gap day memory may dominate the reactivation session and prevent the reconsolidation of instrumental memory. Therefore, the instrumental memory in the control group was intact during the test session.

Overall, such data could not confirm what the cause of the facilitation effect was. The memantine group showed a clearer lever-pression impairment than the delayed reactivation group, which may be due to memantine-induced amnesia on the gap day. Therefore, using a non-amnesic agent to prevent memory loss may reveal the mechanism of the gap day. For the saline control, the results may be due to the handling effect, or the stress caused by injection. However, it is worth noting that those two hypotheses may simultaneously exist. Consequently, a control measure for handling the gap day will be necessary for the next study.

Conclusion

This study confirmed the previous study's results, which showed that delayed reactivation was the best parameter to destabilise the well-learned instrumental memory with the VR5 protocol. However, the mechanism of the facilitative effect is still unclear and further experimentation with different drugs to prevent memory loss or impair reconsolidation is required to support the memantine results. A control with handling but no injection is needed to prove the hypothesis of the saline groups. The double reactivation was not found to have any effect on lever pressing, so whether the well-learned instrumental memory followed the trace dominant theory was unclear. Further studies may also be required to assess the zif268 expression as a marker to identify whether destabilisation occurred after each reactivation, or whether it was destabilised in the rats under different contexts.

Chapter 3

Introduction

Instrumental learning, which pairs a specific action with an outcome, was first studied by Thorndike and Woodworth (1901). Further study indicated that instrumental learning is a dual process: a manifested response that shifts from goal-directed Action-Outcome (A-O) to habitual Stimuli-Response (S-R) association through training (Dickinson et al., 1995). Reconsolidation, which was suggested as a memory-updating progress, was observed in both Pavlovian appetitive and aversive memory (Reichelt & Lee, 2013a); this process includes long-term memory that is destabilised into a labile stage by a reactivation session, before being reconsolidated back to a persistent form (Lewis, 1979; Nader, 2003). The destabilisation and reconsolidation are suggested to involve different neural circuits, as the ventral tegmental area (VTA) which responds to prediction error signal was shown only to be involved in destabilisation but not reconsolidation (Milton et al., 2013).

The reactivation session is normally an extinction session, but this reactivation method fails when concerning habitual instrumental memory (Hernandez & Kelley, 2004; Lee & Everitt, 2008a; Lee & Everitt, 2008b; Lee & Everitt, 2008c; Milton et al., 2008). A new reactivation method, which induces a contingency change, was shown to be successful at triggering A-O memory reconsolidation (Exton-McGuinness & Lee, 2015), but such a method had a non-consistent effect on the habitual S-R memory in the same setting (see Chapter One). By introducing a control lever, the boundary of habitual memory reconsolidation was much clearer, and delayed reactivation was found to be the most reliable reactivation method (see Chapter Two).

There was no study about the neural circuit that may be involved in well-learned instrumental memory destabilisation or reconsolidation. However, several studies had shown that blocking nucleus accumbens (Nac) disrupts appetitive memory consolidation (Dalley et al., 2005; Smith-Roe & Kelley, 2000). Further study showed Nac involvement in conditioned

place preference (CPP) memory reconsolidation. For example, one study shows nucleus accumbens shell (AcbSh) but not core blocked by baclofen disrupted the CCP memory reconsolidation (Wang et al., 2020), but the other study showed infusion of glutamate receptor interacting protein 1 inhibitor in Nac core but not shell disrupted CPP memory reconsolidation (Liang et al., 2017). However, the involvement of Nac in memory destabilisation was unclear. As a major output of VTA's dopamine neurons (DA) which responded to prediction error signals (Watabe-Uchida et al., 2017), Nac was likely to be involved in instrumental memory destabilization. As the infusion of NMDA receptor antagonist MK-801, AP-5 or dopamine receptor D1 receptor antagonist SCH-23390 in Nac alone did not disrupt the instrumental memory reconsolidation, but did when co-infused with AP-5/SCH23390 (Exton-McGuinness & Lee, 2015), the authors suggested that the infusion which did not disrupt memory reconsolidation may however disrupt memory destabilisation, and suggested Nac may be involved in instrumental memory destabilisation. As a follow-up study, our recent result shows blocking Nac core but not shell with $GABA_{A/B}$ receptor agonist baclofen/muscimol (B/M) impaired well-learned instrumental memory destabilisation (Ramon, unpublished data). To further investigate which neural circuit may relate to memory destabilisation, the ventral hippocampus (vHPC) which had glutamatergic projection to Nac was chosen as the target.

Though the ventral hippocampus was not targeted by any memory reconsolidation studies, vHPC may be involved in memory destabilisation. During the non-signalled overtraining instrumental learning, only the vHPC CA3 – but not the CA1 - was activated; this may be due to the CA1 being more related to the incentive value, and that overtraining the S-R association caused an insensitivity to the reward value (Faure et al., 2006). Furthermore, the DA in substantial nigra-ventral tegmental area complex (SNc/VTA) and the vHPC were active following overtime non-signalled training (Faure et al., 2006). The dopaminergic

projection from VTA is found active in multiple reward-related behaviours and A8 and A10 groups suggest involvement in prediction error signalling (Glimcher, 2011; Reichelt et al., 2013; Takahashi et al., 2009). One of the hypotheses in memory reconsolidation is that prediction error is required to trigger memory destabilisation (Lee, 2009; Lee et al., 2017). Therefore, the vHPC may be involved in instrumental memory destabilisation.

As the vHPC may be involved in habitual instrumental memory destabilisation, for this experiment we infused the baclofen/muscimol (B/M) – a $GABA_A$ and $GABA_B$ receptor agonist – immediately before the reactivation session. We hypothesised that the vHPC is involved in habitual memory destabilisation; if this were true, should this prevent the reactivation-dependent amnesia, the results would be identical to the direct reactivation group in experiment 1 of Chapter Two, in which the MK-801 group did not show any difference in active lever pressings when compared to the saline group. There was a chance that the B/M infusion inactivated the vHPC which impaired the restabilisation/reconsolidation process (Wells et al., 2011) since destabilisation and restabilisation had a double-dissociation requirement (Milton et al., 2013). If this is true, the B/M infusion should impair the lever pressing regardless of the MK-801 treatment.

Method

Subjects

The subjects were 27 male experimental-naive Lister-Hooded rats from Charles River, weighing 200-350g at the beginning of the experiment. The rats were housed four per cage in a room with a constant 21°C temperature and a 12-hour light/dark cycle (lights on at 07:00). Each rat was provided with 20g of rat chow per day upon acclimation in the holding room (48-72 hours). Water was available ad libitum throughout except during behavioural sessions. All procedures followed the United Kingdom Animals (Scientific Procedures) Act 1986, Amendment Regulations 2012, and the act under project licence PPL P3B19B9D2.

Behavioural Apparatus

Training sessions, the context extinction session, and the memory retrieval session took place in eight operant boxes, each of which is in a sound-attenuating chamber (MedAssociates, VT). All boxes were the same size: 25 x 32 x 25.5cm. The boxes consisted of two Perspex walls (a door and a rear wall), a Perspex ceiling, two steel walls (the right featured a food magazine and two levers, the left featured a light), as well as a grid floor with 19 stainless steel rods (4.8mm diameter, positioned 1.6cm centre to centre). Each food magazine had an infrared detector to measure nosepokes. All rats received the same training.

Surgery

All surgeries were performed aseptically in consonance with the LASA guiding principles for aseptic surgery (LASA, 2017). Rats were anaesthetised using isoflurane (5% for induction, 2-3% for maintenance), and were administered peri-operative buprenorphine. Post-surgery, rats were housed individually with Puracel bedding overnight, before being rehoused with their

cage mates the next morning. The rats' diet was also supplemented with the non-steroidal anti-inflammatory Carprofen for two days postoperatively. A minimum of five days of recovery was allowed before experimental procedures began.

All rats were implanted with chronic indwelling stainless-steel cannulae (12.5mm, 22 gauge, Coopers Needleworks, UK). Bilateral cannulae were located at the ventral hippocampus. The coordinates for cannula implantation were anteroposterior (-5.3 mm and mediolateral ±4.5mm [relative to bregma]) and dorsoventral (-4.0 mm [relative to the skull; final coordination is -6.0mm]). A recovery period of seven days was imposed before the commencement of behavioural training and testing.

Behavioural Procedure

Training

The rats were trained for ten sessions. In each session, two levers were presented in the box. One lever was the active lever, while the other one was the inactive lever. The active lever (left or right) was assigned pseudo-randomly and counterbalanced across groups. Once assigned, the active lever did not change place during the ten-day training period. The training session started with an illumination of the house light and the presentation of levers. When the active lever was depressed, a 45mg sucrose pellet was delivered to the food magazine (FR1 schedule). The lever did not retract and no stimulus was paired with the reward. No programmed consequences occurred if the inactive lever was depressed. The training session lasted for 30 minutes or when 60 sucrose pellets were obtained - whichever occurred first. Only one training session was given to each rat per day for a total of ten days.

Infusion

Before the reactivation session (see figure 1), infusions were performed using a syringe pump and 5 µl Hamilton syringes, connected to injectors (28 gauge, projecting 2mm beyond the guide cannulae) by polyethylene tubing. Infusions of the baclofen /muscimol (0.1 mM muscimol/1.0 mM baclofen, $GABA_{A/B}$ receptor agonists, Sigma-Aldrich, dose based on a previous goal-tracking memory study (Reichelt et al., 2013) or sterile PBS vehicle (0.5 µl/side, 0.25 µl/min) were begun 30 seconds following the insertion of the injectors and were performed over two minutes. One minute of waiting time was imposed from the end of the infusion to the removal of injectors to allow diffusion of the solution away from the infusion site.

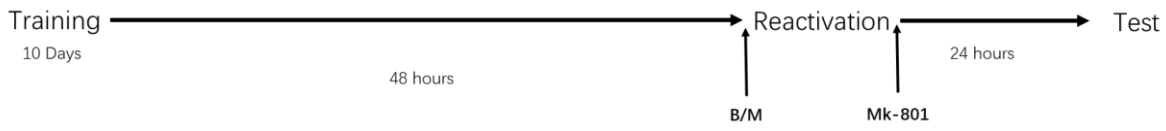


Figure 1. Schematic diagram of the experimental protocol.

Reactivation

The animals stayed in the cage for a 48-hour interval between training and reactivation. The reactivation session commenced immediately following the completion of the infusion. The reactivation session was similar to the training session, except the reinforcement schedule was shifted to the VR5 protocol (between one and nine presses for one pellet; mean = five presses). The session lasted for 20 minutes, or when 20 sucrose pellets were obtained – whichever occurred first. Half the animals received the systemic Non-competitive NMDA receptor antagonist MK-801 (0.1 mg/kg, AbCam, UK) injection immediately following the reactivation sessions; the remained animals received a saline injection (0.1ml/kg).

Post-reactivation Testing

Behavioural groups were placed into the chamber 24 hours following the reactivation for a 30-minute session. The levers were extended and the house lights were illuminated during the session. However, no sucrose pellet was delivered when the lever was pressed. No limitation was imposed upon the lever pressing; CO_2 was used to cull the rats following the experiment.

Cresyl violet staining and verification of the cannulae placements

Rats' brains were carefully removed after CO_2 procedure and stored in 4% paraformaldehyde (PFA) solution overnight at 4^{o}C. The brains were cut into 40 μm slices at -20°C. The slide-mounted brain sections were put into 95% ethanol for 15 minutes, then switched to 70% ethanol for 1 minute, and then into two distilled water tanks for 2 minutes and 1 minute. The slices were stained in 0.1% cresyl violet (Sigma) solution for 3-10 minutes, and rinsed in a third distilled water tank for 1 minute. Then the brain slice was decolorised in 50% ethanol for 1 minute, 70% ethanol for 2 minutes, 95% ethanol for 2 minutes, and finally in 100% ethanol for 1 minute. The slices were left in xylene until mounted with DePeX mounting medium in a fume hood. Brain slices were observed using an Olympus BX50 light microscope attached to a Leica DFC425 camera. The cannulae placements were marked in figure 2.

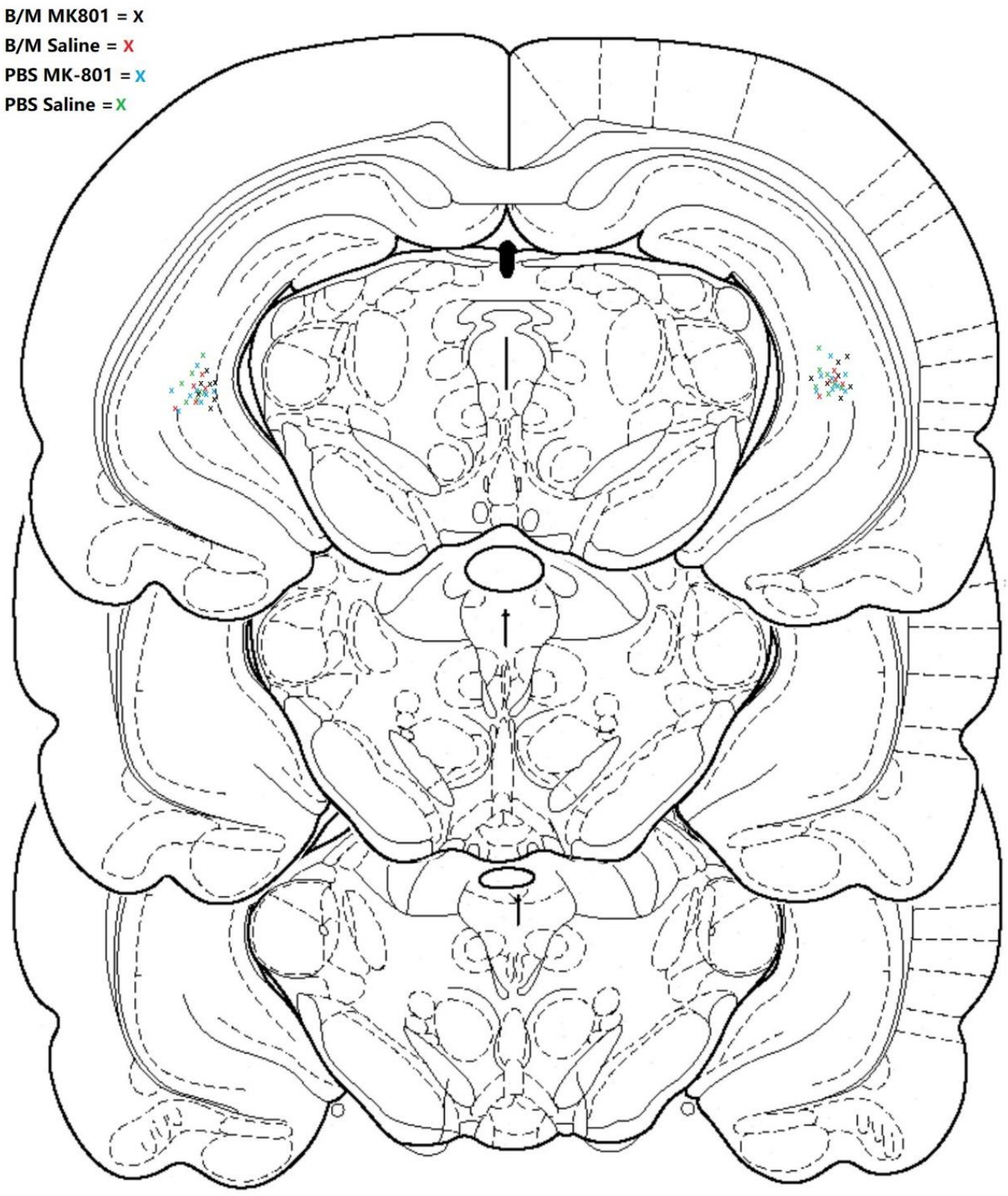


Figure 2. Schematic of the brain. Black crosses indicate the location of injector tips.

Statistical Analysis

Data were presented as mean + S.E.M. for the number of lever presses at each training session, retrieval session, and test session. All data were checked for consistency, and any data lying more than two standard deviations from the mean were treated as outliers and excluded. All analysis was conducted using the IBM SPSS statistic 25 program. The training data were analysed by repeated measures ANOVA to access whether the tasks were learned and whether all experimental groups (B/M-MK-801 n =7, B/M-saline n= 5, PBS-MK-801 n=8, PBS-saline n = 7) performed similarly; the lever, training days, lever, B/M treatment, and MK-801 treatment were used as factors. Reactivation data and test data were analysed using a three-way ANOVA, with lever (active vs. inactive), b/w treatment (B/M or PBS), and drug treatment (MK-801 vs. saline) used as factors. The reactivation and the test session's data were further analysed within each infusion group, using lever and MK-801 treatment as factors in a two-way ANOVA.

Results

To investigate the potential role of vHPC in well-learned instrumental memory reconsolidation, we infused B/M in vHPC before reactivation. The result was as follows: (figure 3).

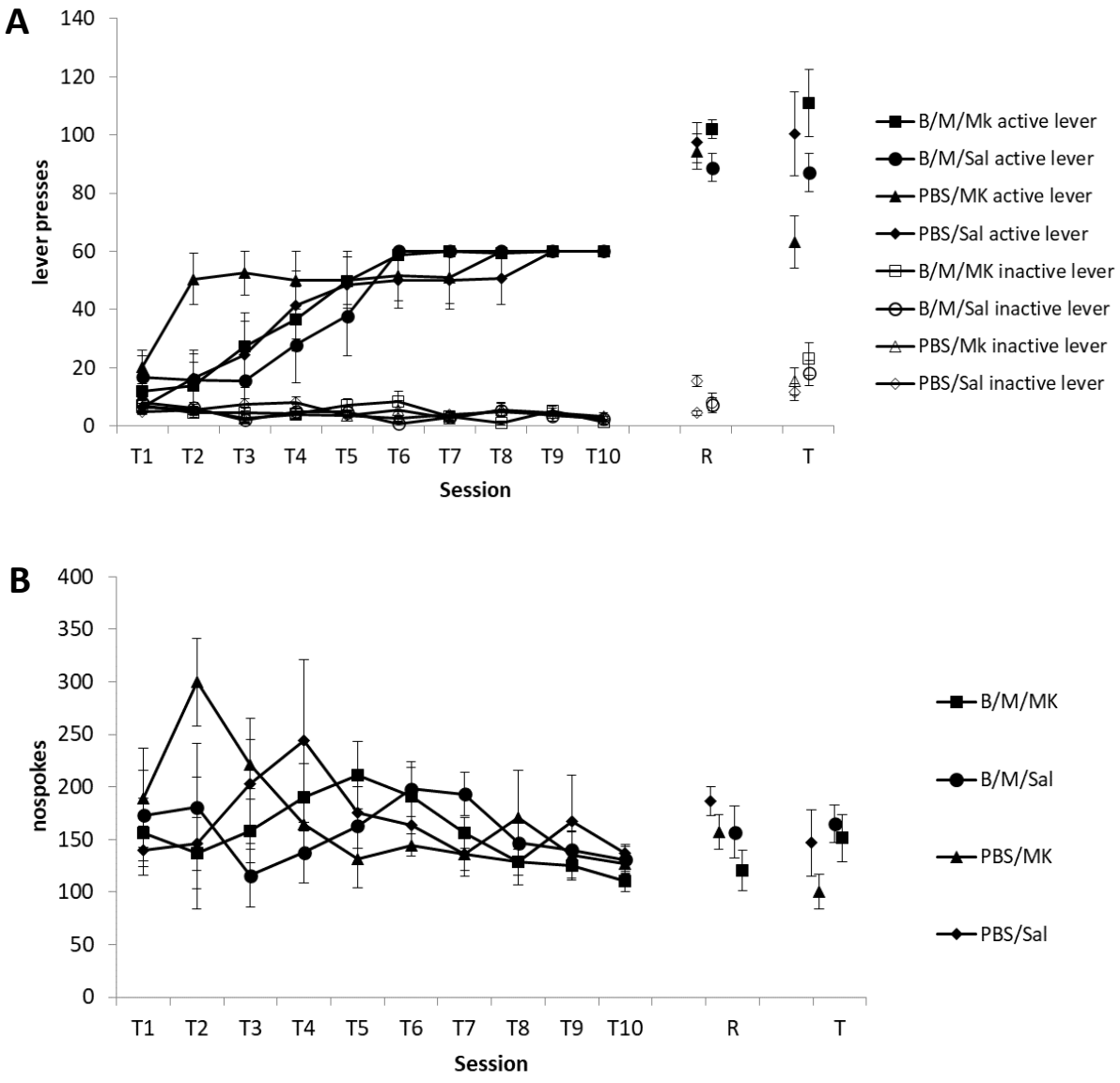


Figure 3. A. B/M group did not have a lever press difference in the test, but the PBS group did (B/M-MK-801 n =7, B/M-saline n= 5, PBS-MK-801 n=8, PBS-saline n = 7). B. There was no difference in nosepokes.

All groups exhibited a similar performance in the training session. The ANOVA revealed there was no significant interaction between lever x training x infusion treatment x MK-801 treatment ($F_{(3.4, 65.1)}$ =1.38, p=0.256). As a planned comparison, no lever x MK-801 treatment

x training interaction was found within the B/M group ($F_{(2.5, 22.9)}$ =0.47, p=0.678), but there was a significant difference found in the PBS group ($F_{(3.0, 30.0)}$ =3.20, p=0.037). Further analysis showed the MK-801 treatment offered no effect upon active lever pressing in the PBS group ($F_{(2.8, 28.0)}$ =2.41, p=0.092) (see Fig. 3a). The number of nosepokes did not differ in training (training x infusion treatment x MK-801 treatment, $F_{(4.4, 78.6)}$ =1.53, p=0.198) and no difference was found in the planned comparison (B/M, $F_{(3.3, 29.3)}$ =0.71, p=0.567; PBS, $F_{(3.5, 31.5)}$ =1.13, p=0.357) (see Fig. 3b).

The MK-801 group after B/M infusion finished the session with higher lever pressings than the saline group. No behavioural difference was found in the groups with PBS infusion. All animals acquired all rewards during the reactivation sessions. In reactivation, neither had any effect on lever presses in the reactivation (lever x infusion treatment x MK-801 treatment, $F_{(1, 20)}$ =2.27, p=0.148; active lever, infusion treatment x MK-801 treatment, $F_{(1, 20)}$ =2.34, p=0.142; inactive lever, infusion treatment x MK-801 treatment, $F_{(1, 20)}$ =0.003, p=0.954). As a planned comparison, the MK-801 group exhibited significantly higher lever pressings than the saline group within the B/M group. The MK-801 treatment also affected the active lever (lever x MK-801 treatment, $F_{(1, 10)}$ =6.09, p=0.033; MK-801 treatment on the active lever, $F_{(1,10)}$ =6.09, p=0.033). There was no difference in lever pressings in the PBS group (lever x MK-801 treatment, $F_{(1, 10)}$ =0.16, p=0.701; MK-801 treatment on the active lever, $F_{(1,10)}$ =0.11, p=0.748) (see Fig. 3a). No significant effect was found in nosepokes in the reactivation session (infusion treatment x MK-801 treatment, $F_{(1, 20)}$ =0.04 p=0.841; B/M, $F_{(1, 10)}$ =1.43, p=0.260; PBS, $F_{(1, 10)}$ =1.78, p=0.212) (see Fig. 3b). Following the B/M infusion, although the MK-801 group had higher active lever pressings, all rats finished the session before the time expired; they received the same amount of reinforcement. Therefore, this difference was due to chance and unlikely to affect the results of the test.

A significant difference in lever pressing was seen in the test. There was an overall effect on

the lever pressing (lever x infusion treatment x MK-801 treatment, $F_{(1, 20)}=6.65$, $p=0.018$). Infusion treatment and MK-801 treatment had a significant interaction on active lever pressing (infusion treatment x MK-801 treatment, $F_{(1, 20)}=7.24$, $p=0.014$), but not on inactive lever pressing (infusion treatment x MK-801 treatment, $F_{(1, 20)}=0.02$, $p=0.904$). However, neither infusion nor MK-801 treatment showed any simple main effect on active lever pressing (infusion treatment, $F_{(1, 20)}=2.34$, $p=0.142$; MK-801 treatment, $F_{(1, 20)}=0.34$, $p=0.566$).

Planned comparisons based on the infusion group were subsequently conducted. The ANOVA revealed that the MK-801 group had no significant lever-pression differences when compared to the saline group in the B/M group, and the MK-801 treatment had no effect on active lever pressings (lever x MK-801 treatment, $F_{(1, 10)}=1.32$, $p=0.277$; MK-801 treatment on the active lever, $F_{(1, 10)}=2.58$, $p=0.139$). The PBS group showed significantly reduced lever pressings after the MK-801 treatment, and the MK-801 treatment on the boundary of a simple effect for active lever pressing (lever x MK-801 treatment, $F_{(1, 10)}=6.31$, $p=0.031$; MK-801 treatment on the active lever, $F_{(1,10)}=4.72$, $p=0.055$) (see Fig. 3a). As a control parameter, the nosepoke data revealed no significant difference in each group (infusion treatment x MK-801 treatment, $F_{(1, 20)}=0.68$ $p=0.419$; B/M, $F_{(1, 10)}=0.07$, $p=0.802$; PBS, $F_{(1, 10)}=1.72$, $p=0.219$) (see Fig. 3b).

The data showed that lever-pression ability was only impaired in the PBS group. The MK-801-induced impairment was found in the PBS group but not in the B/M group. This may indicate that the B/M infusion prevents the impairment which suggests that the vHPC may be involved in the memory destabilisation process.

Discussion

The present data indicated that an infusion of B/M prevented MK-801-induced lever-pression impairment in the MK-801 group. Therefore, the inactivation of the vHPC was found to reverse the lever-pression behaviour in the MK-801 group which may be due to the inactivation of vHPC impairing the instrumental memory destabilisation.

Behavioural impairment was reactivation-dependent.

The first research question asks whether the impairment of active lever pressing was reactivation-dependent. Although we did not include the non-reactivation group, the non-reactivation controls in the previous chapter (Chapter Two, Experiment 1) were utilised as a control for the current experiment. It is common for destabilisation studies not to include non-reactivation controls; they tend to use groups that have failed to trigger the reconsolidation as controls (Debiec et al., 2002; Lee et al., 2004; Reichelt et al., 2013; Wells et al., 2011). In this study, the B/M groups that did not show any sign of impairment could be the control groups for the PBS groups, which showed lever-pression impairment. Hence, the impairment of active lever pressing in the PBS-MK-801 group was reactivation-dependent.

Another perspective of the results may be whether the difference in active lever pressing in the reactivation session would affect the test result. This counter-argument is offered since the B/M-MK-801 group exhibited higher active lever pressing and all subjects completed the reactivation session before the allotted time elapsed. Therefore, it was not the saline group that gave up on the lever pressing: it was that the session ended before the time expired; this also meant that all subjects received the same number of reinforcements. Consequently, this difference was unlikely to be caused by any behavioural differences during the test.

As the lever-pression difference was reactivation dependent, the most reasonable explanation for the B/M groups' result was that the inactivation of the vHPC resulted in the impairment of

destabilisation of the well-learned instrumental memory. The reconsolidation process includes the memory being destabilised into a labile stage, before being reconsolidated to persist in the brain (Lewis, 1979; Nader, 2003). The B/M-MK-801 group, which showed little evidence of impairment, suggested that the reconsolidation was not triggered; this was likely to be due to the impairment of destabilisation. If the destabilisation was impaired, the memory would not be destabilised; this means the memory would be intact, which should have a similar lever pressing to the PBS-saline group. As no statistical difference was found between the groups, the inactivation of vHPC was likely to be the cause of the destabilisation impairment during the reactivation. On the other hand, if the reconsolidation is impaired, this will cause amnesia, as seen in the PBS-MK-801 group, in which the active lever pressing was impaired. As this finding was not observed in the B/M-saline group, the inactivation of the vHPC was unlikely to be the cause of the reconsolidation impairment. Therefore, this showed that the vHPC function may be destabilisation related.

Potential role of vHPC in instrumental memory destabilisation

There were several ways to explain the potential role played by vHPC in instrumental memory reconsolidation. One of these possibilities is based on the prediction error hypothesis. Several studies suggest that in triggering the reconsolidation, the reactivation parameter should be different from the training parameter (Exton-McGuinness et al., 2015; Gisquet-Verrier et al., 2015; Lee 2009; Reichelt & Lee, 2013a; Waelti, Dickinson, & Schultz, 2001). In further studies, the no-reward extinction session which is normally used as a reactivation parameter was shown to be unable to destabilise well-learned instrumental memory (Flavell & Lee, 2013; Hernandez & Kelley, 2004; Mierzejewski et al., 2009). This may be due to insufficient prediction error signalling because no reward was given during the session. With a change of reward contingency, a variant ratio protocol but not a fixed ratio

with a similar number of total rewards was shown to successfully trigger the destabilisation of the well-learned instrumental memory (Exton-McGuinness et al., 2014). This was explained as a Temporal Difference (TD) error signal which sees the prediction error as the prediction of the possibility of future reward rather than the total number of accumulated rewards (Glimcher, 2011; Sutton & Barto, 2018). It is suggested that this prediction error signal is related to dopamine neurons in VTA and in SNc (Glimcher, 2011). The impairment of destabilisation may relate to the prediction error signal.

However, there was little evidence of neuroanatomy between VTA and vHPC. The dorsal hippocampus/BLA-vHPC-Nac circuit is the suggested response for contextual and spatial information processing, whereas the A8 and A10 DA in VTA which is the suggested response for prediction error signal is projected to vmPFC and Nac (Cooper et al., 2017; Glimcher, 2011). However, some instrumental behavioural studies show that vHPC may directly or indirectly affect the VTA-vmPFC/Nac circuit. First, vHPC and VTA are active in extensive instrumental training, and both are involved in habit formation (Faure et al., 2006). In instrumental extinction learning, the vHPC-IL circuit was required for the renewal of heroin-seeking (Wang et al., 2018). Asymmetric lesion of vHPC and mPFC or vHPC and BLA impairs the renewal of conditioned freezing (Orsini et al., 2011). Further evidence shows those aversive and appetitive behaviours are also modulated by the vHPC-Nac circuit (Pascoli et al., 2014; Vezina et al., 1989). Together, this may suggest the vHPC is required for a prediction error signal circuit and may be able to indirectly affect the circuit by modulating mPFC and Nac.

There is an alternative explanation for the B/M group result: vHPC inactivation may negate the facilitation effect and shift the behaviour toward direct reactivation. In chapter 2, there is the hypothesis that any protocol during the gap day will disrupt the facilitation effect. However, the B/M infusion was 30 minutes before reactivation which is the same as the MK-

801 injection in the delayed reaction group in Chapter 2, so it is unlikely that this will disrupt the facilitation effect. Furthermore, the direct reactivation group had lower active lever pressing than the delayed reactivation group in the test in Chapter 2. If the PBS group can be thought of as a delayed reactivation group, such a difference in active lever pressing was not found in this study. Therefore, vHPC inactivation is unlikely to affect the facilitation effect.

Conclusion

This may be the first study to show the vHPC to be involved in memory reconsolidation. The vHPC suggests the new role, which is a response to instrumental memory destabilisation apart from the integration of contextual and spatial information. This may be due to vHPC indirectly modulating VTA-Nac circuit. But further studies are needed to confirm whether this hypothesis is true or whether vHPC is required for other memory reconsolidation.

General Discussion

Memory can be destabilised, updated, and reconsolidated back to a stable state. In this study, we noted the well-learned instrumental memory can be destabilised in both one-lever and two-lever models by several different procedures. These results supported the prediction error hypothesis (Exton-McGuinness & Lee, 2015; Exton-McGuinness et al., 2015; Lee, 2009) and showed the VR20 procedure (12–28 lever presses for a sucrose pellet) is not the only procedure that can destabilise well-learned instrumental memory (Exton-McGuinness et al., 2014), whereas the normal extinction session cannot destabilise such memory (Flavell & Lee, 2013; Hernandez & Kelley, 2004; Mierzejewski et al., 2009). Through our experiments, we found the VR5 procedure (1–9 lever presses for a sucrose pellet) with delayed reactivation which takes a gap day after training is the most reliable method to destabilise the well-learned instrumental memory. This presumes that the gap day facilitates instrumental memory destabilization. We also found several different procedures, like inserting a reactivation session or context extinction session or even handling and giving a saline injection on the gap day may disrupt the facilitation effect, but a memantine injection preserves the effect. Overall, the mechanism of this facilitation effect is still unclear and needs further study. We also investigated the neural circuits that may be involved in well-learned memory reconsolidation. Our results suggest the ventral hippocampus (vHPC) may be involved in instrumental memory destabilization. Our immunohistochemistry data may suggest the prelimbic (PL) and infralimbic (IL) may be involved in instrumental memory reconsolidation, but this experiment is low on statistical power, so the results need further experiments to support them.

The two-lever model is the most suitable for the study of well-learned instrumental memory

In this study, we used two models: the one-lever model, and the two-lever model, to study well-learned instrumental memory reconsolidation. The one lever model was used in the Exton-McGuinness et al (2014) studies and was shown to be capable of destabilising the well-learned instrumental memory. Cheng et al.'s (2022) study showed that context extinction can destabilise a well-learned instrumental memory, but direct reactivation cannot; chapter 1 experiment 2 showed that delayed reactivation can destabilise a well-learned instrumental memory, but context extinction cannot, and experiment 3 showed that direct reactivation can destabilise a well learned instrumental memory but delayed reactivation cannot. The same procedure does not have a consistent result for destabilizing instrumental memory in the one-lever model. Thus, the one-lever model may be not suitable for the VR5 procedure in further well-learned instrumental memory reconsolidation studies.

For the two-lever model, the VR5 delayed reactivation procedure produced a very consistent result. In chapter 2 experiment 1, only the delayed reactivation group destabilised the instrumental memory. In experiment 3, the memantine group, which also followed the procedure of delayed reactivation, destabilised the instrumental memory and in chapter 3, the PBS infusion group, which also used delayed reactivation, destabilized the instrumental memory. Those results show that delayed reactivation can consistently destabilise instrumental memory whereas other procedures such as double reactivation, direct reactivation, and context extinction cannot.

It is worth noting that with the PBS injection during the gap day the animals did not show signs of instrumental memory destabilization (chapter 2 experiment 3), and this may be explained by the disruption of the delayed reactivation procedure rather than the inconsistent result of delayed reactivation. First, the memantine group also undertook delayed reactivation in the same experiment to destabilise the instrumental memory and the PBS infusion before the reactivation did not prevent instrumental memory destabilization (chapter 3). So, it is more likely that the time point of the PBS injection disturbs the memory destabilization rather than the PBS injection itself disturbing the memory destabilization. Hence this memantine experiment (chapter 2 experiment 3) is more likely to be interpreted as the memantine preserving the gap day facilitation effect but handling may disrupt it rather than the delayed reactivation having inconsistent results.

Overall, the two-lever model is appropriate for further studies of well-learned instrumental memory reconsolidation and boundary conditions using a VR5 reactivation after a delay (gap day).

A gap day between training and reactivation influences instrumental memory destabilization

The reason that delayed reactivation can destabilise the well-learned instrumental memory is assumed to be the delay (gap day) facilitating the instrumental memory destabilization. Thus, the likely explanation is that the gap day facilitates instrumental memory destabilization. The exact mechanism for this effect is still unknown, but we can rule out several possibilities through our results. However, this effect seems easy to disrupt; the procedures carried out on gap day, even just handling and the giving of a saline injection (chapter 2 experiment 3), can

disrupt the effect and be unable to trigger the instrumental memory destabilisation. But a memantine injection during the gap day did not prevent the destabilisation; rather it preserves or facilitates the destabilization (chapter 2 experiment 3). This may suggest handling and/or injection of the animal during the gap day prevents the instrumental memory destabilisation, but the memantine injection interferes with the effect of handling and/or injection or facilitates the instrumental memory destabilisation. Furthermore, as memantine is an NMDA receptor antagonist, this may suggest NMDA receptor activation is important for instrumental memory destabilisation not only during the reactivation session but also during the interval before the reactivation session.

In our null hypothesis that context extinction can destabilise the instrumental memory because the context extinction session weakens the context-reward memory which may compete with instrumental memory. This hypothesis is rejected by the double reactivation experiment (chapter 2 experiment 2); that there was no sign of instrumental memory destabilization may suggest the direct reactivation of VR5 was unable to destabilise the competitive memory or that there was no competitive memory for the instrumental memory. In either case, we can exclude the trace dominance hypothesis in the well-learned instrumental memory reconsolidation.

Overall, our results may rule out the trace dominance hypothesis and suggest the ongoing activity in the gap day may interfere with destabilisation, but the mechanism for this effect remains unclear and the involvement of NMDA may need further studies to investigate. The further experiment probably should first rule out the possibility of the memantine having a co-effect with MK-801. This may be done by using a non-NMDA receptor antagonist amnesic agent, like anisomycin or midazolam, before reactivation. To investigate the mechanism, as the results suggest the NMDA receptor activity is important, it may be worth infusing the memantine into specific brain regions, like vmPFC, vHPC, Nac, and VTA. This

may be able to facilitate the mapping of the neural circuit involved and deduce the mechanism of this facilitation effect.

Either this effect was unique to instrumental memory, or a generalisation effect applying to all memories was unknown. The application of the drug-seeking study showed the delayed reactivation VR5 procedure was able to reactivate the memory (Exton-McGuinness et al., 2019), but there was no result regarding whether a direct reactivation VR5 was able to reactivate this memory.

Analysis of neural circuit's base on instrumental memory reconsolidation

In the general introduction, we introduced several brain regions that may be involved in the well-learned instrumental memory reconsolidation. Combining the results from chapter 1 experiment 1 and chapter 3, several brain regions may be involved in instrumental memory destabilization and reconsolidation. The only reliable result within those is that the vHPC is likely to be involved in the instrumental memory destabilization. This is in contrast to our forecast, as the common role of vHPC is suggested as the integration of contextual and spatial information from the dorsal hippocampus (dHPC) and basal lateral amygdala (BLA) (French et al., 2003; French & Totterdell, 2003; Oleskevich et al., 1989).

For the destabilization of appetitive memory, it is suggested that the ventral tegmental area (VTA) and nucleus accumbens (Nac) are involved in this (Exton-McGuinness & Lee, 2015; Reichelt et al., 2013). The dopaminergic projection from VTA to Nac is important for the prediction error signal (Watabe-Uchida et al., 2017), and this prediction error is suggested in different studies to be required to trigger memory destabilization (Exton-McGuinness et al., 2015; Exton-McGuinness et al., 2014; Reichelt et al., 2013). In instrumental memory, the Nac

core but not the shell was suggested to be required for destabilization (Ramon, unpublished date). As the vHPC and Nac core may be involved in the instrumental memory stabilisation, it may suggest that the vHPC and Nac core are in a neural circuit required for instrumental memory destabilization. The vHPC has a glutamatergic projection to Nac, but the current result does not suggest any direct projection between VTA and vHPC (Cooper et al., 2017; Watabe-Uchida et al., 2017). However, some pieces of evidence show that both VTA and vHPC project to the ventral mediate prefrontal cortex (mvPFC) (Cooper et al., 2017; Watabe-Uchida et al., 2017). vmPFC is suggested as important for instrumental learning (Kalivas & Volkow, 2005; McLaughlin & See, 2003; Peters et al., 2009) and the VTA-vmPFC-Nac circuit is important for prediction error signalling (Watabe-Uchida et al., 2017). So, the involvement of vHPC on instrumental memory destabilization may be direct or indirect (like via vmPFC) modulate Nac, (figure 1).

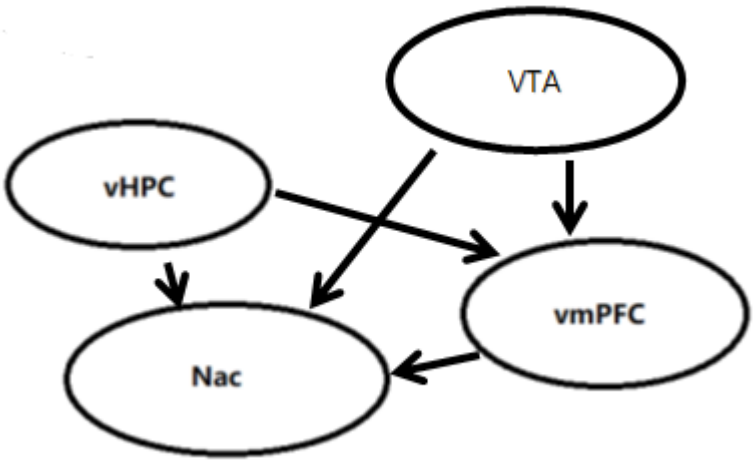


Figure 1. The neural circuit may be involved in well-learned instrumental memory reconsolidation.

For immunohistochemistry data, the expression of zif268 is highly overlapped between the context extinction group and the direct reactivation group, but due to the inconsistent results of direct reactivation and context extinction in the one-lever model, we cannot have a reliable explanation for this data. But taking into account the previous discussion, ongoing neural

activity during the gap day may be important for instrumental memory reconsolidation and reactivation sessions on the gap day may disrupt the effect. The result of the non-reactivation group can be seen as perfused at a similar time as the memantine injection may suggest some brain regions play a role in the facilitation effect. As the striatum is the only region to have no difference in zif268 expression among the three groups, it may be involved in this facilitation effect. It is not surprising if the striatum is involved in such an effect, as elucidated in the general discussion, both the dorsal striatum and Nac are found important in different instrumental learning studies. Furthermore, the Nac core is also required for instrumental memory destabilization (Ramon, unpublished data), so the immunohistochemistry data may support those findings. But it is worth noting that these immunohistochemistry data have low statistical power and further studies are required to confirm the hypothesis.

There is a need to study the neural circuits that may be involved in instrumental memory destabilization and reconsolidation. First, we have a hypothesis about whether the vHPC may directly or indirectly modulate Nac for instrumental memory destabilization. This may be examined by using the asymmetric lesion of vHPC and Nac versus the lateral lesion. Apart from the lesion, optogenetic tools may be a good way to further investigate the involvement of vHPC. As the neural circuits can be specifically turned on and off in response to the colour of the light (Adamantidis et al., 2011; Duebel et al., 2015; Stuber et al., 2012; Wang et al., 2014), the animal may maintain normal brain function during the training and test, but the target brain region can be turned off during the reactivation. This may be further confirmation of the target brain region involved in destabilization and/or reconsolidation. Furthermore, since the optogenetic tools can direct control of a specific subset of neurons (like D1- or D2-like dopaminergic neurons) (Duebel et al., 2015; Tsai et al., 2009), we can use this to figure out the role of the striatum in the reactivation. Since we do not spot any zif268 expression difference between the test groups and non-reactivation control (chapter 1 experiment 1), but

the Nac shell is shown to be required for instrumental memory destabilization, so there may be a change of activated cell type in the striatum during the reactivation session. Though our immunohistochemistry result has low statistical power, it suggests the vmPFC may be involved in instrumental memory reconsolidation. It is also suggested that the vmPFC may be involved in destabilization, so the vmPFC is a valuable target. It is worth noting that just as the IL and PL are suggested as having different functions in other instrumental learning studies (see general introduction), they may also have different roles in destabilization and reconsolidation. In simple terms, the PL is mainly projected to the Nac core and IL mainly projects to the Nac shell (Bouton et al., 2021; Cooper et al., 2017; Sesack & Grace, 2010). As the Nac core was shown to be required for destabilization but the shell was not (Ramon, unpublished data), PL may be more involved in destabilization and IL may be more involved in reconsolidation.

For further immunohistochemistry experiments, the delayed reactivation group in the two-lever model may be used as the test group and the direct reactivation group and non-reactivation group may be used as negative controls. The target proteins may use both zif268 and c-fos, as the single protein expression may not reflect the neural activity, but co-activation of zif268 and c- is more likely to indicate neural activity. As our experiments suggest both vHPC and Nac core are likely to be involved in instrumental memory destabilization, those two regions may be used as a positive control factor for the experiment. As suggested above, the VTA, vmPFC, and dorsal hippocampus may be good targets for further studies.

The reactivation session may be a boundary condition for instrumental memory reconsolidation

The boundary conditions of memory reconsolidation are normally considered as memory age and memory strength (Lee, 2009). The older memory or stronger memory is harder to destabilise (see the general introduction for detail). This is also observed in instrumental memory reconsolidation. In the one-lever model, the weak 2-days-trained instrumental memory can be destabilized by direct VR5 reactivation (Exton-McGuinness & Lee, 2015) and the well-trained (10 days) instrumental memory cannot consistently destabilised by the VR5 procedure (Cheng et al., 2022). By increasing the reactivation session strength, i.e. increasing the exposure length for context fear memory (Suzuki et al., 2004), and increasing the contingency change (to VR20) for well-learned instrument memory (Exton-McGuinness et al., 2014), the memory is not subject to destabilisation. For well-trained instrumental memory, this may be due to its generating a large prediction error signal (compare FR20 to VR20), see the general introduction for detail, (Exton-McGuinness et al., 2014), or the lower reward per unit time diminished the context-reward association, thus favouring the destabilisation of the instrumental memory (compare VR20 to VR5, (Cheng et al., 2022).

As a larger contingency change can destabilise a well-learned instrumental memory and a mild contingency with the same condition cannot, the reactivation session length/strength may be also a boundary condition for memory reconsolidation (see the general introduction for detail). In the conditioned reinforcement experiment by increasing the lever presses in the reactivation session, the conditioned reinforcement memory will be triggered from memory reconsolidation, then to an intermediate state, which shows no sign of memory reconsolidation or generation of extinction memory, and will finally generate the extinction memory (CS-no reward) (Flavell & Lee, 2013). There is no result to show whether this can

be applied to instrumental memory reconsolidation, but the same lever pressings in the extinction reactivation session that would destabilise the conditioned reinforcement memory (10 presses) or generate the extinction memory (50 presses) do not destabilise or generate extinction memory for the five-day trained instrumental memory (Flavell & Lee, 2013). This may be due to a minimum number of exposures required to destabilise the memory (Reichelt & Lee, 2013b; Suzuki et al., 2004). Hence, a certain number of lever-pressings associated with rewards may be required to destabilise the instrumental memory. So, the VR5 with a lower number of lever presses (around 100) can destabilise weak-learned instrumental memory (two-day training) (Exton-McGuinness & Lee, 2015), whereas the well-learned instrumental memory (ten-day training) needs a higher number of lever presses (around 300) to destabilise (Exton-McGuinness et al., 2014). However, in the two-lever model, the MK-801 group with VR20 had a similar number of active lever presses (around 350 active lever presses, 20 inactive lever presses) but did not show signs of instrumental memory destabilization and did not show the sensitivity of devaluation as in the one-lever model (Cheng, unpublished data). One of the possibilities of this result is that inactive lever presses reducing the possibility of reward delivery make VR20 more prone to promote an extinction session. Nor can the direct reactivation of VR5 destabilise the well-learned instrumental memory in the two-lever model, but by inserting a gap day, VR5 can consistently destabilise the well-learned instrumental memory (chapter 2 experiment 1). As the inactive lever presses in the two-lever model can be seen as additional lever presses in the reactivation session, the VR20 cannot destabilise the instrumental memory, possibly due to its shift to the intermediate state, whereas the delayed VR5 reactivation is shifted to a suitable state which is able to trigger the instrumental memory destabilization (figure 2).

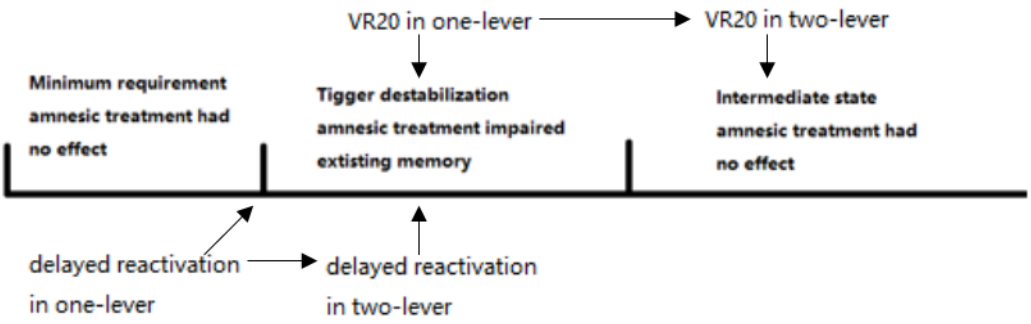


Figure 2. The hypothesis of boundary conditions may be in the one-lever and two-lever models.

If this hypothesis is correct and the gap day is facilitating the instrumental memory destabilisation, there may be a contingency change between the VR5 and VR20 that can direct destabilise the well-learned instrumental memory. Thus, further studies may begin with a direct VR10 reactivation in a two-lever model to determine whether this can destabilise the instrumental memory. But worth noting, our VR5 procedure is semi-random, the lever presses per reward are randomly chosen from 1 to 9 but not 20% per lever press. This means the rat will get a reward with a maximum of 9 lever presses. Otherwise, with the 20% possibility, some rats may press the lever too many times before getting the first reward, which would make the procedure more like an extinction session.

Conclusion

In this study, we found out that delayed VR5 reactivation where the VR5 reactivation session takes place 48 hours after training can consistently destabilise the well-learned instrumental memory. This may be a model for further research for well-learned instrumental memory reconsolidation. The 48-hour interval may facilitate instrumental memory destabilization. This is unlikely to be due to competitive memory interference and likely to be due to ongoing neuronal plasticity, especially inversely related to NMDA receptor activity. We do not have a clear account of the process and mechanism underpinning this facilitation effect, but we found this effect can be disrupted by handling and injection of PBS during the interval. Furthermore, we found the destabilization of well-learned instrumental memory is likely to be required for ventral hippocampus activity. This could be a positive control for further immunohistochemistry studies or a target for neuroanatomy study.

Acknowledgements

I'd like to thank my tutor Jonathan Lee for all his effort in helping me in this project. I would also like to thank my secondary supervisor Charlotte Law (Charlotte Flavell) for her help with my immunohistochemistry experiment. I want specially to thank Marc Exton-McGuinness for his collaborative research and his help since my master's degree. We had a lot of constructive discussion through my whole project. David Barber gave me a lot of technical support in this project. Specially thank for Anne Nicholls who helped me for proofreading my thesis. Finally, I want to thank my parents who always support me.

References

Adams, C. D. (1982). Variations in the sensitivity of instrumental responding to reinforcer devaluation. *The Quarterly Journal of Experimental Psychology B: Comparative and Physiological Psychology, 34B*(2), 77-98. doi:10.1080/14640748208400878

Adamantidis, A. R., Tsai, H. C., Boutrel, B., Zhang, F., Stuber, G. D., Budygin, E. A., Touriño, C., Bonci, A., Deisseroth, K., & de Lecea, L. (2011). Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. J Neurosci, 31(30), 10829-10835. https://doi.org/10.1523/jneurosci.2246-11.2011

Agranoff, B. W., Davis, R. E., & Brink, J. J. (1966). *Chemical studies on memory fixation in goldfish* doi:https://doi.org/10.1016/0006-8993(66)90095-3

Agren, T. (2014). *Human reconsolidation: A reactivation and update* doi:https://doi.org/10.1016/j.brainresbull.2013.12.010

Alaghband, Y., & Marshall, J. F. (2013). Common influences of non-competitive NMDA receptor antagonists on the consolidation and reconsolidation of cocaine-cue memory. *Psychopharmacology, 226*(4), 707-719. doi:10.1007/s00213-012-2793-y [doi]

Amaral, D. G. (1995). The rat nervous system. *Hippocampal Formation, ,* 443-493.

Ambroggi, F., Ishikawa, A., Fields, H. L., & Nicola, S. M. (2008). Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. *Neuron, 59*(4), 648-661. doi:10.1016/j.neuron.2008.07.004 [doi]

Anagnostaras, S. G., Gale, G. D., & Fanselow, M. S. (2001). Hippocampus and contextual fear conditioning: Recent controversies and advances. *Hippocampus, 11*(1), 8-17. doi:10.1002/1098-1063(2001)11:13.0.CO;2-7

Anderson, M. C., Bjork, R. A., & Bjork, E. L. (1994). Remembering can cause forgetting: Retrieval dynamics in long-term memory. *Journal of Experimental Psychology.Learning, Memory, and Cognition, 20*(5), 1063-1087. doi:10.1037//0278-7393.20.5.1063 [doi]

Balleine, B. W., & Killcross, S. (2006). Parallel incentive processing: An integrated view of amygdala function. *Trends in Neurosciences, 29*(5), 272-279.

Balleine, B. W., & Dickinson, A. (1998). Goal-directed instrumental action: Contingency and incentive learning and their cortical substrates. *Neuropharmacology, 37*(4-5), 407-419. doi:10.1016/s0028-3908(98)00033-1 [doi]

Balleine, B. W., Killcross, A. S., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 23*(2), 666-675. doi:23/2/666 [pii]

Balleine, B. W., & Dickinson, A. (1998). The role of incentive learning in instrumental outcome revaluation by sensory-specific satiety. *Animal Learning & Behavior, 26*(1), 46-59. doi:10.3758/BF03199161

Baxter, M. G., & Murray, E. A. (2002). The amygdala and reward. *Nature Reviews Neuroscience, 3*(7), 563.

Belin, D., & Everitt, B. J. (2008). Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. *Neuron, 57*(3), 432-441. doi:10.1016/j.neuron.2007.12.019 [doi]

Ben Mamou, C., Gamache, K., & Nader, K. (2006). NMDA receptors are critical for unleashing consolidated auditory fear memories. *Nature Neuroscience, 9*(10), 1237-1239. doi:nn1778 [pii]

Besnard, A., Laroche, S., & Caboche, J. (2014). Comparative dynamics of MAPK/ERK signalling components and immediate early genes in the hippocampus and amygdala following contextual fear conditioning and retrieval. Brain Struct Funct, 219(1), 415-430. https://doi.org/10.1007/s00429-013-0505-y

Besnard, A., Caboche, J., & Laroche, S. (2012). Reconsolidation of memory: A decade of debate. Progress in Neurobiology, 99(1), 61-80. https://doi.org/https://doi.org/10.1016/j.pneurobio.2012.07.002

Bianchin, M. M., Spanis, C. W., Roesler, R., McGaugh, J. L., & Izquierdo, I. (2000). (+/-)-Alpha-methyl-4-carboxyphenylglycine, a metabotropic glutamate receptor blocker, impairs retention of an inhibitory avoidance task in rats when infused into the basolateral nucleus of the amygdala. *Brain Research, 852*(2), 436-443. doi:S0006-8993(99)02220-9 [pii]

Bjordahl, T. S., Dimyan, M. A., & Weinberger, N. M. (1998). Induction of long-term receptive field plasticity in the auditory cortex of the waking guinea pig by

stimulation of the nucleus basalis. *Behavioral Neuroscience, 112*(3), 467-479. doi:10.1037//0735-7044.112.3.467 [doi]

Blaiss, C. A., & Janak, P. H. (2007). *Post-training, but not post-reactivation, administration of amphetamine and anisomycin modulates pavlovian conditioned approach* doi:https://doi.org/10.1016/j.nlm.2006.12.007

Bossert, J. M., Marchant, N. J., Calu, D. J., & Shaham, Y. (2013). The reinstatement model of drug relapse: Recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology, 229*(3), 453-476. doi:10.1007/s00213-013-3120-y

Bossert, J. M., & Stern, A. L. (2014). Role of ventral subiculum in context-induced reinstatement of heroin seeking in rats. Addiction biology, 19(3), 338-342. https://doi.org/10.1111/adb.12015

Bossert, J. M., Stern, A. L., Theberge, F. R. M., Cifani, C., Koya, E., Hope, B. T., & Shaham, Y. (2011). Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. Nature neuroscience, 14(4), 420-422. https://doi.org/10.1038/nn.2758

Bouton, M. E., Maren, S., & McNally, G. P. (2021). BEHAVIORAL AND NEUROBIOLOGICAL MECHANISMS OF PAVLOVIAN AND INSTRUMENTAL EXTINCTION LEARNING. Physiological reviews, 101(2), 611-681. https://doi.org/10.1152/physrev.00016.2020

Bozon, B., Davis, S., & Laroche, S. (2003). A requirement for the immediate early gene zif268 in reconsolidation of recognition memory after retrieval. Neuron, 40(4), 695-701. https://doi.org/10.1016/s0896-6273(03)00674-3

Brown, T. E., Lee, B. R., & Sorg, B. A. (2008). The NMDA antagonist MK-801 disrupts reconsolidation of a cocaine-associated memory for conditioned place preference but not for self-administration in rats. *Learning & Memory (Cold Spring Harbor, N.Y.), 15*(12), 857-865. doi:10.1101/lm.1152808 [doi]

Brown, T. E., Lee, B. R., & Sorg, B. A. (2008). The NMDA antagonist MK-801 disrupts reconsolidation of a cocaine-associated memory for conditioned place preference but not for self-administration in rats. *Learning & Memory (Cold Spring Harbor, N.Y.), 15*(12), 857-865. doi:10.1101/lm.1152808

Bruel-Jungerman, E., Davis, S., & Laroche, S. (2007). Brain plasticity mechanisms and memory: A party of four. *Neuroscientist, 13*(5), 492-505. doi:10.1177/1073858407302725

Bush, R. R., & Mosteller, F. (1951a). A mathematical model for simple learning. *Psychological Review, 58*(5), 313-323. doi:10.1037/h0054388

Bush, R. R., & Mosteller, F. (1951b). A model for stimulus generalization and discrimination. *Psychological Review, 58*(6), 413-423. doi:10.1037/h0054576

Cenquizca, L. A., & Swanson, L. W. (2007). Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. *Brain Research Reviews, 56*(1), 1-26. doi:S0165-0173(07)00073-2 [pii]

Ceretta, A. P., Camera, K., Mello, C. F., & Rubin, M. A. (2008). Arcaine and MK-801 make recall state-dependent in rats. *Psychopharmacology, 201*(3), 405-411. doi:10.1007/s00213-008-1304-7 [doi]

Chaaya, N., Battle, A. R., & Johnson, L. R. (2018). An update on contextual fear memory mechanisms: Transition between Amygdala and Hippocampus. Neurosci Biobehav Rev, 92, 43-54. https://doi.org/10.1016/j.neubiorev.2018.05.013

Charlier, Y., & Tirelli, E. (2011). Differential effects of histamine H(3) receptor inverse agonist thioperamide, given alone or in combination with the N-methyl-d-aspartate receptor antagonist dizocilpine, on reconsolidation and consolidation of a contextual fear memory in mice. *Neuroscience, 193*, 132-142. doi:10.1016/j.neuroscience.2011.07.034 [doi]

Cheng, C., Exton-McGuinness, M. T. J., & Lee, J. L. C. (2022). Procedures between training and reactivation influence the destabilization of instrumental sucrose memory [Original Research]. Frontiers in Behavioral Neuroscience, 16. https://doi.org/10.3389/fnbeh.2022.953629

Cheval, H., Chagneau, C., Levasseur, G., Veyrac, A., Faucon-Biguet, N., Laroche, S., & Davis, S. (2012). Distinctive features of Egr transcription factor regulation and DNA binding activity in CA1 of the hippocampus in synaptic plasticity and consolidation and reconsolidation of fear memory. Hippocampus, 22(3), 631-642. https://doi.org/10.1002/hipo.20926

Chiba, T. (2000). Collateral projection from the amygdalo--hippocampal transition area and CA1 to the hypothalamus and medial prefrontal cortex in the rat. *Neuroscience Research, 38*(4), 373-383. doi:S0168010200001838 [pii]

Coons, E. E., & Miller, N. E. (1960). Conflict versus consolidation of memory traces to explain "retrograde amnesia" produced by ECS. *Journal of Comparative and Physiological Psychology, 53,* 524-531. doi:10.1037/h0045920 [doi]

Cooper, R. M., & Koppenaal, R. J. (1964). Suppression and recovery of a one-trial avoidance response after a single ECS. *Psychon.Sci., 1,* 303-304.

Cooper, S., Robison, A. J., & Mazei-Robison, M. S. (2017). Reward Circuitry in Addiction. Neurotherapeutics, 14(3), 687-697. https://doi.org/10.1007/s13311-017-0525-z

Corbit, L. H., & Balleine, B. W. (2003). The role of prelimbic cortex in instrumental conditioning. *Behavioural Brain Research, 146*(1-2), 145-157. doi:S0166432803003474 [pii]

Costa, D. S. J., & Boakes, R. A. (2009). *Context blocking in rat autoshaping: Sign-tracking versus goal-tracking* doi:https://doi.org/10.1016/j.lmot.2008.11.001

Coutureau, E., Esclassan, F., Di Scala, G., & Marchand, A. R. (2012). The role of the rat medial prefrontal cortex in adapting to changes in instrumental contingency. *PloS One, 7*(4), e33302. doi:10.1371/journal.pone.0033302 [doi]

Coutureau, E., & Killcross, S. (2003). Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats. *Behavioural Brain Research, 146*(1-2), 167-174. doi:S0166432803003498 [pii]

Cowan, N., Beschin, N., & Della Sala, S. (2004). Verbal recall in amnesiacs under conditions of diminished retroactive interference. *Brain : A Journal of Neurology, 127*(Pt 4), 825-834. doi:10.1093/brain/awh107 [doi]

Creed, M., Ntamati, N. R., Chandra, R., Lobo, M. K., & Lüscher, C. (2016). Convergence of Reinforcing and Anhedonic Cocaine Effects in the Ventral Pallidum. Neuron, 92(1), 214-226. https://doi.org/10.1016/j.neuron.2016.09.001

Cullen, P. K., Ferrara, N. C., Pullins, S. E., & Helmstetter, F. J. (2017). Context memory formation requires activity-dependent protein degradation in the hippocampus. *Learning & Memory (Cold Spring Harbor, N.Y.), 24*(11), 589-596. doi:10.1101/lm.045443.117 [doi]

Danker, J. F., & Anderson, J. R. (2010). The ghosts of brain states past: Remembering reactivates the brain regions engaged during encoding. *Psychological Bulletin, 136*(1), 87-102. doi:10.1037/a0017937 [doi]

Dalley, J. W., Lääne, K., Theobald, D. E., Armstrong, H. C., Corlett, P. R., Chudasama, Y., & Robbins, T. W. (2005). Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. Proc Natl Acad Sci U S A, 102(17), 6189-6194. https://doi.org/10.1073/pnas.0502080102

de Carvalho Myskiw, J., Furini, C. R., Schmidt, B., Ferreira, F., & Izquierdo, I. (2015). Extinction learning, which consists of the inhibition of retrieval, can be learned without retrieval. *Proceedings of the National Academy of Sciences of the United States of America, 112*(2), E230-3. doi:10.1073/pnas.1423465112 [doi]

de la Fuente, V., Medina, C., Falasco, G., Urrutia, L., Kravitz, A. V., Urbano, F. J., . . . Romano, A. (2019). The lateral neocortex is critical for contextual fear memory reconsolidation. *Scientific Reports, 9*(1), 12157-019-48340-9. doi:10.1038/s41598-019-48340-9 [doi]

Debiec, J., Diaz-Mataix, L., Bush, D. E., Doyere, V., & LeDoux, J. E. (2013). The selectivity of aversive memory reconsolidation and extinction processes depends on the initial encoding of the pavlovian association. *Learning & Memory (Cold Spring Harbor, N.Y.), 20*(12), 695-699. doi:10.1101/lm.031609.113 [doi]

Debiec, J., LeDoux, J. E., & Nader, K. (2002). Cellular and systems reconsolidation in the hippocampus. *Neuron, 36*(3), 527-538. doi:S0896627302010012 [pii]

Delaney, P. F., Verkoeijen, P. P. J. L., & Spirgel, A. (2010). In Ross B. H. (Ed.), *Chapter 3 - spacing and testing effects: A deeply critical, lengthy, and at times discursive review of the literature* Academic Press. doi:https://doi.org/10.1016/S0079-7421(10)53003-2

Diaz-Mataix, L., Ruiz Martinez, R. C., Schafe, G. E., LeDoux, J. E., & Doyere, V. (2013). Detection of a temporal error triggers reconsolidation of amygdala-dependent memories. *Current Biology : CB, 23*(6), 467-472. doi:10.1016/j.cub.2013.01.053 [doi]

Dickinson, A. (1985). Actions and habits: The development of behavioural autonomy. *Philosophical Transactions of the Royal Society of London.Series B, Biological Sciences, 308*(1135), 67-78.

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., & Boakes, R. A. (1995). Motivational control after extended instrumental training. *Animal Learning & Behavior, 23*(2), 197-206. doi:10.3758/BF03199935

Dickinson, A., Nicholas, D. J., & Adams, C. D. (1983). The effect of the instrumental training contingency on susceptibility to reinforcer devaluation. The

Quarterly Journal of Experimental Psychology Section B, 35(1), 35-51. https://doi.org/10.1080/14640748308400912

Diergaarde, L., Schoffelmeer, A. N. M., & De Vries, T. J. (2006). *B-adrenoceptor mediated inhibition of long-term reward-related memory reconsolidation* doi:https://doi.org/10.1016/j.bbr.2006.02.014

Duebel, J., Marazova, K., & Sahel, J. A. (2015). Optogenetics. Curr Opin Ophthalmol, 26(3), 226-232. https://doi.org/10.1097/icu.0000000000000140

Duncan, C. P. (1949). The retroactive effect of electroshock on learning. *Journal of Comparative and Physiological Psychology, 42*(1), 32-44. doi:10.1037/h0058173

Ebbinghaus, M. Über das Gedächtnis (Buehler, Leipzig, 1885).

Eddy, M. C., Todd, T. P., Bouton, M. E., & Green, J. T. (2016). Medial prefrontal cortex involvement in the expression of extinction and ABA renewal of instrumental behavior for a food reinforcer. Neurobiology of learning and memory, 128, 33-39. https://doi.org/10.1016/j.nlm.2015.12.003

Eisenberg, M., & Dudai, Y. (2004). Reconsolidation of fresh, remote, and extinguished fear memory in medaka: Old fears don't die. *The European Journal of Neuroscience, 20*(12), 3397-3403. doi:EJN3818 [pii]

Eisenberg, M., Kobilo, T., Berman, D. E., & Dudai, Y. (2003). Stability of retrieved memory: Inverse correlation with trace dominance. *Science (New York, N.Y.), 301*(5636), 1102-1104. doi:10.1126/science.1086881 [doi]

Elsey, J. W. B., & Kindt, M. (2017). *Tackling maladaptive memories through reconsolidation: From neural to clinical science* doi:https://doi.org/10.1016/j.nlm.2017.03.007

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nature Neuroscience, 8*(11), 1481-1489. doi:nn1579 [pii]

Everitt, B. J., Dickinson, A., & Robbins, T. W. (2001). *The neuropsychological basis of addictive behaviour* doi:https://doi.org/10.1016/S0165-0173(01)00088-1

Exton-McGuinness, M. T., & Lee, J. L. (2015). Reduction in responding for sucrose and cocaine reinforcement by disruption of memory reconsolidation. *eNeuro, 2*(2), 10.1523/ENEURO.0009-15.2015. eCollection 2015 Mar-Apr. doi:10.1523/ENEURO.0009-15.2015 [doi]

Exton-McGuinness, M. T., Lee, J. L., & Reichelt, A. C. (2015). Updating memories--the role of prediction errors in memory reconsolidation. *Behavioural Brain Research, 278*, 375-384. doi:10.1016/j.bbr.2014.10.011 [doi]

Exton-McGuinness, M. T., Patton, R. C., Sacco, L. B., & Lee, J. L. (2014). Reconsolidation of a well-learned instrumental memory. *Learning & Memory (Cold Spring Harbor, N.Y.), 21*(9), 468-477. doi:10.1101/lm.035543.114 [doi]

Exton-McGuinness, M. T. J., Drame, M. L., Flavell, C. R., & Lee, J. L. C. (2019). On the resistance to relapse to cocaine-seeking following impairment of instrumental cocaine memory reconsolidation. *Frontiers in Behavioral Neuroscience, 13*, 242. doi:10.3389/fnbeh.2019.00242 [doi]

Fanselow, M. S., & Dong, H. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron, 65*(1), 7-19. doi:10.1016/j.neuron.2009.11.031

Faure, A., Conde, F., Cheruel, F., & el Massioui, N. (2006). Learning-dependent activation of fra-1: Involvement of ventral hippocampus and SNc/VTA complex in learning and habit formation. *Brain Research Bulletin, 68*(4), 233-248. doi:S0361-9230(05)00361-8 [pii]

Flavell, C. R., & Lee, J. L. (2013). Reconsolidation and extinction of an appetitive pavlovian memory. *Neurobiology of Learning and Memory, 104*, 25-31. doi:10.1016/j.nlm.2013.04.009 [doi]

Flint, R. W.,Jr, & Marino, C. L. (2007). Cycloheximide impairs reconsolidation of a contextually reactivated memory in a conditioned taste aversion paradigm. *Behavioral Neuroscience, 121*(2), 433-438. doi:2007-04812-021 [pii]

Flint, R. W.,Jr, Noble, L. J., & Ulmen, A. R. (2013). NMDA receptor antagonism with MK-801 impairs consolidation and reconsolidation of passive avoidance conditioning in adolescent rats: Evidence for a state dependent reconsolidation effect. *Neurobiology of Learning and Memory, 101*, 114-119. doi:10.1016/j.nlm.2013.01.009 [doi]

French, S. J., & Totterdell, S. (2003). *Individual nucleus accumbens-projection neurons receive both basolateral amygdala and ventral subicular afferents in rats* doi:https://doi.org/10.1016/S0306-4522(03)00150-7

French, S. J., Hailstone, J. C., & Totterdell, S. (2003). *Basolateral amygdala efferents to the ventral subiculum preferentially innervate pyramidal cell dendritic spines* doi:https://doi.org/10.1016/S0006-8993(03)03017-8

Fuchs, R. A., Bell, G. H., Ramirez, D. R., Eaddy, J. L., & Su, Z. I. (2009). Basolateral amygdala involvement in memory reconsolidation processes that facilitate drug context-induced cocaine seeking. *The European Journal of Neuroscience*, *30*(5), 889-900. doi:10.1111/j.1460-9568.2009.06888.x [doi]

Fuchs, R. A., Eaddy, J. L., Su, Z.-I., & Bell, G. H. (2007). Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats. The European journal of neuroscience, 26(2), 487-498. https://doi.org/10.1111/j.1460-9568.2007.05674.x

Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., & See, R. E. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology*, *30*(2), 296-309. doi:10.1038/sj.npp.1300579

Galván, V. V., Chen, J., & Weinberger, N. M. (2001). Long-term frequency tuning of local field potentials in the auditory cortex of the waking guinea pig. *Journal of the Association for Research in Otolaryngology, 2*(3), 199-215. doi:10.1007/s101620010062

Geller, A., Robustelli, F., & Jarvik, M. E. (1970). A parallel study of the amnesic effects of cycloheximide and ECS under different strengths of conditioning. *Psychopharmacologia, 16*(4), 281-289. doi:10.1007/BF00404734

Gerard, R. W. (1949). Physiology and psychiatry. *Ajp, 106*(3), 161-173. doi:10.1176/ajp.106.3.161

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., Jr., & Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science, 250(4986), 1429-1432. https://doi.org/10.1126/science.2147780

Gerfen, C. R., & Surmeier, D. J. (2011). Modulation of striatal projection systems by dopamine. Annu Rev Neurosci, 34, 441-466. https://doi.org/10.1146/annurev-neuro-061010-113641

Gibbon, J., & Balsam, P. (1981). Spreading association in time. *Autoshaping and Conditioning Theory, ,* 219-253.

Gilman, T. L., Dutta, S., Adkins, J. M., Cecil, C. A., & Jasnow, A. M. (2018). Basolateral amygdala Thy1-expressing neurons facilitate the inhibition of contextual fear during consolidation, reconsolidation, and extinction. *Neurobiology of Learning and Memory, 155,* 498-507. doi:S1074-7427(18)30234-X [pii]

Gisquet-Verrier, P., Lynch, J. F.,3rd, Cutolo, P., Toledano, D., Ulmen, A., Jasnow, A. M., & Riccio, D. C. (2015). Integration of new information with active memory accounts for retrograde amnesia: A challenge to the consolidation/reconsolidation hypothesis? *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 35*(33), 11623-11633. doi:10.1523/JNEUROSCI.1386-15.2015 [doi]

Gisquet-Verrier, P., & Riccio, D. C. (2018). Memory integration: An alternative to the consolidation/reconsolidation hypothesis. *Progress in Neurobiology, 171*, 15-31. doi:S0301-0082(17)30217-4 [pii]

Glimcher, P. W. (2011). Understanding dopamine and reinforcement learning: The dopamine reward prediction error hypothesis. *Proc Natl Acad Sci USA, 108*, 15647. doi:10.1073/pnas.1014269108

Gold, P. E., & Van Buskirk, R. B. (1975). Facilitation of time-dependent memory processes with posttrial epinephrine injections. *Behavioral Biology, 13*(2), 145-153. doi:10.1016/s0091-6773(75)91784-8 [doi]

Gold, P. E., & King, R. A. (1974). Retrograde amnesia: Storage failure versus retrieval failure. *Psychological Review, 81*(5), 465-469. doi:10.1037/h0036949

Gordon, W. C., & Spear, N. E. (1973). Effect of reactivation of a previously acquired memory on the interaction between memories in the rat. *Journal of Experimental Psychology, 99*(3), 349-355. doi:10.1037/h0035301 [doi]

Gordon, W., & Mowrer, R. (1980). An extinction trial as a reminder treatment following electroconvulsive shock. *Animal Learning and Behavior, 8*, 363-367. doi:10.3758/BF03199618

Gray, J. A. (1982). Précis of the neuropsychology of anxiety: An enquiry into the functions of the septo-hippocampal system. *Behavioral and Brain Sciences, 5*(3), 469-484. doi:10.1017/S0140525X00013066

Graybiel, A. M., & Grafton, S. T. (2015). The striatum: where skills and habits meet. Cold Spring Harb Perspect Biol, 7(8), a021691. https://doi.org/10.1101/cshperspect.a021691

Grice, G. R. (1948). The relation of secondary reinforcement to delayed reward in visual discrimination learning. *Journal of Experimental Psychology*, *38*(1), 1-16. doi:10.1037/h0061016 [doi]

Gutman, A. L., Nett, K. E., Cosme, C. V., Worth, W. R., Gupta, S. C., Wemmie, J. A., & LaLumiere, R. T. (2017). Extinction of Cocaine Seeking Requires a Window of Infralimbic Pyramidal Neuron Activity after Unreinforced Lever Presses. The Journal of neuroscience : the official journal of the Society for Neuroscience, 37(25), 6075-6086. https://doi.org/10.1523/jneurosci.3821-16.2017

Haber, S. N., Fudge, J. L., & McFarland, N. R. (2000). Striatonigrostriatal pathways in primates form an ascending spiral from the shell to the dorsolateral striatum. J Neurosci, 20(6), 2369-2382. https://doi.org/10.1523/jneurosci.20-06-02369.2000

Hall, G., & Pearce, J. M. (1982). Restoring the associability of a pre-exposed CS by a surprising event. *The Quarterly Journal of Experimental Psychology Section B, 34*(3), 127-140. doi:10.1080/14640748208400881

Hall, J., Thomas, K. L., & Everitt, B. J. (2001a). Fear memory retrieval induces CREB phosphorylation and fos expression within the amygdala. *The European Journal of Neuroscience, 13*(7), 1453-1458. doi:ejn1531 [pii]

Hall, J., Thomas, K. L., & Everitt, B. J. (2001b). Cellular imaging of zif268 expression in the hippocampus and amygdala during contextual and cued fear memory

retrieval: selective activation of hippocampal CA1 neurons during the recall of contextual memories. J Neurosci, 21(6), 2186-2193. https://doi.org/10.1523/jneurosci.21-06-02186.2001

Hamlin, A. S., Clemens, K. J., & McNally, G. P. (2008). Renewal of extinguished cocaine-seeking. Neuroscience, 151(3), 659-670. https://doi.org/10.1016/j.neuroscience.2007.11.018

Hardt, O., Nader, K., & Nadel, L. (2013). Decay happens: The role of active forgetting in memory. *Trends in Cognitive Sciences, 17*(3), 111-120. doi:10.1016/j.tics.2013.01.001 [doi]

Heath, F. C., Jurkus, R., Bast, T., Pezze, M. A., Lee, J. L., Voigt, J. P., & Stevenson, C. W. (2015). Dopamine D1-like receptor signalling in the hippocampus and amygdala modulates the acquisition of contextual fear conditioning. *Psychopharmacology, 232*(14), 2619-2629. doi:10.1007/s00213-015-3897-y [doi]

Heinsbroek, J. A., Neuhofer, D. N., Griffin, W. C., 3rd, Siegel, G. S., Bobadilla, A. C., Kupchik, Y. M., & Kalivas, P. W. (2017). Loss of Plasticity in the D2-Accumbens Pallidal Pathway Promotes Cocaine Seeking. J Neurosci, 37(4), 757-767. https://doi.org/10.1523/jneurosci.2659-16.2016

Hernandez, P. J., & Kelley, A. E. (2004). Long-term memory for instrumental responses does not undergo protein synthesis-dependent reconsolidation upon retrieval. *Learning & Memory (Cold Spring Harbor, N.Y.), 11*(6), 748-754. doi:lm.84904 [pii]

Hernandez, P. J., Sadeghian, K., & Kelley, A. E. (2002). Early consolidation of instrumental learning requires protein synthesis in the nucleus accumbens. *Nature Neuroscience, 5*(12), 1327-1331. doi:10.1038/nn973 [doi]

Heroux, N. A., Horgan, C. J., Pinizzotto, C. C., Rosen, J. B., & Stanton, M. E. (2019). *Medial prefrontal and ventral hippocampal contributions to incidental context learning and memory in adolescent rats* doi:https://doi.org/10.1016/j.nlm.2019.107091

Hinderliter, C. F., Webster, T., & Riccio, D. C. (1975). Amnesia induced by hypothermia as a function of treatment-test interval and recooling in rats. *Animal Learning & Behavior, 3*(3), 257-263. doi:10.3758/BF03213441

Hock, B. J.,Jr, & Bunsey, M. D. (1998). Differential effects of dorsal and ventral hippocampal lesions. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 18*(17), 7027-7032.

Holland, P. C. (2004). Relations between pavlovian-instrumental transfer and reinforcer devaluation. *Journal of Experimental Psychology: Animal Behavior Processes, 30*(2), 104.

Hollerman, J. R., & Schultz, W. (1998). Dopamine neurons report an error in the temporal prediction of reward during learning. Nature Neuroscience, 1(4), 304-309. https://doi.org/10.1038/1124

Hoover, W. B., & Vertes, R. P. (2007). Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. *Brain Structure & Function, 212*(2), 149-179. doi:10.1007/s00429-007-0150-4 [doi]

Ishikawa, A., & Nakamura, S. (2006). Ventral hippocampal neurons project axons simultaneously to the medial prefrontal cortex and amygdala in the rat. *Journal of Neurophysiology, 96*(4), 2134-2138. doi:00069.2006 [pii]

Ishikawa, R., Fukushima, H., Frankland, P. W., & Kida, S. (2016). Hippocampal neurogenesis enhancers promote forgetting of remote fear memory after hippocampal reactivation by retrieval. *eLife, 5*, 10.7554/eLife.17464. doi:10.7554/eLife.17464 [doi]

James, M. H., & Aston-Jones, G. (2016). The Ventral Pallidum: Proposed Integrator of Positive and Negative Factors in Cocaine Abuse. Neuron, 92(1), 5-8. https://doi.org/10.1016/j.neuron.2016.09.042

James, M. H., McGlinchey, E. M., Vattikonda, A., Mahler, S. V., & Aston-Jones, G. (2018). Cued Reinstatement of Cocaine but Not Sucrose Seeking Is Dependent on Dopamine Signaling in Prelimbic Cortex and Is Associated with Recruitment of Prelimbic Neurons That Project to Contralateral Nucleus Accumbens Core. Int J Neuropsychopharmacol, 21(1), 89-94. https://doi.org/10.1093/ijnp/pyx107

Janak, P. H., & Tye, K. M. (2015). From circuits to behaviour in the amygdala. Nature, 517(7534), 284-292. https://doi.org/10.1038/nature14188

Johnson, A. W., Gallagher, M., & Holland, P. C. (2009). The basolateral amygdala is critical to the expression of pavlovian and instrumental outcome-specific reinforcer devaluation effects. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 29*(3), 696-704. doi:10.1523/JNEUROSCI.3758-08.2009 [doi]

Jones, M. W., & Wilson, M. A. (2005). Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. *PLoS Biology, 3*(12), e402. doi:05-PLBI-RA-0687R2 [pii]

Kalivas, P. W., Volkow, N., & Seamans, J. (2005). Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron, 45(5), 647-650. https://doi.org/10.1016/j.neuron.2005.02.005

Kalivas, P. W., & Volkow, N. D. (2005). The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry, 162(8), 1403-1413. https://doi.org/10.1176/appi.ajp.162.8.1403

Kelley, A. E., & Berridge, K. C. (2002). The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci, 22(9), 3306-3311. https://doi.org/10.1523/jneurosci.22-09-03306.2002

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral Cortex (New York, N.Y.: 1991), 13*(4), 400-408. doi:10.1093/cercor/13.4.400 [doi]

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*(5057), 675-677. doi:10.1126/science.1585183

Kindt, M., Soeter, M., & Vervliet, B. (2009). Beyond extinction: Erasing human fear responses and preventing the return of fear. *Nature Neuroscience, 12*(3), 256-258. doi:10.1038/nn.2271

Kishi, T., Tsumori, T., Ono, K., Yokota, S., Ishino, H., & Yasui, Y. (2000). Topographical organization of projections from the subiculum to the hypothalamus in

the rat. *The Journal of Comparative Neurology, 419*(2), 205-222. doi:10.1002/(SICI)1096-9861(20000403)419:23.0.CO;2-0 [pii]

Kjelstrup, K. G., Tuvnes, F. A., Steffenach, H. A., Murison, R., Moser, E. I., & Moser, M. B. (2002). Reduced fear expression after lesions of the ventral hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 99*(16), 10825-10830. doi:10.1073/pnas.152112399 [doi]

Klanker, M., Fellinger, L., Feenstra, M., Willuhn, I., & Denys, D. (2017). Regionally distinct phasic dopamine release patterns in the striatum during reversal learning. *Neuroscience, 345*, 110-123. doi:S0306-4522(16)30160-9 [pii]

Koh, M. T., & Bernstein, I. L. (2003). Inhibition of protein kinase A activity during conditioned taste aversion retrieval: Interference with extinction or reconsolidation of a memory? *Neuroreport, 14*(3), 405-407. doi:10.1097/00001756-200303030-00021 [doi]

Land, C., Bunsey, M., & Riccio, D. C. (2000). Anomalous properties of hippocampal lesion-induced retrograde amnesia. *Psychobiology, 28*(4), 476-485. doi:10.3758/BF03332005

Landfield, P. W., Mcgaugh, J. L., & Tusa, R. J. (1972). Theta rhythm: A temporal correlate of memory storage processes in the rat. *Science, 175*(4017), 87-89. doi:10.1126/science.175.4017.87

Laroche, S. (2010). Cellular and molecular mechanisms of memory. *Biologie Aujourd'Hui, 204*(2), 93-102.

LASA (2017) Guiding principles for preparing for and undertaking aseptic surgery. A report by the LASA Education, Training and Ethics section. (Jennings M, Berdoy M, eds). Available at: http://www.lasa.co.uk/publications.html.

Lasseter, H. C., Xie, X., Ramirez, D. R., & Fuchs, R. A. (2010). Sub-region specific contribution of the ventral hippocampus to drug context-induced reinstatement of cocaine-seeking behavior in rats. Neuroscience, 171(3), 830-839. https://doi.org/10.1016/j.neuroscience.2010.09.032

Laurent, V., & Westbrook, R. F. (2008). Distinct contributions of the basolateral amygdala and the medial prefrontal cortex to learning and relearning extinction of context conditioned fear. *Learning & Memory (Cold Spring Harbor, N.Y.), 15*(9), 657-666. doi:10.1101/lm.1080108 [doi]

Lee, J. L. (2008). Memory reconsolidation mediates the strengthening of memories by additional learning. *Nature Neuroscience, 11*(11), 1264-1266. doi:10.1038/nn.2205 [doi]

Lee, J. L. (2009). Reconsolidation: Maintaining memory relevance. *Trends in Neurosciences, 32*(8), 413-420. doi:10.1016/j.tins.2009.05.002 [doi]

Lee, J. L. (2010). Memory reconsolidation mediates the updating of hippocampal memory content. *Frontiers in Behavioral Neuroscience, 4*, 168. doi:10.3389/fnbeh.2010.00168 [doi]

Lee, J. L., Di Ciano, P., Thomas, K. L., & Everitt, B. J. (2005). Disrupting reconsolidation of drug memories reduces cocaine-seeking behavior. *Neuron, 47*(6), 795-801. doi:S0896-6273(05)00656-2 [pii]

Lee, J. L., Dickinson, A., & Everitt, B. J. (2005). Conditioned suppression and freezing as measures of aversive pavlovian conditioning: Effects of discrete amygdala lesions and overtraining. *Behavioural Brain Research, 159*(2), 221-233. doi:S0166-4328(04)00405-X [pii]

Lee, J. L., & Everitt, B. J. (2008a). Appetitive memory reconsolidation depends upon NMDA receptor-mediated neurotransmission. *Neurobiology of Learning and Memory, 90*(1), 147-154. doi:10.1016/j.nlm.2008.02.004 [doi]

Lee, J. L., & Everitt, B. J. (2008b). Reactivation-dependent amnesia for appetitive memories is determined by the contingency of stimulus presentation. *Learning & Memory (Cold Spring Harbor, N.Y.), 15*(6), 390-393. doi:10.1101/lm.976108 [doi]

Lee, J. L., & Everitt, B. J. (2008c). Reactivation-dependent amnesia in pavlovian approach and instrumental transfer. *Learning & Memory (Cold Spring Harbor, N.Y.), 15*(8), 597-602. doi:10.1101/lm.1029808 [doi]

Lee, J. L., Everitt, B. J., & Thomas, K. L. (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science (New York, N.Y.), 304*(5672), 839-843. doi:10.1126/science.1095760 [doi]

Lee, J. L., Milton, A. L., & Everitt, B. J. (2006a). Cue-induced cocaine seeking and relapse are reduced by disruption of drug memory reconsolidation. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 26*(22), 5881-5887. doi:26/22/5881 [pii]

Lee, J. L., Milton, A. L., & Everitt, B. J. (2006b). Reconsolidation and extinction of conditioned fear: Inhibition and potentiation. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 26*(39), 10051-10056. doi:26/39/10051 [pii]

Lee, J. L. C., Nader, K., & Schiller, D. (2017). *An update on memory reconsolidation updating* doi:https://doi.org/10.1016/j.tics.2017.04.006

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science (New York, N.Y.), 315*(5814), 961-966. doi:315/5814/961 [pii]

Lewis, D. J. (1979). Psychobiology of active and inactive memory. *Psychological Bulletin, 86*(5), 1054-1083.

Lewis, D. J., Misanin, J. R., & Miller, R. R. (1968). Recovery of memory following amnesia. *Nature, 220*(5168), 704-705. doi:10.1038/220704a0 [doi]

Li, Y., Ge, S., Li, N., Chen, L., Zhang, S., Wang, J., . . . Wang, X. (2016). NMDA and dopamine D1 receptors within NAc-shell regulate IEG proteins expression in reward circuit during cocaine memory reconsolidation. *Neuroscience, 315*, 45-69. doi:10.1016/j.neuroscience.2015.11.063 [doi]

Liang, J., Li, J. L., Han, Y., Luo, Y. X., Xue, Y. X., Zhang, Y., Zhang, Y., Zhang, L. B., Chen, M. L., Lu, L., & Shi, J. (2017). Calpain-GRIP Signaling in Nucleus Accumbens Core Mediates the Reconsolidation of Drug Reward Memory. J Neurosci, 37(37), 8938-8951. https://doi.org/10.1523/jneurosci.0703-17.2017

Lin, H. C., Mao, S. C., & Gean, P. W. (2006). Effects of intra-amygdala infusion of CB1 receptor agonists on the reconsolidation of fear-potentiated startle. Learn Mem, 13(3), 316-321. https://doi.org/10.1101/lm.217006

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., Heitland, I., Hermann, A., Kuhn, M., Kruse, O., Meir Drexler, S., Meulders, A., Nees, F., Pittig, A., Richter, J., Römer, S., Shiban, Y., Schmitz, A., Straube, B., . . . Merz, C. J. (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. Neuroscience & Biobehavioral Reviews, 77, 247-285. https://doi.org/https://doi.org/10.1016/j.neubiorev.2017.02.026

Lonsdorf, T. B., & Merz, C. J. (2017). More than just noise: Inter-individual differences in fear acquisition, extinction and return of fear in humans - Biological, experiential, temperamental factors, and methodological pitfalls. Neuroscience & Biobehavioral Reviews, 80, 703-728. https://doi.org/https://doi.org/10.1016/j.neubiorev.2017.07.007

Mackintosh, N. J. (1974). The psychology of animal learning. Academic Press.

Mactutus, C. F., Riccio, D. C., & Ferek, J. M. (1979). Retrograde amnesia for old (reactivated) memory: Some anomalous characteristics. *Science (New York, N.Y.)*, *204*(4399), 1319-1320. doi:10.1126/science.572083 [doi]

Mactutus, C. F., Concannon, J. T., & Riccio, D. C. (1982). *Nonmonotonic age changes in susceptibility to hypothermia-induced retrograde amnesia in rats* doi:https://doi.org/10.1016/0031-9384(82)90218-9

Maddox, S. A., Monsey, M. S., & Schafe, G. E. (2011). Early growth response gene 1 (Egr-1) is required for new and reactivated fear memories in the lateral amygdala. Learn Mem, 18(1), 24-38. https://doi.org/10.1101/lm.1980211

Madsen, M. C., & McGaugh, J. L. (1961). The effect of ECS on one-trial avoidance learning. *Journal of Comparative and Physiological Psychology*, *54*(5), 522-523. doi:10.1037/h0046887

Maroteaux, M., Valjent, E., Longueville, S., Topilko, P., Girault, J. A., & Hervé, D. (2014). Role of the plasticity-associated transcription factor zif268 in the early phase of instrumental learning. PLoS One, 9(1), e81868. https://doi.org/10.1371/journal.pone.0081868

Martin, S. J., Grimwood, P. D., & Morris, R. G. (2000). Synaptic plasticity and memory: An evaluation of the hypothesis. *Annual Review of Neuroscience, 23*, 649-711. doi:10.1146/annurev.neuro.23.1.649 [doi]

McClelland, J. L., McNaughton, B. L., & O'Reilly, R. C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: Insights from the successes and failures of connectionist models of learning and memory. *Psychological Review, 102*(3), 419-457. doi:10.1037/0033-295X.102.3.419 [doi]

McCloskey, M., & Cohen, N. J. (1989). In Bower G. H. (Ed.), *Catastrophic interference in connectionist networks: The sequential learning problem* Academic Press. doi:https://doi.org/10.1016/S0079-7421(08)60536-8

McFarland, K., & Kalivas, P. W. (2001). The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. The Journal of neuroscience : the official journal

of the Society for Neuroscience, 21(21), 8655-8663. https://doi.org/10.1523/jneurosci.21-21-08655.2001

McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science, 153*(3742), 1351-1358. doi:10.1126/science.153.3742.1351

McGaugh, J. L. (2000). Memory--a century of consolidation. *Science (New York, N.Y.), 287*(5451), 248-251. doi:8182 [pii]

McHugh, T. J., Jones, M. W., Quinn, J. J., Balthasar, N., Coppari, R., Elmquist, J. K., . . . Tonegawa, S. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science (New York, N.Y.), 317*(5834), 94-99. doi:1140263 [pii]

McLaughlin, J., & See, R. E. (2003). Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology (Berl), 168(1-2), 57-65. https://doi.org/10.1007/s00213-002-1196-x

McNaughton, N., & Gray, J. A. (2000). Anxiolytic action on the behavioural inhibition system implies multiple types of arousal contribute to anxiety. *Journal of Affective Disorders, 61*(3), 161-176. doi:S016503270000344X [pii]

Merhav, M., & Rosenblum, K. (2008). Facilitation of taste memory acquisition by experiencing previous novel taste is protein-synthesis dependent. *Learning & Memory (Cold Spring Harbor, N.Y.), 15*(7), 501-507. doi:10.1101/lm.986008 [doi]

Merlo, E., Milton, A. L., Goozée, Z. Y., Theobald, D. E., & Everitt, B. J. (2014). Reconsolidation and extinction are dissociable and mutually exclusive processes:

behavioral and molecular evidence. J Neurosci, 34(7), 2422-2431. https://doi.org/10.1523/jneurosci.4001-13.2014

Mierzejewski, P., Korkosz, A., Rogowski, A., Korkosz, I., Kostowski, W., & Scinska, A. (2009). Post-session injections of a protein synthesis inhibitor, cycloheximide do not alter saccharin self-administration. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33*(2), 286-289. doi:10.1016/j.pnpbp.2008.11.015 [doi]

Milekic, M. H., Brown, S. D., Castellini, C., & Alberini, C. M. (2006). Persistent disruption of an established morphine conditioned place preference. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 26*(11), 3010-3020. doi:26/11/3010 [pii]

Milekic, M. H., Pollonini, G., & Alberini, C. M. (2007). Temporal requirement of C/EBPbeta in the amygdala following reactivation but not acquisition of inhibitory avoidance. *Learning & Memory (Cold Spring Harbor, N.Y.), 14*(7), 504-511. doi:14/7/504 [pii]

Milekic, M. H., & Alberini, C. M. (2002). Temporally graded requirement for protein synthesis following memory reactivation. *Neuron, 36*(3), 521-525. doi:10.1016/S0896-6273(02)00976-5

Millan, E. Z., & McNally, G. P. (2011). Accumbens shell AMPA receptors mediate expression of extinguished reward seeking through interactions with basolateral amygdala. Learning & memory (Cold Spring Harbor, N.Y.), 18(7), 414-421. https://doi.org/10.1101/lm.2144411

Millan, E. Z., Reese, R. M., Grossman, C. D., Chaudhri, N., & Janak, P. H. (2015). Nucleus Accumbens and Posterior Amygdala Mediate Cue-Triggered Alcohol Seeking and Suppress Behavior During the Omission of Alcohol-Predictive Cues. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 40(11), 2555-2565. https://doi.org/10.1038/npp.2015.102

Miller, R. R., & Matzel, L. D. (2000). Memory involves far more than 'consolidation'. *Nature Reviews.Neuroscience, 1*(3), 214-216. doi:10.1038/35044578 [doi]

Miller, R. R., Ott, C. A., Berk, A. M., & Springer, A. D. (1974). Appetitive memory restoration after electroconvulsive shock in the rat. *Journal of Comparative and Physiological Psychology, 87*(4), 717-723. doi:10.1037/h0036972

Miller, R. R., & Springer, A. D. (1971). *Temporal course of amnesia in rats after electroconvulsive shock* doi:https://doi.org/10.1016/0031-9384(71)90031-X

Miller, R. R., & Springer, A. D. (1972). *Induced recovery of memory in rats following electroconvulsive shock* doi:https://doi.org/10.1016/0031-9384(72)90089-3

Miller, R. R., & Springer, A. D. (1973). Amnesia, consolidation, and retrieval. *Psychological Review, 80*(1), 69-79. doi:10.1037/h0033897

Milton, A. L., & Everitt, B. J. (2012). The persistence of maladaptive memory: Addiction, drug memories and anti-relapse treatments. *Neuroscience and Biobehavioral Reviews, 36*(4), 1119-1139. doi:10.1016/j.neubiorev.2012.01.002 [doi]

Milton, A. L., Lee, J. L., Butler, V. J., Gardner, R., & Everitt, B. J. (2008). Intra-amygdala and systemic antagonism of NMDA receptors prevents the reconsolidation of drug-associated memory and impairs subsequently both novel and previously acquired

drug-seeking behaviors. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 28*(33), 8230-8237. doi:10.1523/JNEUROSCI.1723-08.2008 [doi]

Milton, A. L., Merlo, E., Ratano, P., Gregory, B. L., Dumbreck, J. K., & Everitt, B. J. (2013). Double dissociation of the requirement for GluN2B- and GluN2A-containing NMDA receptors in the destabilization and restabilization of a reconsolidating memory. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 33*(3), 1109-1115. doi:10.1523/JNEUROSCI.3273-12.2013 [doi]

Milton, A. L., Schramm, M. J., Wawrzynski, J. R., Gore, F., Oikonomou-Mpegeti, F., Wang, N. Q., . . . Everitt, B. J. (2012). Antagonism at NMDA receptors, but not beta-adrenergic receptors, disrupts the reconsolidation of pavlovian conditioned approach and instrumental transfer for ethanol-associated conditioned stimuli. *Psychopharmacology, 219*(3), 751-761. doi:10.1007/s00213-011-2399-9 [doi]

Milton, A. (2013). *Drink, drugs and disruption: Memory manipulation for the treatment of addiction* doi:https://doi.org/10.1016/j.conb.2012.11.008

Misanin, J. R., Miller, R. R., & Lewis, D. J. (1968). Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science (New York, N.Y.), 160*(3827), 554-555. doi:10.1126/science.160.3827.554 [doi]

Morris, R. G. M., Inglis, J., Ainge, J. A., Olverman, H. J., Tulloch, J., Dudai, Y., & Kelly, P. A. T. (2006). Memory reconsolidation: Sensitivity of spatial memory to inhibition of protein synthesis in dorsal hippocampus during encoding and retrieval. *Neuron, 50*(3), 479-489. doi:10.1016/j.neuron.2006.04.012

Moser, M., & Moser, E. I. (1998). Functional differentiation in the hippocampus. *Hippocampus, 8*(6), 608-619. doi:10.1002/(SICI)1098-1063(1998)8:63.0.CO;2-7

Muller, W. E., Mutschler, E., & Riederer, P. (1995). Noncompetitive NMDA receptor antagonists with fast open-channel blocking kinetics and strong voltage-dependency as potential therapeutic agents for alzheimer's dementia. *Pharmacopsychiatry, 28*(4), 113-124. doi:10.1055/s-2007-979603 [doi]

Müller, G., Pilzecker, A., 1900. Experiental contributions to the theory of memory. Z.Psychol. Z. Angew. Psychol. 1, 1–300.

Murchison, C. F., Zhang, X., Zhang, W., Ouyang, M., Lee, A., & Thomas, S. A. (2004). *A distinct role for norepinephrine in memory retrieval* doi:https://doi.org/10.1016/S0092-8674(04)00259-4

Murray, E. A. (2007). The amygdala, reward and emotion. *Trends in Cognitive Sciences, 11*(11), 489-497.

Murray, J. E., Belin, D., & Everitt, B. J. (2012). Double dissociation of the dorsomedial and dorsolateral striatal control over the acquisition and performance of cocaine seeking. Neuropsychopharmacology, 37(11), 2456-2466. https://doi.org/10.1038/npp.2012.104

Nadel, L., & Hardt, O. (2011). Update on memory systems and processes. *Neuropsychopharmacology, 36*(1), 251-273. doi:10.1038/npp.2010.169

Nader, K. (2003). Memory traces unbound. *Trends in Neurosciences, 26*(2), 65-72. doi:S0166-2236(02)00042-5 [pii]

Nader, K., & Hardt, O. (2009). A single standard for memory: The case for reconsolidation. *Nature Reviews.Neuroscience, 10*(3), 224-234. doi:10.1038/nrn2590 [doi]

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*(6797), 722-726. doi:10.1038/35021052 [doi]

Nakashiba, T., Cushman, J. D., Pelkey, K. A., Renaudineau, S., Buhl, D. L., McHugh, T. J., . . . Tonegawa, S. (2012). Young dentate granule cells mediate pattern separation, whereas old granule cells facilitate pattern completion. *Cell, 149*(1), 188-201. doi:10.1016/j.cell.2012.01.046 [doi]

Nandhra, H. S., Murphy, C. L., & Sule, A. (2013). Novel pharmacological agents targeting memory and cognition in the treatment of anxiety disorders. *Human Psychopharmacology, 28*(6), 538-543. doi:10.1002/hup.2348 [doi]

Naneix, F., Marchand, A. R., Di Scala, G., Pape, J. R., & Coutureau, E. (2009). A role for medial prefrontal dopaminergic innervation in instrumental conditioning. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 29*(20), 6599-6606. doi:10.1523/JNEUROSCI.1234-09.2009 [doi]

Nicoll, R. A. (2017). *A brief history of long-term potentiation* doi:https://doi.org/10.1016/j.neuron.2016.12.015

Oleskevich, S., Descarries, L., & Lacaille, J. -. (1989). Quantified distribution of the noradrenaline innervation in the hippocampus of adult rat. *Journal of Neuroscience, 9*(11), 3803-3815.

Oliver, C. F., Kabitzke, P., Serrano, P., Egan, L. J., Barr, G. A., Shair, H. N., & Wiedenmayer, C. (2016). Repeated recall and PKMzeta maintain fear memories in juvenile rats. *Learning & Memory (Cold Spring Harbor, N.Y.), 23*(12), 710-713. doi:10.1101/lm.042549.116 [doi]

Orsini, C. A., Kim, J. H., Knapska, E., & Maren, S. (2011). Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. J Neurosci, 31(47), 17269-17277. https://doi.org/10.1523/jneurosci.4095-11.2011

Ostlund, S. B., & Balleine, B. W. (2005). Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 25*(34), 7763-7770. doi:25/34/7763 [pii]

Ostlund, S. B., & Balleine, B. W. (2008). Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 28*(17), 4398-4405. doi:10.1523/JNEUROSCI.5472-07.2008 [doi]

Otani, S., & Abraham, W. C. (1989). *Inhibition of protein synthesis in the dentate gyrus, but not the entorhinal cortex, blocks maintenance of long-term potentiation in rats* doi:https://doi.org/10.1016/0304-3940(89)90222-X

Overton, D. A. (1964). Statedependent or "dissociated" learning produced with pentobarbital. *Journal of Comparative and Physiological Psychology, 57*(1), 3-12. doi:10.1037/h0048023

Packard, M. G., & Knowlton, B. J. (2002). Learning and memory functions of the basal ganglia. *Annual Review of Neuroscience, 25*, 563-593. doi:10.1146/annurev.neuro.25.112701.142937 [doi]

Pascoli, V., Terrier, J., Espallergues, J., Valjent, E., O'Connor, E. C., & Lüscher, C. (2014). Contrasting forms of cocaine-evoked plasticity control components of relapse. Nature, 509(7501), 459-464. https://doi.org/10.1038/nature13257

Passino, E., & Ammassari-Teule, M. (1999). Visual discrimination in inbred mice: Strain-specific involvement of hippocampal regions. *Physiology & Behavior, 67*(3), 393-399. doi:S0031-9384(99)00076-1 [pii]

Peak, J., Chieng, B., Hart, G., & Balleine, B. W. (2020). Striatal direct and indirect pathway neurons differentially control the encoding and updating of goal-directed learning. Elife, 9. https://doi.org/10.7554/eLife.58544

Pearce, J. M., & Hall, G. (1980). A model for pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review, 87*(6), 532-552. doi:10.1037/0033-295X.87.6.532

Pedreira, M. E., & Maldonado, H. (2003). Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron, 38*(6), 863-869. doi:10.1016/S0896-6273(03)00352-0

Pedroso, T. R., Jobim, P. F., Carvalho, L. M., Christoff, R. R., Maurmann, N., Reolon, G. K., . . . Roesler, R. (2013). Inhibition of protein synthesis or mTOR in the basolateral amygdala blocks retrieval-induced memory strengthening. *Journal of Neural*

Transmission (Vienna, Austria : 1996), 120(11), 1525-1531. doi:10.1007/s00702-013-1032-y [doi]

Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem, 16(5), 279-288. https://doi.org/10.1101/lm.1041309

Petrovich, G. D., Canteras, N. S., & Swanson, L. W. (2001). Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Research.Brain Research Reviews, 38*(1-2), 247-289. doi:S0165017301000807 [pii]

Pitkanen, A., Pikkarainen, M., Nurminen, N., & Ylinen, A. (2000). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. *Annals of the New York Academy of Sciences, 911*, 369-391. doi:10.1111/j.1749-6632.2000.tb06738.x [doi]

Piva, A., Gerace, E., Di Chio, M., Padovani, L., Paolone, G., Pellegrini-Giampietro, D. E., & Chiamulera, C. (2019). Reconsolidation of sucrose instrumental memory in rats: The role of retrieval context. *Brain Research, 1714*, 193-201. doi:S0006-8993(19)30129-5 [pii]

Piva, A., Gerace, E., Di Chio, M., Osanni, L., Padovani, L., Caffino, L., Fumagalli, F., Pellegrini-Giampietro, D. E., & Chiamulera, C. (2018). The metaplastic effects of NMDA receptors blockade on reactivation of instrumental memories in rats. Neurobiol Learn Mem, 154, 87-96. https://doi.org/10.1016/j.nlm.2018.01.007

Plosker, G. L. (2015). Memantine extended release (28 mg once daily): A review of its use in alzheimer's disease. *Drugs, 75*(8), 887-897. doi:10.1007/s40265-015-0400-3 [doi]

Przybyslawski, J., Roullet, P., & Sara, S. J. (1999). Attenuation of emotional and nonemotional memories after their reactivation: Role of beta adrenergic receptors. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 19*(15), 6623-6628.

Reed, P., & Reilly, S. (1990). Context extinction following conditioning with delayed reward enhances subsequent instrumental responding. *Journal of Experimental Psychology.Animal Behavior Processes, 16*(1), 48-55.

Reichelt, A. C., Exton-McGuinness, M. T., & Lee, J. L. (2013). Ventral tegmental dopamine dysregulation prevents appetitive memory destabilization. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 33*(35), 14205-14210. doi:10.1523/JNEUROSCI.1614-13.2013 [doi]

Reichelt, A. C., & Lee, J. L. (2012). Appetitive pavlovian goal-tracking memories reconsolidate only under specific conditions. *Learning & Memory (Cold Spring Harbor, N.Y.), 20*(1), 51-60. doi:10.1101/lm.027482.112 [doi]

Reichelt, A. C., & Lee, J. L. (2013a). Memory reconsolidation in aversive and appetitive settings. *Frontiers in Behavioral Neuroscience, 7*, 118. doi:10.3389/fnbeh.2013.00118 [doi]

Reichelt, A. C., & Lee, J. L. (2013b). Over-expectation generated in a complex appetitive goal-tracking task is capable of inducing memory reconsolidation. *Psychopharmacology, 226*(4), 649-658. doi:10.1007/s00213-012-2934-3 [doi]

Ren, Z., Liu, M., Xue, Y., Ding, Z., Xue, L., Zhai, S., & Lu, L. (2013). A critical role for protein degradation in the nucleus accumbens core in cocaine reward memory. *Neuropsychopharmacology, 38*(5), 778-790. doi:10.1038/npp.2012.243

Roberts, A. C., Tomic, D. L., Parkinson, C. H., Roeling, T. A., Cutter, D. J., Robbins, T. W., & Everitt, B. J. (2007). Forebrain connectivity of the prefrontal cortex in the marmoset monkey (callithrix jacchus): An anterograde and retrograde tract-tracing study. *The Journal of Comparative Neurology, 502*(1), 86-112. doi:10.1002/cne.21300 [doi]

Robbins, T. W., & Everitt, B. J. (1999). Drug addiction: bad habits add up. Nature, 398(6728), 567-570. https://doi.org/10.1038/19208

Rodriguez-Ortiz, C., Garcia-DeLaTorre, P., Benavidez, E., Ballesteros, M. A., & Bermudez-Rattoni, F. (2008). Intrahippocampal anisomycin infusions disrupt previously consolidated spatial memory only when memory is updated. *Neurobiology of Learning and Memory; Protein Synthesis and Memory, 89*(3), 352-359. doi:https://doi.org/10.1016/j.nlm.2007.10.004

Rodriguez-Ortiz, C. J., De la Cruz, V., Gutierrez, R., & Bermudez-Rattoni, F. (2005). Protein synthesis underlies post-retrieval memory consolidation to a restricted degree only when updated information is obtained. *Learning & Memory (Cold Spring Harbor, N.Y.), 12*(5), 533-537. doi:lm.94505 [pii]

Rolls, E. T., & Treves, A. (1994). Neural networks in the brain involved in memory and recall. *Progress in Brain Research, 102*, 335-341. doi:S0079-6123(08)60550-6 [pii]

Roozendaal, B. (2002). *Stress and memory: Opposing effects of glucocorticoids on memory consolidation and memory retrieval* doi:https://doi.org/10.1006/nlme.2002.4080

Rosenblum, K., Meiri, N., & Dudai, Y. (1993). Taste memory: The role of protein synthesis in gustatory cortex. *Behavioral and Neural Biology, 59*(1), 49-56.

Rudy, J. W., Biedenkapp, J. C., Moineau, J., & Bolding, K. (2006). Anisomycin and the reconsolidation hypothesis. *Learning & Memory (Cold Spring Harbor, N.Y.), 13*(1), 1-3. doi:13/1/1 [pii]

Sachser, R. M., Santana, F., Crestani, A. P., Lunardi, P., Pedraza, L. K., Quillfeldt, J. A., . . . Alvares Lde, O. (2016). Forgetting of long-term memory requires activation of NMDA receptors, L-type voltage-dependent Ca2+ channels, and calcineurin. *Scientific Reports, 6*, 22771. doi:10.1038/srep22771 [doi]

Sadler, R., Herzig, V., & Schmidt, W. J. (2007). Repeated treatment with the NMDA antagonist MK-801 disrupts reconsolidation of memory for amphetamine-conditioned place preference. *Behavioural Pharmacology, 18*(7), 699-703. doi:10.1097/FBP.0b013e3282effb81 [doi]

Sakurai, S., Yu, L., & Tan, S. E. (2007). Roles of hippocampal N-methyl-D-aspartate receptors and calcium/calmodulin-dependent protein kinase II in amphetamine-produced conditioned place preference in rats. *Behavioural Pharmacology, 18*(5-6), 497-506. doi:10.1097/FBP.0b013e3282ee7b62 [doi]

Saunders, R. C., Rosene, D. L., & Van Hoesen, G. W. (1988). Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: II. reciprocal and non-reciprocal connections. *The Journal of Comparative Neurology, 271*(2), 185-207. doi:10.1002/cne.902710203 [doi]

Schachtman, T. R., Reed, P., & Hall, G. (1987). Attenuation and enhancement of instrumental responding by signals for reinforcement on a variable interval schedule. *Journal of Experimental Psychology: Animal Behavior Processes, 13*(3), 271-279. doi:10.1037/0097-7403.13.3.271

Seif el Nasr, M., Peruche, B., Rossberg, C., Mennel, H. D., & Krieglstein, J. (1990). Neuroprotective effect of memantine demonstrated in vivo and in vitro. *European Journal of Pharmacology, 185*(1), 19-24. doi:0014-2999(90)90206-L [pii]

Sesack, S. R., & Grace, A. A. (2010). Cortico-Basal Ganglia reward network: microcircuitry. Neuropsychopharmacology, 35(1), 27-47. https://doi.org/10.1038/npp.2009.93

Sevenster, D., Beckers, T., & Kindt, M. (2013). Prediction error governs pharmacologically induced amnesia for learned fear. *Science (New York, N.Y.), 339*(6121), 830-833. doi:10.1126/science.1231357 [doi]

Shadmehr, R., & Holcomb, H. H. (1997). Neural correlates of motor memory consolidation. *Science (New York, N.Y.), 277*(5327), 821-825. doi:10.1126/science.277.5327.821 [doi]

Shinohara, K., & Hata, T. (2014). Post-acquisition hippocampal NMDA receptor blockade sustains retention of spatial reference memory in morris water maze. *Behavioural Brain Research, 259*, 261-267. doi:10.1016/j.bbr.2013.11.016 [doi]

Shipman, M. L., Trask, S., Bouton, M. E., & Green, J. T. (2018). Inactivation of prelimbic and infralimbic cortex respectively affects minimally-trained and extensively-trained goal-directed actions. Neurobiol Learn Mem, 155, 164-172. https://doi.org/10.1016/j.nlm.2018.07.010

Smith-Roe, S. L., & Kelley, A. E. (2000). Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. J Neurosci, 20(20), 7737-7742. https://doi.org/10.1523/jneurosci.20-20-07737.2000

Smith, K., & Graybiel, A. (2013). A dual operator view of habitual behavior reflecting cortical and striatal dynamics. *Neuron, 79*(2), 361-374. doi:10.1016/j.neuron.2013.05.038

Smith, K. S., Virkud, A., Deisseroth, K., & Graybiel, A. M. (2012). Reversible online control of habitual behavior by optogenetic perturbation of medial prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America, 109*(46), 18932-18937. doi:10.1073/pnas.1216264109

Smith, R. J., Lobo, M. K., Spencer, S., & Kalivas, P. W. (2013). Cocaine-induced adaptations in D1 and D2 accumbens projection neurons (a dichotomy not necessarily synonymous with direct and indirect pathways). Curr Opin Neurobiol, 23(4), 546-552. https://doi.org/10.1016/j.conb.2013.01.026

Sokolov, E. N. (., 1920-, & Vinogradova, O. S. (1975). In FAO of t. U. (Ed.), *Neuronal mechanisms of the orienting reflex; NeiÌ†ronnye mekhanizmy orientirovochnogo refleksa* L. Erlbaum Associates.

Song, Z., Chen, H., Xu, W., Wu, S., & Zhu, G. (2018). Basolateral amygdala calpain is required for extinction of contextual fear-memory. *Neurobiology of Learning and Memory, 155*, 180-188. doi:S1074-7427(18)30188-6 [pii]

Spear, N. E. (1973). Retrieval of memory in animals. *Psychological Review, 80*(3), 163-194. doi:10.1037/h0034326

Squire, L. R., & Barondes, S. H. (1972). Variable decay of memory and its recovery in cycloheximide-treated mice. *Proceedings of the National Academy of Sciences of the United States of America, 69*(6), 1416-1420. doi:10.1073/pnas.69.6.1416 [doi]

Stern, C. A., Gazarini, L., Vanvossen, A. C., Hames, M. S., & Bertoglio, L. J. (2013). Activity in prelimbic cortex subserves fear memory reconsolidation over time. *Learning & Memory (Cold Spring Harbor, N.Y.), 21*(1), 14-20. doi:10.1101/lm.032631.113 [doi]

Stern, E., Chinnakkaruppan, A., David, O., Sonenberg, N., & Rosenblum, K. (2013). Blocking the eIF2alpha kinase (PKR) enhances positive and negative forms of cortex-dependent taste memory. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 33*(6), 2517-2525. doi:10.1523/JNEUROSCI.2322-12.2013 [doi]

Stuber, G. D., Britt, J. P., & Bonci, A. (2012). Optogenetic modulation of neural circuits that underlie reward seeking. Biol Psychiatry, 71(12), 1061-1067. https://doi.org/10.1016/j.biopsych.2011.11.010

Sutton, R. S., & Barto, A. G. (1998). Reinforcement learning: An introduction MIT press. *Cambridge, MA,*

Sutton, R. S., & Barto, A. G. (2018). Reinforcement learning: An introduction. MIT press.

Suurmond, R., van Rhee, H., & Hak, T. (2017). Introduction, comparison, and validation of meta-essentials: A free and simple tool for meta-analysis. *Research Synthesis Methods, 8*(4), 537-553. doi:10.1002/jrsm.1260

Suzuki, A., Josselyn, S. A., Frankland, P. W., Masushige, S., Silva, A. J., & Kida, S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 24*(20), 4787-4795. doi:10.1523/JNEUROSCI.5491-03.2004 [doi]

Takahashi, Y. K., Roesch, M. R., Stalnaker, T. A., Haney, R. Z., Calu, D. J., Taylor, A. R., . . . Schoenbaum, G. (2009). The orbitofrontal cortex and ventral tegmental area are necessary for learning from unexpected outcomes. *Neuron, 62*(2), 269-280. doi:10.1016/j.neuron.2009.03.005

Takeuchi, T., Duszkiewicz, A. J., & Morris, R. G. M. (2014). The synaptic plasticity and memory hypothesis: Encoding, storage and persistence. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*(1633), 20130288. doi:10.1098/rstb.2013.0288

Tallot, L., Diaz-Mataix, L., Perry, R. E., Wood, K., LeDoux, J. E., Mouly, A. M., . . . Doyere, V. (2017). Updating of aversive memories after temporal error detection is differentially modulated by mTOR across development. *Learning & Memory (Cold Spring Harbor, N.Y.), 24*(3), 115-122. doi:10.1101/lm.043083.116 [doi]

Tanaka, K., Pevzner, A., Hamidi, A., Nakazawa, Y., Graham, J., & Wiltgen, B. (2014). Cortical representations are reinstated by the hippocampus during memory retrieval. *Neuron, 84*(2), 347-354. doi:10.1016/j.neuron.2014.09.037

Tedesco, V., Roquet, R. F., DeMis, J., Chiamulera, C., & Monfils, M. H. (2014). Extinction, applied after retrieval of auditory fear memory, selectively increases zinc-finger protein 268 and phosphorylated ribosomal protein S6 expression in prefrontal cortex and lateral amygdala. *Neurobiology of Learning and Memory, 115*, 78-85. doi:10.1016/j.nlm.2014.08.015 [doi]

Theberge, F. R., Milton, A. L., Belin, D., Lee, J. L., & Everitt, B. J. (2010). The basolateral amygdala and nucleus accumbens core mediate dissociable aspects of drug memory reconsolidation. *Learning & Memory (Cold Spring Harbor, N.Y.), 17*(9), 444-453. doi:10.1101/lm.1757410 [doi]

Thierry, A. M., Gioanni, Y., Degenetais, E., & Glowinski, J. (2000). Hippocampo-prefrontal cortex pathway: Anatomical and electrophysiological characteristics. *Hippocampus, 10*(4), 411-419. doi:10.1002/1098-1063(2000)10:43.0.CO;2-A [doi]

Thomas, K. L., Hall, J., & Everitt, B. J. (2002). Cellular imaging with zif268 expression in the rat nucleus accumbens and frontal cortex further dissociates the neural pathways activated following the retrieval of contextual and cued fear memory. *The European Journal of Neuroscience, 16*(9), 1789-1796. doi:2247 [pii]

Thompson, C. I., & Grossman, L. B. (1972). Loss and recovery of long-term memories after ECS in rats: Evidence for state-dependent recall. *Journal of Comparative and Physiological Psychology, 78*(2), 248-254. doi:10.1037/h0032178 [doi]

Thorndike, E. L., & Woodworth, R. S. (1901). The influence of improvement in one mental function upon the efficiency of other functions. II. the estimation of magnitudes. *Psychological Review, 8*(4), 384-395. doi:10.1037/h0071280

Tran-Tu-Yen, D. A., Marchand, A. R., Pape, J. R., Di Scala, G., & Coutureau, E. (2009). Transient role of the rat prelimbic cortex in goal-directed behaviour. *The European Journal of Neuroscience, 30*(3), 464-471. doi:10.1111/j.1460-9568.2009.06834.x [doi]

Tronson, N. C., Schrick, C., Guzman, Y. F., Huh, K. H., Srivastava, D. P., Penzes, P., . . . Radulovic, J. (2009). Segregated populations of hippocampal principal CA1 neurons mediating conditioning and extinction of contextual fear. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 29*(11), 3387-3394. doi:10.1523/JNEUROSCI.5619-08.2009 [doi]

Tronson, N. C., & Taylor, J. R. (2007). Molecular mechanisms of memory reconsolidation. *Nature Reviews.Neuroscience, 8*(4), 262-275. doi:nrn2090 [pii]

Tsai, H. C., Zhang, F., Adamantidis, A., Stuber, G. D., Bonci, A., de Lecea, L., & Deisseroth, K. (2009). Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science, 324(5930), 1080-1084. https://doi.org/10.1126/science.1168878

Tucci, P., Mhillaj, E., Morgese, M. G., Colaianna, M., Zotti, M., Schiavone, S., . . . Trabace, L. (2014). Memantine prevents memory consolidation failure induced by

soluble beta amyloid in rats. *Frontiers in Behavioral Neuroscience, 8*, 332. doi:10.3389/fnbeh.2014.00332 [doi]

Ungerer, A. (1973). *Nature et ampleur des effets de l'acétoxycycloheximide sur la rétention d'un apprentissage instrumental chez la souris* doi:https://doi.org/10.1016/0031-9384(73)90007-3

Veyrac, A., Besnard, A., Caboche, J., Davis, S., & Laroche, S. (2014). The transcription factor Zif268/Egr1, brain plasticity, and memory. Prog Mol Biol Transl Sci, 122, 89-129. https://doi.org/10.1016/B978-0-12-420170-5.00004-0

Vezina, P., Giovino, A. A., Wise, R. A., & Stewart, J. (1989). Environment-specific cross-sensitization between the locomotor activating effects of morphine and amphetamine. Pharmacol Biochem Behav, 32(2), 581-584. https://doi.org/10.1016/0091-3057(89)90201-3

Villarreal, D. M., Do, V., Haddad, E., & Derrick, B. E. (2002). NMDA receptor antagonists sustain LTP and spatial memory: Active processes mediate LTP decay. *Nature Neuroscience, 5*(1), 48-52. doi:10.1038/nn776 [doi]

Volbracht, C., van Beek, J., Zhu, C., Blomgren, K., & Leist, M. (2006). Neuroprotective properties of memantine in different in vitro and in vivo models of excitotoxicity. *The European Journal of Neuroscience, 23*(10), 2611-2622. doi:EJN4787 [pii]

Vyklicky, V., Korinek, M., Smejkalova, T., Balik, A., Krausova, B., Kaniakova, M., . . . Vyklicky, L. (2014). Structure, function, and pharmacology of NMDA receptor channels. *Physiological Research, 63 Suppl 1*, S191-203. doi:932678 [pii]

Waelti, P., Dickinson, A., & Schultz, W. (2001). Dopamine responses comply with basic assumptions of formal learning theory. *Nature, 412*(6842), 43-48. doi:10.1038/35083500 [doi]

Waelti, P., Dickinson, A., & Schultz, W. (2001). Dopamine responses comply with basic assumptions of formal learning theory. *Nature, 412*(6842), 43-48. doi:10.1038/35083500

Wang, L., Shen, M., Yu, Y., Tao, Y., Zheng, P., Wang, F., & Ma, L. (2014). Optogenetic activation of GABAergic neurons in the nucleus accumbens decreases the activity of the ventral pallidum and the expression of cocaine-context-associated memory. Int J Neuropsychopharmacol, 17(5), 753-763. https://doi.org/10.1017/s1461145713001570

Wang, N., Ge, F., Cui, C., Li, Y., Sun, X., Sun, L., Wang, X., Liu, S., Zhang, H., Liu, Y., Jia, M., & Yang, M. (2018). Role of Glutamatergic Projections from the Ventral CA1 to Infralimbic Cortex in Context-Induced Reinstatement of Heroin Seeking. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 43(6), 1373-1384. https://doi.org/10.1038/npp.2017.279

Wang, R. C., Xiao, L. F., Zhang, C., Sun, T., & Sun, K. S. (2020). [Intra-nucleus accumbens shell injection of baclofen blocks the reconsolidation of conditioned place preference in morphine-addicted mice]. Sheng Li Xue Bao, 72(2), 255-261.

Wang, S. H., Ostlund, S. B., Nader, K., & Balleine, B. W. (2005). Consolidation and reconsolidation of incentive learning in the amygdala. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 25*(4), 830-835. doi:25/4/830 [pii]

Wang, S., de, O. A., & Nader, K. (2009). Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nature Neuroscience, 12*(7), 905-912. doi:10.1038/nn.2350

Warren, B. L., Mendoza, M. P., Cruz, F. C., Leao, R. M., Caprioli, D., Rubio, F. J., Whitaker, L. R., McPherson, K. B., Bossert, J. M., Shaham, Y., & Hope, B. T. (2016). Distinct Fos-Expressing Neuronal Ensembles in the Ventromedial Prefrontal Cortex Mediate Food Reward and Extinction Memories. The Journal of neuroscience : the official journal of the Society for Neuroscience, 36(25), 6691-6703. https://doi.org/10.1523/jneurosci.0140-16.2016

Wassum, K. M., Ostlund, S. B., Maidment, N. T., & Balleine, B. W. (2009). Distinct opioid circuits determine the palatability and the desirability of rewarding events. *Proceedings of the National Academy of Sciences of the United States of America, 106*(30), 12512-12517. doi:10.1073/pnas.0905874106 [doi]

Watabe-Uchida, M., Eshel, N., & Uchida, N. (2017). Neural Circuitry of Reward Prediction Error. Annu Rev Neurosci, 40, 373-394. https://doi.org/10.1146/annurev-neuro-072116-031109

Weatherly, J. N., Arthur, E. I., & Nurnberger, J. T. (2006). Is induction produced by upcoming food-pellet reinforcement the outcome of an increase in overall activity or in operant responding? *The Journal of General Psychology, 133*(1), 97-111. doi:10.3200/GENP.133.1.97-111 [doi]

Wells, A. M., Lasseter, H. C., Xie, X., Cowhey, K. E., Reittinger, A. M., & Fuchs, R. A. (2011). Interaction between the basolateral amygdala and dorsal hippocampus is critical for cocaine memory reconsolidation and subsequent drug context-induced

cocaine-seeking behavior in rats. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *18*(11), 693-702. doi:10.1101/lm.2273111 [doi]

White, N. M., & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. Neurobiol Learn Mem, 77(2), 125-184. https://doi.org/10.1006/nlme.2001.4008

Williams, B. A. (1976). The effects of unsignalled delayed reinforcement. *Journal of the Experimental Analysis of Behavior, 26*(3), 441-449. doi:10.1901/jeab.1976.26-441 [doi]

Wilson, P. N., Boumphrey, P., & Pearce, J. M. (1992). Restoration of the orienting response to a light by a change in its predictive accuracy. *The Quarterly Journal of Experimental Psychology Section B, 44*(1), 17-36. doi:10.1080/02724999208250600

Wittman, T. K., & DeVietti, T. L. (1980). Heart-rate reactivity to reminder treatment predicts test performance in rats given ECS following training. *Physiological Psychology, 8*(4), 515-521. doi:10.3758/BF03326486

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci, 19(1), 181-189. https://doi.org/10.1111/j.1460-9568.2004.03095.x

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2005). Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. *The European Journal of Neuroscience, 22*(2), 505-512. doi:EJN4219 [pii]

Yin, H. H., Ostlund, S. B., Knowlton, B. J., & Balleine, B. W. (2005). The role of the dorsomedial striatum in instrumental conditioning. *The European Journal of Neuroscience, 22*(2), 513-523. doi:EJN4218 [pii]

Zajaczkowski, W., Frankiewicz, T., Parsons, C. G., & Danysz, W. (1997). Uncompetitive NMDA receptor antagonists attenuate NMDA-induced impairment of passive avoidance learning and LTP. *Neuropharmacology, 36*(7), 961-971. doi:S0028390897000701 [pii]

Zapata, A., Minney, V. L., & Shippenberg, T. S. (2010). Shift from goal-directed to habitual cocaine seeking after prolonged experience in rats. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 30*(46), 15457-15463. doi:10.1523/JNEUROSCI.4072-10.2010 [doi]