A Psychological Analysis of the Effects of Memory Retrieval Prior to Extinction on the Reacquisition of a Conditioned Fear Association

Melissa Allison Wood

Downing College, Cambridge

September 2010

Department of Experimental Psychology

University of Cambridge

Cambridge

United Kingdom

This dissertation is submitted for the degree of

Doctor of Philosophy

PREFACE

The following work was carried out at the Department of Experimental Psychology, University of Cambridge, under the supervision of Prof Barry J. Everitt.

I hereby declare that this dissertation has not been submitted, in whole or in part, for any other degree, diploma or qualification at any other University.

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

The length of this dissertation does not exceed the 60 000 word limit set by the Degree Committee of the Faculty of Biology.

ACKNOWLEDGEMENTS

There are many people to whom I am deeply grateful for making my time at Cambridge so valuable and memorable.

To being with, I would like to thank my supervisor, Professor Barry J. Everitt, for his guidance, support, patience and encouragement throughout the duration of my PhD training. I am deeply grateful for the opportunity to have studied under his supervision and for the invaluable experience this has provided.

My sincere thanks go to Dr Jonathan L.C. Lee for his advice and support in all aspects of my PhD project. I would also like to express my deep gratitude to Dr Gonzalo Urcelay who has been an enthusiastic and dedicated source of scholarship and encouragement.

To all members of the lab, I consider myself very fortunate to have worked with each one of you. Thanks go to David Theobald and Alan Lyon for technical and histological assistance, as well as Alan Graham, Julie Gautrey, and Chris Cardinal for their dedication to animal care. Also, a special thanks to all those who, at various times, have stepped in to help me with running experiments including Dr Amy Milton, Dr Yann Pelloux, Dr Florence Théberge, Dr Gonzalo Urcelay, Anushka Fernando and Moritz Schramm.

The wonderful friends I have had in Cambridge have all contributed to making these last years so rewarding and enjoyable. Among these, I would like to give a special mention to Laura Haynes who has been a vital part of this adventure from the very beginning.

To my family, whose love and support has made this all possible, and to Balázs, who has been my strength and inspiration, I will be forever grateful.

AUTHOR'S NOTE

Financial Support

The work presented in this thesis was funded by a Medical Research Council Programme Grant. Research conducted within the Behavioural and Clinical Neuroscience Institute is funded by a joint award from the MRC and Wellcome Trust. The author was supported by an Overseas Research Studentship, an award from the Poynton Cambridge Commonwealth Trust and an Oon Khye Beng Ch'hia Tsio Bursary.

SUMMARY

The successful reduction of fear is the aim of clinicians treating people with anxiety disorders such as post-traumatic stress disorder or phobias. Existing treatments for these conditions, however, require many treatment sessions and are prone to relapse. A new technique, first demonstrated in rats by Monfils, Cowansage, Klann, & LeDoux (2009) and later shown to be effective in humans (Schiller et al., 2010), provides a method of efficiently reducing fear in a manner which is resistant to various known triggers of relapse. This procedure involves a single presentation of the fear-inducing stimulus one hour prior to extinction training. This procedure produces extinction learning that is resistant to the return of fear resulting from a change of context, the passage of time, exposure to the unconditioned stimulus, and even further conditioning of the stimulus with an aversive stimulus.

This dissertation focuses on one particular property of this procedure: that a stimulus extinguished using this procedure is resistant to subsequent retraining of the fear association. The first four experiments presented here are aimed at replicating this phenomenon and determining whether prediction error at retrieval is necessary for the effect to occur.

Following on from these studies, the next chapter presents three experiments which investigate whether trial spacing effects could explain the enhanced extinction and highlights conditions under which the effect is weakened, or possibly reversed.

The next three experiments compare the properties of a stimulus extinguished under these conditions with a stimulus extinguished under normal conditions. These studies focus on explanations involving inhibition, inattention and the disruption of stimulus representations.

In the final three experiments, the possibility of reversing the effect is investigated. These studies look at the effect of memory retrieval prior to retraining of the stimulus to determine the conditions under which the stimulus can again come to elicit a fear response.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BDNF	brain-derived neurotrophic factor
BLA	basolateral amygdala
CS	conditioned Stimulus
Cxt	context
DCS	D-cycloserine
ECS	electroconvulsive shock
ET	Exposure Therapy
Ext	extinction
LI	latent inhibition
LTM	long-term memory
LTP	long-term potentiation
M	mean
NMDA	*N*-methyl-*D*-aspartate
NoRet	no retrieval
Ret	retrieval
SD	standard deviation
SEM	standard error of the mean
SOP	Sometimes Opponent Process
US	unconditioned stimulus
βlac	clasto-lactacystin-β-lactone

I. GENERAL INTRODUCTION

The study of the processes involved in fear and anxiety is of critical importance for understanding the mechanisms underlying emotional regulation of behaviour, and as a consequence developing treatments for anxiety disorders. Many of the treatments for anxiety disorders such as phobias or post-traumatic stress disorder (PTSD) show similarities with methods of fear reduction in non-human animals (Bouton, 1988). One of the more common treatments for anxiety disorders is exposure therapy in which a fear-eliciting stimulus is presented repeatedly in a safe environment until anxiety subsides (e.g., Foa & Kozak, 1986). Similarly, in experimental extinction of fear, a stimulus which has been trained to produce a fear response is presented to the subject in the absence of any aversive outcome and fear responding declines (e.g., Rescorla, 2002).

Unfortunately, high rates of relapse have been reported following such treatments for anxiety disorders (Rachman, 1989). Other treatments too have met with similar problems (Paunovic & Öst, 2001). As a consequence, much effort has gone into developing more effective methods for producing long-term reductions in fear (Craske et al., 2008; Hofmann, 2008). Much of this research has focussed on identifying factors which influence the success of fear extinction, including contextual, temporal and pharmacological effects on fear learning and responding (Bouton, Westbrook, Corcoran, & Maren, 2006).

In recent years, however, there has been a resurgence in investigation of the mechanisms of memory storage and retrieval. The discovery that amnesia for a previously stable memory can be induced by a variety of manipulations administered after memory retrieval has led to a reconceptualisation of memory processes (Misanin, Miller, & Lewis, 1968; Nader, Schafe, & LeDoux, 2000). Moreover, this finding represents another, potentially more efficient and more permanent, method of ameliorating the effects of unwanted or maladaptive memories.

This thesis explores a new technique for the reduction of fear in an experimental context. The experiments presented investigate the interaction between learning and memory systems and examine potential clinical applications of this technique in treating anxiety.

Pavlovian Conditioning

In the laboratory, fear is commonly studied within the context of Pavlovian fear conditioning. In this paradigm, a neutral stimulus is paired temporally with a naturally aversive stimulus [unconditioned stimulus (US)] with the result that the previously neutral stimulus, now a conditioned stimulus (CS), comes to elicit a conditioned response (CR). In the case of fear conditioning, this CR may take the form of fear-responses such as freezing (Fanselow, 1994), fear-potentiated startle (Davis & Astrachan, 1978), or autonomic changes, e.g., in blood pressure or heart rate (LeDoux, Iwata, Cicchetti, & Reis, 1988). Following Pavlovian fear conditioning, the capacity of the CS to elicit a CR can be reduced through extinction: the repeated presentation of the CS in the absence of the US. While the present discussion will focus on fear conditioning, the same principles of Pavlovian association formation apply to learning involving appetitive reinforcers such as food or drug (Blaiss, 2008; Pavlov, 1927).

Requirements for Associative Learning

The success of conditioning depends greatly upon the temporal relationship between the CS and the US. The precise timing of CS and US events required to produce optimal learning varies widely across behavioural paradigms (Rescorla, 1988). Eye-blink conditioning, for example, in which a CS predicts a puff of air to the cornea or a mild shock to the skin at the edge of the eye socket, is most effective with an interval between CS onset and US onset of around 200 milliseconds (Smith, Coleman, & Gormezano, 1969). For conditioned taste aversion, in comparison, a CS-US offset of approximately one hour has been shown to be the most effective in producing an association between a novel flavour and

illness (Barker & Smith, 1974). In the context of fear conditioning, the most commonly used arrangement of CS and US is one in which the onset of the US is later than the onset of the CS but prior to CS termination. Conditioning conducted in this manner is referred to as delay conditioning.

However, mere concurrence of CS and US presentations is not sufficient for learning. In fact, under certain conditions the pairing of CS and US can lead to little or no change in conditioned responding (blocking; Kamin, 1968), or even in a loss of conditioned responding (overexpectation; Rescorla, 1970). More important than simple temporal contiguity is a requirement for predictive power. In other words, for a CS to become associated with the US, the occurrence of the CS must provide some information about the likelihood of the occurrence of the US, that is, the CS-US contingency. With consistent temporal contingency between CS and US, variations in the effectiveness of conditioning can be obtained through varying the CS-US contingency. In other words, learning is influenced not only by the probability of the US in the presence of the CS, but also by the probability of the US in the absence of the CS (Rescorla, 1968). An association between the CS and the US can only form if the US is more likely to occur in the presence of the CS than in its absence. If the likelihood of the US is unchanged by the occurrence of the CS, then no learning will occur. Moreover, if the US is more likely in the absence of the CS than in its presence, this can lead to the development of an inhibitory association between the CS and the US.

Prediction Error in Pavlovian Conditioning

A demonstration of the requirement for predictive information was provided by Kamin (1968) with a phenomenon termed *blocking*. In the first phase of a standard blocking procedure, one CS (A) is initially paired with the US such that A comes to elicit a CR. Training on this phase continues until responding to A reaches asymptote. In the second phase, the target CS (X) is introduced and the compound presentation of the two stimuli, AX,

is then paired with the US. If temporal contiguity were sufficient for learning, then X should become associated with the US by virtue of the two stimuli having been presented in close temporal proximity to each other during the second phase of the experiment. However, the prior training of A results in an impairment in learning about X. Kamin (1968) attributes this impairment to the fact that the US was already fully predicted by A, and so X provided no new information about the outcome of the trial. In other words, learning about X was impaired by the absence of "prediction error" (Schultz & Dickinson, 2000).

Prediction error is defined as the discrepancy between what is expected and what actually occurs. An error term is commonly derived from this relationship which is represented as $(\lambda - V)$ where λ is the actual outcome of the trial and V is the expected outcome (Bush & Mosteller, 1955). This error term is central to most contemporary theories of learning with increments in associative strength generally being proportional to the value of this discrepancy. The presentation of the US on a given trial allows the allocation of an arbitrary positive value to λ. This value represents the maximal learning possible across repeated trials with the same US. When the US presentation is completely unexpected, the value of V will be 0, and so the error term will be at a maximum. Once the US is well predicted, V will approach the value of λ such that the error term will approach 0. At this point learning will be minimal.

Theories of Pavlovian Association Formation

One of the most influential theories of Pavlovian association formation, the Rescorla-Wagner model (Rescorla & Wagner, 1972), relies heavily on the concept of prediction error. The Rescorla-Wagner model can be represented by the following equation:

$$\Delta V_A = \alpha.\beta.(\lambda - V_T) \qquad (1)$$

where ΔV_A is the change in associative strength of stimulus A during the trial, α is the CS learning rate parameter, β is the US learning rate parameter, λ is the asymptote of learning,

and V_T is the total associative strength of all CSs present during the trial. The curve described by this model is negatively accelerated, reaching asymptote at λ. According to this model, changes in associative strength of a CS are a function of the discrepancy between perfect prediction of the US (λ) and the degree to which the US is currently predicted by the existing CSs (V_T). The largest changes in associative strength occur when an event is surprising, and thus the discrepancy ($\lambda - V_T$) is large. This may occur during the first conditioning trial when the total associative strength of the CSs is 0. Similarly, when the CSs are strongly conditioned (V_T is large) and the US is omitted ($\lambda = 0$), the omission of the US will be surprising and thus the discrepancy will again be large.

An important feature of the Rescorla-Wagner model is the implications it has for compound stimuli. The importance of this feature of the model can be highlighted through consideration of the phenomenon of blocking (Kamin, 1968). During the first phase of the blocking paradigm, stimulus A acquires associative strength with the US such that V_A approaches the value of λ. At the conclusion of phase one training, therefore, the error term ($\lambda - V_T$) has approached 0, indicative of the US being well predicted by the CS. The two stimuli, A and X, are then paired with the US in phase two and the amount of learning about X is proportional to the discrepancy between λ and the associative strengths of all stimuli present on the trial. As a result of phase one training, stimulus A becomes a good predictor of the occurrence of the US and so the value of V_A approaches λ. Since V_T is equal to the sum of V_A and V_X, the error term ($\lambda - V_T$) at the start of phase two training is minimal. Therefore, the amount of learning that can accrue to X is also limited. According to the Rescorla-Wagner model, therefore, stimulus X could not become associated with the US since it did not predict anything that was not already predicted by the stimuli (i.e., A) present on that trial.

An alternative account of the blocking effect was proposed by Mackintosh (1975a). Mackintosh's (1975a) model is summarised by the following equation:

$$\Delta V_A = \alpha.\beta.(\lambda - V_A) \qquad (2)$$

The terms of this equation are equivalent to their respective terms in the Rescorla-Wagner model with the exception that α is now defined as a variable reflecting the amount of attention given to the CS. At first glance, the primary difference between the two models seems to be that while Rescorla and Wagner (1972) focus on the error in prediction between λ and all stimuli present on the trial (V_T), Mackintosh (1975a) identifies prediction error of a given stimulus with the discrepancy between λ and the associative strength of only that stimulus, much as how it was originally proposed by Bush and Mosteller (1955). What allows Mackintosh's (1975a) model to account for the effects of compound stimuli is variations in the value of α which correspond to the relationships between the target CS (A) and other stimuli (X) present at the time of reinforcement. Specifically:

$$\alpha_A \text{ increases when } |\lambda - V_A| < |\lambda - V_X| \qquad (3a)$$

$$\alpha_A \text{ decreases when } |\lambda - V_A| > |\lambda - V_X| \qquad (3b)$$

This model was based on the intuitive notion that animals will attend most readily to stimuli that predict events of significance in the environment. The success with which a stimulus predicts the occurrence of a biologically significant US on a given trial will determine the degree of attention that is paid to that stimulus on the subsequent trial. If a CS proves to be a good predictor of the US (or more specifically, a better predictor than other available stimuli) on trial *n*, then more attention will be paid to that CS on trial *n* + 1. The value of α for a good predictor will approach 1 whereas for a poor predictor, α will tend towards 0. Attention (α) to the CS affects learning in a multiplicative fashion (see Equation 2), meaning that the rate of learning about a specific CS will be modulated by the attention

paid to that stimulus. Higher values of α will permit more learning while low values will result in little change in associative strength.

In the case of the phenomenon of blocking, Mackintosh's (1975) theory claims that attention to the target CS on the first compound conditioning trial will be of an intermediate value on account of the novelty of the stimulus. However, after this trial, attention to this CS will be reduced due to the failure of the stimulus to provide any new information. As a result of this reduction in α, learning about the target stimulus on the next trial will be reduced. Interestingly, this leads Mackintosh (1975) and Rescorla and Wagner (1972) to make differing predictions about blocking in the case of a single compound conditioning trial. Given that the redundancy of the target stimulus is not determined until after the first trial, Mackintosh (1975) predicts that attention to, and therefore learning about, the CS and US on this trial should proceed normally. Blocking, therefore, should only be observed when phase two conditioning consists of more than one trial. The Rescorla-Wagner model, in contrast, predicts that learning will be reduced (i.e., blocked) on the very first trial since learning cannot proceed in the absence of prediction error. Although Mackintosh (1975b) provided evidence in support of the absence of one-trial blocking, the interpretation of these results was later questioned (Dickinson & Mackintosh, 1979), and ultimately the weight of evidence lies in favour of the Rescorla-Wagner model in predicting blocking after a single trial (Balaz, Kasprow, & Miller, 1982).

One important feature of the Mackintosh model is its ability to account for latent inhibition. The term *latent inhibition* (LI) refers to the observation that repeated nonreinforced exposure to a CS prior to conditioning retards the emergence of conditioned responding when that CS is subsequently paired with a US. The Rescorla-Wagner model cannot account for this effect since the absence of prediction error during pre-exposure should not result in a change in any of the parameters in the model, and so should not have

any effect on subsequent conditioning involving the pre-exposed stimulus. Over the course of pre-exposure, according to Mackintosh (1975), the CS is found to be no better at predicting the absence of reinforcement than the context in which it is presented, and so attention (α) to the stimulus by the end of pre-exposure is only minimal. (This additionally assumes that the situation in which the prediction error relating to the target CS is *equal* to the prediction error relating to other stimuli leads to a reduction in α.) Thus, when the CS later comes to be paired with the US, the rate of learning is diminished and the development of the CS retarded.

Another model which readily accounts for the retarded acquisition seen in the LI preparation is that of Pearce and Hall (1980). Like Mackintosh (1975), the Pearce-Hall model assumes that the α parameter captures attention and permits the value of this parameter to be determined on the basis of the outcome of the previous trial. In contrast to Mackintosh, however, attention is allocated not to stimuli which already serve as reliable predictors of biologically significant events but rather to stimuli about which learning is still required. According to Pearce and Hall (1980), the amount of associative change which occurs on a given trial is a function of the intensity of both the CS (*S*) and the US (λ) as well as, most importantly, the amount of attention conferred upon the CS (α). This relationship can be represented as:

$$\Delta V_A = \alpha \, . \, S \, . \, \lambda \qquad (3)$$

Critical for this model is that α is equal to the magnitude of the prediction error on the previous trial, or mathematically:

$$\alpha_A{}^n = |\lambda_A{}^{n-1} - V_T{}^{n-1}| \qquad (4)$$

The use of the combined associative strength (V_T) allows this model to easily account for effects of compound stimuli, such as in the case of blocking. However, like Mackintosh (1975), factors influencing attention not having their effect until the following trial leads this model to predict no effect of one-trial blocking. The account Pearce and Hall (1980) provide

for latent inhibition, however, allows an examination of the differences between this model and that of Mackintosh (1975). While both models can adequately account for this effect, the Pearce-Hall model does so by focussing on the absence of prediction error during CS pre-exposure. That the US is neither expected nor presented results in the value of α reaching 0 (Equation 4). When the CS is first paired with the US, this will mean that the change in associative strength of the CS (ΔV_A) will be close to 0, i.e., very little learning will occur. Learning about a pre-exposed stimulus, therefore, is delayed by at least one trial relative to conditioning of a novel stimulus for which the starting value of α is greater than zero. (It should be noted that rather than being determined by only the immediately preceding trial, α may in fact adopt a weighted average of values resulting from multiple previous trials, which would allow the predictions of this model to fit more closely with observed data (Pearce & Hall, 1980).)

In summary, while Mackintosh (1975) explains LI as being due to the CS not being a good predictor of anything, the Pearce-Hall model (Pearce & Hall, 1980) claim the effect arises from the CS being a perfect predictor of nothing. This difference is best highlighted in a phenomenon which, for the Pearce-Hall model, is analogous to LI, but that is problematic for Mackintosh's (1975) model. In the Hall-Pearce negative transfer paradigm, rather than establishing the CS as a good predictor of no US, the CS is trained to asymptote to predict the delivery of a weak US (Hall & Pearce, 1982). In the second phase of the experiment, the CS is paired with a stronger shock and fear responding is recorded across these trials. Mackintosh's (1975) model predicts that, since the CS is a good predictor of the weak shock, attention to that stimulus should be high and so learning during the second phase of training should, if anything, be facilitated. The Pearce-Hall model in contrast anticipates that once conditioning to the CS has reached asymptote, attention will be low and so phase two training should be retarded. The results of this experiment supported the predictions of the Pearce-

Hall model (Hall & Pearce, 1979). Furthermore, the effect could be reversed by introducing prediction error prior to conditioning with the stronger US, supporting the claim that attention is directed towards surprising rather than predictable events (Hall & Pearce, 1982).

The models outlined here represent three of the most successful accounts of the psychology of learning. Unfortunately, no single model currently conceived can explain the full range of psychological phenomena reported in the literature. While the Rescorla-Wagner model provided an elegant account of many of the properties of learning, its failure to accommodate the role of attention limited its ability to explain a number of phenomena, including the deceptively simple LI effect. This shortcoming was addressed by both Mackintosh (1975) and Pearce and Hall (1980) with their attentional models, yet with one-trial blocking being among a handful of preparations which they could not account for.

Extinction

Another phenomenon which has presented problems for learning theorists over the last century is extinction (Mackintosh, 1974; Delamater, 2004). The reduction in conditioned responding resulting from the repeated presentation of a CS in the absence of the associated US was reported by Pavlov (1927). Despite the simplicity of the procedure, the underlying psychological mechanisms are yet to be fully explained by existing theories of associative learning. Accounts of extinction have variously appealed to a range of processes, including unlearning, new learning, and non-associative processes. Theories which emphasise unlearning processes propose a breakdown in the association between the CS and the US. The Rescorla-Wagner model (Rescorla & Wagner, 1972), for example, suggests that reductions in conditioned responding represent a loss of associative strength between CS and US. These changes in associative strength occur as a function of the discrepancy between the actual and expected outcomes, and serve to correct for errors in prediction of the US. According to the Rescorla-Wagner theory, extinction occurs when an expected reinforcer is

omitted (effectively setting λ, or the amount of associative strength the US can support, to 0) such that the error term becomes $(0 - V_T)$ and, therefore, negative. As a result, changes in associative strength (ΔV_A) on extinction trials will be negative, i.e., associative strength will be reduced from one trial to the next. Reductions in associative strength continue across extinction trials until the CS is no longer able to elicit a CR.

There is, however, strong evidence that extinction does not involve complete erasure of the CS-US association. A change of context, presentation of the US or simply the passage of time have been shown to restore an extinguished CR, thus providing evidence that the original association has, at least to some extent, remained intact (Bouton, 1993; Pavlov, 1927; Rescorla & Heth, 1975). 'New learning' theories of extinction propose that extinction involves the learning of new inhibitory associations that oppose the original excitatory associations yet leave these original associations intact (Konorski, 1967; Pearce & Hall, 1980; Bouton, 1993). Konorski (1967) proposed that the reduction in conditioned responding resulting from extinction was due to the formation of a new inhibitory association between the CS and the US that opposed the original excitatory association.

Bouton (1993) proposes that during extinction, the CS forms an inhibitory association with the US to compete with the excitatory association, but, in addition, suggests that this inhibitory association is gated by contextual cues. The consequence of such gating is that the extinction memory can only be retrieved in the extinction context. Removal from the extinction context will then lead to an unmasking of (or failure to retrieve) the excitatory association, and so the conditioned responding will be restored. This model is well suited to explain phenomena such as renewal and spontaneous recovery that are not readily explained by pure unlearning models of extinction. Renewal is the return of a CR after extinction that occurs as a result of a removal from the extinction context. According to Bouton (1993), the return of the CR occurs because, in the absence of the extinction context, the inhibitory

association cannot be accessed and thus the excitatory CS-US association is again observed. This model can similarly explain spontaneous recovery, the return of a CR resulting from the passage of time following extinction. Bouton (1993) explains spontaneous recovery as a change in temporal context that impairs retrieval of the extinction memory in much the same way as a change in physical context would impair retrieval. A third way in which conditioned responding may be recovered is through reinstatement, i.e., re-exposure to the US following extinction. Bouton's theory explains that reinstatement occurs through conditioning of the context in which the unpaired US is presented, which then elicits a fear response when the animal is tested in this same context (Bouton & Bolles, 1979). While this account falls short of explaining reinstatement in the case of a signalled shock presentation (Rescorla & Heth, 1975), the observation that reinstatement effects are observed only in when reinstatement and testing occur in the same context is uniquely accounted for by this model.

Other theories of extinction have appealed to non-associative processes in the loss of conditioned responding which occurs across nonreinforced presentations of the CS. Non-associative accounts of extinction involve changes in the processing of either the CS or the US rather than a change in the association between them. Pavlov (1927) suggested that extinction may occur through a reduction in CS processing or attention to the CS. If little attention is focussed on the CS it becomes unable to produce a CR. Inattention to the CS may also allow the conditioned association to remain intact.

Alternatively, it has been suggested that extinction involves, at least in part, changes in the US representation. Rescorla & Heth (1975) suggest that along with changes in associative strength between CS and US, modification of the US representation may occur in the absence of the US. These modifications interfere with the ability of the retrieved US representation to elicit a CR. Rescorla & Heth (1975) demonstrate that it is possible to restore the US representation through re-exposure to the US (reinstatement). Rescorla & Heth (1975) did not

speculate as to the nature of the modification. It may be that the US comes to be perceived as less intense over time through a process akin to US devaluation, or that the representation of the US deteriorates such that the threshold for its activation is increased.

Evidence exists to support unlearning, new learning and non-associative accounts of extinction. It is clear that extinction does not involve complete erasure of the CS-US association as this association can be readily observed as the result of renewal (Bouton, 1993), reinstatement (Rescorla & Heth, 1975) or spontaneous recovery (Pavlov, 1927). However, incomplete recovery of conditioned responding under these conditions may indicate some degree of unlearning (Richardson, Ledgerwood & Cranney, 2004). A few models exist which provide a means of uniting unlearning and new learning accounts of extinction (e.g., Gershman, Blei, & Niv, 2010; Kehoe, 1988; Redish, Jensen, Johnson, & Kurth-Nelson, 2007). A model presented by Redish et al. (2007) outlines two processes proposed to operate during learning: a value-learning process and a state-classification process. The value-learning process resembles the temporal difference learning algorithm of Sutton and Barto (1998), which incorporates error correction processes in a similar manner to Rescorla and Wagner (1972). In short, values assigned to actions or stimuli are adjusted by increments proportional to the discrepancy between the expected and actual reinforcement. The second process, the state-classification process, identifies the current state of the animal and determines when it is necessary to create a new state. Each state is associated with a collection of observations which identify that state and differentiate it from other states. A new state will be formed when the observation statistics deviate sufficiently from the prototype of the current state. This can occur particularly in the case of a tonically negative prediction error. (Interestingly, this was implemented in the model most successfully by assuming an increase in attention to the stimuli when an expected reward was omitted, a assumption consistent with the basis of the Pearce-Hall model; Pearce & Hall, 1980)

Importantly for this model, a stimulus can have different values in different states. The value which will be applied when that stimulus is encountered will depend upon which of the previous states most resembles the current state (Redish et al., 2007).

The Redish et al. model was developed primarily to explain patterns in appetitive learning. However, the authors state that the principles can be readily applied to Pavlovian fear learning. To provide an example, consider the case of Pavlovian fear conditioning. As a result of excitatory conditioning, the CS will acquire a value representing its associative strength with the US. Additionally, the state classification process will categorise the situation according to a range of observations made at the time of conditioning. For the purposes of this example, we will identify the state which the animal was in during conditioning as State A. If the CS is later subjected to extinction, the negative prediction error present on the initial trials will lead to a loss of associative strength, in much the same way as would be predicted by the Rescorla-Wagner model. Therefore, some unlearning of the CS-US association will occur and this will be reflected in an amended value for the CS in State A. However, the persistence of the negative prediction error is likely at some point to trigger the state-classification process to generate a new state, State B. From this point, no further changes in the value of the CS in State A will occur and the value of the CS in State B will depend on experiences of that stimulus gained in the new state. Ultimately, responding to the stimulus will be determined by which state is inferred at the time of testing. Factors that influence the classification of states include, but are not limited to, the physical context of training. Therefore, testing in the extinction context will increase the likelihood that the conditions present at test will be classified as belonging to State B, the extinction state. Responding to the CS, then, will be influenced by the value assigned to the CS in State B. In this case, this would translate to a low level of conditioned responding. On the other hand, if testing was carried out in the conditioning context, this would bias the state-classification

process towards retrieving the value of the CS relevant to State A. Recall that the associative strength of the CS in State A was comprised of that acquired during excitatory conditioning as well as a certain amount of loss of associative strength which occurred prior to the formation of State B. Therefore, responding to the CS in the conditioning context is likely to show a substantial but incomplete return to pre-extinction levels.

While the authors identify tonically negative prediction error as a factor contributing to the creation of a new state, they allow also that other factors may influence the propensity to form a new state. Certain conditions, therefore, the omission of reinforcement may create a bias towards unlearning. Others conditions, meanwhile, may encourage state splitting and allow extinction training to proceed as a new learning episode.

Consolidation

In order to obtain an observable change in behaviour through conditioning, it is not enough to satisfy the requirements for learning. Behavioural changes that occur through learning require successful storage of a memory in which the learning episode is represented, as well as conditions that allow for that memory to be retrieved at a later stage. The process by which learning is translated into a stable memory is termed *consolidation* after Müller and Pilzecker's (1900; Lechner, Squire, & Byrne, 1999). On a cellular level, memory consolidation is a time-dependent process involving protein synthesis through which relatively permanent changes in synaptic transmission between neurons are established (Davis & Squire, 1984).

Evidence for a time-dependent consolidation process comes from a large body of research demonstrating, in a wide range of species and behavioural paradigms, that the strength of a memory can be modulated by treatments given immediately after the learning episode has ended. In particular, much of the support for the consolidation hypothesis was derived from studies demonstrating amnesia for a learning episode through physical,

chemical or behavioural interventions administered after training (Brashers-Krug, Shadmehr, & Bizzi, 1996; Duncan, 1949; Schafe & LeDoux, 2000). One of the earliest demonstrations of retroactive amnesia was by Duncan (1949) who trained rats on an active avoidance task and then administered electroconvulsive shock (ECS) at varying intervals after the learning episode. In the active avoidance paradigm, animals are placed in a compartment where, if they remain after a set period of time, they receive an aversive outcome, usually a foot shock. This aversive event can be avoided if the animal moves quickly to a safe compartment. Using this preparation, Duncan (1949) found that ECS administered shortly (i.e., 20 s to 15 min) after each training trial impaired subsequent performance on the avoidance task. Animals receiving ECS one hour or more after training did not differ from controls that were trained but not given ECS. On the basis of this finding, Duncan (1949) concluded that ECS administered in close temporal proximity to the learning event interfered with time-dependent neuronal processes required to permanently store the memory. An alternative account of these data might have been that ECS close in time to the end of the trial constituted an aversive US which conditioned fear to the compartment the animals were in at the conclusion of each trial: after a few trials, this was more likely to be the safe compartment than the shock compartment and so the latency to move from the shock compartment to the safe compartment may have been due not to a failure of active avoidance, but rather the expression of passive avoidance. However, this interpretation was subsequently ruled out by a number of studies including that of Madsen & McGaugh (1961) who demonstrated similar effects of ECS for memory of a one-trial passive avoidance procedure where an explanation in terms of punishment would predict, if anything, more robust avoidance. Other studies have produced retroactive amnesia through the use of protein synthesis inhibitors such as puromycin (Flexner, Flexner, & Stellar, 1963) and anisomycin (Schafe & LeDoux, 2000), as well as through behavioural interference (Brashers-Krug et al., 1996). The deficits in

performance produced cannot easily be explained as the result of permanent damage to structures necessary for learning, retrieval or performance since normal acquisition and responding can be observed after amnesia (Schafe & LeDoux, 2000). Further evidence for a consolidation process has been obtained in which memories can be facilitated by post-training administration of stimulants such as strychnine, nicotine or amphetamine (Gordon & Spear, 1973; M. E. Hall, 1969; Garg & Holland, 1968; for a review, see Izquierdo, 1989).

Important to all studies of retroactive interference with memory consolidation is the time-dependent nature of the effects. Amnestic treatments are only effective if administered within a critical time window after learning. Treatments administered outside of this time window are ineffective in modulating memory (Madsen & McGaugh, 1961). Therefore it is possible to conclude that memories pass through a phase during which they are susceptible to disruption or enhancement, and that after this period the memory becomes resistant to such manipulations. The memory is then considered to be consolidated.

Reconsolidation

A consolidated memory is, by definition, resistant to disruption by amnestic agents (McGaugh, 1966, 2000). There are situations, however, in which a consolidated memory can return to a state in which it is again susceptible to amnestic treatments and requires protein synthesis in order to restabilise. This process is known as reconsolidation. The phenomenon of reconsolidation was first reported by Misanin, Miller, & Lewis (1968) in rats conditioned to fear an auditory stimulus in a conditioned suppression paradigm. In this study, rats received electroconvulsive shock (ECS) 24 hours after conditioning, an interval at which the memory would ordinarily be insensitive to disruption leading to retrograde amnesia. Indeed, animals receiving only ECS at this time were not impaired in conditioned responding at test. However, rats in the experimental group, which were re-exposed to the CS prior to ECS, displayed amnesia for the CS similar to that seen when ECS is administered immediately

after initial training. This loss of conditioned responding could not be explained simply as extinction, since no deficits were observed in animals receiving the stimulus re-exposure without ECS: amnesia was dependent on the combination of CS re-exposure and ECS. These results were interpreted as evidence that a memory trace becomes unstable and sensitive to disruption not only immediately after initial learning, but any time the memory is retrieved. Since the initial demonstration by Misanin et al. (1968), reconsolidation has been demonstrated in a broad range of species from humans to honey bees (Anokhin, Tiunova, & Rose, 2002; Eisenberg, Kobilo, Berman, & Dudai, 2003; Judge & Quartermain, 1982; Nader, Schafe, & LeDoux, 2000; Pedreira & Maldonado, 2003; Stollhoff, Menzel, & Eisenhardt, 2005; Walker, Brakefield, Hobson, & Stickgold, 2003). The phenomenon has also employed a variety of paradigms to assess both aversive and appetitive Pavlovian memories, as well as spatial memory, motor learning and object recognition memory (Bozon, Davis, & Laroche, 2003; Dębiec & LeDoux, 2004; Duvarci & Nader, 2004; Lee, Everitt, & Thomas, 2004; Lee, Milton, & Everitt, 2006a; Morris et al., 2006; Walker et al., 2003). Disruption of reconsolidation typically results in deficits in long-term retention of the retrieved memory while leaving short-term memory intact and importantly is dependent on both reactivation of the memory and administration of the amnestic agent. Amnesia should not be observed when the memory retrieval occurs without amnestic interference, so as to exclude extinction as an explanation for the loss of responding. Furthermore, it is always important in these studies to show that the effect occurs only in the wake of memory retrieval to rule out non-specific effects of the amnestic manipulation (Duvarci & Nader, 2004).

The precise physiological mechanisms responsible for the reconsolidation of memory are still the subject of intense research and speculation. However, a number of pharmacological and molecular systems have already been implicated. For instance, there is a large body of evidence demonstrating the critical role of glutamate transmission in memory

reconsolidation. In particular, inhibition of NMDA-receptor activation via both competitive (APV) and non-competitive (MK-801) antagonists has been shown to produce amnesia following retrieval for a variety of memories including spatial (Przybyslawski & Sara, 1997), conditioned place preference (Kelley, Anderson, & Itzhak, 2007), object recognition (Akirav & Maroun, 2006), and fear memories (Lee, Milton, & Everitt, 2006b; Pedreira, Pérez-Cuesta, & Maldonado, 2002; Suzuki et al., 2004). Conversely, facilitation of NMDA transmission using the partial agonist D-cycloserine (DCS) has been shown to produce effects consistent with an enhancement of reconsolidation of conditioned fear (Lee et al., 2006b). However, the role of the NMDA receptor in reconsolidation is still a topic of debate with at least one study suggesting that amygdala NMDA receptors were necessary for the destabilisation, but not the restabilisation of a conditioned fear memory (Ben Mamou, Gamache, & Nader, 2006). While this finding appears in contrast to the literature just mentioned, the authors suggest that differences in the behavioural paradigms and/or the route of administration of the drug may account for these discrepancies. For example, while amygdala NMDA receptors may have a specific role in the destabilisation of fear memories, NMDA receptors elsewhere in the brain, which would be affected by the systemic administration used in the majority of studies, may be involved in fear memory restabilisation. The mechanisms involved in other paradigms may also differ from those reported by Ben Mamou et al. (2006).

Another neurotransmitter that has been shown to be important in memory reconsolidation is noradrenaline. The β-adrenergic receptor antagonist propranolol can induce amnesia when administered following retrieval of cued fear memories (Dębiec & LeDoux, 2004), spatial memories (Przybyslawski, Roullet, & Sara, 1999; Roullet & Sara, 1998), conditioned place preference (Bernardi, Lattal, & Berger, 2006; Robinson & Franklin, 2007) and context-induced sucrose seeking (Diergaarde, Schoffelmeer, & De Vries, 2006; Milton, Lee, & Everitt, 2008). Furthermore, a study in humans diagnosed with PTSD found that

administration of propranolol at the time of recall of the traumatic event resulted in reduced anxiety-related physiological responses when asked to recall the traumatic event one week later (Brunet et al., 2008).

It is likely that the effects of NMDA and β-adrenergic receptor modulation arise from their actions on a signalling cascade known to be involved in the consolidation of at least some forms of long-term memory (Bozon et al., 2003). Indeed, it has been shown that reconsolidation can be disrupted by pharmacological agents that block this pathway at any of a variety of levels, including MAPK/ERK (Duvarci, Nader, & LeDoux, 2005; Miller & Marshall, 2005; Valjent, Corbillé, Bertran-Gonzalez, Hervé, & Girault, 2006), cAMP response-element binding protein (CREB; Kida et al., 2002) and zif268 (Bozon et al., 2003; Lee, Di Ciano, Thomas, & Everitt, 2005; Lee et al., 2004; Lee et al., 2006a).

Evidence for Reconsolidation

Despite the growing volume of research findings on the topic, reconsolidation as a property of normal memory processing has struggled to gain acceptance. To assess the strength of this hypothesis, it may be helpful to examine the basis for the general acceptance of McGaugh's (1966) consolidation hypothesis. The evidence put forward in support of the original consolidation hypothesis can be summarised in the following central observations: (1) that memories could be retrospectively disrupted or enhanced by amnestic agents, even when these treatments are administered after the learning episode has ended; (2) that these effects would diminish with increasing time between training and the administration of the amnestic agent; and (3) that impairments resulting from post-retrieval amnestic manipulations are not due to non-specific neuronal damage. Together these findings were interpreted as strong evidence for a time-dependent consolidation process. Using these same criteria, it is possible to argue for a similar process being engaged after memory retrieval (Nader & Hardt, 2009). In the case of reconsolidation, it must additionally be demonstrated that the memory

had indeed consolidated at the time of retrieval. This requirement is generally satisfied in studies of reconsolidation by the inclusion of a control group receiving identical training as the experimental group but without receiving a retrieval session prior to administration of the amnestic agent. If consolidation was on-going at the time of the amnestic treatment, then amnestic agents should be capable of disrupting the memory regardless of whether the memory had been first retrieved. Retrieval-dependant amnesia is therefore necessary to conclude that the treatment is acting to disrupt reconsolidation rather than a prolonged consolidation process.

Performance on a behavioural task can be profoundly disrupted by the administration of amnestic agents after retrieval of a consolidated memory (Misanin et al., 1968; Nader et al., 2000). In fact, amnesia following retrieval can be induced by many of the same manipulations used to produce amnesia after initial learning, such as ECS (Misanin et al., 1968), protein synthesis (Nader et al., 2000) and new learning (Monfils et al., 2009; M. P. Walker et al., 2003). It is also possible to enhance memories after retrieval with Lee et al. (2006b) demonstrating stronger fear responding when the NMDA partial agonist DCS was administered after a brief reminder of the CS. Enhancement of retrieved fear memory has also been reported following activation of protein kinase A (PKA), an enzyme required for the induction of LTP in the amygdala (Huang & Kandel, 1998). Thus, in the same way that it is possible to manipulate new memories after initial learning, old but reactivated memories are also subject to retrospective modification by physical, chemical or behavioural interventions.

The second criterion was that the effects of post-retrieval manipulations should be time-dependent. Consistent with data from the consolidation literature, it has been confirmed that post-retrieval manipulations are only effective in producing amnesia if given in close temporal proximity to the retrieval session (Nader et al., 2000; Pedreira & Maldonado, 2003).

For example, in a cued fear conditioning paradigm, Nader et al. (2000) saw behaviour indicative of an amnestic effect when the protein synthesis inhibitor, anisomycin, was administered immediately after retrieval. However, if the anisomycin infusion was delayed by six hours, no deficit in subsequent fear responding was seen. These data, along with other reports of time-dependency in post-retrieval amnestic treatment (e.g., Dębiec, LeDoux, & Nader, 2002; Pedreira & Maldonado, 2003), give further support to the claim that memory retrieval initiates a time-dependent reconsolidation process.

Finally, it must be shown that the deficits in performance resulting from post-retrieval amnestic treatments are not due to non-specific effects of the manipulation such as damage to the brain structure necessary for conditioned responding. The inclusion of control groups that receive the amnestic treatment in the absence of retrieval largely satisfies this criterion. Additionally, Duvarci & Nader (2004) demonstrate successful reacquisition of fear responding after anisomycin-induced amnesia for a CS-shock association. Although it is possible for learning to proceed via alternative pathways following neuronal damage (Lee, Dickinson, & Everitt, 2005), this finding would suggest that the pathways involved in learning, consolidation, retrieval and performance were not permanently compromised by the inhibition of protein synthesis.

On the basis of these behavioural data, the case for reconsolidation appears at least as strong as that for initial consolidation. However, before any behavioural model can be considered to reflect real neural processes, there should be some indication of how the brain might generate the observed patterns in behaviour. Changes in behaviour resulting from initial learning are likely to depend on the relatively stable changes in connectivity between neurons which occur with the establishment long-term potentiation (LTP) of synaptic communication (Martin, Grimwood, & Morris, 2000). In support of this claim is a wealth of data demonstrating a necessary (if not sufficient) role for LTP in a variety of behavioural

tasks, particularly Pavlovian fear conditioning of which the neuronal circuit is fairly well understood (Sah, Westbrook, & Lüthi, 2008). Sah et al. (2008) highlight the following findings as being central to the hypothesis that LTP underlies learning and memory in the brain. The first is that synaptic transmission of sensory inputs into the amygdala (the primary anatomical locus of fear learning and memory) is facilitated by fear conditioning. Secondly, facilitation of synaptic transmission in the amygdala can also be achieved by stimulation of afferent sensory pathways. Finally, pharmacological and molecular manipulations that block LTP also impair learning in a fear conditioning preparation. Together these observations provide a strong case for the involvement of amygdala LTP in fear learning.

If we take LTP as the process by which learning experiences are translated into stable changes in synaptic communication between cells, then one prediction from the behavioural data would be that the presentation of a conditioned stimulus after the establishment of LTP would result in destabilisation of the synapse and a protein-synthesis dependent restabilisation process. Consistent with this prediction is data showing that late-phase LTP (L-LTP) is unaffected by pre-synaptic stimulation 2 h after the induction of L-LTP, but if that stimulation occurs in the presence of a protein-synthesis inhibitor, potentiation of the synapse begins to decline over the following few hours (Fonseca, Nägerl, & Bonhoeffer, 2006). The administration of the protein synthesis inhibitor without synaptic activation had no effect on the potentiation of the synapse and, thus, these findings show a reconsolidation-like process in which the maintenance of LTP requires protein synthesis after synaptic activation. Further evidence for reconsolidation on a cellular level comes from a fear conditioning study demonstrating reconsolidation deficits for one of two auditory stimuli trained to predict delivery of a foot shock US (Doyère, Dębiec, Monfils, Schafe, & LeDoux, 2007). Following successful induction of amnesia, the learning-induced increases in field potentials for the reactivated stimulus, but not the non-reactivated stimulus, were attenuated. The loss of

conditioned fear responding which occurs when stimulus reactivation occurs in the presence of an amnestic agent therefore appears to be accompanied by a corresponding loss of synaptic potentiation.

Together these results present a strong case for the existence of a protein synthesis-dependent reconsolidation process which occurs over a period of minutes to hours after memory retrieval. While the reconsolidation process appears to share many properties of the consolidation process, differences between the two have been recorded (Alberini, 2005). Most notable is the double-dissociation between the roles of the brain-derived neurotrophic factor (BDNF) and the immediate early gene, zif268 in consolidation and reconsolidation respectively (Lee et al., 2004). In a contextual fear conditioning preparation, Lee et al. (2004) showed that blockade of BDNF via intra-hippocampal infusion of BDNF antisense oligodeoxynucleotide (ASO) during consolidation impaired long-term memory for the conditioning phase. Infusion of BDNF ASO after retrieval of a consolidation memory, however, had no effect on subsequent fear responding. In contrast, post-reactivation infusion of zif268 ASO produced an amnestic effect at a post-reactivation long-term memory test while infusion of the same compound during initial memory consolidation had no effect on retention. Thus, Lee et al. (2004) demonstrated a critical role for BDNF in memory consolidation and a role for zif268 in reconsolidation, while also providing a unique molecular signature to differentiate the otherwise very similar processes of consolidation and reconsolidation.

Reconsolidation as a Mechanism for Memory Updating

This double-dissociation has been applied more recently to a study aiming to test the hypothesis that reconsolidation exists as a mechanism for allowing new information to be incorporated into existing memories (Lee, 2008). While it may appear at first that the potential for memories to be disrupted whenever they are retrieved would represent a serious

weakness in a system designed to store memories for later access, it should be pointed out that the conditions under which post-retrieval amnesia is induced, such as ECS and protein-synthesis inhibition, are unlikely to be encountered in a natural environment. Instead, it has been suggested that the destabilisation and reconsolidation of memories at retrieval could serve an adaptive purpose: to allow memories to be updated with new information (Dudai & Eisenberg, 2004; Lee, 2009; Sara, 2000). In a recent study by Lee (2008), it was shown that while consolidation mechanisms are required to store a new association, the activation of reconsolidation mechanisms was also necessary when memories are strengthened by additional learning. Using a contextual fear learning paradigm, Lee (2008) demonstrated a requirement for zif268 on the second conditioning session when the memory from the first session was retrieved and further training was taking place. Furthermore, this additional learning was not dependent on BDNF, showing that the learning which occurred on the second conditioning session modified the original memory rather than forming a new association. This was the first to study to demonstrate a requirement for reconsolidation mechanisms in the addition of new information to an existing memory and so gave strong support to theories of reconsolidation as an adaptive process directed at memory updating (Lee, 2009; Nader & Einarsson, 2010).

The conceptualisation of reconsolidation as a component of a process dedicated to memory updating is consistent with reports of the conditions under which it is possible to interfere with reconsolidation. As mentioned previously, evidence of reconsolidation has been obtained in a wide range of learning paradigms across a wide variety of species (for a review, see Duvarci & Nader, 2004). However, a number of studies have emerged which have reported failures to observe reconsolidation under certain conditions (see Lee, 2009 and Nader & Einarsson, 2010 for reviews of this literature). While it is difficult, given the huge variations between studies, to identify strict boundary conditions on reconsolidation (Nader &

Hardt, 2009), the constraints appear to correspond to situations in which it is unnecessary to update an existing memory, or in which it is easier or more adaptive to form a new memory (Lee, 2009). For example, if the process of reconsolidation is dedicated to incorporating new information into an existing memory, then a retrieval trial which presents no new information relevant to the memory would not be expected to initiate memory updating. This may explain the absence of reconsolidation effects in situations where the stimuli are already highly predictable, such as in a water-maze task where the location of the target platform is fixed compared to a task in which the location of the platform is changed for each trial (Morris et al., 2006). Circumstances under which reconsolidation is not observed despite the availability of new information are likely to be those which favour new learning. Situations which favour new learning may be those in which the physical or temporal context is sufficiently distinct from that of the original learning that it may be more adaptive to maintain two memories which can be utilised selectively depending on the context in which they are retrieved (e.g., Bouton, 1993). This may account for the absence of reconsolidation effects under conditions in which extinction is observed (Eisenberg et al., 2003), the requirement for retrieval in the same context as training (Misanin et al., 1968), as well as the resistance to reconsolidation observed for old memories (Eisenberg & Dudai, 2004; Milekic & Alberini, 2002).

Clinical Applications of Extinction and Reconsolidation

The study of reconsolidation has generated a great deal of interest from researchers in diverse fields due to the implications of the phenomenon for our understanding of the mechanisms of memory storage and retrieval. This interest has been further fuelled by the potential for the paradigm to be applied to clinical settings in which the aim of treatment is to attenuate unwanted memories. This is especially true for the treatment of anxiety disorders where patients may experience an uncontrollable fear reaction when they encounter objectively harmless stimuli or situations.

Post-traumatic stress disorder is a chronic and debilitating condition that affects a significant proportion of individuals following a traumatic event. Around 8% of individuals who show initial signs of trauma will go on to develop PTSD, which can persist for many years and cause significant disruption of day-to-day life (American Psychiatric Association, 1994; Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995). PTSD is characterised by persistent re-experiencing of the traumatic event, avoidance of stimuli associated with the trauma, and increased arousal (American Psychiatric Association, 1994).

It has been suggested that Pavlovian conditioning may provide a framework in which to understand the development of at least some PTSD symptoms (Foa, Steketee, & Rothbaum, 1989; Mowrer, 1960). According to Mowrer (1960), neutral stimuli that happen to be present at the time of a traumatic event can become associated with the trauma through Pavlovian learning. Subsequent exposure to these stimuli can then elicit intense fear. If avoidance of such stimuli successfully reduces exposure to fearful situations, then this avoidance behaviour will be perpetuated through a process of negative reinforcement (i.e., the reinforcement of a response by the omission of an expected aversive outcome). This 'two-stage' theory can, thus, explain the emergence of anxiety responses to trauma-related stimuli and the consequent avoidance of these stimuli. While this model may have shortcomings in not adequately accounting for symptoms involving re-experiencing of trauma (Foa et al., 1989), the suggestion that the disorder may develop through Pavlovian mechanisms raises the possibility that the power of trauma-related stimuli to elicit fear may, in turn, be alleviated by the application of Pavlovian theory. More specifically, therapy based on a fear extinction paradigm may be useful in attenuating the conditioned associations formed between neutral stimuli and the traumatic event. Indeed, Exposure Therapy (ET), one of the most popular and effective treatments for anxiety disorders including PTSD, is based on a model of Pavlovian fear extinction (Paunovic & Öst, 2001; Rothbaum & Davis, 2003). ET for the treatment of

PTSD involves the repeated presentation of stimuli which had previously become associated with trauma until fear responding subsides.

Exposure to trauma-related stimuli in a safe therapeutic environment is analogous to the nonreinforced presentation of a previously conditioned fear CS during experimental extinction. Unfortunately, the similarities between the two procedures do not stop there: just as a recovery of responding can be seen following extinction in the laboratory with changes in context, time or as a result of re-exposure to the US, ET too can be susceptible to the return of fear under similar conditions (Rachman, 1989). The clinical reports discussed by Rachman (1989) included cases in which patients reported a resurgence of anxiety after leaving the therapists' office (renewal), after a prolonged period of time (spontaneous recovery) or after a stressful or aversive encounter (reinstatement). The long-term success of the therapy was therefore compromised by factors which were largely inevitable.

As a consequence of the fragility of extinction learning, the investigation of techniques for facilitating reductions in fear responding has attracted a great deal of attention. One line of research in this area involves pharmacological facilitation of fear extinction. For instance, the administration of DCS prior to or following extinction training has been shown to facilitate the extinction of conditioned fear (Ledgerwood, Richardson, & Cranney, 2003; Lee et al., 2006b; Walker, Ressler, Lu, & Davis, 2002). Furthermore, rats treated with DCS show resistance to reinstatement (Ledgerwood, Richardson, & Cranney, 2004) and show reduced fear to non-extinguished stimuli when another stimulus previously paired with the same US has been extinguished (Ledgerwood, Richardson, & Cranney, 2005). On the basis of these findings, it has been suggested that DCS may represent a useful adjunct to behavioural treatments for anxiety disorders as it may encourage more robust extinction of fear (Richardson, Ledgerwood, & Cranney, 2004). Consistent with this suggestion, (Ressler

et al., 2004) have shown that DCS administration in conjunction with ET enhances the reduction of fear for sufferers of acrophobia.

More recently, however, researchers have begun to investigate clinical applications of the disruption of reconsolidation. The potential to produce amnesia for reactivated memories may provide a means for bringing about rapid and robust reductions in fear. The amount of exposure required to induce memory labilisation is, by definition, less than that required for extinction (Eisenberg et al., 2003), and so a treatment based on pharmacological or molecular disruption of reconsolidation would in all probability require less time than standard extinction-based therapies. Additionally, patients would likely welcome a treatment in which exposure to the anxiety-provoking stimuli is minimised.

A first step towards the use of reconsolidation blockade in the clinic is to replicate the results of animal studies in humans. To this end, Kindt and her colleagues (Kindt, Soeter, & Vervliet, 2009; Soeter & Kindt, 2010) have investigated the effects of administration of propranolol prior to memory retrieval for human participants previously trained in a fear conditioning procedure. When they tested responses to the fear CS one day later they observed a marked reduction in fear for those participants who had received propranolol prior to memory reactivation (Kindt et al., 2009). This effect was then shown to persist to a follow-up test one month later (Soeter & Kindt, 2010). Of additional interest in these studies was the reported dissociation between the conditioned fear response (in this case, the fear-potentiated startle response) and declarative knowledge of the CS-US relationship. It would seem, therefore, that post-reactivation amnesia may involve a disruption of the emotional components of the memory while sparing cognitive components (Soeter & Kindt, 2010).

Disruption of fear memory reconsolidation with propranolol has also been investigated in a population of PTSD sufferers. In the study by Brunet et al. (2008), memory reactivation was achieved by asking subjects to describe the traumatic incident which led them to develop

PTSD. Immediately afterwards, subjects were administered either propranolol or an inert placebo substance. When descriptions of the trauma were read back to them one week later, those who had received propranolol exhibited significantly weaker physiological responses than those who had received the placebo (Brunet et al., 2008). Although these results are still preliminary, they are encouraging for those hoping to develop reconsolidation-based clinical interventions for anxiety disorders.

With further clinical trials, the use of propranolol to disrupt reconsolidation of fear memories may develop into a useful intervention in the treatment of anxiety disorders. However, one important practical limitation is the risks involved with the use of propranolol, a drug primarily used as a treatment for hypertension. There are a number of cases in which the use of propranolol is inadvisable such as during pregnancy or in patients with diabetes, asthma or a range of heart conditions. There will be many people, therefore, for whom this form of treatment may not be suited.

Behavioural Modification of Fear Memories

A study published last year by Monfils et al. (2009) identified a method of producing profound and persistent reductions in fear without the use of pharmacological or surgical intervention. The technique is entirely behavioural and involves simply presenting the fear-inducing CS one hour or (even just 10 minutes) prior to extinction. The result is extinction learning that is resistant to spontaneous recovery, renewal and reinstatement. Moreover, at least in the case of spontaneous recovery, no effect on the persistence of extinction could be seen if the interval between retrieval and extinction was 6 hours. Thus, it appears that a window of time exists sometime between 3 minutes and 6 hours after memory retrieval during which extinction trials must be presented if facilitation of the extinction learning is to be observed.

Importantly, the effect of pre-extinction retrieval on spontaneous recovery can also be demonstrated in humans after conditioning of a visual CS with shock to the wrist (Schiller et al., 2010). Participants having received a single CS presentation one hour prior to extinction training displayed significantly less recovery of responding from the last trial of extinction to the first trial of the test session whether this test occurred one day or one year after extinction training. Although Schiller et al. (2010) did not investigate the effects on renewal or reinstatement, the data are promising in regard to the application of the pre-extinction retrieval procedure in humans. If applied to a clinical setting, this procedure could mean a more effective treatment for anxiety disorders without the need for drugs. Given that the common causes of relapse to anxiety parallel the conditions under which fear responding can be recovered in a laboratory setting, a method which alleviates these factors may help to make treatment also more resistant to relapse. Without the requirement of drugs, this technique could also be administered by a therapist without the involvement of medical staff or any unwanted drug side-effects.

In both the Monfils et al. (2009) and the Schiller et al. (2010) studies, the authors discuss their findings in terms of reconsolidation mechanisms. The presentation of the CS at retrieval is said to initiate a reconsolidation period during which additional training with the CS can disrupt or modify the original memory. Retrieval of the CS may destabilise the original fear memory, opening the memory up to modification by new, relevant information. The extinction trials could then be interpreted as part of the existing memory rather than being stored as a new and competing memory. An alternative view is that the conflicting information about the CS contained in the extinction trials (i.e., that the CS does not predict the US) serves as interference to the reconsolidation process in a similar manner to the disruption of a learning motor response by retrieval prior to learning a conflicting response (Walker et al., 2003). Rather than updating the memory, the new learning occurring within

the reconsolidation window might prevent restabilisation of the acquisition memory. In either case, the result is a persistent reduction in CS-elicited fear responding due to the absence of any strong excitatory association between the CS and the US.

The results of the experiments by Monfils et al. (2009) showing a lack of spontaneous recovery, renewal and reinstatement are consistent with the idea that the pre-extinction retrieval led to an updating of the original memory, revaluing the CS as less aversive. These data could also be explained as the result of new learning of a conflicting association or response interfering with the reconsolidation of the destabilised acquisition memory. However, the results of the experiment showing retardation of reacquisition, in particular, demonstrate an impairment in the ability of the CS to acquire a fear response, suggesting that the CS is not rendered neutral, but rather may have become a safety signal. This proposal we will revisit in Chapter V. These results are therefore unique in suggesting that the consequence of extinction within the reconsolidation window is not simply to prevent restabilisation of the memory, but that some form of extinction learning also takes place. Whether the extinction learning is incorporated into the original memory or forms alongside the original memory which then fails to restabilise is as yet not clear. In either case, the reluctance with which the CS again enters into an association with the US is worthy of further examination, especially if this paradigm is to be applied to a clinical setting. Stimuli that once predicted danger, while perhaps not warranting the degree of fear they elicit, nevertheless merit caution. Should those stimuli come to signal safety and encourage approach, this is liable to increase the likelihood of re-encountering the dangerous situation.

The experiments presented in this thesis investigate the impairment in reacquisition observed after extinction with prior retrieval. The goal of these experiments is to gain an understanding of the phenomenon in terms of the necessary conditions for producing impairment in reacquisition, the effect of this treatment on the CS memory, and the potential

of reversing the effect to allow relearning of the CS-US association. The general methods employed throughout these experiments are briefly outlined in Chapter II. Chapter III in concerned with the replication of the Monfils et al. (2009) data on the impairments in reacquisition of the CS-US association as a result of extinction following retrieval. Additionally, it is investigated whether this effect is dependent on prediction error at retrieval. In Chapter IV, a series of experiments is presented to assess the possibility of an explanation in terms of trial spacing effects on extinction. Following this, an attempt is made in Chapter V to better understand the nature of the impairment in reacquisition by analysing properties of the CS after extinction. The last series of experiments, presented in Chapter VI, looks at manipulations which may allow the CS to again enter into association with the US. The final chapter of this thesis brings together the results of these studies and discusses their implications for our understanding of learning and memory processes.

II. GENERAL METHODS

This chapter provides descriptions of the experimental subjects, apparatus and procedures that will form the basis of the experiments to follow. These details will hold for the majority of the experiments reported in this thesis and any digression from the details provided in this section will be explicitly stated.

Subjects

The subjects used in the following experiments were adult male Lister Hooded rats sourced from Charles River, UK. Animals were housed in groups of four and maintained on a 12 h reverse light/dark cycle (lights on at 1900). Food and water were available *ad libitum* for the duration of the procedures. Animals were well handled prior to the start of any experimentation.

Apparatus

All behavioural procedures took place in four identical conditioning chambers (Paul Fray, Cambridge, UK) contained within four sound-attenuating boxes. The chambers had three stainless steel walls with the door and ceiling made of clear Perspex. The floor of each chamber comprised steel rods (0.5 mm diameter) spaced 15 mm apart (centre to centre). The chambers were illuminated by a red houselight. The conditioned stimulus (CS) was a 70 dB, 20 Hz clicker presented via a speaker lodged within the top of each conditioning chamber. The experimental room was illuminated by red fluorescent lights.

Behavioural Scoring

All behavioural procedures were recorded via cameras mounted within the sound-attenuating boxes. The recordings were played back at normal speed while an electronic beeper sounded at 2 s intervals. Animals were scored two at a time by a trained observer (the author) who was not aware of group allocation at the time of scoring. At the exact time of each beep, the behaviour of each animal was scored as either freezing or not freezing.

Freezing was defined as the absence of all movement other than that related to respiration (Fanselow, 1994). An exception to this criterion was applied in the present studies if it was clear that the animal was asleep.

Statistical Analyses

Data analysis was carried out using SPSS version 17 statistical software. All statistical tests were conducted so as to maintain a maximum familywise error rate of $\alpha = .05$. Standard sphericity corrections were applied for analyses involving within-subjects factors (Cardinal & Aitken, 2006). Where specific hypotheses were to be tested for a given set of data, planned contrast analyses were applied using the Šidák correction. Where no differences were expected, or where the effects were not easily predicted, overall Analysis of Variance (ANOVA) was carried out on the data followed, where appropriate, by post-hoc contrast analysis. Analyses involving a single between-subjects factor and no within-subjects variables were analysed using the One-Way ANOVA procedure. Where multiple trials or multiple stimuli were compared within an analysis, the repeated-measures ANOVA was applied with groups forming the between-subjects factors and trials or stimuli as the within-subjects factor. Performance across trials was typically analysed through the application of a linear trend transform so as to assess rate of change. When the pattern of responding across trials was not predictable, or no differences across trials were expected, within-subjects effects were assessed using a repeated-measures ANOVA adjusting the degrees of freedom by application of either the Greenhouse-Geisser (G-G) or the Huynh-Feldt (H-F) coefficients. The choice of coefficient was determined by the value of the G-G coefficient: if this value was less than .75, then the G-G correction was applied; if G-G was greater than .75, then the H-F correction was used (Cardinal & Aitken, 2006; Howell, 2007). In the case where more than one within-subjects factor was to be compared across groups, a Multivariate ANOVA (MANOVA) was used. Within-subjects factors were transformed into a set of orthogonal

contrasts and analyses performed on the contrasts of interest in the testing of the experimental hypotheses.

Where overall analyses produced a significant *F* statistic, post-hoc tests were carried out to determine the source of variance. In the case of a simple between-groups effect with three groups or less, Fisher's Least Significant Difference Procedure was applied (Howell, 2007). Where more than three groups were involved, the Scheffé procedure for post-hoc contrasts was utilised. In cases where variance between groups differed (as indexed by a significant Levene's *F* statistic), the significance of effects was judged with reference to the Welch correction for unequal variances (Howell, 2007).

III. IMPAIRMENT IN REACQUISITION

In their last two experiments, Monfils et al. (2009) saw that animals given extinction one hour after retrieval of the target CS showed retarded emergence of fear to the CS with further pairings of the CS and US relative to groups not exposed to the CS prior to extinction. The first of these experiments assessed the recovery of conditioned fear following a single pairing of the CS with the US. The authors suggested an interpretation of this in terms of savings, suggesting that the retrieval group fails to show the rapid reacquisition of fear following extinction that has often been taken as evidence of survival of a portion of the original memory trace. On the basis of studies of reacquisition after extinction, Macrae and Kehoe (1999) found support for a 'hybrid' connectionist model of extinction (Kehoe, 1988). According to this model, CS and US inputs gain control over a conditioned response via serially associated units. During extinction the activation of CS inputs in the absence of US inputs weakens the connections between CS input and subsequent units in the series. Importantly, once connections early in the chain are weakened sufficiently to prevent the CS eliciting a CR, downstream connections cannot be further degraded. Thus, while extinction results in loss of the CR, a portion of the chain of connections from CS to CR remains intact. When the CS and US are then presented together again, only the degraded connections need to be reformed and so learning will proceed faster than learning in a naive group. That is, learning after extinction shows a savings effect.

The data presented by Monfils et al. (2009) might then suggest that retrieval prior to extinction allows more of the chain to degrade and, therefore, results in a weaker savings effect. However, in order to draw this conclusion, it is necessary first to assess whether savings is observed in the group given extinction only. To do this requires comparison between a group conditioned following extinction and a group conditioned without previous experience of the CS-US association. Monfils et al. (2009) in fact did include such a group in

their experiment, but did not report the data from this group. It is therefore impossible to determine whether the extinction-only group displayed savings in reacquisition, which was prevented by retrieval, or whether the retrieval actually suppressed learning about the CS during the reacquisition phase. Their final experiment, in fact, gave support to the latter explanation. Animals given retrieval prior to extinction displayed a persistent impairment in learning across five reacquisition trials relative to both the extinction-only group and a naive control group. Little evidence of savings, on the other hand, was observed, as the extinction group did not differ from naïve controls.

In any case, the reluctance with which the CS becomes reconditioned to the US may pose a problem for clinical application of the procedure. A young man suffering trauma after having been robbed at knife point should not be afraid to leave his apartment, but should still perhaps remain wary of darkened alleyways. A treatment which results in a dark alleyway signalling safety may in fact lead to a repetition of the traumatic event, with perhaps even this not discouraging the victim from frequenting such areas. Ideally, the treatment of an anxiety-provoking stimulus or situation would, through treatment, be rendered neutral or even just less anxiety-provoking to a degree that normal functioning and quality of life can be restored. If the impairment in reacquisition seen after extinction with retrieval is an effect on savings, then this would be less problematic since it may simply indicate that the CS has returned to neutral. Of course, if it is important to be able to relearn fear, then the extinction-only group are in a better position to do so. However, the susceptibility of the CS in this case to renewal, reinstatement and spontaneous recovery is likely to represent a greater threat to the quality of life of a patient undergoing treatment. If, on the other hand, the effect is due to suppressed learning or safety learning, then this treatment may ultimately be detrimental to the patient. The final experiment of Monfils et al. (2009) suggests that this latter explanation is more likely. However, it is not possible to draw any strong conclusions from these data.

The experiments in this chapter represent a first step towards testing the contrasting hypotheses for the retardation of reacquisition following retrieval and extinction. These experiments initially aimed to replicate the effects on reacquisition reported by Monfils et al. (2009) to confirm that these effects are reliable when applied in a different laboratory with a different strain of rat. Following successful replication of the retardation of reacquisition effect, the final experiment examined whether memory strength or the predictiveness of the US might represent boundary conditions on the capacity for retrieval to facilitate extinction learning.

Experiment 1.1

The aim of this experiment was to replicate the observation of Monfils et al. (2009) showing that reacquisition of fear to an extinguished CS would be retarded in a group given a reactivation trial 1 hr prior to extinction training. In addition to the original study, however, rats in the present experiment were tested 24 hr following reacquisition training to confirm that any differences observed during reacquisition persist and are evident on non-reinforced CS presentations. The stimulus employed as the CS in this study was a tone, as was used in the original study. On the basis of pilot studies, however, the parameters for acquisition and reacquisition were altered slightly as these parameters were found to produce the most robust conditioned responding for the particular combination of equipment and strain of rat available in our laboratory.

The design of the experiment is outlined in Table 1. Two groups received pairings of a tone and foot shock. These groups then underwent extinction with or without retrieval of the CS one hour prior to extinction training. On the following day, these groups along with a third, naive, group were given further pairings of the CS and US. Fear responding to the CS was assessed during these trials and during non-reinforced CS presentations given after a further 24 h.

Table 1: Design of Experiment 1.1

Group	Acquisition	Ret	Extinction	Reacquisition	Test
Ret	3 x T+	T	18 x T	5 x T+	3 x T
NoRet	3 x T+	-	19 x T	5 x T+	3 x T
Naive	-	-	-	5 x T+	3 x T

N.B. Ret = retrieval; NoRet = no retrieval; T = tone CS; "+" indicates a reinforced CS presentation (no "+" means CS presentations were not reinforced); "-" indicates that rats remained in their home cages.

Methods

Subjects

The subjects were 33 adult male Lister Hooded rats (Charles River, UK) housed in groups of two but otherwise maintained under the conditions outlined in Chapter II.

Apparatus

All behavioural procedures took place in the conditioning chambers described in Chapter II. The CS was a 72 dB, 2.9 kHz tone, 20 s in duration, presented via a speaker lodged within the top of each conditioning chamber.

Behavioural Procedures

Acquisition. After a 10 min adaptation period in the conditioning chamber, rats in the retrieval group (Ret) and the no retrieval group (NoRet) were given 3 trials of a 20 s CS co-terminating with a 1 s, 0.5 mA foot-shock (US) with an inter-trial interval of 180 s. Each rat was removed from the conditioning chamber 1 min following the last trial and returned to its home cage.

Retrieval. Rats in Group Ret were returned to the conditioning chambers one day following conditioning. After 150 s in the context, the CS was presented once for 20 s. The rats were removed from the chambers one minute later and returned to their home cages.

Extinction. One hour following retrieval, rats in Group Ret were returned to the conditioning chambers for extinction. The extinction session comprised 18 non-reinforced presentation of the CS with an ITI of 120 s. Rats in the NoRet group received extinction training with 19 trials such that the total number of non-reinforced CS presentations would equal that of Group Ret.

Reacquisition. 24 hours following extinction training, Ret and NoRet rats were returned to the conditioning chambers for further excitatory conditioning of the CS and US. An additional group of rats that had not received any prior training (Naïve) were also conditioned at this time. The reacquisition session consisted of a 10 min adaptation period following by 5 CS-US pairings with a 180 s ITI.

LTM Test. A retention test was given 24 h after reacquisition consisting of four non-reinforced presentations of the CS with an ITI of 180 s.

Behavioural Scoring

All behavioural procedures were scored as described in Chapter II.

Statistical Analyses

The data were analysed by way of repeated measures ANOVAs in SPSS with trials (where relevant) as within-subject factors and conditions (Ret, NoRet, and Naïve) as between-subjects factors for each of the relevant stages: acquisition, extinction, reacquisition and LTM test. A within-subjects linear contrast was applied to the extinction data to determine whether the extinction training resulted in a significant decrement in conditioned freezing. For the reacquisition and test phases, the group effects were assessed using planned contrasts, which were designed to assess whether Group Ret was different to Group NoRet,

and whether Group Ret was different to Group Naïve. Linear trend analyses were additionally applied to the reacquisition phase to assess whether any differences in the rates of reacquisition were observed. To control the family-wise error rate at 0.05, the Šidák correction was used for the reacquisition and test phases such that a significant effect was inferred when $\alpha < .025$.

Results

Acquisition

The mean (± *SEM*) percentage of observations scored as freezing within each of the three CS periods during acquisition is shown in Figure 1. A linear contrast analysis revealed a significant increase in freezing behaviour across the course of conditioning, $F(1, 20) = 43.43$, $p < .001$, indicating that the procedure was successful in conditioning fear to the CS. No significant overall effect of group was observed at this stage of the experiment, $F(1, 20) < 1$, and neither was the interaction significant, $F(1, 20) < 1$.

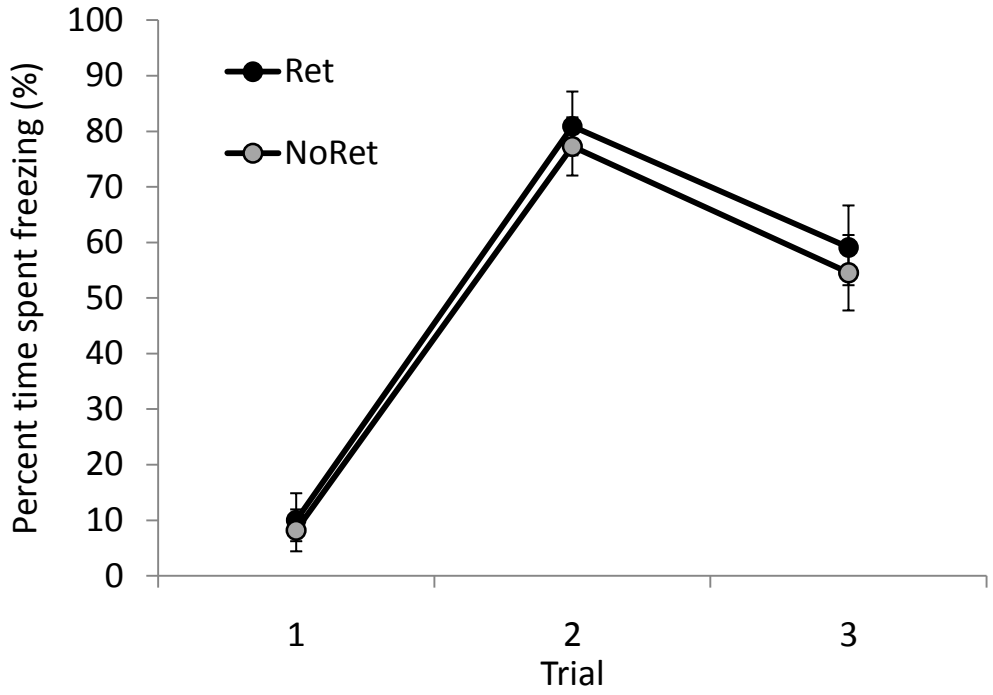

Figure 1. Freezing to the CS across three conditioning trials. Circles represent group means ± *SEM*.

Retrieval and Extinction

For the purposes of analysis, the retrieval trial was treated as the first extinction trial for Group Ret such that the two groups would be compared in their responding over an equivalent number of non-reinforced trials. The data are presented in Figure 2. Overall levels of freezing of the two groups did not differ significantly, $F(1, 20) < 1$. A significant linear decrease in conditioned freezing was observed across extinction trials confirmed that the extinction training successfully reduced levels of freezing for both groups, $F(1, 20) = 28.81$, $p < .001$. This linear decrement was not significantly different between two groups, $F(1, 20) < 1$, indicating that conditioned freezing extinguished at a similar rate in the Ret group as in the NoRet group.

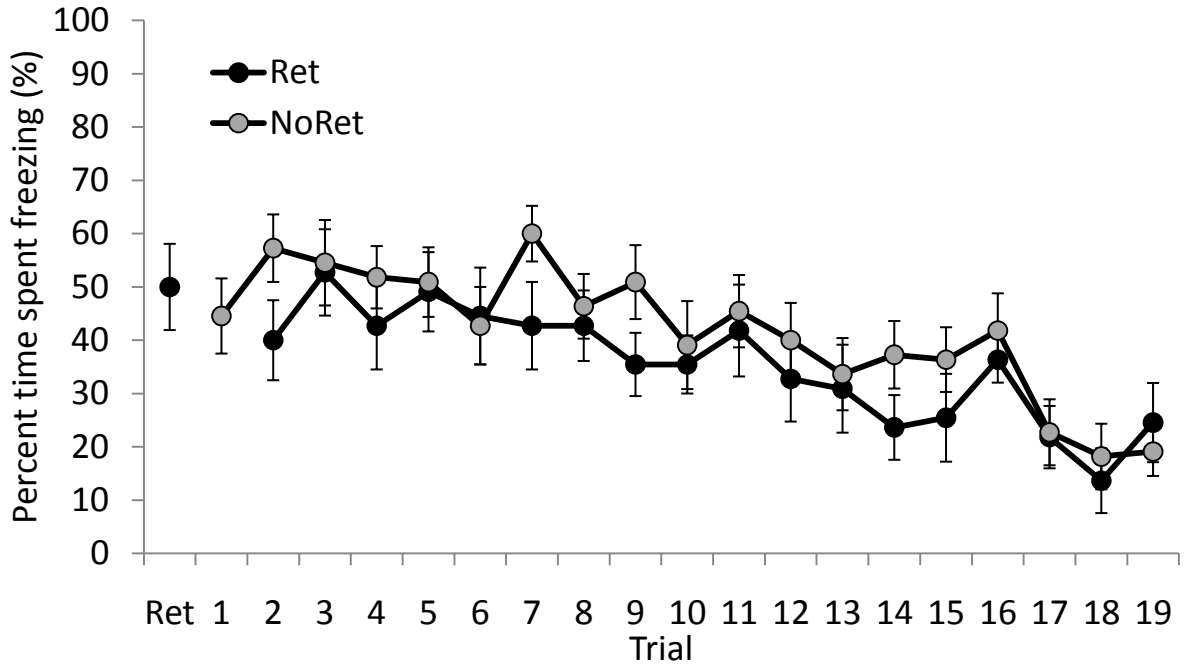

Figure 2. Percentage freezing to the CS during retrieval and extinction. Circles represent group means ± *SEM*.

Reacquisition

The levels of freezing across the five reacquisition trials for the three groups can be seen in Figure 3. Averaging across trials, Group Ret displayed significantly less freezing when compared to Group NoRet, $F(1, 30) = 14.35$, $p < .025$ (.001). No significant difference was observed between the NoRet group and the Naïve group, $F(1, 30) = 4.20$, $p > .025$ (.049). Overall, no significant linear trend was observed, $F(1, 30) < 1$. The value of the linear trend was not statistically difference between Groups Ret and NoRet suggesting these groups were not different in terms of the rate of reacquisition. There was, however, a significant difference in reacquisition rate between Ret and Naïve, which reflects an overall linear increase in freezing of the Naïve group ($M = 47.24$, $SD = 66.95$) in contrast to the overall linear decrease in responding of the Ret group ($M = -51.82$, $SD = 53.07$).

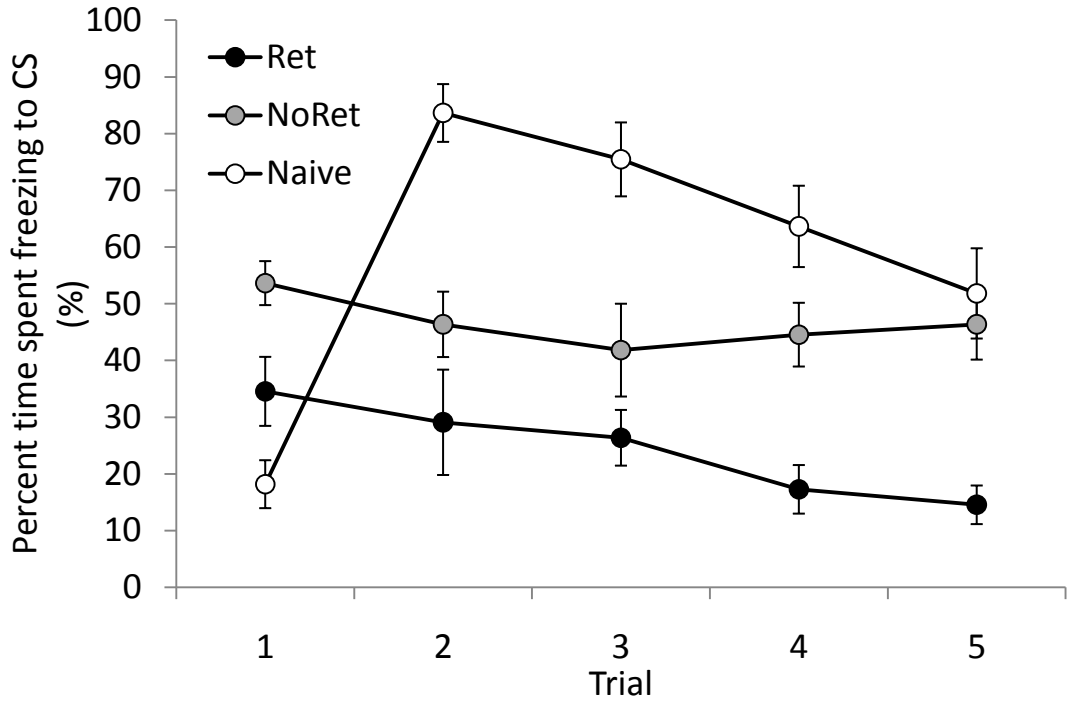

Figure 3. Freezing to the CS during reacquisition training. Circles represent group means ± *SEM*.

LTM Test

The first two trials of the test were analysed as these were the least likely to be affected by extinction occurring during the test session. The average levels of freezing across these two trials (CS), and for the three minute period before presentation of the first CS (Cxt), are shown in Figure 4. No significant effect of group on freezing to the context prior to the CS was observed $F(2, 32) < 1$. There was no significant difference between the Ret group and the No Ret group in terms of CS freezing during this phase, $F(1, 30) = 2.91$, $p > .025$. There remained, however, a significant difference between the Ret and Naïve groups, F (1, 30) = 11.627, $p < .025$. This analysis reveals a persistence of the deficit in freezing displayed by Group Ret relative to the naïve control group. The difference between Ret and NoRet, however, did not persist through to the LTM Test.

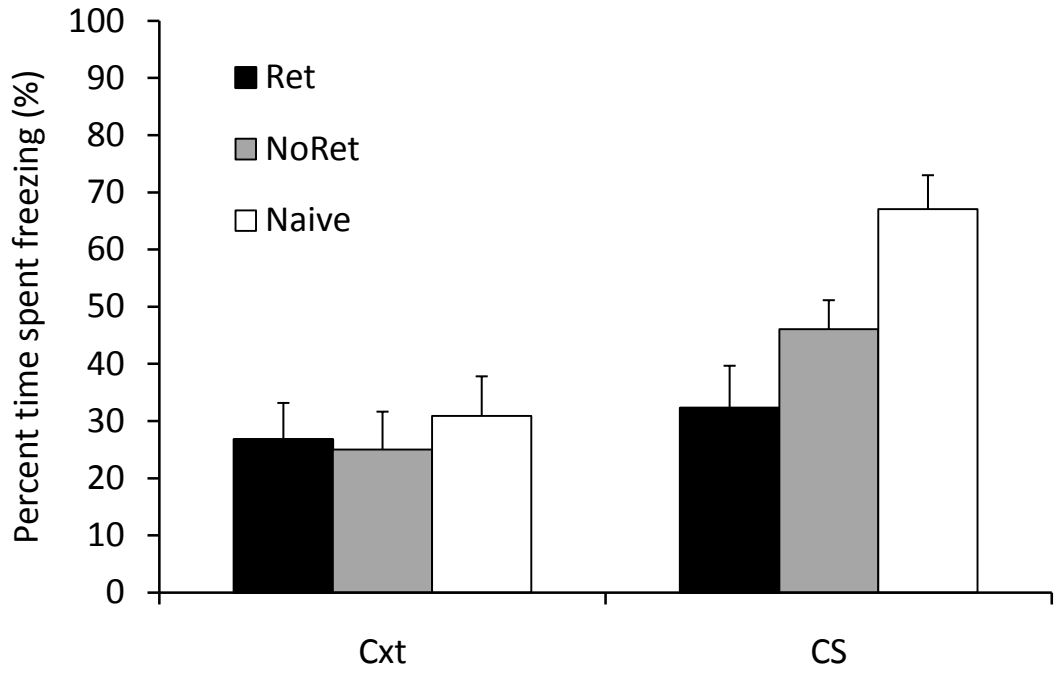

Figure 4. Average freezing during the three minutes preceding the first onset of the CS (Cxt) and to the CS. Bars represent group means ± *SEM*.

Discussion

The results presented here support the claim by Monfils et al. (2009) that a brief retrieval trial prior to extinction results in retardation of reacquisition of the conditioned response to the CS. Furthermore, a persistent reduction in freezing in these animals relative to a naïve control group was observed during a test for long-term memory given 24 hr after reacquisition training. The difference between Ret and NoRet animals, however, was not significant at test. This may suggest that retrieval prior to extinction influences responding to the CS when that stimulus is again presented, but that these animals are still capable of learning the CS-US association. When the stimulus is presented again 24 h after reacquisition, the new learning will have had a chance to consolidate such that the CS is again capable of eliciting a conditioned response. Alternatively, this discrepancy between freezing during reacquisition and test may reflect a convergence of learning by the end of

reacquisition training, or simply insensitivity in the dependent measure or the experimental parameters.

One concern within the current data was the quadratic nature of the acquisition curve. Freezing to the CS appeared to reach a peak on the second trial after which there appeared to be a substantial reduction in responding. The final level of responding was still reliably above zero, but the observation that fear responding declined after a certain point in training casts doubt upon the assumed linear relationship between learning and responding. Possibly, the physical properties of the tone stimulus were not ideal to induce reliable freezing. It has been reported previously that certain frequencies of tone can, in fact, increase locomotor behaviour in Lister hooded rats (Commissaris et al., 2000) and that this effect was not seen with other forms of auditory stimulus. The propensity towards this locomotor response interfered with the ability of these rats to display freezing behaviour compared to an equivalent group of Wistar rats. Although the frequency of the tone used in this study was set well below the 7-20 kHz levels at which these effects were observed, it is possible that some higher frequencies, unintended and undetectable by the experimenter, may have been transmitted through the speakers. Within the current apparatus, then, the use of stimuli other than the tone may be preferred.

Experiment 1.2:

The following experiment was designed simply to assess the acquisition of fear responding to three distinct stimuli. This experiment had two aims. Firstly, to confirm that the unstable levels of freezing to the CS in Experiment 1.1 were specific to the tone stimulus and, secondly, to identify a stimulus with which more reliable conditioned responding could be observed for future experiments. The three stimuli examined in this experiment are the tone used previously, along with an auditory clicker stimulus and a white light.

Materials and Methods

Subjects

The subjects were 12 adult male Lister Hooded rats (Charles River, UK) maintained under the conditions outlined in Chapter II.

Apparatus

All behavioural procedures took place in the conditioning chambers described in Chapter II. The chambers were illuminated by a red houselight. Three distinct conditioned stimuli (CSs) were used: tone, light and clicker. The tone CS was a 72 dB, 2.9 kHz tone presented via a speaker lodged within the top of each conditioning chamber. The light CS was a white stimulus light lodged within one of the stainless steel side walls of the chamber. The clicker CS was digitally generated with a click frequency of 20 Hz and was presented at 70 dB via the same speakers as used for the tone CS. The duration of the CS in all phases of the experiment was 60 s.

Behavioural Procedures

Acquisition. After a 30 min adaptation period in the conditioning chamber, rats were given 3 trials of a 1 min CS co-terminating with a .05 s, 0.5 mA foot-shock (US) with inter-trial intervals of 4 min and 6 min in a randomised order. Groups differed in the nature of the CS with one group being conditioned to the tone used in Experiment 1.1, a second group receiving conditioning to the light, and a third group to the clicker. Each rat was removed from the conditioning chamber 1 min following the last trial and returned to its home cage.

Test. A retention test was given 24 h after acquisition consisting of three non-reinforced presentations of the CS with an adaptation period and ITI of 180 s.

Statistical Analyses

The data from the acquisition session were analysed by way of a repeated measures ANOVA with Trial as the within-subject factor and Stimulus (Tone, Light, and Clicker) as

the between-subjects factor. For the test phase, responses were averaged across the three presentations of the CS and these values were analysed across the three groups. In each of the two phases of the experiments, post-hoc contrasts were run for those analyses where the overall test produced a significant *F* statistic.

Results

Acquisition

Averaged across the three stimuli, a significant linear increase in freezing to the CS over the three conditioning trials was observed, $F(1, 9) = 34.99$, $p < .001$, indicating successful acquisition of fear to the stimuli (see Figure 5). No overall differences were seen between the three stimuli when averaged across the three trials, $F(2, 9) < 1$. However, the stimuli did differ in the degree to which they acquired fear over during the course of conditioning, $F(2, 9) = 4.56$, $p = .043$. Follow-up contrasts revealed that this difference arose from the relatively weak increase in freezing to the tone compared with the light and the clicker, $F(1, 9) = 8.99$, $p = .015$. No significant difference in rate of acquisition was observed between the light and the clicker, $F(1, 9) < 1$. These data replicate the previous pattern of results with acquisition to the tone stimulus showing an initial increase in freezing followed by a decrease on the third trial. Furthermore, the present data indicate that with the parameters used in this experiment the light CS and the clicker CS are better able to support acquisition of conditioned freezing than the tone CS.

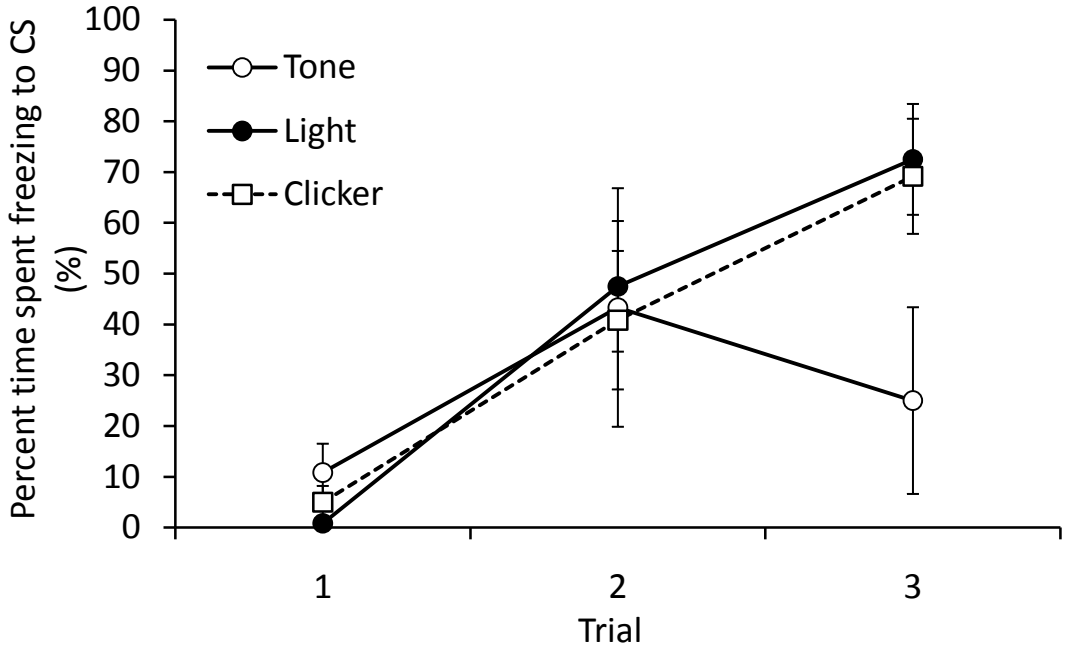

Figure 5: Freezing to each of the three stimuli across three reinforced presentations. Circles and squares represent group means ± *SEM*.

Test

Freezing to the three stimuli at test is shown in Figure 6. An overall effect of stimulus was observed on levels of freezing during CS presentations at test, $F(2, 9) = 7.04$, $p = .014$. Follow-up tests revealed that freezing to the tone was significantly lower than to the light and clicker, $F(1, 9) = 14.02$, $p = .005$. No significant difference was observed between the light and the clicker, $F(1, 9) < 1$. From these data we can see that the differences in the rate of acquisition of the freezing response to the stimuli were still evident 24 h after conditioning when the stimuli were presented in extinction.

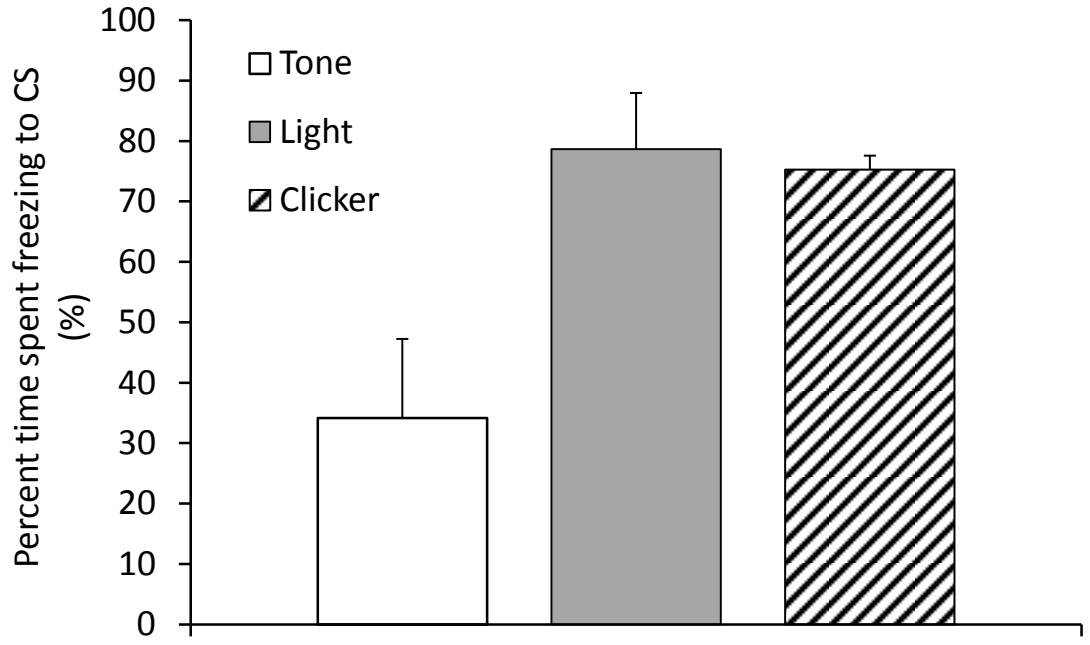

Figure 6: Freezing to each of the three stimuli averaged across three presentations of the non-reinforced stimuli at test. Circles represent group means ± *SEM*.

Discussion

The data in this experiment replicate the acquisition curves seen in Experiment 1.1 when the tone CS was used. Given the same history of reinforcement as the light and the clicker, the tone failed to elicit a comparable level of freezing. The reasons for this weaker response to the tone are uncertain. It may be that the tone has a lower salience than the other stimuli and thus fails to become associated with the US, or that, having been successfully associated with the US, the tone CS elicits a response which competes with the freezing response. In fact, in observing the acquisition session on video after the conclusion of experimentation it was noticed that the rats began to exhibit an alternative response to the tone stimulus after pairing with the US in which they rapidly jerked their heads from side to side. This behaviour is similar to that described for rats trained with pairings of an auditory stimulus with an appetitive reinforcer (Holland, 1977). Responding to an auditory stimulus, as opposed to a visual stimulus, was observed to comprise rapid movement of the head in a

horizontal or vertical plane. It was proposed that this response was made in an effort to localise the source of the auditory stimulus so as to orientate towards it, a response which develops readily to localisable visual stimuli. If sufficient fear accrues to a stimulus, it may be that the animal attempts to escape that stimulus. In order to escape the stimulus, it must first be located, and thus the head movements may be an attempt at localising the source of the threat. The fact that this response is seen for the tone and not for the clicker may result from the tone being of a higher pitch, thus requiring the use of head movements to disambiguate the direction of the source (Blauert, 1997). Alternatively, the tone may generate more fear and so more escape-like behaviour.

Whatever the reason for the lower levels of freezing to the tone, it is clear that the level of freezing to the tone does not reliably reflect the learning history of the animals. For this reason, subsequent experiments avoided use of the tone and instead employed the clicker and/or light as the CSs.

Experiment 1.3

Employing the clicker as the CS, a second effort was made to replicate the finding of Monfils et al. (2009) that reacquisition of the CS-US association is impaired when extinction is carried out one hour after retrieval of the CS memory. In this experiment, however, only one trial of reacquisition was given so as to avoid ceiling effects in reacquisition and so increase the likelihood that any differences would still be observable at a long-term retention test. This is important as it avoids the risk that although rats in the retrieval group may display a retardation in reacquisition, this group may converge on the no-retrieval group by the end of the reacquisition training. Thus no group differences would remain to be observed at test. Additionally, the observation of differences 24 h after reacquisition would help to distinguish between deficits in responding to the CS after extinction and deficits learning about the CS.

An additional control group was included in this experiment (Ret-Cxt; see Table 2) to rule out the possibility that the loss of responding and subsequent impairment in reacquisition could be due to interference with memory reconsolidation resulting from the animals being removed from the context, transported to the home cages, interacting with cage mates and then being returned to the experimental context all within the reconsolidation window. If these events were sufficient to disrupt the reconsolidation of the CS memory, this may have led to amnesia for the original memory. In this situation, the repeated presentation of the CS during extinction training may have led to the development of latent inhibition in learning about the CS at reacquisition.

Table 2: Design of Experiment 1.3

Group	Acquisition	Ret	Extinction	Reacquisition	Test
Ret-Ext	3 x C+	C	18 x C	1 x C+	2 x C
Cxt-Ext	3 x C+	Cxt	19 x C	1 x C+	2 x C
Ret-Cxt	3 x C+	C	Cxt	1 x C+	2 x C
Naive	-	-	-	1 x C+	2 x C

N.B. Ret = retrieval; NoRet = no retrieval; Ext = extinction; Cxt = context exposure; C = clicker CS; "+" indicates a reinforced CS presentation (no "+" means CS presentations were not reinforced); "-" indicates that rats remained in their home cages.

Methods

Subjects

The subjects were 32 adult male Lister hooded rats (Charles River, UK) maintained under the conditions previously described. Each subject was assigned to one of the four groups (n = 8 for each group): Ret-Ext, Cxt-Ext, Ret-Cxt and Naive.

Apparatus

All behavioural procedures took place in the conditioning chambers described in Chapter II. The CS was a 60 s presentation of the clicker stimulus.Behavioural Procedures

Habituation. All animals were exposed to the context for one hour per day for two days prior to the start of training. During this period the house light remained on and no stimuli were presented. These sessions were included in this and subsequent experiments in an attempt to minimise the potential for conditioning of fear to contextual stimuli.

Acquisition. After a 30 min adaptation period in the conditioning chamber, rats in the retrieval-extinction group (Ret-Ext), the no retrieval-extinction group (Cxt-Ext) and the retrieval only group (Ret-Cxt) were given 3 trials of the CS co-terminating with a 0.5 s, 0.5 mA foot shock (US) with a variable intertrial interval with an average of 300 s. Each rat was removed from the conditioning chamber 1 min following the last trial and returned to its home cage.

Retrieval. One day following acquisition, rats in groups Ret-Ext and Ret-Cxt were returned to the conditioning chambers given one presentation of the CS after 2 min in the context. No shock was delivered during this session. The rats were then removed from the chambers and returned to their home cages. Rats in Group Cxt-Ext were placed in the context for an equivalent period of time and then returned to their home cages.

Extinction. One hour following the retrieval session, rats in Group Ret-Ext were returned to the conditioning chambers for extinction. The extinction session comprised 18 non-reinforced presentations of the CS with an ITI of 120 s. Rats in the Cxt-Ext group received extinction training with 19 trials such that the total number of non-reinforced CS presentations would equal that of Group Ret-Ext. Rats in Group Ret-Cxt were placed in the context for a period of time equivalent to Group Ret-Ext, i.e. 54 min, during which time the houselight remained on but no nominal stimuli were presented.

Reacquisition. After 24 hours, Ret-Ext, Cxt-Ext and Ret-Cxt rats were returned to the conditioning chambers for further excitatory conditioning of the CS and US. An additional group of rats that had been habituated to the context but had not received any prior training with the CS or US (Naïve) were also conditioned at this time. The reacquisition session consisted of a 10 min adaptation period following by a single CS-US pairing. Rats were returned to their home cages one minute after the offset of the CS.

Test. Animals were returned to the context for testing 24 h after reacquisition. This test comprised a 180 s adaptation period followed by two non-reinforced presentations of the CS with an ITI of 180 s.

Statistical Analyses

The data were analysed by way of repeated measures ANOVAs in SPSS with trials (where relevant) as within-subject factors and conditions (Ret-Ext, Cxt-Ext, Ret-Cxt, and Naïve) as between-subjects factors for each of the relevant stages: acquisition, extinction, reacquisition and LTM test. A within-subjects linear contrast was applied to the extinction data to determine whether the extinction training resulted in a significant decrement in conditioned freezing. For the reacquisition and test phases, the group effects were assessed using planned, orthogonal contrasts. For reacquisition, these contrasts were designed to test the following predictions: (1) that Group Ret-Cxt would show higher levels of freezing than the other groups as a result of having been conditioned to the CS but not having received extinction, and (2) that the groups having received extinction would show some residual fear that, although low, would be greater than for the Naive group. A third contrast was designed to detect any differences in freezing between Groups Ret-Ext and Cxt-Ext in responding to the CS that could account for any subsequent differences in freezing levels. For the test phase, contrasts were designed to assess three distinct hypotheses: (1) that the brief presentation of the CS prior to extinction (Group Ret-Ext) will result in impaired

reacquisition of fear to the CS compared with the three control groups (Groups Cxt-Ext, Ret-Cxt and Naive), (2) whether the retrieval trial alone could account for this effect (Ret-Cxt Group Ret-Ext differed from the three control conditions, whether Group Ret-Cxt was different to Group Naïve. Linear trend analyses were additionally applied to the reacquisition phase to assess whether any differences in the rates of reacquisition were observed. An alpha level of .05 was applied to all statistical tests.

Results

Acquisition

The two minutes immediately preceding onset of the first CS were taken as a measure of baseline freezing. A between-subjects ANOVA found no evidence of group differences in baseline freezing at this stage of the experiment, $F(2, 21) = 2.49$, $p = .107$; *M*s (*SEM*) of percent time freezing (%): Ret-Ext = 1.0 (0.4), Cxt-Ext = 0.2 (0.2), Ret-Cxt = 0.2 (0.2).

Levels of freezing to the CS across the three acquisition trials for Group Ret-Ext, Cxt-Ext and Ret-Cxt are shown in Figure 7. No overall group differences in freezing during CS presentations were observed, $F(2, 21) < 1$. Freezing to the CS across the three trials showed a significant linear increase, $F(1, 21) = 262.9$, $p < .001$. The magnitude of this effect did not differ between the three groups, $F(2, 21) < 1$. From these data it can be concluded that the fear acquisition was successful, and that the three groups have no pre-existing differences as they go into the next phase of the experiment.

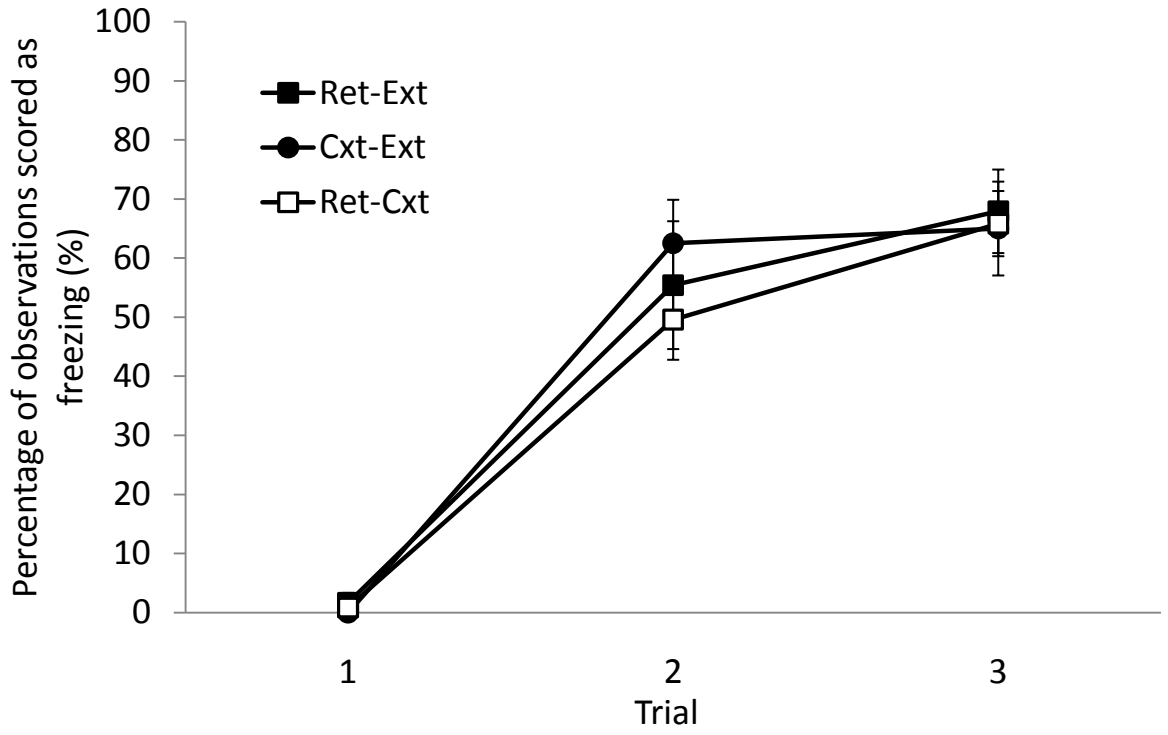

Figure 7. Freezing to the CS during acquisition training. Circles and squares represent group means ± *SEM*.

Retrieval

Freezing to the context during the first two minutes of the retrieval session was analysed to assess any contextual fear acquired in the course of conditioning of the CS. Average levels of freezing to the context for all groups was less than 1% and no group differences were observed on this measure, $F(2, 21) = 1.10$, $p = .352$; *M*s (*SEM*) of percent time freezing (%): Ret-Ext = 0.4 (0.4), Cxt-Ext = 0.8 (0.5), Ret-Cxt = 0.0 (0.0).

Freezing to the CS for Groups Ret-Ext and Ret-Cxt is shown at the left of Figure 8. The two groups did not differ significantly at this point, $F(2, 21) = 1.66$, $p = .218$.

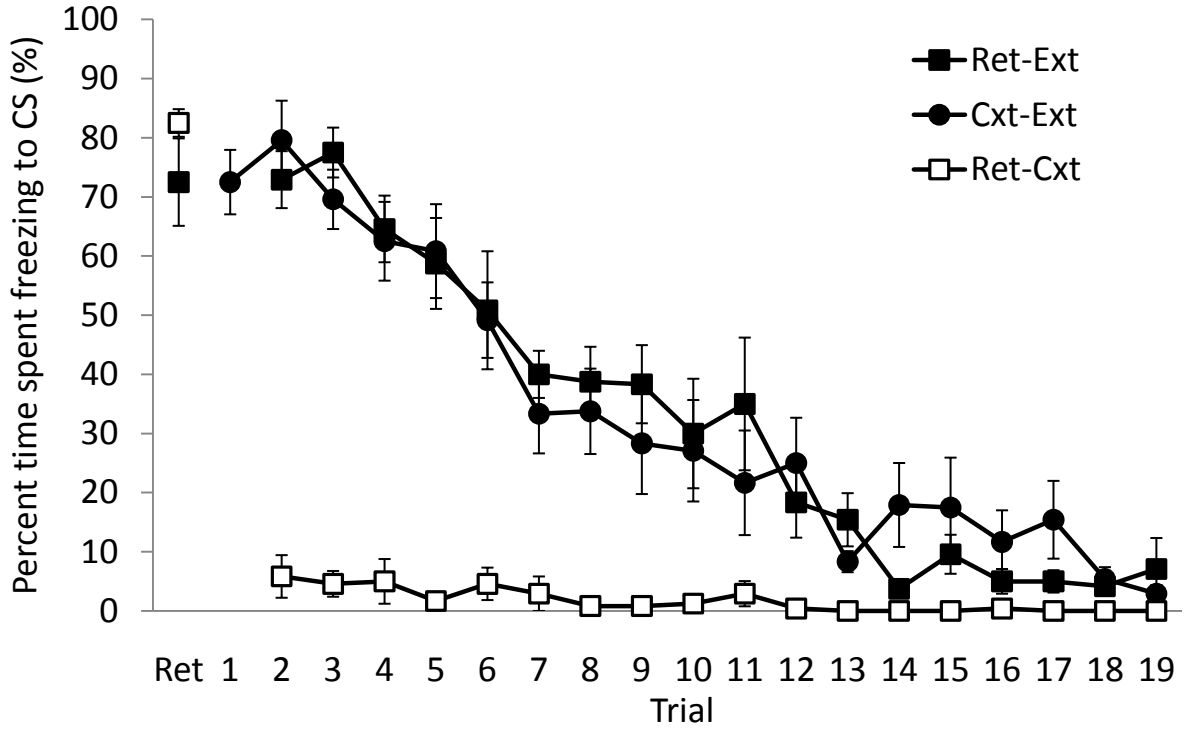

Figure 8. Freezing to the CS during retrieval (Groups Ret-Ext and Ret-Cxt) and extinction (Groups Ret-Ext and Cxt-Ext). Group Ret-Cxt were exposed to the context for the same period of time as Group Ret-Ext and freezing was recorded at the same time intervals, although no CS presentations were given. Circles and squares represent group means ± *SEM*.

Extinction

Freezing to the context during the first two minutes of the session, the period immediately preceding onset of the first CS for groups receiving extinction training, did not differ between the three groups, $F(2, 21) < 1$; *M*s (*SEM*): Ret-Ext = 0.6 (0.6), Cxt-Ext = 0.0 (0.0), Ret-Cxt = 6.5 (6.2).

Freezing to the CS during extinction training for Groups Ret-Ext and Cxt-Ext are shown in Figure 8. Freezing was recorded for Group Ret-Cxt during equivalent time periods corresponding with presentations of the CS for Group Ret-Ext. For the purposes of analysis,

the last 18 of 19 extinction trials for Group Cxt-Ret were compared with the 18 trials of Group Ret-Ext and data from the equivalent time points for Group Ret-Cxt.

A planned, orthogonal contrast analysis revealed an overall linear decrease in freezing across the 18 CS presentations or during the equivalent time periods, $F(1, 21) = 462.1$, $p < .001$, and that the magnitude of the reduction in freezing over trials was greater in groups receiving extinction training compared with Group Ret-Cxt, which received no CS presentations, $F(1, 21) = 183.8$, $p < .001$. Groups Ret-Ext and Cxt-Ext did not differ during extinction, $F(1, 21) = 1.69$, $p = .208$. Thus, freezing reduced across extinction training, with the effect being specific to presentation of the CS. Furthermore, no effect of the retrieval trial could be seen on the rate of subsequent extinction.

Reacquisition

The two minutes prior to CS presentation were taken as a baseline measure of freezing (see left panel of Figure 9). Means and standard deviations of percent time spent freezing were zero for all four groups (Ret-Ext, Cxt-Ext, Ret-Cxt and Naive), and so no further analysis was carried out on these data.

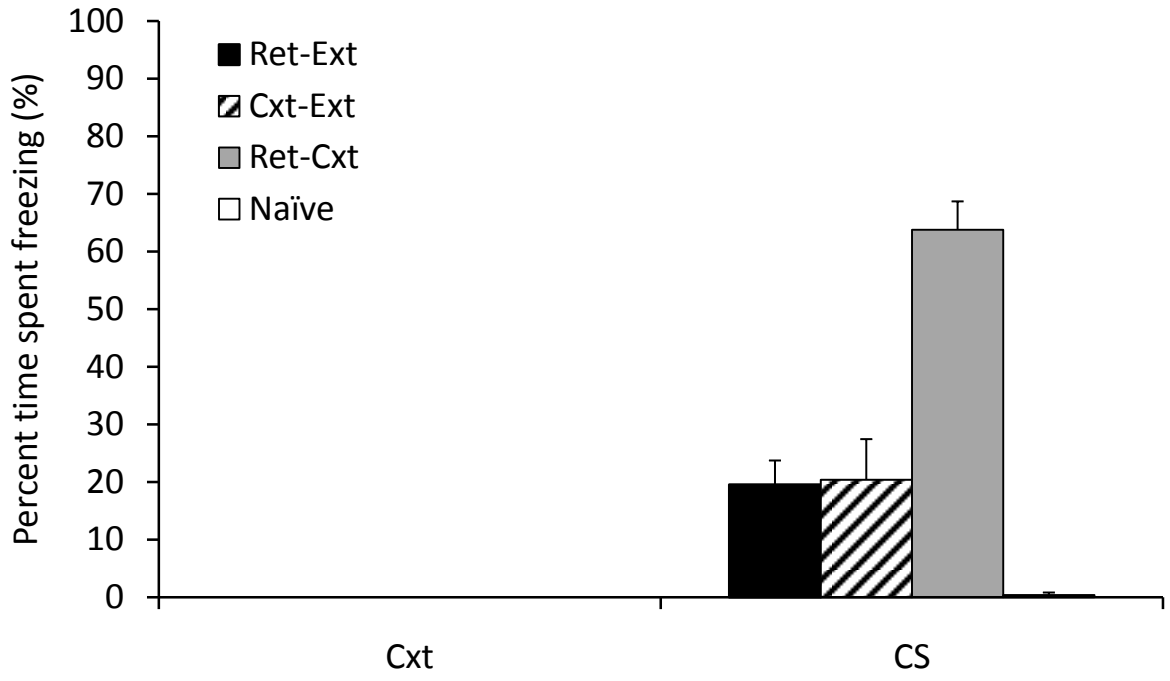

Figure 9. Freezing to the context and to the CS during reacquisition training. Bars represent group means ± *SEM*.

The results of the analysis of planned, orthogonal contrasts revealed higher freezing in Group Ret-Cxt in comparison to groups that had either been conditioned and extinguished, or never conditioned, $F(1, 28) = 79.41$, $p < .001$. Groups that had been conditioned and extinguished (Groups Ret-Ext and Cxt-Ext) displayed residual levels of freezing that were significantly higher than in the Naive groups, $F(1, 28) = 22.80$, $p < .001$. Groups Ret-Ext and Cxt-Ext did not differ, $F(1, 28) < 1$, showing that any effects of the pre-extinction retrieval trial were not apparent in terms of residual freezing to the CS after extinction.

Test

Data from the retention test can be seen in Figure 10. Freezing during the three minute adaptation period prior to first CS onset (left panel of Figure 10) did not differ between groups, $F(3,28) < 1$.

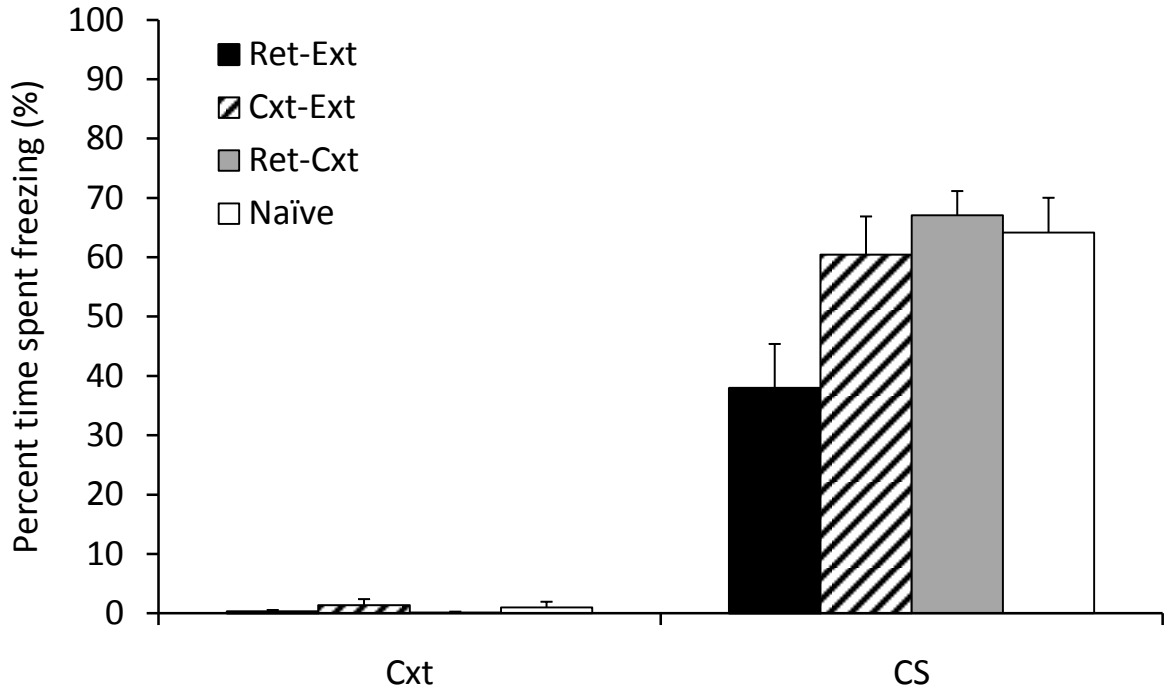

Figure 10. Freezing to the context and to the CS at test 24 h after reacquisition training. Bars represent group means ± *SEM*.

Freezing to the CS at test for each of the four groups is shown in the right panel of Figure 10. The planned, orthogonal contrast analysis revealed that the group receiving a retrieval trial prior to extinction (Ret-Ext) displayed significantly less fear in response to the CS at test than to the remaining control groups, $F(1, 28) = 13.66$, $p = .001$. This effect cannot be attributed to the retrieval trial alone, as the group receiving only the retrieval trial (Ret-Cxt) does not differ from the extinction-only (Cxt-Ext) and Naive groups, $F(1, 28) < 1$. No savings in reacquisition was detected for the extinction-only group (Cxt-Ext) as this group did not differ from the Naive group, $F(1, 28) < 1$.

Discussion

The results of this experiment reveal a significant effect of retrieval prior to extinction on subsequent (re)learning of an association between the CS and US. As expected, animals presented with the CS one hour prior to extinction showed less fear of the CS at test than

animals conditioned after normal extinction, after only retrieval and no extinction, and animals naive to the CS at the time of conditioning. Furthermore, this effect was dependant on both retrieval and extinction, since animals receiving only retrieval or only extinction reconditioned to the same level as naive animals. Thus the effect of retrieval on the loss of conditioned responding cannot be attributed to disruption of the CS-US memory by simply removing the animal from the experimental chamber, transporting to the home cage, interaction with cage mates, returning to the experimental chamber and exposure to the conditioning context within the reconsolidation window. The presentation of the CS in the context one hour after CS retrieval was necessary to produce the impairment in subsequent reacquisition.

This experiment also supports the suggestion that the effect seen in Monfils et al.'s (2009) experiment with one-trial reacquisition is due not to savings in the no-retrieval group, but rather to impaired relearning in the retrieval group. There was no suggestion that the parameters applied in this replication, at least, permit the observation of savings in reacquisition of the CS-US association. It is possible, however, that rapid acquisition in the novel control group may have produced a ceiling effect such that any facilitation of learning would have been obscured.

Experiment 1.4

It has been suggested by many researchers that the function of reconsolidation may be to allow a memory to be updated (for a review see Lee, 2009). Consistent with this account are studies demonstrating a requirement of a mismatch between what is expected and what actually occurs on the retrieval trial (Morris et al., 2006; Pedreira, Pérez-Cuesta, & Maldonado, 2004). A mismatch between expectation and outcome may open a window of memory lability which could allow for additional information to be incorporated into the memory (i.e., updated), allowing better predictability on subsequent encounters with the

stimulus or situation. In other words, the memory can be destabilised to allow the new information to be incorporated before reconsolidating the updated memory. In the case of no mismatch between expectation and outcome, the memory is already adequate in predicting the relevant events in the environment. Thus, to destabilise the memory may represent an unnecessary risk to the memory trace and a waste of cellular resources.

Within the current paradigm, training of the CS in the first phase would lead to an expectation of foot shock when the CS was again presented at retrieval. However, in previous demonstrations of the pre-extinction retrieval effect, both in this chapter and in those of Monfils et al. (2009), the retrieval trial comprised a single non-reinforced presentation of the CS. The unexpected omission of the US at retrieval may constitute a mismatch which allows the memory to become destabilised and enter a labile state. The extinction learning occurring one hour later may therefore be able to directly interfere with this memory. The following experiment (see Table 3) examines whether a violation in expectation is necessary at retrieval for extinction to produce impairment in reacquisition. As in previous experiments, extinction of the CS is carried out one hour after either a single presentation of the CS, or after exposure to the context only. Again, it is expected that the presentation of the CS prior to extinction will result in impairment of reacquisition. Of particular interest is whether reinforcement of the retrieval trial is capable of producing this same effect. In order to minimise the prediction error present at the time of retrieval, the CS is trained across two sessions with a total of seven (Ret+) or eight (Ret- and NoRet) trials, controlling for the total number of CS-US pairings. Assuming for now that destabilisation of memory requires a mismatch between expectation and outcome, the results of this experiment should determine whether memory destabilisation prior to extinction is necessary in order to observed impairment in subsequent reacquisition of the CS-US association.

Table 3: Design of Experiment 1.4

Group	Acquisition	Ret	Extinction	Reacquisition	Test
Ret+	7 x C+	C+	19 x C	1 x C+	2 x C
Ret-	8 x C+	C	18 x C	1 x C+	2 x C
NoRet	8 x C+	Cxt	19 x C	1 x C+	2 x C

N.B. Ret = retrieval; NoRet = no retrieval; C = clicker CS; "+" indicates a reinforced CS presentation (no "+" means CS presentations were not reinforced).

Methods

Subjects

Subjects for this experiment were 24 Lister hooded rats (Charles River, UK) maintained under the conditions described in Chapter II.

Apparatus

All behavioural procedures took place in the conditioning chambers described in Chapter II. The CS was a 60 s presentation of the clicker stimulus.

Behavioural Procedures

Habituation. All animals were habituated to the experimental chambers for one hour per day for three days prior to the first acquisition session. The houselight remained on for the duration of the session. No other stimuli were presented during these sessions.

Acquisition. Acquisition training took place over two sessions on two consecutive days. On the first day of training, all groups were given four pairings of a 60 s clicker CS and a 0.5 s, 0.5 mA foot-shock US. The first of these was presented after a 30 min adaptation period with subsequent trials occurring after a variable ITI with a mean of 300 s. Animals were removed 60 after the end of the last trial. On the second day of training, animals in

Groups Ret- and NoRet again received four pairings of the CS and US after a 30 min adaptation periods and with a mean ITI of 300 s. Animals in Group Ret+ received training with just three trials such that the total number of CS-US pairings would be equal across groups (Group Ret+ receive one additional pairing at retrieval).

Retrieval. On the next day, all animals were returned to the experimental chambers for a 3 min retrieval session. After a 120 s delay, Group Ret+ received a single presentation of the CS paired with shock, while Group Ret- was presented with the CS alone. Group NoRet was exposed to the context for an equivalent period of time with no stimuli being presented. All animals were returned immediately to their home cages at the termination of the session.

Extinction. One hour following retrieval, all animals were returned to the experimental chambers for extinction training. This session comprised 18 (Group Ret-) or 19 (Groups Ret+ and NoRet) non-reinforced CS presentations with an adaptation period and ITI of 180 s. Animals were returned to the home cages at the conclusion of the session.

Reacquisition. One day following extinction training, all animals were placed in the chambers for 10 min followed by one CS presentation co-terminating with the foot-shock US. Animals were removed from the context after a further 60 s and returned to their home cages.

Test. Retention of the CS-US association was tested after 24 h. Animals were given a 180 s adaptation period after which time the CS was presented twice with a 180 s ITI.

Statistical Analyses

The data from the acquisition, retrieval and extinction phases were analysed as in Experiment 1.3. For the reacquisition and test phases, freezing to both the context and the CS were analysed using a One-Way ANOVA with the LSD post-hoc procedure applied where the overall *F* statistic was found to be significant.

Results

Acquisition

The two minutes immediately preceding onset of the first CS for each of the two acquisition sessions were taken as a baseline. For the first day of acquisition training, freezing during the pre-CS period was consistently low across the three groups; in terms of percent time spent freezing, $F(2, 16) = 1.55$, $p = .242$; *M*s (*SEM*) of percent time freezing (%): Ret+ = 2.29 (3.97), Ret- = 0.00 (0.00), NoRet = 0.63 (1.24). Neither were any differences in pre-CS freezing observed on day two of acquisition, $F(2, 21) = 2.35$, $p = .120$; Ret+ = 14.80 (19.20), Ret- = 1.87 (2.58), NoRet = 4.16 (10.50).

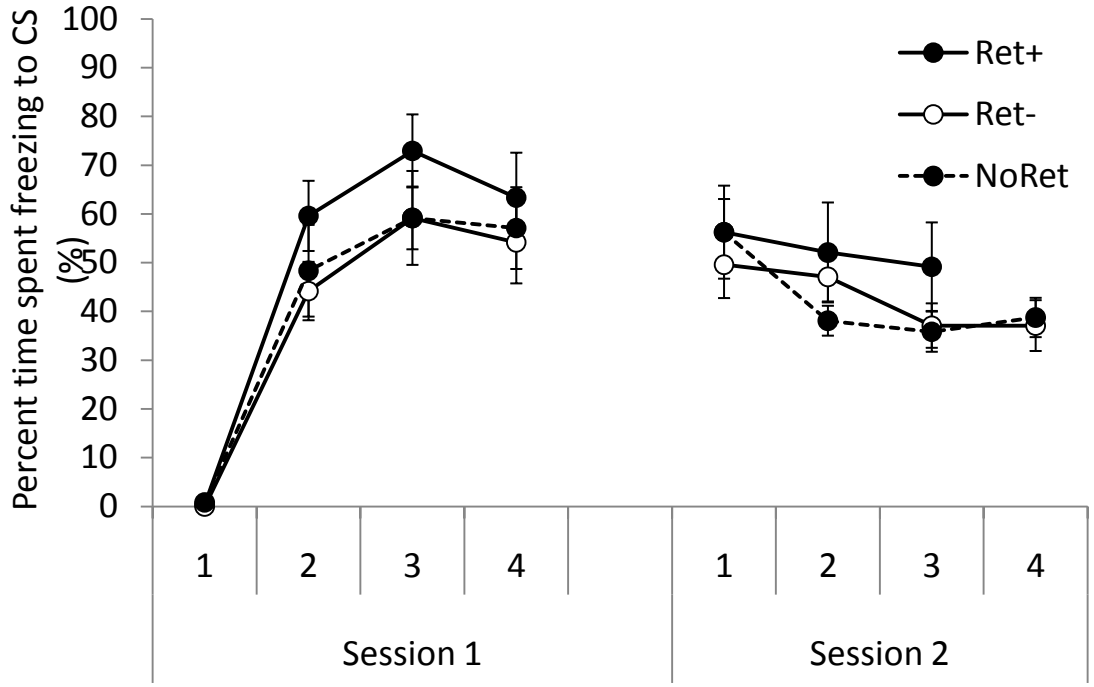

Figure 11. Freezing to the CS across four pairings per session of the CS with a foot-shock US. The numbers 1 to 4 indicate trial numbers within each conditioning session. Circles represent group means ± *SEM*.

Freezing to the CS for the two sessions of acquisition training can be seen in Figure 11. For the purposes of analysis, the first 7 trials (day one plus trials 1-3 of day two) were analysed for all three groups, while the 8th trial (trial 4 of day two) was analysed separately

for Groups Ret- and NoRet, since Group Ret+ did not receive an 8th trial. Due to failure of recording equipment, data from 4 animals in Group Ret- on trials 1-3 were lost. Therefore, the analysis of acquisition data excludes these subjects. Subsequent analyses include these subjects, however, as the conditioning session for these animals was otherwise executed successfully.

A significant linear trend across trials 1-6 indicated successful acquisition of the conditioned fear association, $F(1, 17) = 22.51$, $p < .001$. This effect did not differ between groups, $F(1, 17) < 1$. Groups Ret- and NoRet did not differ in their levels of freezing to the CS on trial 7, $F(1, 14) < 1$. Thus, there is no evidence for any pre-existing differences between groups leading into the retrieval phase.

Retrieval

Data for the retrieval trial are presented at the left of Figure 12. During the pre-CS period of the retrieval session, only Group Ret- displayed any freezing ($M = 2.20$, $SD = 3.68$). Due to this inequality of variance, a nonparametric analogue of the One-Way ANOVA was utilised: the Kruskal-Wallis test. This analysis revealed no significant group differences, $\chi^2(2) = 4.17$, $p = .124$. Freezing during the CS presentation for Groups Ret+ and Ret- was not significantly different, $F(1, 14) < 1$. Thus, no group differences were detected in either contextual fear or fear of the CS at this stage of experimentation.

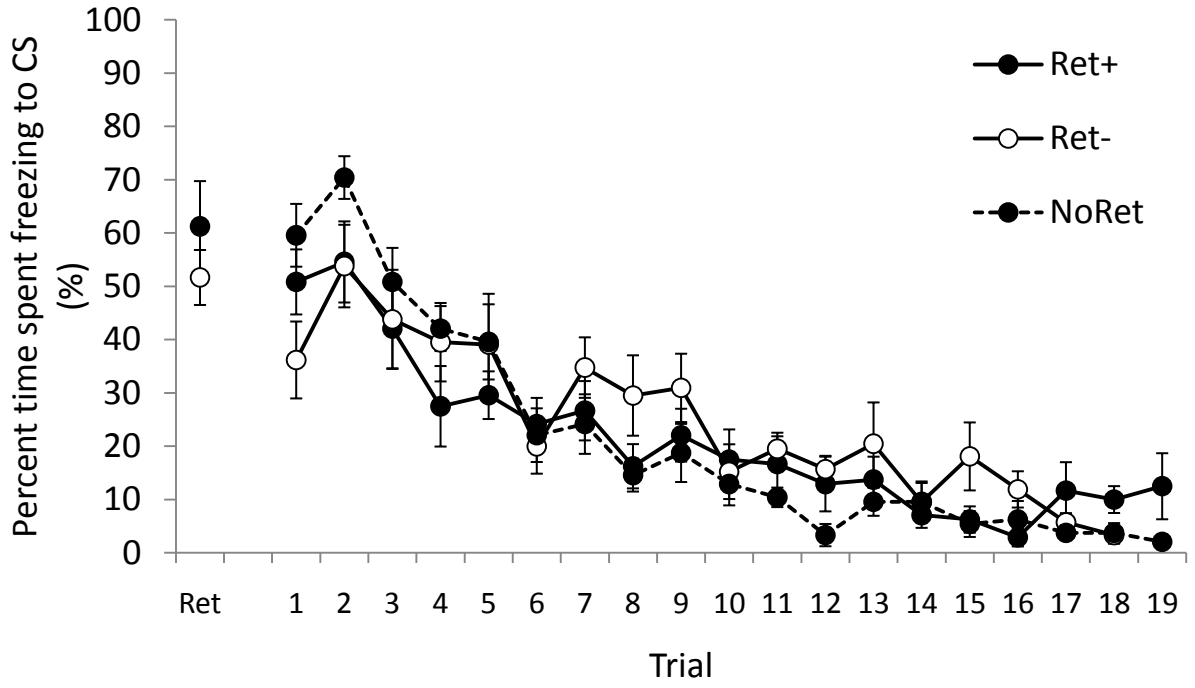

Figure 12. Freezing to the CS during retrieval (Ret) and extinction (trials 1-19). Circles represent group means ± *SEM*.

Extinction

No differences in pre-CS freezing were observed at extinction (Ret+: $M = 1.46$, $SD = 4.14$; Ret-: $M = 1.66$, $SD = 4.40$ and NoRet: $M = 0.00$, $SD = 0.00$; $F(2, 20) < 1$.

The analysis of freezing over extinction trials for Group Ret- included the non-reinforced retrieval trial as trial 1 of extinction followed by the 18 extinction trials, such that all three groups could be compared in their freezing over 19 non-reinforced presentations of the CS. These data are presented above in Figure 12. Freezing across trials decreased in a linear fashion during the course of extinction training, $F(1, 20) = 175.9$, $p < .001$. No significant interaction indicated that the magnitude of the linear effect did not differ across the three groups, $F(2, 20) = 2.62$, $p = .098$.

Reacquisition

The two minutes prior to CS onset at reacquisition were taken as an index of contextual fear. These data can be seen in the left panel of Figure 13. Freezing during this

period was minimal (Ret+: *M* = 1.25, *SD* = 2.91; Ret-: *M* = 1.88, *SD* = 2.86 and NoRet: *M* = 1.46, *SD* = 2.44), with no significant differences between groups detected, $F(2, 21) < 1$.

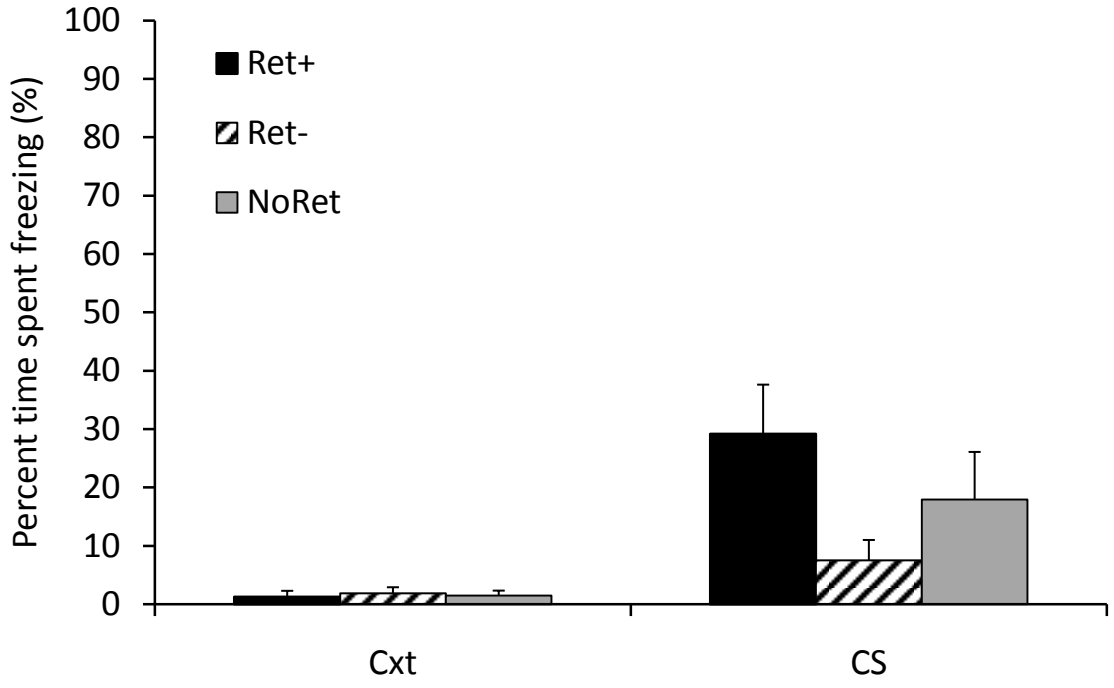

Figure 13. Freezing to the context (Cxt) and CS during reacquisition. Bars represent group means ± *SEM*.

Levels of freezing to the CS at reacquisition are displayed in the right panel of Figure 13. No significant differences in freezing during the single CS presentation were observed, $F(2, 21) = 2.34$, $p = .121$, thus providing no evidence for differences in fear to the CS prior to retraining of the CS-US association.

Test

As can be seen in the left panel of Figure 14, levels of freezing averaged across the three minutes prior to CS onset were minimal, suggesting that little fear accrued to the context in the course of experimental training. These levels were not different between groups, $F(2, 21) = 2.33$, $p = .122$.

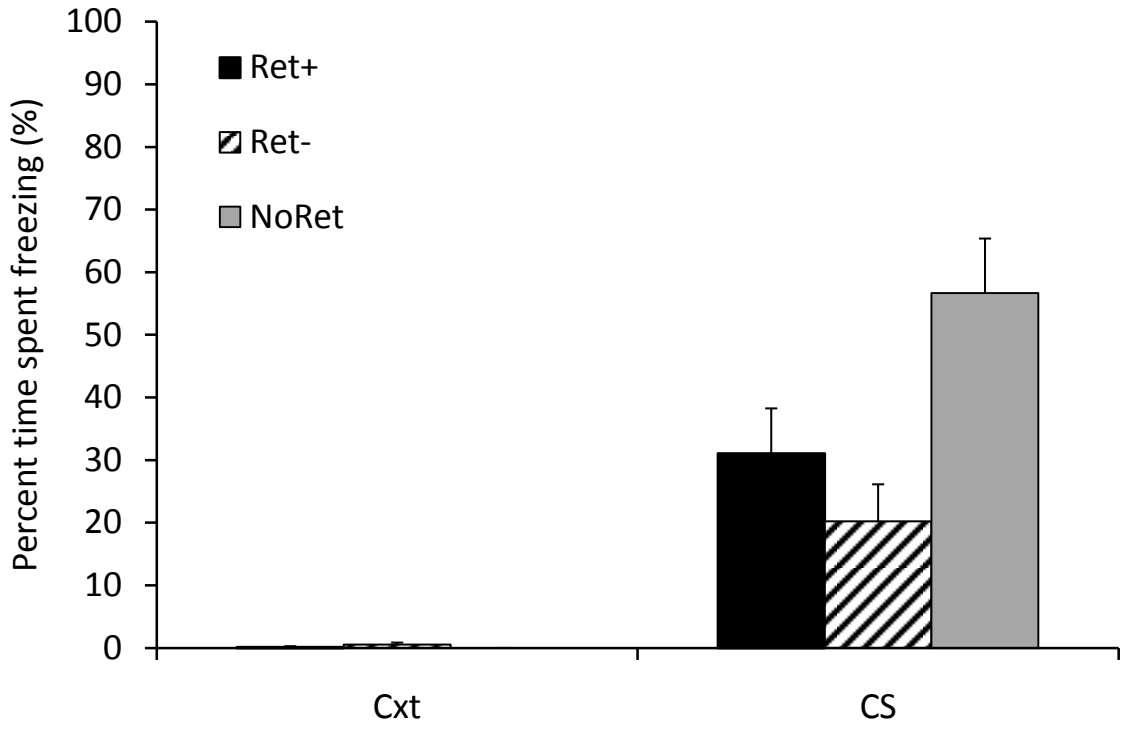

Figure 14. Freezing to the context (Cxt) and CS at a retention test given 24 h after reacquisition training. Bars represent group means ± *SEM*.

An overall ANOVA of freezing data across two presentations of the CS at test revealed a significant effect of retrieval treatment on the reacquisition of fear to the CS, $F(2,21) = 6.46$, $p = .007$ (see Figure 14). An LSD post-hoc analysis revealed that both Ret+, $F(1, 14) = 5.14$, $p = .040$, and Ret-, $F(1, 14) = 11.99$, $p = .004$, displayed reduced levels of fear of the CS in comparison to Group NoRet. Group Ret+ and Ret- did not differ from one another, $F(1, 14) = 1.35$, $p = .265$. Thus, retrieval prior to extinction, whether reinforced or not, renders a single CS-US pairing less effective at re-establishing fear to the CS than for animals extinguished without prior retrieval.

Discussion

The results of this experiment replicated earlier experiments in demonstrating an impairment in reacquisition when extinction was preceded by a single non-reinforced

presentation of the CS. In addition, these data demonstrate the same effect when the retrieval trial was paired with shock. While steps were taken to maximise learning prior to retrieval, it is still possible that the US was not sufficiently predicted at retrieval to prevent the destabilisation of the CS-US memory. Levels of freezing across acquisition trials appeared to be approaching an asymptote; however, responding in this case may reach asymptote earlier than the underlying learning process. Thus, it is possible that prediction error was still present when the CS was again paired with the US at retrieval, and that this was sufficient to destabilise the memory and allow for interference by the extinction training.

Alternatively, the prediction error may indeed have been minimal and insufficient in itself to destabilise the memory, but perhaps the relatively early presentation of CS after placement in the context may have constituted a mismatch. On the first two days of experimentation, the animals were exposed to the context for an hour per day during which time no stimuli were presented. On the third day, they waited in the context 30 min before the first presentation of the CS. The occurrence of the CS after only two minutes may in itself have been surprising and been enough to trigger destabilisation of the memory.

A third explanation is simply that the effect of retrieval prior to extinction on subsequent reacquisition is not dependent upon prediction error on the retrieval trial. This would then lead to two possible conclusions: either that a mismatch at retrieval is not necessary for the destabilisation of a consolidated memory, or that memory destabilisation is not critical for the effect on extinction of prior presentation of the CS.

Finally, there remains the possibility that while Groups Ret+ and Ret- may show the same impairment in reacquisition, the reasons for the effects in these two cases are different. In a study published by Myers, Ressler, & Davis (2006), the authors conditioned rats to fear a discrete light CS in a fear-potentiated startle procedure. The CS was subsequently extinguished 10 min, 1 h, 24 h or 72 hours after acquisition. It was found that at the shorter

acquisition-extinction intervals less recovery of responding was observed when assessing spontaneous recovery, renewal and reinstatement. These results parallel those of the Monfils et al. (2009) study with the exception that these effects were seen after initial conditioning rather than after reactivation of the conditioning memory. It was suggested that extinction at short intervals biases extinction towards an unlearning process, whereas extinction at longer intervals consists largely of new learning. Whether similar processes could be responsible for the effects of extinction after memory retrieval remains to be seen. However, for Group Ret+ in the current experiment, one of the eight acquisition trials was given just one hour prior to extinction training. If the extinction of a CS one hour after conditioning can result in unlearning of the association, then perhaps this group failed to retain the learning from this trial, effectively reducing their training to seven rather than eight trials. Thus the difference in fear observed subsequent to extinction may be due simply to having had fewer effective acquisition trials. On the other hand, Group Ret- may have benefited from the non-reinforced retrieval trial as it effectively increased the spacing of extinction trials, a factor known to influence the success of extinction learning (Li & Westbrook, 2008). This explanation will be examined more closely in the next chapter.

Chapter Discussion

The experiments presented in this chapter replicate the findings of Monfils et al. (2009) in showing that reacquisition after extinction is impaired if the extinction training is given one hour after a single presentation of the CS. In the first experiment, retardation in reacquisition was observed across five pairings of a tone CS with foot-shock after the CS had undergone extinction one hour after a brief retrieval trial. This effect did not persist to a long-term memory test given 24 h later, however, and the freezing response did not appear to be reliable enough for the purpose of examining the effect in detail. The next experiment aimed to confirm that the instability of the freezing response was specific to the tone CS and to

identify a stimulus which would produce a more robust freezing response. It was found that both the light and the clicker supported strong and reliable levels of freezing across three acquisition trials and a test given in extinction after 24 h. It was therefore decided that subsequent experiments would employ these stimuli rather than the tone. The following experiment then assessed the effect of retrieval prior to extinction on reacquisition using the clicker as the CS. In addition to replicating previous findings now with the clicker CS, these results demonstrated the requirement for both retrieval and extinction training since groups given only retrieval or only extinction did not show any evidence for impairment in fear reacquisition subsequent to extinction. This result ruled out the possibility that the deficits observed in previous experiments were due to any disruptive effect of removal to the home cages within the reconsolidation window following reactivation of the CS-US memory. Furthermore, these results support the suggestion that the difference reported by Monfils et al. (2009) in one-trial reacquisition for groups extinguished with and without retrieval was not due merely to a lack of savings in the retrieval group, but active impairment in the re-establishment of the CS-US association. Finally, it was shown that a similar effect on fear learning could be observed with a reinforced retrieval trial when acquisition training was extended to maximise the associative strength of the CS. This result may be interpreted in a number of ways including that prediction error is not required to destabilise a consolidated memory, or that destabilisation of the memory is not necessary to produce the effects of pre-extinction retrieval on reacquisition. These results still cannot rule out the possibility, however, that the effect shown by Monfils et al. (2009) and in the earlier experiments may simply be an effect of trial spacing on extinction learning.

IV. THE ROLE OF TRIAL SPACING IN THE PRE-EXTINCTION RETRIEVAL EFFECT

The effects of a pre-extinction retrieval trial observed so far, both here and in the paper by Monfils et al. (2009), are consistent with the idea that a retrieval trial one hour prior to non-reinforced presentations of the CS somehow strengthens the subsequent extinction learning. This is a curious effect given that this procedure appears simply to increase the interval between the first and second non-reinforced trials on the extinction day. Groups receiving the Monfils treatment receive the first non-reinforced presentation of the CS (i.e., the retrieval trial) one hour prior to the second non-reinforced CS presentation (i.e. the first trial of extinction). The no-retrieval groups are presented with the first CS-alone trial at extinction, with the second trial following after only 2 min. This difference in timing appears the most likely factor responsible for the effects seen on the post-extinction manipulations.

The spacing between trials has long been known to be an important parametric variable in Pavlovian conditioning with learning generally aided by longer ITIs (Barela, 1999; Barnet, Grahame, & Miller, 1993; Gibbon, Baldock, Locurto, Gold, & Terrace, 1977; Lattal, 1999; Rescorla & Durlach, 1987)). Similarly, extinction training tends to benefit from longer ITIs relative to CS length (Bouton & García-Gutiérrez, 2006; Li & Westbrook, 2008; Moody, Sunsay, & Bouton, 2006).

Since the spacing of trials in most preparations is achieved by distributing CS presentations within a longer session, spaced trials generally result in greater overall exposure to the training context. According to most theories, it is this difference in context exposure, rather than the spacing of trials per se, that accounts for the superior acquisition of excitatory associations observed with widely spaced conditioning trials. The Rescorla-Wagner model (Rescorla & Wagner, 1972), for example, treats the context in the same manner as a discrete CS and attributes the trial-spacing effect to competition between the CS and the training

context for associative strength with the US. According to this account, the CS and context, both being present on each acquisition trial, each have the potential to enter into an association with the US. The context however, unlike the CS, is also present during the ITI when the US is not presented. Thus, during this period of non-reinforced exposure, the context undergoes extinction. Spaced trials, therefore, give more opportunity for the context to extinguish its association with the US between trials, and so more associative strength will be available to accrue to the CS.

A second account of the effect comes from the comparator hypothesis of Miller & Matzel (1988). This account again attributes the effect to conditioning of the US to the context, although in this case the effect is not on learning but on performance. According to this model, the amount of associative strength that accrues to the CS (V_{CS}) is proportional to the probability of the US occurring in the presence of the CS. Similarly, associative strength can be established between the context and the US (V_{Cxt}) based on the likelihood of the US occurring while the context is present. Unlike the Rescorla-Wagner model, the CS and context do not compete for associative strength. Rather, these two predictive cues compete for control over fear responding such that a CR will be produced when the ratio V_{CS}:V_{Cxt} reaches a particular threshold. Any manipulation which decreases the associative strength of the context, e.g., context extinction, will therefore increase the response ratio and increase the rate of fear responding. As in the Rescorla-Wagner account, trial spacing allows for extinction of the context between trials, which reduces V_{Cxt} and so increases the response ratio. The key distinction between these two accounts is that Rescorla-Wagner asserts that the CS competes with the context for associative strength at the time of *training*, whereas the comparator hypothesis states that the competition between these two sources of predictive power occurs at the time of *testing*. To address this question, Kasprow, Schachtman, & Miller (1987) paired a CS with foot-shock in one context and tested in another context. Critically,

between the training and testing sessions, rats were exposed to the training context in the absence of any stimulus presentations, effectively to reduce the associative strength of the context. Subsequent responding to the CS in the testing context was higher following extinction of the training context than when the training context was not extinguished. Since the Rescorla-Wagner model attributes conditioned responding to the CS to the amount of associative strength acquired during training, , it struggles to explain how post-training changes in the associative strength of the conditioning context could increase responding to the CS, even when that context is absent.According to each of the models discussed so far, the trial spacing effect is due to the opportunity for the context to extinguish between trials. A consequence of this view is that extinction learning should benefit from short rather than long intervals between trials. With longer exposure to the context between trials, the associative value of the context will be reduced. In terms of the Rescorla-Wagner model, this results in less combined associative strength on the following extinction trial, thus less prediction error and less learning. In the language of the comparator model, the loss of associative strength to the context will increase the V_{CS}:V_{Cxt} ratio, and so increase responding to the CS. One study to show this effect is by Rescorla & Durlach (1987) who trained pigeons in an autoshaping procedure and subsequently extinguished the stimuli with either a short (10 s) or a long (2 min) ITI. The researchers observed that extinction with the short ITI proceeded faster than with the long ITI with this effect persisting when the stimuli were presented outside the extinction context. However, it was not clear from their data whether this effect arose as a result of excitation of the training context since the extinction contexts were not seen to influence responding to stimuli extinguished elsewhere. Thus the role of the context in mediating the trial spacing effect in extinction could not clearly be established.

A number of other studies, however, have addressed this issue by assessing the effect of ITI on extinction while controlling for total exposure to the context. The prediction of most

contemporary learning theories, including Rescorla-Wagner and the comparator hypothesis, would be that controlling for time in the context should eliminate the trial spacing effect. However, many studies controlling for context exposure have shown profound effects of trial spacing, with most showing more robust extinction with long rather than short ITIs (Li & Westbrook, 2008; Morris, Furlong, & Westbrook, 2005; Urcelay, Wheeler, & Miller, 2009; Westbrook, Smith, & Charnock, 1985; but see Cain, Blouin, & Barad, 2003). These effects may best be explained by appealing to Wagner's "sometimes opponent-process" or "SOP" model (Wagner, 1981), one of the only theories of learning capable of explaining the effects of time on Pavlovian association formation. The SOP model states that elements of stimulus nodes can exist in one of three states of activation: A1, A2 and I (inactive; see Figure 15). The important features of these states for the present discussion are: (1) the presentation of a stimulus will result in elements of the stimulus node being activated into A1; (2) with time, during CS presentation or immediately after, CS elements in A1 will decay into A2 and eventually into the inactive state; (3) stimulus elements simultaneously active in A1 will form an excitatory association, while an inhibitory association will form between elements active in A1 and those active in A2; (4) a stimulus node in A1 can result in an associated stimulus node being brought into A2; (5) elements of a stimulus node cannot pass directly from A2 into A1.

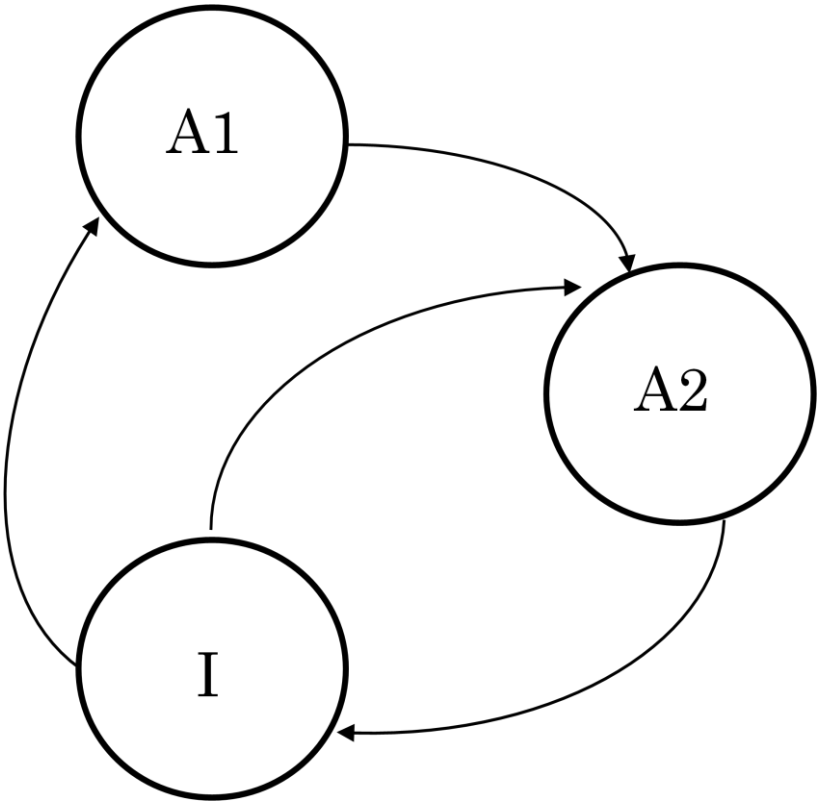

Figure 15. Diagrammatic representation of the three activation states according to Wagner's (1981) SOP model. Stimulus elements can be excited into A1 when in the centre of attention. These elements then degrade into A2, a peripheral attentional state, before degrading into the inactive, I, state. The activation of a stimulus in A1 can also result in the activation of associated stimulus elements from I directly into A2.

On the first trial in a Pavlovian conditioning paradigm, presentation of the CS will excite elements of the stimulus node into the A1 activation state. Shortly afterwards, the US will typically be presented such that elements of the US node will also be activated into A1. The conjoint activation of the CS and US elements in A1 will lead to an association being formed between these elements such that the next time the CS is presented, a portion of the elements of the US node, proportional to the current associative strength between the CS and US, will be retrieved from the inactive state into A2. When the US is then presented, only those elements not already in A2 will be able to enter the A1 state, and thus the amount of excitatory conditioning between the CS and the US will be less than on the previous trial.

This is consistent with the observation of asymptotic learning curves in Pavlovian conditioning. The SOP's account of extinction follows the same principles. Once an association has been established between a CS and a US, presentation of that CS will retrieve a representation of the US into A2. As stated previously, the existence of stimulus elements in A1 and A2 simultaneously will result in the formation of an inhibitory association between them and so the simultaneous activation of CS elements in A1 and US elements in A2 weakens the associative strength of the CS. The CS is consequently less capable of activating the US representation and so less able to elicit a CR.

A unique feature of the SOP model is its ability to account for the effects of timing on conditioning. It should be noted, however, that the model can predict facilitation in learning from both trial spacing and trial massing depending upon the values assigned to the decay rate parameters. While the exact rate at which a stimulus node decays from A1 to A2, or from A2 to I, are not specified in the model, it is theoretically possible to assign values to these variables which could account for the facilitatory effects of trial spacing on learning (acquisition and extinction, among other learning paradigms). Consider the case of extinction of a CS-US association. On the first trial of extinction, presentation of the CS excites its own stimulus elements into A1 (self-generated priming) while causing the retrieval of US elements into A2 (retrieval-generated priming). As discussed already, this arrangement results in the formation of an inhibitory relationship between the CS and US. Importantly, however, following the CS presentation, the CS representation decays into A2 where it remains until finally decaying into the inactive state.

Depending on the rate at which the representation decays from A2 to I, it is possible that some CS elements may still be active in A2 when the next trial begins. If this is the case, remembering that elements cannot move from A2 back to A1, there will be fewer stimulus elements available to be activated into A1. With fewer CS elements active in A1, there are

fewer elements available to retrieve the US. The result of having less of the CS representation active in A1 and less of the US representation primed to A2 is that there is less opportunity for CS and US elements to form an inhibitory association between each other. The shorter the interval between CS trials, the more likely it will be that CS elements remain active in A2 and so the less effective extinction training will be. Somewhat paradoxically, this model also predicts that with given decay rate parameters, ITIs which produce impaired extinction learning (as assessed at a test given at a common interval) will also produce a more rapid decline in conditioned responding within the extinction session. A CS is assumed to elicit conditioned responding via its ability to retrieve a representation of the US. By presenting CS trials in close succession, the potential for a CS presentation to retrieve the US representation is minimised, and so the potential for the CS to elicit a fear response is also impaired.

This prediction was investigated in an elegant study by Li & Westbrook (2008) using a contextual fear extinction paradigm. The authors examined the effects of 4 min versus 24 h ITIs on the extinction of fear to a context previously paired with shock. Critically, the time between extinction trials was spent in the home cages so as to equate the groups on total exposure to the context. They observed that animals extinguished with a short ITI displayed a more rapid loss of conditioned responding across extinction trials. However, these animals also displayed robust recovery of responding when tested after a 24 h period. In contrast, animals extinguished with a 24 h ITI maintained low levels of fear to the context whether test in a massed or a spaced fashion. The authors went on to show that extinction learning occurred on only the first trial of a series of massed trials, such that daily sessions of massed extinction trials proved no more effective in extinguishing conditioned fear than one trial per day. The results of this study were best accounted for by the SOP model and show that the spacing of trials can have profound effects on the progress and success of extinction learning over and above any mediating role of context.

In light of these studies, it seemed plausible to consider whether trial spacing might be involved in the enhancement of extinction seen when the CS is retrieved one hour prior to extinction training. The experiments that follow in this chapter test the hypothesis that the extended period between the first and second non-reinforced presentation of the CS may help in part account for the resistance to reacquisition observed in the previous experiments.

Experiment 2.1

The potential for trial spacing to affect extinction learning independently of the effects of context extinction raises the possibility that the impairment in reacquisition seen following retrieval and extinction could be due to the extended period between the first two CS presentations. It is possible that the relative difficulty with which Group Ret reacquires the CS-US association may be a reflection not of superior extinction in this group, but of inferior extinction in Group NoRet due to a trial massing effect. In other words, the relative ease with which the NoRet groups reacquire fear is due to a failure to extinguish the fear effectively in the first place. This may be due to the elements of the CS node having insufficient time to decay into the inactive state between trials and so interfering with extinction while still allowing the conditioned response to diminish across trials.

The following experiment investigated the effect of different temporal arrangements of CS presentations on subsequent reacquisition, keeping total context exposure constant between groups (see Figure 16). The purpose of this experiment was to assess whether trial spacing effects on extinction could account for the effect of pre-extinction retrieval on reacquisition. Previous studies holding context exposure constant have reported opposing effects of trial spacing on the efficacy of extinction with Urcelay et al. (2009) reporting facilitation of extinction with spaced trials and Cain et al. (2003) reporting stronger extinction with massed trials. The differences between these studies were discussed by Urcelay et al. (2009) who proposed that particularly long intertrial intervals may cause the extinction trials

to act as reminders of the original learning rather than extinction trials. Thus, while trial spacing may in principle aid extinction learning, lengthening of the intertrial interval beyond a certain point may lead the animal to treat each trial as a separate reminder event such that little extinction learning may occur from trial to trial.

In the current experiment, Groups Ret-Ext and NoRet-Ext were treated as in previous experiments except that the time between retrieval and extinction was spent in the conditioning chambers rather than the home cages. A third group (Spaced) was given the same number of trials (19) spaced evenly across the same period of time. Increasing the interval between trials from 120 s to 305 s would allow more time for CS elements to decay from A2 and so should therefore have enhanced extinction. If the pre-extinction retrieval effect is due to the enhancement of extinction through an increase in spacing of trials, then this group should also show impairment in reacquisition, at least relative to the NoRet-Ext group. The purpose of the final group (Ext-Ret) was to assess whether extinction of the context early or late in extinction would affect subsequent reacquisition. This group was similar to Group Ret except that the order of the “retrieval” and “extinction” phases are reversed.

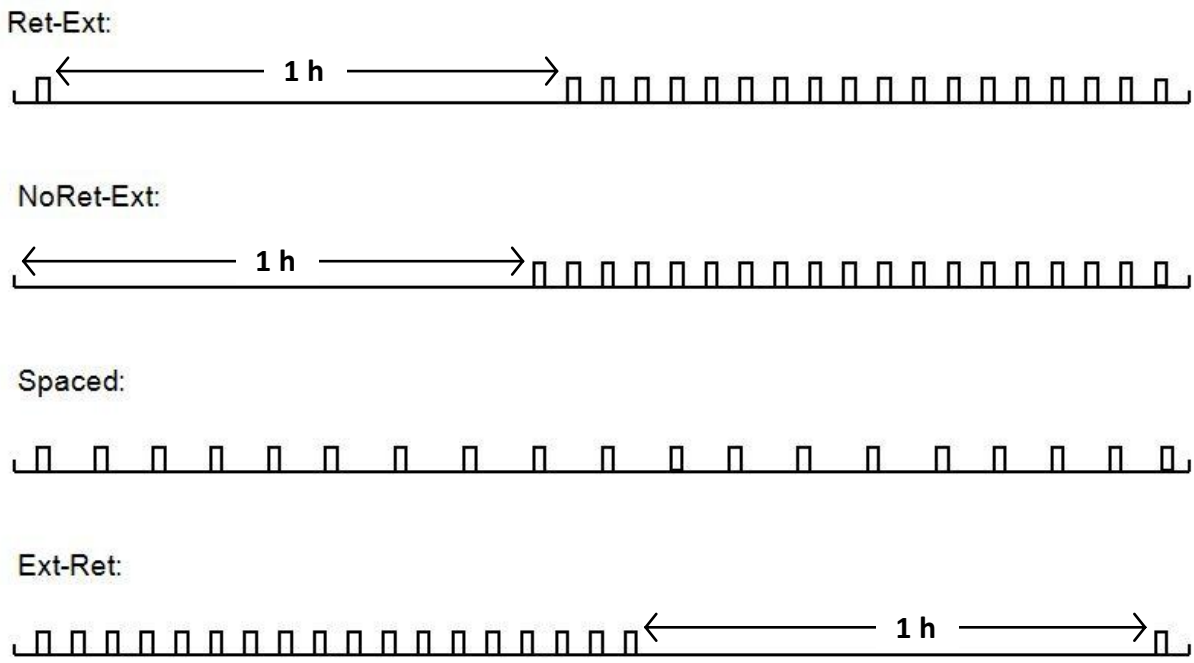

Figure 16: Arrangement of 19 CS presentations within the extinction session for each of the four experimental groups. Open boxes represent CS presentations. Trial spacing for group Spaced is 305 s. Trial spacing for the remaining groups is 120 s except where marked. The total number of CS presentations (19) and total time in the context (116 min) are equivalent across each of the groups.

The Rescorla-Wagner model predicts that early extinction of the context should result in less prediction error at the time of the second CS presentation, and so less learning should occur across the remaining trials. In the case of Group Ext-Ret, extinction of the context occurs only after CS extinction leaving the early trials unaffected by context extinction, and so more extinction learning should occur in this group relative to Group Ret-Ext. Group NoRet-Ext would be more impaired still since all 19 trials would occur after the context extinction.

The comparator hypothesis would predict no difference between any of the groups since total context exposure is held constant. Freezing to the CS after 24 h should be

relatively high, however, since the extinction would be less effective with the longer time spent in the context in this experiment.

According to the SOP model, Groups Ret-Ext, Spaced and Ext-Ret should display stronger extinction compared to Group NoRet-Ext. If the 120 s interval between extinction trials is indeed sufficient to interfere with extinction, the effect of retrieval prior to extinction is essentially to provide two efficacious extinction trials as opposed to one. Spacing the trials at 305 s intervals should allow more of the CS elements to decay from A2 and so should at least be more effective than Group NoRet-Ext, if not also Groups Ret-Ext and Ext-Ret. To the extent that any extinction occurs on trials spaced at 120 s, extinction in Group Ret-Ext should be greater than in Group Ext-Ret since the prediction error at the time of the 1 h ITI will be larger for the former than for the latter. These predictions are summarised in Table 4.

Table 4. Predictions of three models of Pavlovian learning for the amount of fear observed at test following extinction under the conditions outlined in Figure 16.

Model	Prediction
Rescorla-Wagner[1]	Ext-Ret < Spaced < Ret-Ext < NoRet-Ext
Comparator hypothesis[2]	Ret-Ext = NoRet-Ext = Spaced = Ext-Ret
Wagner's SOP[3]	Spaced < Ret-Ext < Ext-Ret < NoRet-Ext

1. Rescorla & Wagner (1972); 2. Miller & Matzel (1988); 3. Wagner (1981).

Methods

Subjects and Apparatus

The subjects were 32 adult male Lister-hooded rats (Charles River, UK), which were divided equally into 4 groups of 8. All experimental procedures were carried out in the

chambers described previously. The stimuli used were a 60 s clicker CS and 0.5 s, 0.5 mA foot-shock US, as described in Experiment 1.2.

Behavioural Procedures

Habituation. Animals were placed in the experimental chambers for one hour a day for two consecutive days. No stimuli were presented during this time and animals were returned directly to the home cages at the conclusion of the session.

Acquisition. On the following day, animals were again placed in the experimental chambers and presented with three pairings of the CS with the foot-shock US. The conditioning parameters were identical to those used in Experiment 1.3.

Extinction. One day following acquisition training, all groups were returned to the experimental chambers and presented with 19 trials of the CS in the absence of the US. The group designations determined the temporal arrangement of these presentations, which are represented graphically in Figure 16. For Group Ret-Ext, one CS trial was given after 2 min in the context, after which a 60 min period passed before presentation of the remaining 18 CS trials with an ITI of 2 min. Animals were removed from the context one min after the final CS presentation. Group NoRet-Ext waited 60 min after being placed in the context for presentation of 19 CS trials with an ITI of 2 min, and was removed one min after the end of the last trial. Group Spaced received the same number of trials spaced evenly (5 min 3 s) across the first 115 min of the session and then removed 1 min and 3s[1] after the final presentation. Finally, Group Ext-Ret, received 18 CS presentations after 2 min in the context, with an ITI of 2 min, and then waited one hour for the final CS presentation. The animals were removed one min after this last trial.

[1] The ITI needed to space the trials evenly across the same time period would be 303.158 s. This was rounded down to 303 s due to limitations of the programming language. The accumulated remainders (.158 s × 19) totalled to 3 s and this time was added to the time after the final CS such that the total time in the context would be equal to that of the other groups.

Reacquisition. A single pairing of the CS with the US was presented during reacquisition training, as described in Experiments 1.3 and 2.1.

Test. The test session comprised two presentations of the CS in the manner described for Experiments 1.3 and 2.1.

Statistical Analyses

For each session, levels of freezing during the pre-CS period were averaged across the time period and analysed using the One-Way ANOVA procedure with Group (Ret-Ext, NoRet-Ext, Spaced, Ext-Ret) as the between-subjects factor. The data for the acquisition and extinction sessions was analysed by way of a mixed ANOVA with Group as the between-subjects factor and Trial as the repeated measures variable. Freezing during the single reacquisition trial was analysed using the One-Way ANOVA procedure with Group as the between-subjects factor. Freezing across the two test trials were analysed with a mixed ANOVA with Group as between-groups factor and Trial as the repeated-measures variable.

Results

Acquisition

Data from 3 animals (one each from Groups Ret-Ext, NoRet-Ext and Spaced) were lost due to a failure of the camera in one of the four chambers. As the session had otherwise proceeded as planned, these animals were retained in the experiment and included in subsequent analyses.

Freezing during the two min prior to first CS onset was consistently low (Ret-Ext: M = 1.19, SD = 3.15; NoRet-Ext and Spaced: Ms = 0.00, SDs = 0.00; Ext-Ret: M = 0.83, SD = 1.26) and no differences between groups were significant, $F(3, 25) < 1$. The percentage of time spent freezing during each of the CS presentations is shown in Figure 17. A significant linear increase in freezing across the three conditioning trials was observed, $F(1, 25) = 369.3$, $p < .001$, indicating successful acquisition of conditioned responding to the CS. No overall

differences between groups were found, nor any significant between × within groups interaction, *F*s < 1.

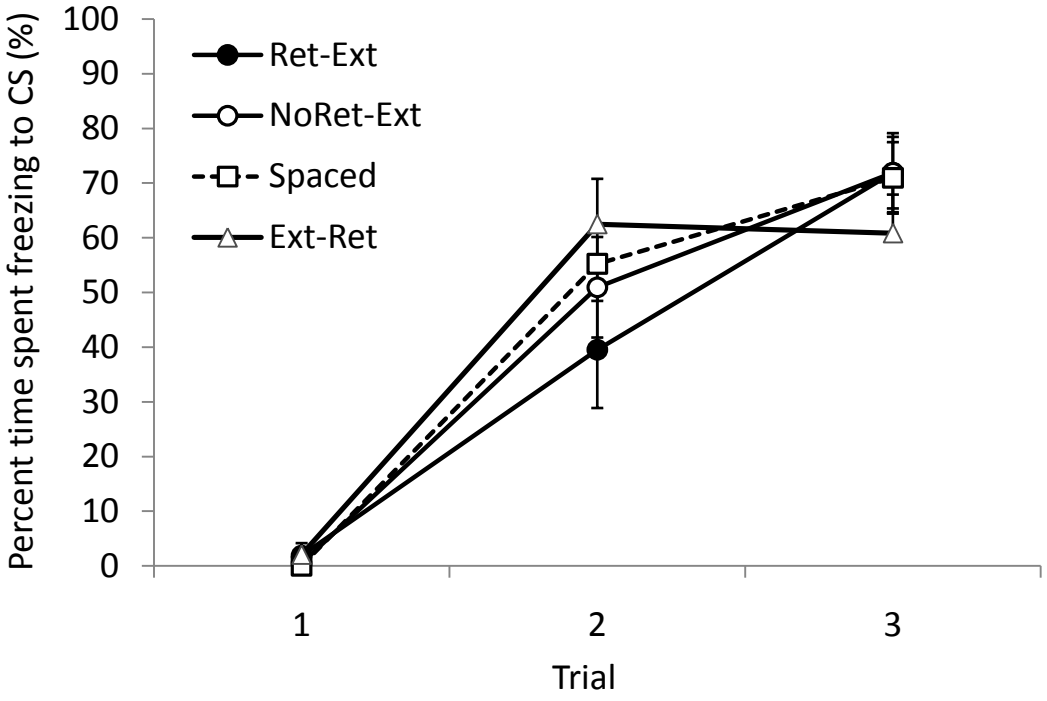

Figure 17. Freezing to the CS during acquisition. Circles and triangles represent group means ± *SEM*.

Extinction

The first two min of context exposure were taken as a measure of contextual fear. No group differences were significant on this measure, $F(3, 28) = 1.15$, $p = .348$, Means (*SEM*s) for Groups Ret-Ext = 0.21 (0.21), NoRet-Ext = 1.46 (0.66), Spaced = 7.29 (5.17) and Ext-Ret = 2.50 (2.50). Extinction training was successful in reducing levels of freezing to the CS, as indexed by a significant linear decrease in freezing across trials, $F(1, 28) = 214.6$, $p < .001$, see Figure 18. The groups did not differ in terms of the rate of extinction, nor did they differ in overall levels of freezing during the session, *F*s < 1.

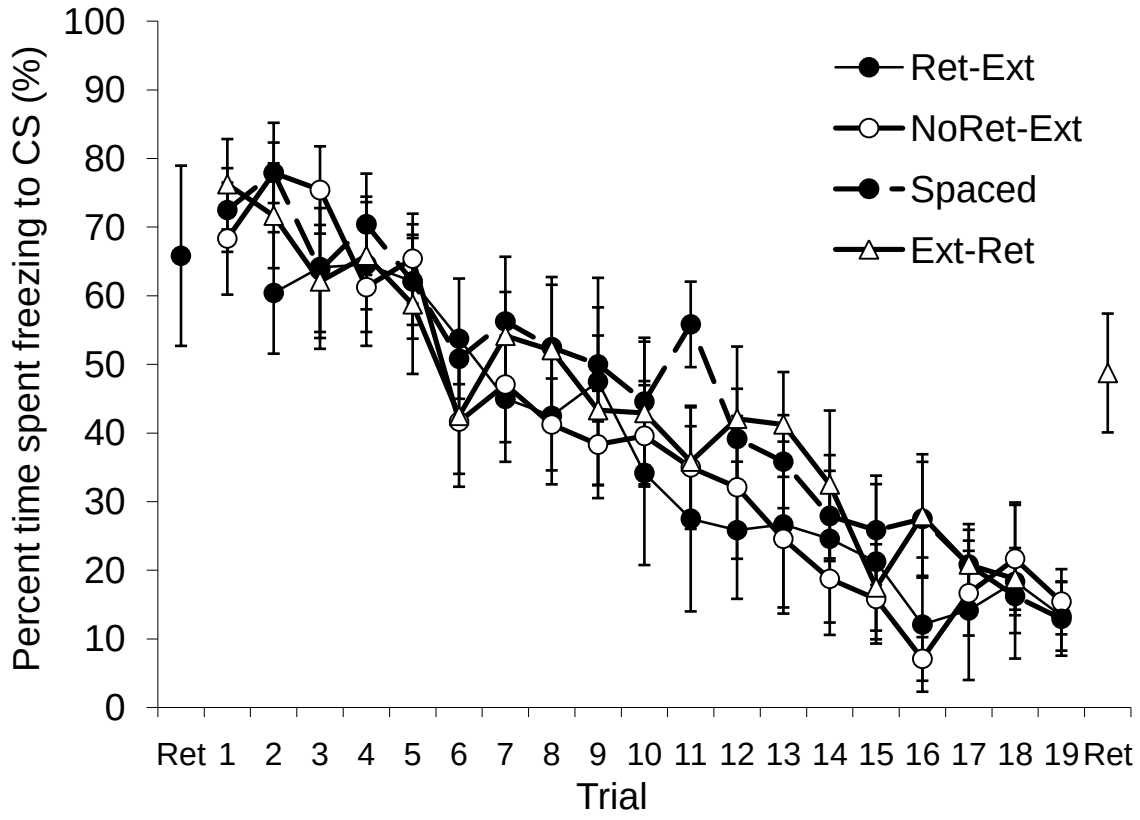

Figure 18. Freezing to the CS across 19 extinction trials. The first trial of Group Ret-Ext is plotted to the left of Trial 1 as there was a one hour delay between this trial and Trial 2 for these animals. Similarly, the last trial for Group Ext-Ret is shown to the right of Trial 19 as this was presented one hour after Trial 18. Circles and triangles represent group means ± *SEM*.

Inspection of the extinction data for Trial 19 (including the final trial of Ext-Ret) suggested a significant recovery in responding for Group Ext-Ret when the CS was presented one hour after the conclusion of the 18-trial extinction in comparison to the other three groups. Post-hoc analysis of multiple comparisons with Scheffé correction for this trial revealed this effect to be significant, such that freezing in Group Ext-Ret was significantly higher than that of the other groups, $F(1, 28) = 14.55$, $p = .004$.

Reacquisition

Data from the pre-CS (Cxt) and CS periods during reacquisition are presented in Figure 19. Groups did not differ either in terms of contextual fear, $F(3, 28) = 2.27$, $p = .102$, nor in terms of fear to the CS, $F(3, 28) < 1$.

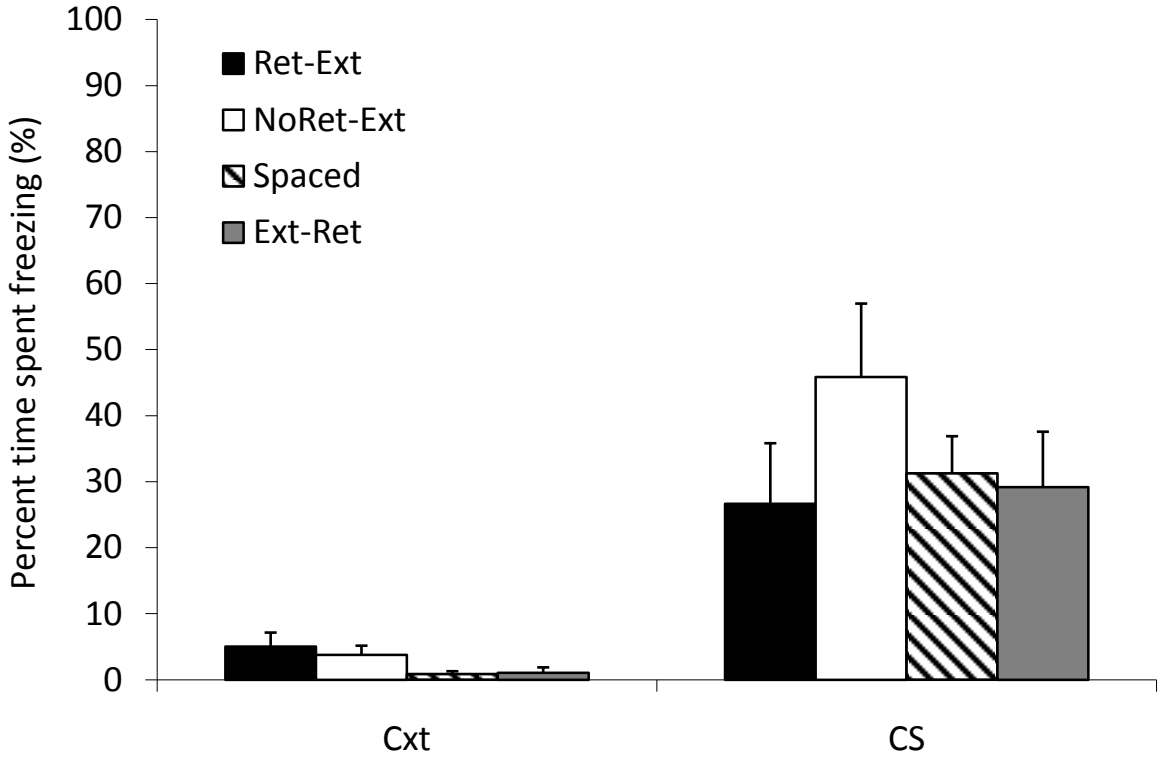

Figure 19. Freezing to the context (Cxt) and CS during the reacquisition session. Bars represent group means ± *SEM*.

Test

Figure 21 shows freezing to the context and CS during the retention test. Only low levels of freezing were observed during the 2 min pre-CS period, suggesting minimal contextual conditioning through the course of the experiment, with no significant group effects, $F(3, 28) = 1.12$, $p = .357$. Due to the relatively high levels of freezing to the CS that remained following acquisition, the degree of learning resulting from the reacquisition session may be better assessed through the analysis of difference scores calculated by subtracting freezing to the CS at reacquisition from CS freezing at test. These data are

presented below in Figure 20. Absolute levels of freezing to the context and CS are presented in Figure 21.

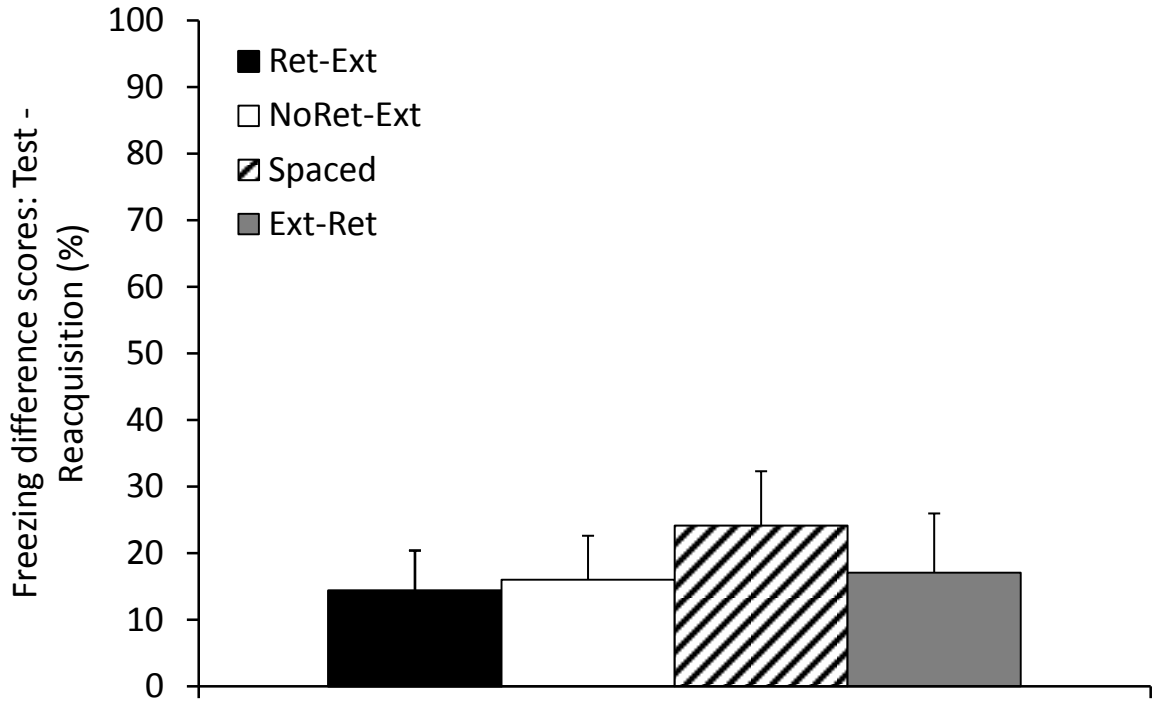

Figure 20. Difference scores showing the increase in freezing to the CS from reacquisition to test. Bars represent group means ± *SEM*.

The increase in freezing from reacquisition to test, in other words, the efficacy of the reacquisition session, was not influenced by group allocation, $F(3, 28) < 1$. Thus, the temporal arrangement of CS presentations during the extinction session did not reliably affect the ability of rats to reacquire fear to the CS.

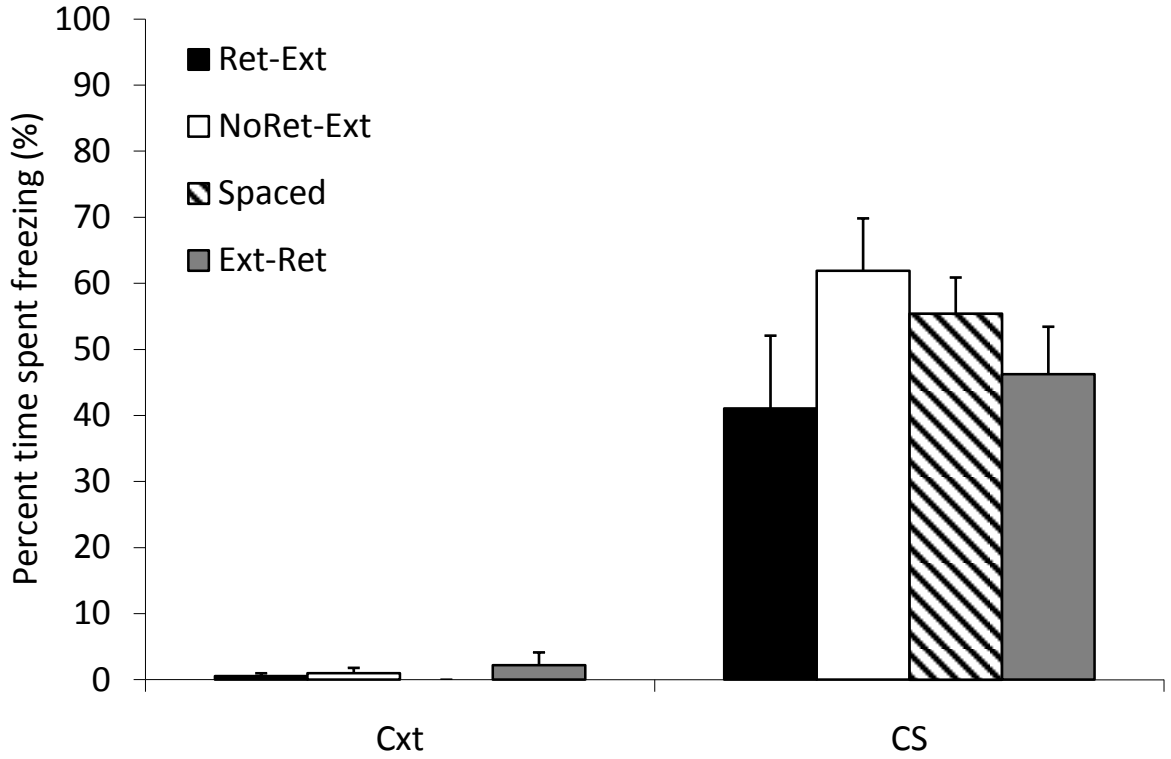

Figure 21. Absolute levels of freezing during the pre-CS (Cxt) and average freezing across the two presentations of the CS periods at test. Bars represent group means ± *SEM*.

Discussion

The results of this experiment reveal little effect on extinction learning and reacquisition of the different temporal distributions of CS trials during extinction training. Using freezing to the CS at reacquisition as an index of the success of extinction training, no significant effects were observed. While this was consistent with the comparator hypothesis, the higher mean of Group NoRet compared with the remaining groups, although non-significant, was consistent with both the Rescorla-Wagner model and Wagner's SOP model. It is thus difficult to draw conclusions as to which of the models best predicted responding to the CS when first presented after extinction. In addition, while it is not statistically valid to draw a direct comparison between the levels of freezing in this experiment compared to previous experiments, the apparently higher degree of freezing to the CS at reacquisition

relative to the experiments of the previous chapter is also consistent with the comparator model.

The amount of learning which occurred as a result of the reacquisition session showed no sign of influence from the temporal distribution of extinction trials. This effect was predicted by the comparator model since the combined exposure to the CS and context was controlled across groups. This theory does not explain, however, why the effect of retrieval prior to extinction should be different in this experiment as compared to Experiments 1.1, 1.3 and 1.4. Apparently, pre-extinction retrieval has differential effects on reacquisition depending on whether animals spend the period between retrieval and extinction in the home cages or in the experimental chambers. Each of the models discussed in this chapter would predict better extinction overall with time spent in the home cages rather than the experimental context. Wagner's SOP additionally predicts better extinction with retrieval than without (under certain conditions). It is not immediately clear, however, how these models would explain an interaction between these two factors.

In summary, the results of this experiment, while consistent at least in part with the comparator model, do not explain the effect of retrieval prior to extinction in the case where the intervening time is spent outside the experimental context.

Experiment 2.2

Since experimental conditions can vary from one experiment to another beyond the control of the experimenter, conclusions drawn by comparing factors present in different experiments can be misleading. The aim of the following experiment was to compare the effect of pre-extinction retrieval with and without removal from the context in a single experiment. This experiment employed a two-factor design to assess the effects of retrieval and context on extinction and reacquisition of conditioned fear to a discrete CS. Animals were conditioned in the first phase 24 h before extinction with or without prior CS retrieval.

The period between retrieval and extinction was spent either in the experimental context or in the home cage. This design aimed to directly assess the observation that remaining in the experimental context between retrieval and extinction attenuates the effect of retrieval on reacquisition.

Methods

Subjects

Subjects were 32 adult male Lister hooded rats (Charles River, UK), each allocated arbitrarily into one of four groups with n = 8.

Apparatus

All sessions took place in the conditioning chambers described in Chapter II. The stimulus used for all training and testing was the clicker with the same temporal and physical characteristics as described for Experiment 1.2.

Behavioural Procedures

Habituation. All animals were exposed to the context for one hour per day for two days prior to the start of training. During this period the houselight remained on. No stimuli were presented.

Acquisition. After a 30 min adaptation period in the conditioning chamber, rats were given 3 trials of a 60 s CS coterminating with a 0.5 s, 0.5 mA foot-shock (US) with a variable intertrial interval with an average of 300 s. Each rat was removed from the conditioning chamber 1 min following the last trial and returned to its home cage.

Retrieval and Extinction. One day following acquisition training, all animals were returned to the context for retrieval and extinction. Figure 22 represents the treatments received by each group in this phase. Animals in the EC condition were placed in the context for 116 min during which time they received 19 non-reinforced CS presentations. Of these animals, those in condition Ret (i.e., Group Ret-EC) were presented with the CS first after 2

min in the context after which a 60 min period passed before presentation of the remaining 18 CS trials with an ITI of 2 min. Those animals in condition NoRet (i.e., NoRet-EC) had a 60 min adaptation period before presentation of 19 CS trials with an ITI of 2 min. Animals in the HC condition were brought to the experimental chambers for the retrieval session and then returned to their home cages until the start of the extinction session. The retrieval session for animals in condition Ret (i.e., Group Ret-HC) were given one presentation of the CS after a 2 min adaptation period. Those in condition NoRet (i.e., Group NoRet-HC) were exposed to the context for an equivalent period of time. Animals in Group Ret-HC were placed back in the experimental chambers for extinction after 57 min such that the period between the retrieval CS and first CS of extinction would be the same as for Group Ret-EC. Animals in Group NoRet-HC waited 54 min in the home cages before returning for the extinction session such that the time from first placement in the context to the first CS presentation would be the same as for Group NoRet-EC.

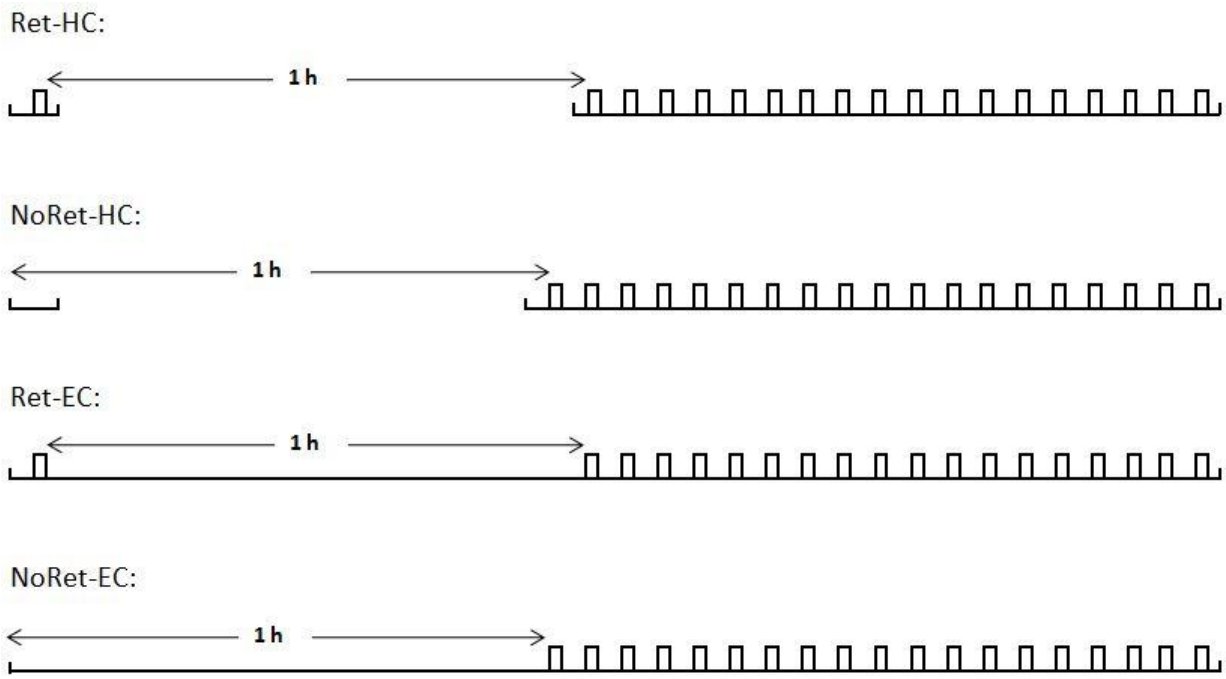

Figure 22. Arrangement of CS presentations during retrieval and extinction sessions. The open rectangles represent CS presentations. The horizontal lines indicate time spent in the experimental chambers (EC). Where there is no line, this indicates time during which animals are removed from the context and returned to the home cages. The time spent in the home cages for the HC groups is adjusted such that the timing of the stimuli matches those of the equivalent EC group.

Reacquisition and Test. Parameters used for the reacquisition and test phases are identical to those used previously in Experiments 1.3 and 2.1.

Statistical Analysis

This experiment employed a 2 × 2 factorial design with Retrieval and Context as between-groups factors. The two levels of the Retrieval factor were Ret (CS at retrieval) and NoRet (context-only retrieval). The two levels of the Context factor were EC (Experiment Chamber) and HC (Home cage). The pre-CS periods of each session, as well as the CS periods for retrieval, reacquisition and test, were analysed using the univariate ANOVA procedure. Where change across trials was of interest, i.e., during acquisition and extinction

training, a repeated-measures ANOVA was employed to analyse the main effects and interactions of the two between-groups factors and the within-subjects factor, i.e. Retrieval × Context × (Trial).

Results

Acquisition

Animals in all conditions showed minimal levels of freezing to the context during the two min prior to the first CS-US pairing with no significant main effects or interactions detected; Retrieval: $F(1, 28) = 2.42$, $p = .131$; Context: $F(1, 28) = 1.58$, $p = .219$; Retrieval × Context: $F(1, 28) = 1.97$, $p = .172$.

Levels of freezing to the CS across the three CS-US trials are presented in Figure 23. A significant linear increase in freezing across trials indicated that the acquisition procedure was successful in conditioning the fear response to the CS, $F(1, 28) = 268.2$, $p < .001$. As expected, no other main effects or interactions were significant at this stage, Fs < 1.

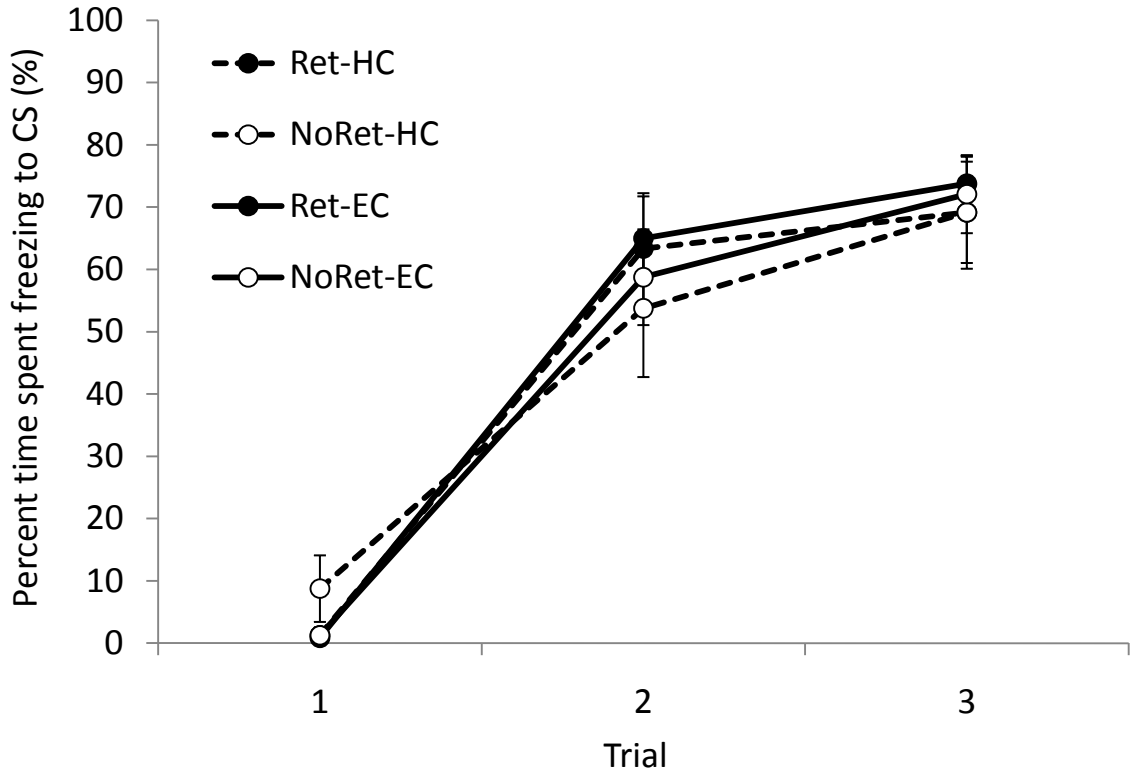

Figure 23: Freezing to the CS during acquisition training. Circles represent group means ± *SEM*.

Retrieval and Extinction

The first two min of context exposure were taken as an index of contextual fear. For animals in condition HC, this period was the first two minutes of the retrieval session, whether followed by a CS presentation or not. For animals in condition EC, this was the first two min of the extended extinction session, regardless of whether the first trial began immediately after this period or one hour after the start of the session. During this period, only one animal in Group Ret-EC displayed any freezing, and thus no group differences were found to be significant, $\chi^2(3) = 3.00$, $p = .392$.

For the purposes of analysis, the retrieval trials for animals in condition Ret were treated as the extinction Trial 1. A linear trend analysis of the extinction 19 trials verified the success of the extinction training, $F(1, 28) = 364.1$, $p < .001$. This trend did not interact with

either of the between-groups factors (Retrieval: $F(1, 28) = 2.14$, $p = .154$; Context: $F(1, 28) < 1$), nor with the interaction of these factors, $F(1, 28) < 1$. No between-groups main effects or interactions were detected; Retrieval: $F(1, 28) = 1.63$, $p = .213$; Context: $F(1, 28) < 1$; Retrieval × Context: $F(1, 28) = 1.25$, $p = .273$.

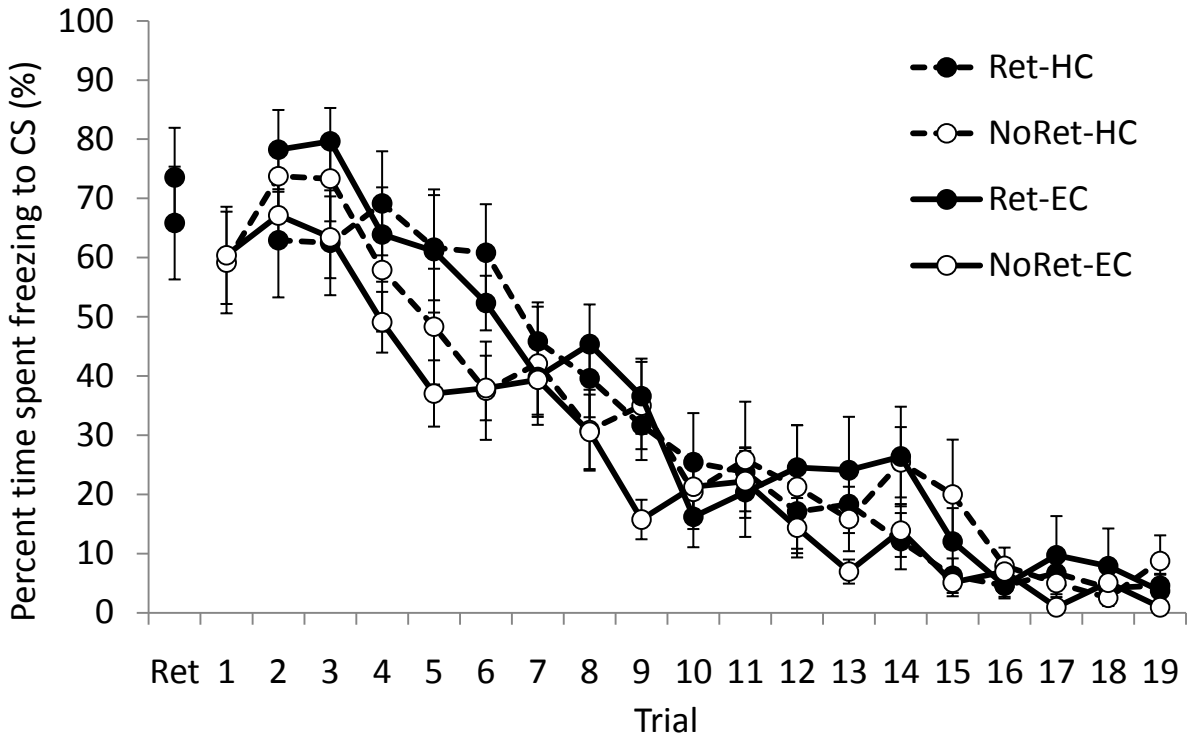

Figure 24. Freezing to the CS during retrieval (Ret) and extinction Trials 1-19. Circles represent group means ± *SEM*.

Reacquisition

Freezing during the 2 min pre-CS period is displayed at the left of Figure 25. Freezing was low in all groups (highest $M = 3.5$, $SD = 4.91$), and no main effects of Retrieval condition or Context condition were detected, $F(1, 28) = 2.65$, $p = .115$, $F(1, 28) < 1$. There was, however, a significant interaction between conditions such that of the animals returned to the home cage between retrieval and extinction, the NoRet animals showed more contextual fear, whereas for those animals which remained in the experimental chambers, this effect was, if anything, reversed, $F(1, 28) = 4.23$, $p = .049$.

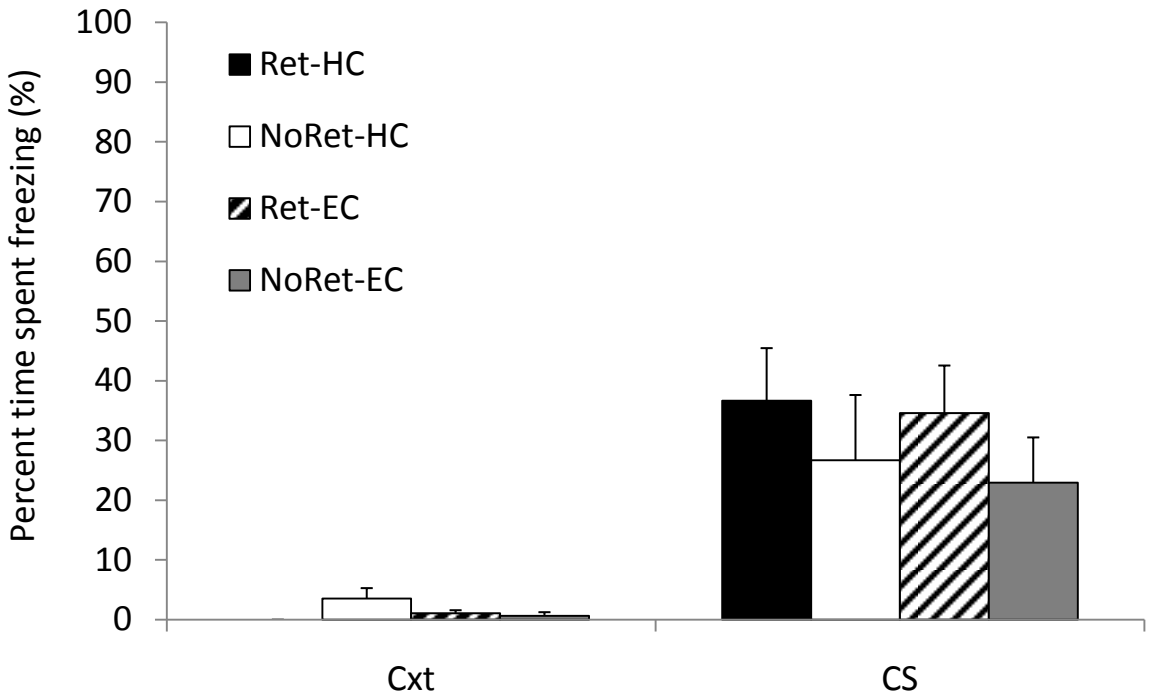

Figure 25. Freezing to the context (Cxt) and the CS during reacquisition. Bars represent means ± *SEM*.

Levels of freezing to the CS can be seen in the right panel of Figure 25. Freezing to the CS at this stage was no affected by either Retrieval condition, $F(1, 28) = 1.47$, $p = .235$, or Context condition, $F(1, 28) < 1$. The interaction between these conditions was also not significant, $F(1, 28) < 1$.

Test

Data from the test session are presented in Figure 27. The left panel of this figure shows freezing to the context during the 3 min period prior to the first CS presentation. No main effects of or interactions between conditions were detected, *F*s < 1. Once again, levels of freezing to the CS at reacquisition were higher than usual and somewhat variable and, therefore, the degree of learning resulting from the reacquisition session was again assessed through the analysis of difference scores calculated by subtracting freezing to the CS at reacquisition from CS freezing at test. These data are presented below in Figure 26. Absolute levels of freezing to the context and CS are presented in Figure 27.

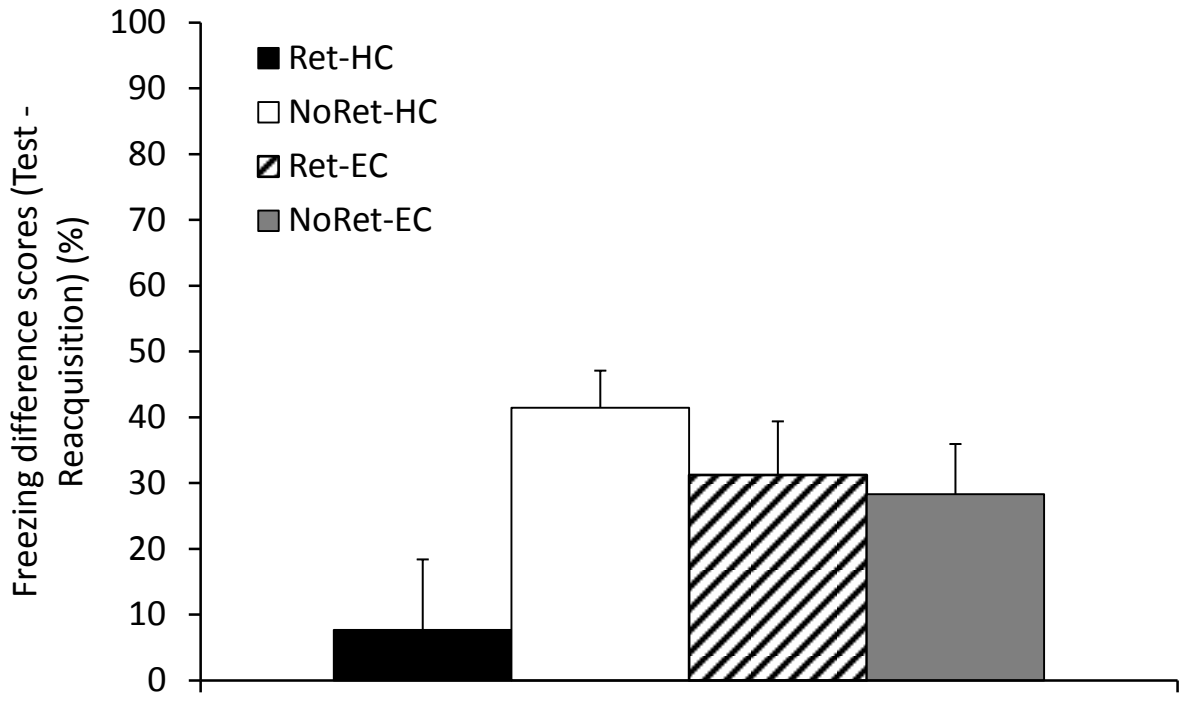

Figure 26. Difference scores showing the increase in freezing to the CS from reacquisition to test. Bars represent group means ± *SEM*.

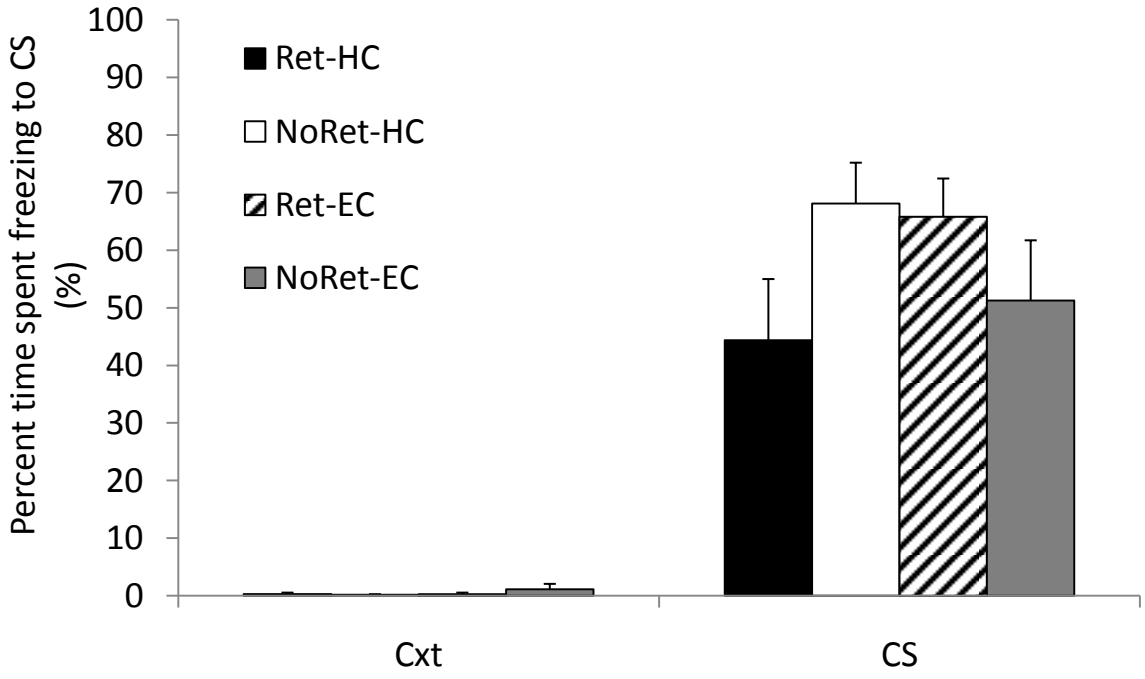

Figure 27. Absolute levels of freezing to the context (Cxt) and CS at test. Bars represent group means ± *SEM*.

While no significant main effects for the Retrieval factor, $F(1, 28) = 3.52$, $p = .071$, or for the Context factor, $F(1, 28) < 1$, were found, a significant interaction between these two factors was detected, $F(1, 28) = 4.98$, $p = .034$. Follow-up comparisons revealed a significant effect of retrieval when animals were removed from the chambers between retrieval and extinction, $F(1, 28) = 8.43$, $p = .042$, but no such effect for animals remaining the context for the intervening period, $F(1, 28) < 1$. This result indicates that the effect of the pre-extinction retrieval trial is dependent upon animals being returned to their home cages, such that remaining in the experimental context between retrieval and extinction appears to abolish the effect of retrieval on subsequent reacquisition.

Discussion

The results of Experiment 3.2 were consistent with previous observations that (a) retrieval of the CS prior to extinction impairs subsequent reacquisition of the CS-US association, and that (b) this effect is not observed if the animals remain in the experimental context during the period between retrieval and extinction. This experiment has demonstrated that removal from the context between retrieval and extinction is critical to the effect on reacquisition of pre-extinction retrieval.

There is evidence from other reports in the literature that removal from the context may be necessary in order to observe certain treatment effects (Revusky, 1971). This suggestion is made in the context of a theory of associative learning proposed by Revusky (1971) in which he argues that a key factor in determining whether two events (stimuli, actions or reinforcers) become associated is the number of intervening events which can interfere with the formation of the target association. Learning an association between a CS and a US, for example, is impaired when those stimulus presentations are separated by a long interval of time, not because learning requires stimulus contiguity, but rather because a long interval provides greater opportunity for interference. Interference may come in the form of

other events (e.g., external stimuli or behaviours elicited by the animal) which may become associated with the CS and/or US and which may, as a consequence, interfere with the formation of a direct CS-US association. In theory, if such intervening events could be eliminated, a CS-US association could potentially form over indefinitely long interstimulus delays.

In some circumstances, however, learning can occur over long delays. Revusky (1971) cites a number of examples of learning which occurs with long interstimulus intervals. The taste aversion paradigm (Garcia, Kimeldorf, & Koelling, 1955) is one classic example in which rats are able to learn an association between saccharin and radiation-induced illness even when the interval between consumption of the saccharin solution and the induction of illness was separated by up to 12 hours. A second example cited by Revusky (1971) is what he refers to as the Capaldi Effect (Capaldi, 1967) in which a rat's performance on a runway task can be determined by the presence or absence of reinforcement on the previous trial even when the interval between trials is 24 h. In these situations it is argued that the formation of an association is possible due to the presence of factors which reduce the ability of intervening events to become associated with the target events. One such factor is stimulus relevance (Revusky, 1971). There is strong evidence to support the claim that certain types of stimuli more readily enter association with each other than with other types of stimuli. This was demonstrated convincingly by Garcia and Koelling (1966) when they found that associations between stimuli could be acquired readily when the two stimuli were from the same sensory "system", but learning was minimal when stimuli from different "systems" were used. Specifically, it was shown that rats could learn an association between a flavour and illness (internal system) or between a light-sound compound and electric foot-shock (external system), but rats did not learn to associate flavour and foot-shock (internal-external) or between the light-sound compound and illness (external-internal). Thus, the relevance of a

stimulus influences its capacity to form an association with another stimulus. According to Revusky (1971), this may explain why conditioned taste aversion learning can occur across long interstimulus delays: while many events may occur between the consumption of the flavoured water and the onset of illness, few of these would be relevant to either the CS or the US and so the chances of forming interfering associations would be minimal.

Importantly for the current discussion, Revusky (1971) also suggests that the situation in which events occur can influence their ability to enter into an association with other stimuli. Stimuli are more likely to enter into an association with one another if they both occur in the same or a similar situation. This may, therefore, explain how the animals in Capaldi's (1967) experiments were able to associate events occurring on different trials of the runway task despite the 15 min intervening period. The time between trials was spent outside the runway apparatus and so events which occurred during this time may have been less relevant to events which occurred in the experimental situation. If association formation depends, at least in part, on situational relevance, then these intervening events would be less likely to form associations with the events occurring on the trials, and so less likely to interfere with learning of the target association. Revusky (1971) therefore makes the suggestion that this effect, along with other similar cases (Capaldi & Spivey, 1964; Petrinovich & Bolles, 1957), is dependent upon removal from the experimental context during the ITI. In the case of the present study, it may be the case that removal from the context allows the experience of the CS during the retrieval session to have its influence on the subsequent extinction only when interference from stimuli or behaviours occurring during the one-hour intervening period is minimised by removing the animal from the experimental chambers. This analysis further raises the question as to whether the Monfils et al. (2009) effects would still be observed if retrieval and extinction manipulations were carried out in different contexts.

An alternative perspective on these data is to focus not on the termination of the session, but the total exposure to the context across retrieval and extinction. While the role of the context in Pavlovian learning is generally considered to be important, there is less agreement on what that role is. Learning theories typically treat context in the same manner as a discrete cue, albeit a less salient one (e.g., Mackintosh, 1975; Miller & Matzel, 1988; Pearce & Hall, 1980; Rescorla & Wagner, 1972; Wagner, 1981). In this way, the context competes for associative strength along with all other stimuli present on a given trial. Other perspectives, however, recognise the capacity of the context to aid memory retrieval such as the encoding specificity hypothesis of Tulving & Thomson (1973; see also Spear, 1973). This position is similar to that taken by Bouton (1993) who emphasises the role of the context as a retrieval cue when ambiguity arises in the reinforcement history of the CS. Urcelay & Miller (2010) present data consistent with the idea that the context can fulfil both these roles, with trial spacing at least one of the factors that determine its role. By examining the effects on trial spacing of behavioural phenomena known to be differentially influenced by context in its roles as cue and occasion setter, the authors provide evidence that massed trials encourage the context to act as a cue, whereas the spacing of trials (or the longer time spent in the context) allows the context to behave as an occasion setter. The present data suggest that the pre-extinction retrieval induced impairment in reacquisition may be dependent on the context acting as a cue. Prolonged exposure to the context might encourage the context to take on the role of occasion setter and it may be for this reason that the effect on reacquisition was not observed in these groups. However, the differences in context exposure applied by Urcelay and Miller (2010) were typically much greater than the one-hour difference applied in these experiments. For example, context extinction comprised 10 hours of context exposure compared to 25 min for control groups.

To assume that the effect requires the context to act as a Pavlovian cue does not solve the question as to why the effect occurs in the first place, but rather to suggest one way in which an interaction between retrieval and context might arise. Treating the context as a cue would in fact predict for most theories, if anything, better extinction in the case of the NoRet rather than Ret. Presenting an excitatory CS in the context without reinforcement will trigger extinction of the CS and the context, and the degree of extinction to the context will be greater than if the CS were not present. Therefore, the context will have less associative strength with the US at the start of the extinction session, which will result in a weaker comparator (Miller & Matzel, 1988) or a smaller decrement in associative strength of the CS (Rescorla & Wagner, 1972).

Experiment 2.3

The term *latent inhibition* (LI) refers to the observation that acquisition of a Pavlovian response is slower following repeated non-reinforced presentations of the CS. The term is somewhat misleading as there is little evidence to suggest that the CS acquires inhibitory properties as a result of this treatment (Reiss & Wagner, 1972). Rather, the effect has often been explained as a loss of attention to the CS resulting from training in which a lack of prediction error (Pearce & Hall, 1980) or the fact that the CS was no better than the context at predicting no US (Mackintosh, 1975a) led to the CS being ignored during subsequent pairings with the US.

An alternative view of LI is that of Wagner's SOP model, which claims the effect is mediated by context-US associations such that elements of the CS node are primed into A2 by exposure to the context at acquisition, interfering with the ability of the CS node to be co-active with the US in A1 (Wagner, 1981). This model is consistent with observations of context specificity in LI (Lovibond, Preston, & Mackintosh, 1984), as well as the facilitatory

effect of the increased spacing of CS presentations in producing a retardation of acquisition (Lantz, 1973).

The following experiment employs the LI paradigm to assess whether Wagner's SOP model can account for the effect of pre-extinction retrieval on reacquisition. Assuming that the decay rate of a given CS is constant regardless of its reinforcement history, the temporal arrangement of trials should have a similar effect on the success of the procedure whether the training takes place before or after conditioning. In other words, if the presentation of the CS one hour prior to extinction can account for the facilitative effect on extinction learning, then a similar effect should be observable on LI such that one trial presented one hour before pre-exposure training should lead to a stronger retardation of acquisition across subsequent pairings of the CS with the US.

The design of the experiment is outlined in Table 5. Three groups of rats were conditioned with pairings of the clicker CS with shock. For Group Naive, this was the first exposure to the stimulus and it was expected that they would acquire fear to the CS readily. For Groups Ret-LI and NoRet-LI, the CS had already been presented in the context 12 times prior to acquisition. (The total number of trials was reduced for this experiment since a pilot experiment showed robust LI with 19 trials from which it may have been difficult to observe any additional retardation.) The manipulation of interest was for Group Ret-LI the presentation of the first CS pre-exposure trial within a separate session one hour prior to pre-exposure of the remaining 11 trials. If self-generated priming of the CS into A2 on trial *n* can block learning two minutes later on trial *n + 1*, then the context should form a stronger association with the CS for Group Ret-LI when the CS elements have a greater opportunity to decay from A2 between trials 1 and 2.

Table 5: Experimental Design for Experiment 2.3

Group	Ret	Pre-Exposure	Acquisition	Test
Ret-LI	C-	11 x C-	3 x C+	3 x C-
NoRet-LI	-	12 x C-	3 x C+	3 x C-
Naive	-	-	3 x C+	3 x C-

N.B. Ret = retrieval; NoRet = no retrieval; LI = latent inhibition; C = clicker CS; "+" indicates a reinforced CS presentation; "-" indicates a non-reinforced trial.

Methods

Subjects

The subjects used in this experiment were 24 adult male Lister hooded rats (Charles River, UK).

Apparatus

The experimental chambers used in this experiment are those described for the previous experiments in this thesis. The experimental room and the chambers were illuminated by red light during all experimental procedures. The CS used in all phases was a 60 s presentation of the clicker stimulus.

Behavioural Procedures

Habituation. On the first two days of experimentation, animals were first brought to the conditioning chambers for a one-hour habituation session during which no events were scheduled.

CS Presentation. On the third day, animals in Groups Ret and NoRet were first brought to the experimental chambers for a three-minute session. For Group Ret, the clicker

CS was presented for the last minute of the session, while no stimuli were presented for Group NoRet. Group Naive remained in their home cages during this time.

CS Pre-Exposure. One hour after the retrieval session, Groups Ret and NoRet returned to the chambers for a CS pre-exposure session. This session comprised 11 (Ret) or 12 (NoRet) CS trials presented with an ITI of 2 min.

Acquisition. After 24 hours, all three groups were brought to the experimental room and placed in the conditioning chambers. An adaptation period of 30 min was followed by three pairings of the CS with a 0.5 s, 0.5 mA foot-shock US with a variable ITI of average 5 min.

Test. A retention test was given after a further 24 hours, which consisted of two presentations of the CS with an ITI of three minutes. Freezing to the context and during the two CS periods was measured as an index of fear.

Statistical Analyses

CS Presentation and CS Pre-Exposure. CS presentations at retrieval and pre-exposure were combined into a single analysis for Groups Ret and NoRet with the 12 CS trials forming a repeated-measures variable in a 2 × (12) repeated-measures ANOVA.

Acquisition. Freezing to the CS across the three trials of acquisition was subjected to both a linear and a quadratic trend analysis. The linear trend was designed to determine the success of the conditioning in establishing fear responding to the CS. Since linear trends tend to emphasise the beginning and end points of a trend, and the effects of pre-exposure might possibly be more evident at intermediate stages of learning, the quadratic trend was employed to test for differences in the shape of the acquisition curves. The between-groups effects were analysed by way of two planned orthogonal contrasts, the first assessing the effect of the pre-exposure treatment (Groups Ret and NoRet v Group Naive), and the second assessing the

effect of the retrieval trial prior to pre-exposure (Group Ret v Group NoRet). The interactions of these contrasts with the linear and quadratic trends were also assessed.

Results

CS Presentation and CS Pre-Exposure

No freezing was recorded in any of the groups during the first two minutes of the retrieval session. CS-induced freezing at retrieval was included in the analysis of the data from the pre-exposure session.

Data from the single CS presentation and during CS pre-exposure is presented below in Figure 28. No significant differences in pre-CS freezing were observed during the first two minutes of the pre-exposure session, $F(1, 14) = 1.00$, $p = .334$. Freezing to the CS across the 12 presentations (including CS presentation for Group Ret-LI) revealed no significant main effects of group, $F(1, 14) < 1$, or trial, $F(3.7, 51.5) = 2.01$, $p = .112$, nor any significant interaction between these factors, $F(3.7, 51.5) < 1$ (Greenhouse-Geisser corrected *df*s).

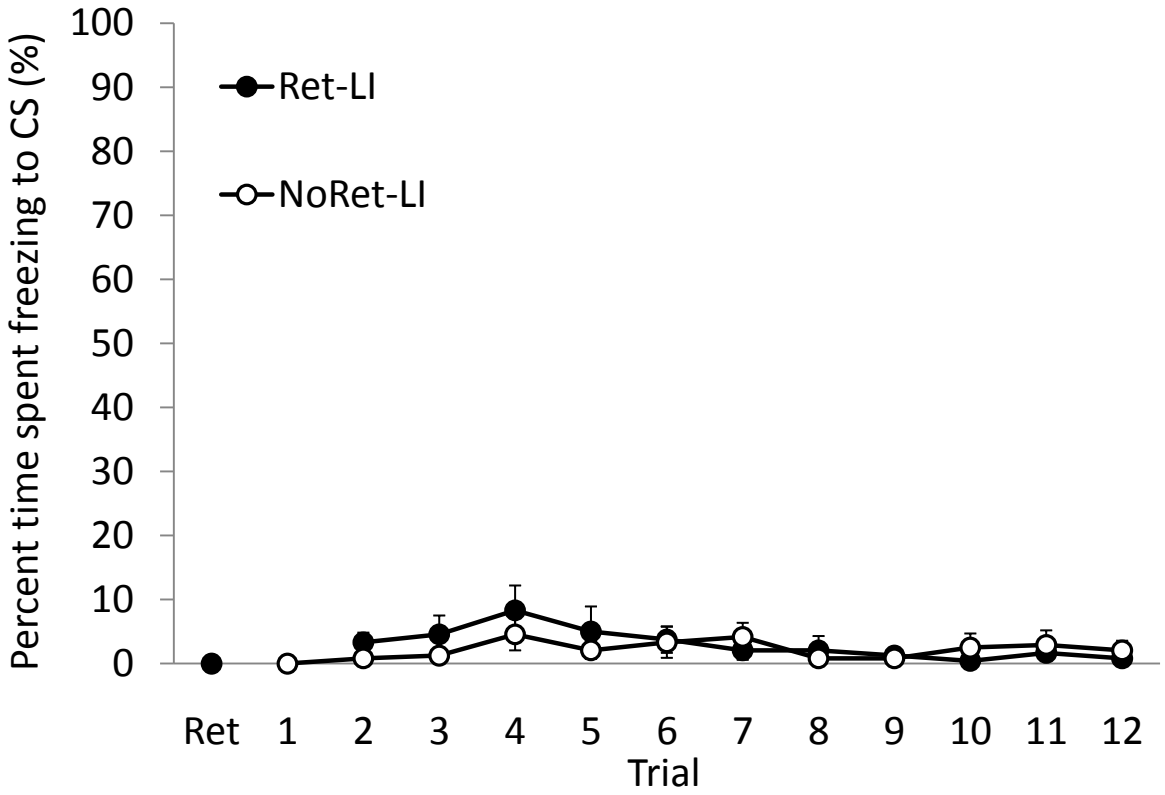

Figure 28. Freezing to the CS during CS presentation (Ret) and CS pre-exposure trials 1-12. Circles represent means ± *SEM*.

Acquisition

Freezing during the two minutes prior to onset of the first trial was taken as a measure of contextual fear. No group differences were detected during this period, $F(2, 21) < 1$, Ms (*SEMs*) of percent time freezing (%): Ret-LI = 1.3 (0.6), NoRet-LI = 0.6 (0.6).

Freezing to the CS across each of the three acquisition trials is presented in Figure 29. The within-subjects contrast analysis revealed a significant linear increase in responding across trials, $F(1, 21) = 166.0$, $p < .001$, indicative of successful acquisition of the fear response.

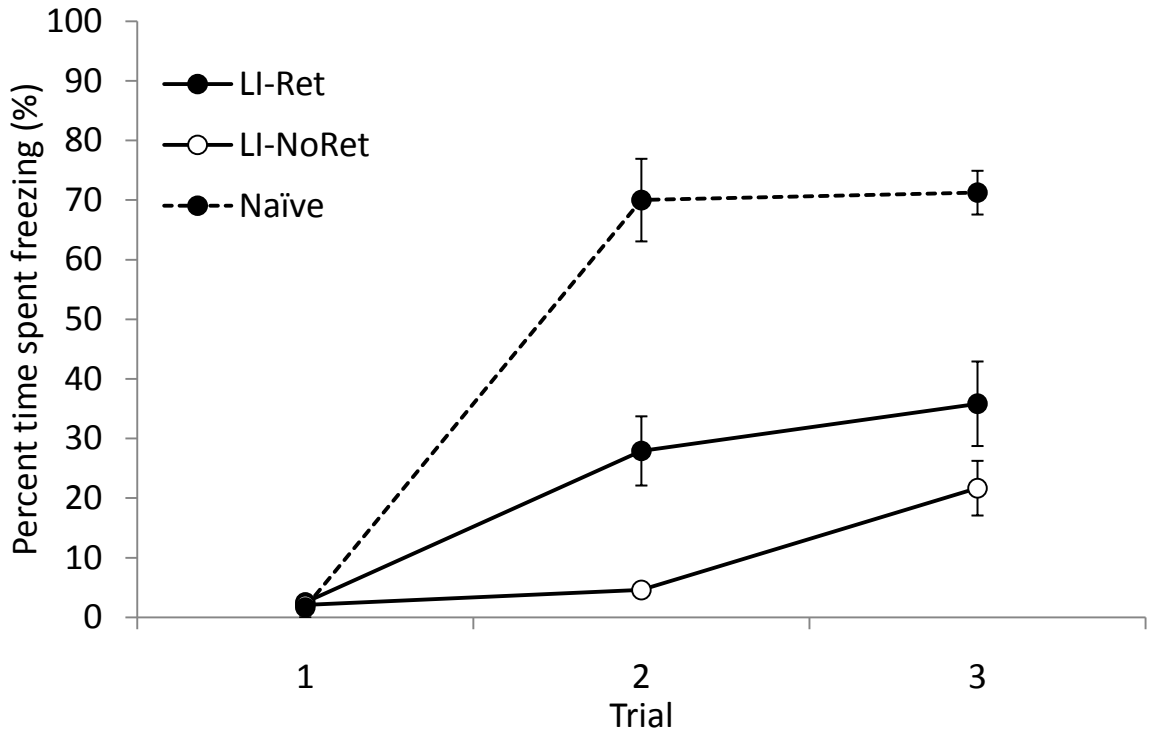

Figure 29. Acquisition of fear to the CS across three conditioning trials. Circles represent means ± *SEM*.

Unexpectedly, the group given retrieval prior to LI training displayed a weaker retardation effect than the group given all pre-exposure trials within the same session. Averaging across trials, animals in Groups Ret-LI and NoRet-LI exhibited significantly less fear in response to the CS when compared to the group having been naive to the CS at the time of conditioning, $F(1, 21) = 95.16$, $p < .001$. Furthermore, these groups showed a significantly weaker linear trend, $F(1, 21) = 41.16$, $p < .001$, a sign of latent inhibition (LI). Responding across trials for Group Naive followed a significantly stronger quadratic trend, $F(1, 21) = 23.09$, $p < .001$, than the LI groups, demonstrating that these animals reached a level of responding which appears to have approached asymptote after the second trial.

The retrieval trial given prior to pre-exposure also was seen to have an effect on overall levels of fear during acquisition, with animals given retrieval showing more fear than those given the 19 CS presentations within a single session, $F(1, 21) = 11.19$, $p = .003$.

However, no differences could be seen between these groups in terms of the magnitude of either the linear, $F(1, 21) = 3.13$, $p = .091$, or the quadratic trend, $F(1, 21) = 4.15$, $p = .054$ suggesting that the two groups acquired fear at similar rates.

Test

Freezing to the context during the first three minutes of the test session (see Figure 30, left panel) was consistently low across all groups, $F(2, 21) < 1$. No significant effect of trial was observed for across the three presentations of the CS at test, $F(1, 21) < 1$, and thus the mean freezing across trials is presented for reasons of clarity (Figure 30, right panel).

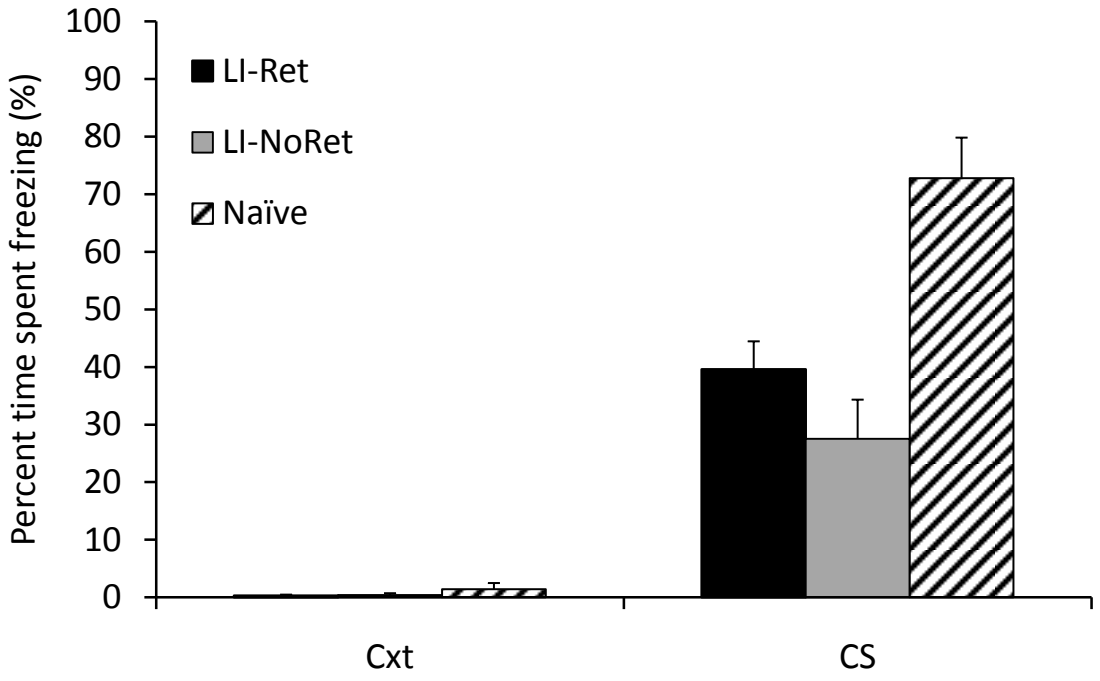

Figure 30. Freezing during the pre-CS and CS periods at test. Bars represent means ± *SEM*.

Consistent with the data from acquisition, groups having received 19 presentations of the CS prior to acquisition displayed less fear to the CS at test than the group having been naive to the CS at the time of conditioning, $F(1, 21) = 25.73$, $p < .001$. The presentation of the CS once prior to the pre-exposure session appears to have had no lasting effect on the

ability of the CS to acquire fear, as no significant difference was detected between Groups Ret-LI and NoRet-LI, $F(1, 21) = 1.83$, $p = .190$.

Discussion

Pre-exposure of the CS prior to acquisition produced a substantial LI effect such that the rate of acquisition was lower compared to a group of naive animals. This effect carried over to a long term retention test demonstrating that the effect was on learning, not just performance. The presentation of the CS one hour prior to CS pre-exposure did not facilitate the progression of LI and, if anything, may have reduced the intensity of the effect. This finding is contrary to the prediction that the temporal arrangement of CS pre-exposure trials would increase the impairment in conditioning in an analogous manner to the facilitation of extinction seen when the CS trials are similarly scheduled. While it is not clear why an additional CS presentation given one hour prior to pre-exposure training should impair the development of latent inhibition, this result clearly fails to support the hypothesis that such training should facilitate latent inhibition.

As mentioned before, the predictions of Wagner's SOP model are highly dependent upon the values assigned to certain parameters in the model, particularly the decay rate parameters. In order for this model to account for the effect of retrieval on extinction, the decay rate must be such that at the end of the 120 s intertrial interval the majority of CS elements will have decayed from A1 and a significant proportion will remain primed in the A2 state. Thus, when the CS is presented on the next trial, the proportion of elements of the CS available to enter into A1 will be reduced and so the ability of the CS to retrieve the US into A2 is also reduced. Furthermore, the lack of opportunity for the CS and US to be concurrently present in A1 and A2 respectively prevents the formation of an inhibitory CS-US association. By this account, the presentation of one trial with an intertrial interval of one hour would allow more of the CS elements to decay from A2 and so partially overcome the

impairment in extinction of the relatively massed trials. In as much as successful decay of the CS from A2 to I is required for an association to form between the CS and the context, it would be expected that a spacing of CS trials during pre-exposure training equivalent to that employed in extinction training would also lessen the LI effect by interfering with the ability of the CS and context to be concurrently active in A1. Therefore, presentation of one trial with a one-hour interval before the next trial would aid in the development of context-CS associations and so should produce a stronger LI effect i.e., more retardation of acquisition. The data from the current experiment fail to support this hypothesis and instead suggest that the temporal arrangement of CS trials employed in the experiments of this chapter cannot alone account for the different rates of learning seen when the CS is later paired with the US.

Chapter Discussion

The three experiments presented in this chapter investigated the role of trial spacing in the pre-extinction retrieval effect. Experiment 2.1 failed to show an effect on reacquisition of various arrangements of 19 non-reinforced CS presentations across a fixed period of time in the experimental chambers. Among these arrangements were two that corresponded to the timings of the CS presentations in the Ret and NoRet groups of the previous experiments. The only difference between the groups in this experiment and previous experiments was that the rats remained in the context between retrieval and extinction. Since no effect of retrieval was seen in this experiment, the next experiment investigated whether removal from the context was necessary in order to observe an effect of retrieval. An interaction was observed between the pre-extinction retrieval effect and whether the animals remained in the context or were returned to the home cages. This result was consistent with all previous data and suggests a role for context exposure in mediating the effect of retrieval on extinction, or that the termination of the retrieval trial is required to trigger a process of destabilisation before extinction trials can be used in updating the original memory. The final experiment assessed

the ability of context-CS associations to account for differences in the strength of extinction learning. This experiment tested the notion that the effect on reacquisition of pre-extinction retrieval may reflect deficits in extinction due to self-generated priming of the CS which are partially overcome by the long interval between the first and second non-reinforced presentations of the CS. This explanation draws on Wagner's (1981) SOP model and relies on a decay rate of the CS node from A2 being such that a large proportion of elements would remain in this state of activation after 120 s. Should this be the case, then the development of latent inhibition should also be impaired by a 120 s ITI, an impairment which should be partially overcome by presenting the first two CS trials with an ITI of one hour.

This conclusion, however, is based on the assumption that the rate of decay of elements of a specific stimulus (in this case, a clicker with fixed duration, frequency, intensity, etc.) is constant regardless of its reinforcement history. This is perhaps an unrealistic assumption. In fact, in Wagner's model, decay of a representation from either A1 or A2 is said to be driven by limitations in capacity of these states. More rapid decay is required when the capacity of a state is limited. It is possible, therefore, that other stimulus representations active during the session may influence the rate of decay of the clicker node. During extinction, as opposed to CS pre-exposure, presentations of the CS will also excite elements of the US node into A2. The capacity of A2 will be reduced by the presence of the US elements and so the rate of decay would not necessarily be the same as during CS pre-exposure. However, this reduction in A2 capacity would be expected to increase the rate of decay of the CS elements, which should help to overcome any effects of self-generated priming on extinction. Thus, if anything, less benefit of the early CS presentation would be expected in extinction than in LI.

Any explanation in terms of trial spacing will also meet difficulty when faced with the data of Monfils et al. (2009) who, in their experiment on spontaneous recovery following

extinction, show strong effects of retrieval when given one hour or even just 10 min prior to extinction, yet no effects if the retrieval trial was given six hours or more prior to extinction.

That the effect of pre-extinction retrieval on reacquisition was not observed when the animals remained in the experimental context between retrieval and extinction suggests that removal from the context is important to this phenomenon. Groups which were removed from the experimental contexts differed from the other groups not only in their physical location, but also in having been removed from the experimental chambers, transported back to the home cages and having had the opportunity to interact with cage mates in the intervening period. In the language of reconsolidation theory, the presentation of the CS during the retrieval session is likely to have resulted in destabilisation of the CS-US memory. If, during this labile period, the animal is taken from the context and returned to the home cage, this may in itself be disruptive to the reconsolidation process such that the CS-US memory does not successfully restabilise. This would imply then that the extinction training given one hour later is redundant since the CS-US memory is no longer functional. The 18 trials then given during the extinction session would function as latent inhibition trials and so account for the retardation in reacquisition observed when the CS is again paired with the US. However, the data from Experiment 1.3 would suggest that this is not the case. Recall that in this study, one group was given a single CS presentation during the retrieval session, and then returned to the home cages for one hour prior to returning the experimental context for one hour without any further presentations of the CS. Had the retrieval session and subsequent return to the home cages been sufficient to disrupt the original CS-US memory, then this group should have shown minimal freezing to the CS at the next exposure. The animals in this group, however, still displayed robust freezing to the CS when it was presented 24 h later at reacquisition, a finding which argues against the idea that events occurring between retrieval and extinction interfere with reconsolidation of the CS-US memory.

Alternatively, the critical factor differentiating groups remaining in the context and those returned to the home cages is simply that in the latter condition the retrieval session was explicitly terminated. It is possible that termination of the retrieval session and removal from the context is necessary in order to signal the end of the reminder phase and to start the process of memory destabilisation. Animals left in the context had no such signal and so the retrieved memory may not have been destabilised and, therefore, could not be updated or replaced by the new information provided during extinction training. This hypothesis is not dissimilar to that proposed by Pedreira et al. (2004) who show that a long-term memory is only destabilised after the conclusion of the trial at which time it can be confirmed that a mismatch between the expected and actual outcome of the CS presentation (normally reinforced at the very end of the trial) has in fact occurred. In this case, the CS was a context in which a visual danger signal (the US) had previously been presented such that subsequent exposure to this context elicited a conditioned freezing response. The labilisation-reconsolidation process was triggered by removal from the context before presentation of the US. It is at this point in time that the violation of the expectation of a US presentation in the presence of the CS can be confirmed and so it is at this point that the system can identify a need to update the existing memory. In the present study, the CS itself was terminated for Ret animals in both the HC and EC conditions. Thus it would be expected that a violation of expectation would occur in both cases. However, it is possible that in the case of a discrete cue paired with a US, the context in which these pairings occur is an important component of the learning event such that consolidation or reconsolidation processes are only activated after removal from the context. As long as the animal remains in the experimental context, there is potential for new information to be obtained relevant to the original CS-US association and its relationship to the context.

The role of the context, or the removal from the context, in the current experiments remains unclear. Additional studies would be required to identify the minimal requirements for the pre-extinction retrieval effect such as by removing all animals from the experimental context, placing some back into the experimental context immediately while placing others in an alternative context where they are not permitted to interact with other animals. Those animals replaced into the experimental context would then be briefly removed and replaced just prior to the start of the extinction phase at the time when the other groups would return from the alternative context. In this way it may be possible to delineate the effects of the conditioning context and its reinforcement history from the potentially disruptive effects of a return to the home cage. If the effect is due simply to disruption resulting from a return to the home cage, then the effect of retrieval should be abolished and no difference would be observed between groups given retrieval prior to extinction and those given extinction without retrieval. In contrast, if the effect of retrieval is dependent on an explicit termination of the retrieval session, then a facilitation of extinction should be seen for animals given retrieval regardless of which context they spend the intervening period.

V. MECHANISMS OF REACQUISITION IMPAIRMENT

In the previous chapter, three experiments were presented which assessed an explanation of the reacquisition impairment resulting from pre-extinction retrieval in terms of an effect of trial spacing. The results of this chapter did not support the hypothesis that the increased spacing between the first and second extinction trials could account for the enhancement of extinction observed in Chapter III and in the study by Monfils et al. (2009), at least through the mechanisms proposed by the Rescorla-Wagner model (Rescorla & Wagner, 1972), the comparator hypothesis (Miller & Matzel, 1988) or Wagner's SOP model (Wagner, 1981). The purpose of this next chapter is to explore the nature of the reacquisition impairment in order to elucidate the mechanisms responsible for the effect of pre-extinction retrieval on reacquisition of conditioned fear. In approaching this question, these experiments assessed the effect of pre-extinction retrieval on the state of the CS memory after extinction training.

Monfils et al. (2009) showed that memory retrieval prior to extinction produced extinction learning that was resistant to manipulations that ordinarily result in a recovery of conditioned fear, namely a change of context (renewal; Bouton & King, 1983), the unsignalled presentation of the US (reinstatement; Rescorla & Heth, 1975) and the passage of time (spontaneous recovery; (Pavlov, 1927). The occurrence of each of these phenomena has been taken as evidence that extinction does not result in complete unlearning (i.e., erasure) of the CS-US association. That responding can be restored without further pairings of the CS and US demonstrates instead that at least some of the association between these stimuli survives extinction but that the expression of that association is prevented by the learning that occurs during extinction. Thus, the absence of these effects has, in many cases, been taken as a sign that the excitatory association has in fact been unlearned (Barad, Gean, & Lutz, 2006), or in the case of studies of amnesia, that the original memory has been erased (Duvarci &

Nader, 2004). The fact that Monfils et al. (2009) show a lack of renewal, reinstatement and spontaneous recovery in their rats extinguished within a putative reconsolidation window is suggestive of erasure of the original memory. If the CS no longer retains an association with the US, then conditioned responding should not be restored by any of these treatments.

The finding that memory retrieval one hour prior to extinction produces a retardation in reacquisition of the conditioned response, however, points to more than simply erasure. The reluctance with which the CS again comes to elicit a fear response suggests that the stimulus has not only lost its excitatory link with the US, but that the memory has perhaps been updated to reflect the new association present during extinction: that the CS predicts nothing, or perhaps the absence of the US (i.e., a CS-noUS association). It is this question that forms the focus of this chapter. In determining what is learned or unlearned when extinction occurs one hour after memory retrieval, it is hoped that the mechanisms by which these effects arise can be better understood.

The first experiment of this chapter investigated the possibility that the post-retrieval extinction training results in the formation of an inhibitory association between the CS and US. This experiment followed the recommendations of Rescorla (1969) and Hearst (1972) that evidence of conditioned inhibition is best obtained by the use of two complimentary procedures: the retardation test and the summation test. The effect of retardation has already been demonstrated in previous experiments. Therefore, the first experiment of this chapter applies the summation test to a CS extinguished under the same conditions as those shown to produce the retardation effect. If the retardation of reacquisition previously observed is due to conditioned inhibition, then a CS trained in this way should also be able to suppress responding to a second, excitatory stimulus when the two are presented in compound.

Following on from this study, the ability of the CS to enter into a new association was examined. If retrieval prior to extinction encourages learning that the CS predicts nothing of

importance, then it is likely that animal will cease to attend to the stimulus and so any subsequent learning will be impaired (e.g., Mackintosh, 1975; Pearce & Hall, 1980). After acquisition and extinction of a conditioned fear response, the CS was paired with the delivery of a sucrose pellet to a magazine and the frequency of magazine entries during the CS presentation is recorded. Assuming that attention is a necessary prerequisite for learning (Mackintosh, 1975a; Pearce & Hall, 1980), inattention to the CS would be expected to result in retardation in the emergence of the new conditioned approach response. This prediction, of course, additionally assumes that reductions in the value of α are not specific to the reinforcer used during training. This point is raised by Mackintosh (1975a) as well as Revusky (1971), although assumption of reinforcer specificity would lead attentional models to difficulty in explaining effects, such as latent inhibition, which are independent of a specific reinforcer (Mackintosh, 1975a).

The final experiment examined components of the retardation in the CS-US association that are independent of the CS. Following acquisition and extinction with one stimulus, the US was then paired with a distinct and novel stimulus. The rate of acquisition of the conditioned response to the new CS was examined so as to assess the role of the US representation in the formation of a new association following post-retrieval extinction.

Experiment 3.1

The retardation of reacquisition reported by Monfils et al. (2009), replicated here in Experiment 1.1, and consistent with the observations of impairment in reacquisition seen in Experiments 1.3, 1.4 and 2.2 may be indicative of an inhibitory association having formed between the CS and US over the course of extinction following retrieval. For the purposes of the following discussion, the term “inhibition” will be used to refer to a property of stimuli which predict the absence or omission of a particular reinforcer. Such a stimulus is said to have an “inhibitory” association with that specific reinforcer. According to the Rescorla-

Wagner model, the formation of an inhibitory association between a CS and US is equivalent to the CS acquiring negative associative strength. While the Rescorla-Wagner model would not ordinarily predict such effects in extinction, a CS can acquire negative associative strength under certain conditions, such as when the CS predicts the omission of expected reinforcement for a different, excitatory stimulus. If a CS with such inhibitory properties is then paired with the US, the learning curve begins below zero and so the emergence of the conditioned response is delayed (see Figure 31, panel A). Thus, retardation of acquisition (or in this case, reacquisition) of responding to a CS may be a sign of an inhibitory association between the CS and the US.

However, this is not the only condition which can give rise to retardation in the development of a conditioned response. The rate of acquisition of conditioned responding depends upon a number of factors which, in the Rescorla-Wagner model (Rescorla & Wagner, 1972) as well as in attentional models such as Mackintosh (1975a) and Pearce-Hall (1980) models, include the salience or associability of the CS. This variable is usually designated as α and in the models just mentioned is given a multiplicative role in determining the changes in associative strength from trial to trial. The rate of acquisition, therefore, will be proportional to the value of α such that a stimulus with low associability will acquire conditioned responding at a slower rate than a stimulus with high associability (see Figure 31, panel B). In contrast to the Rescorla-Wagner model in which the value of α is fixed, Mackintosh (1975) and Pearce and Hall (1980) allow this parameter to vary across trials. One factor thought to be related to the associability of a stimulus is attention (Mackintosh, 1975; Pearce & Hall, 1980). Hence, according to these attentional models, any manipulation which reduces attention to the CS will impair subsequent learning about that stimulus.

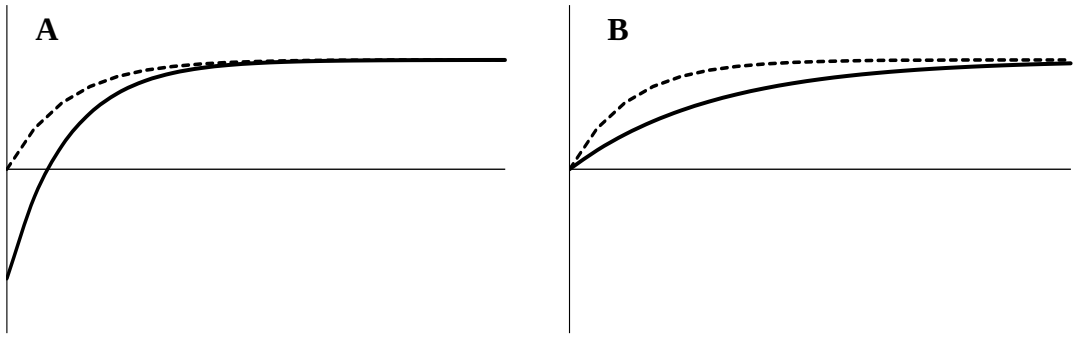

Figure 31. Hypothesised changes in associative strength during acquisition for an inhibitory CS (A) and for a CS with low associability (B). The dotted line represents learning to a novel, neutral CS.

The observation of retardation, then, is not unambiguously a sign of inhibition; the effect could equally come about as the result of inattention to the CS. In order to discriminate between these two alternatives, Rescorla (1969) and Hearst (1972) suggested the use of the summation test in conjunction with the retardation test to identify true inhibitory stimuli. If the slower rate of acquisition on the retardation test is due to inhibition, then presenting that same stimulus in compound with a known excitatory stimulus should result in reduced responding to the compound than to the excitor alone. If, however, the retardation is due to inattention to the CS, then the stimulus would not be expected to suppress responding to an excitatory CS. Conversely, the suppression of responding on the summation test is not in itself evidence for inhibition as it may simply reflect attention to the target stimulus at the expense of the concurrently presented excitor. Should this be the case, learning about the target stimulus should actually be facilitated rather than retarded. In short, attentional explanations can account for both retardation and summation but under different sets of assumptions. Should a particular treatment result in a CS which passes both the retardation test and the summation test, these effects are more likely to be the result of inhibition. It should be noted, however, that some authors have pointed out limitations of this "two-test"

strategy in terms of the availability of alternative explanations (Papini & Bitterman, 1993). Therefore, in the event of a stimulus passing both the retardation and summation tests, it is recommended that these alternative accounts be examined.

The impairment in reacquisition observed in previous experiments, therefore, may be the result of the CS acquiring inhibitory properties, but may equally be due to inattention to the CS. To disambiguate these alternatives, the experiment that follows employed the same parameters previously shown to produce impairment in reacquisition in order to assess whether this treatment results in a CS capable of passing the summation test. In other words, this experiment investigated whether a CS extinguished during the labile state after retrieval will inhibit responding to a known excitatory stimulus (i.e. a transfer excitor) when the two stimuli are tested in compound. The experimental design employed to answer this question is presented in Table 6. In the first phase of the experiment, each group was trained with two distinct stimuli, A and X. On the following day stimulus X was extinguished, with or without prior retrieval of the same stimulus. After a further 24 h, both groups were tested for responding to the non-extinguished stimulus, A, as well as the compound AX. Inhibitory properties of X would be reflected in lower levels of responding to AX than to A. Whether such an effect would be expected for the no-retrieval group is a matter of debate with some studies reporting inhibitory summation after extinction (Calton, Mitchell, & Schachtman, 1996) but others suggesting that this result is more likely due to generalisation decrements than inhibition (Aguado, de Brugada, & Hall, 2001). Aguado et al. (2001) maintain that the retardation of reacquisition seen after extinction is best explained as a latent inhibition effect. It should also be noted, however, that evidence of inhibition has been observed after extinction given intense extinction training. Denniston and Miller (2003) demonstrated both summation and retardation effects after 1000 extinction trials.

Table 6: Design for Experiment 3.1

Group	Acquisition	Ret	Extinction	Test
Ret	3 x A+ 3 x X+	X	18 x X-	2 x A- 2 x AX-
NoRet	3 x A+ 3 x X+	-	19 x X-	2 x A- 2 x AX-

N.B. Ret = retrieval; NoRet = no retrieval; A = non-extinguished CS; X = extinguished CS; "+" indicates a reinforced CS presentation; "-" indicates a non-reinforced trial.

On the basis of the reacquisition data reported so far, there is no evidence of inhibition following extinction without retrieval since these animals appear to acquire the conditioned response at an equivalent rate to naive animals. The central focus of this experiment, however, was to test whether the conditions which produce impairment in reacquisition for Ret versus NoRet animals would also lead to inhibitory strength as assessed with a summation test. If the effect of pre-extinction retrieval on reacquisition is due to the CS becoming an inhibitor, then the associative strength of X will be less than zero ($V_X < 0$). Thus, the sum of the associative strengths of A and X together will be less than that of stimulus A alone (A > AX). On the basis of an inhibition account of the Monfils et al. (2009) effect, therefore, it would be hypothesised that the compound AX should elicit less freezing that A alone. If, on the other hand, retrieval facilitates retrieval of extinction by enhancing the latent inhibition component of the extinction process rather than by inducing inhibition, the associative strength of X should be greater than or equal to zero ($V_X \geq 0$). Therefore, if retrieval prior to extinction encourages inattention to the extinguished CS (X), the presentation of X in compound with A would not be expected to suppress responding relative to A alone ($A \leq AX$).

Methods

Subjects

The subjects used in this experiment were 16 adult male Lister hooded rats (Charles River, UK).

Apparatus

All experimental procedures were conducted in the chambers described in Chapter II. For this experiment, two distinct stimuli from different modalities were used in a counter-balanced fashion. The stimuli used were the clicker (auditory) and light (visual) with a duration of 60 s, as described in Experiment 1.2. One of the two stimuli was designated as stimulus A and the other as stimulus X. The physical identity of the stimuli was counter-balanced within group and order of training.

Behavioural Procedures

Habituation. All animals were habituated to the experimental context of one hour per day for two days prior to the first conditioning session.

Acquisition. On the first day following habituation, animals were conditioned to both stimulus A and stimulus X. The stimuli were trained in separate sessions, one in the morning and one in the afternoon with a minimum of three hours between the end of the first session and the start of the second. The order of training was counter-balanced across stimuli. Each training session comprised a 30 min adaptation period followed by three presentations of the CS co-terminating with the foot-shock US with an average ITI of 5 min. Animals were removed from the chambers one min after the last CS presentation of the session.

Retrieval. One day following acquisition training, animals were returned to the experimental chambers for a retrieval session. Animals in Group Ret were given two minutes context exposure followed by a single nonreinforced presentation of stimulus X. Animals

were then removed and returned to their home cages. Animals in Group NoRet were given 3 min exposure to the context before being returned to the home cages.

Extinction. One hour following the retrieval session, all animals were brought back to the experimental chambers for extinction training. Animals in Group Ret were given 18 presentations of X with an ITI of 120 s. Animals in Group NoRet were received 19 presentations of X such that the total number of CS presentations including the retrieval session would be equal to Group Ret. All animals were removed from the chambers one minute after the last CS presentation.

Summation Test. A summation test for inhibition was given 24 h after extinction training. This session comprised two presentations each of A and the compound stimulus AX (simultaneous presentation of stimuli A and X). The order of presentation was either "A, AX, AX, A" or "AX, A, A, AX" with the order counter-balanced across groups, order of training and stimulus identity (clicker or light). A 3 min adaptation period preceded each stimulus presentations, with each stimulus presentation lasting 1 min.

Statistical Analyses

Acquisition. Pre-CS freezing during acquisition was analysed using a repeated-measures ANOVA with Group (Ret, NoRet) as the between-groups factor and Stimulus (A or X) as the repeated-measures factor.

Data from the two conditioning sessions was analysed using the Multivariate Analysis of Variance (MANOVA) with Group (Ret, NoRet) as a between-groups factor and Stimulus (A, X) and Trial (Trial 1, Trial 2, Trial 3) as within-subjects factors.

Retrieval. The first two minutes immediately before the retrieval trial, when both groups were being exposed to the context, were compared between groups by way of a One-way ANOVA. The period during which the CS was presented for Group Ret was analysed with the extinction data as Trial 1.

Extinction. Freezing during the two min prior to first CS onset was analysed for Group with a One-Way ANOVA and taken as an index of contextual fear. The 19 trials of extinction (including retrieval for Group Ret) were analysed as a linear contrast with 19 levels to obtain an indicator of reduction in freezing across extinction training. The value of this contrast was compared between groups to ascertain whether the groups differed in their rate of extinction.

Summation Test. The first three minutes of the test session were taken as the pre-CS period with average freezing during this time reflecting contextual fear. This measure was analysed using a One-Way ANOVA with Group as the between-subjects factor. Data from the X and AX trials during test were analysed using a repeated-measures ANOVA with Group as a between-subjects factor and Stimulus (A, AX) as a within-subjects factor with the interaction between Group and Stimulus serving as an index of a differential effect of summation for Group Ret compared with Group NoRet.

Results

Acquisition

Freezing during the two min prior to first CS onset during conditioning of both stimulus A and stimulus X did not differ as a function of group, $F(1, 14) < 1$. Pre-CS freezing did not differ between stimulus A and stimulus X, $F(1, 14) = 1.69$, $p = .215$. No significant interaction between group and stimulus was found at this stage either, $F(1, 14) = 3.49$, $p = .083$; *M*s (*SEM*s) of percent time freezing to Stimulus A (%): Ret = 23.3 (12.7), NoRet = 4.8 (2.0); *M*s (*SEM*s) of percent time freezing to Stimulus X (%): Ret = 3.5 (1.3), NoRet = 8.3 (2.8).

Figure 32 shows the acquisition curves for Groups Ret and NoRet on each of the two stimuli. A significant effect of trial was detected, $F(1, 15) = 452.2$, $p < .001$, indicating successful acquisition of fear to the two stimuli. No main effects of Group (Ret, NoRet) or Stimulus (A, X) were significant, nor any interactions between the three variables, *F*s < 1.

From these data we can conclude that no pre-existing differences between groups or stimuli were present at the time of acquisition training.

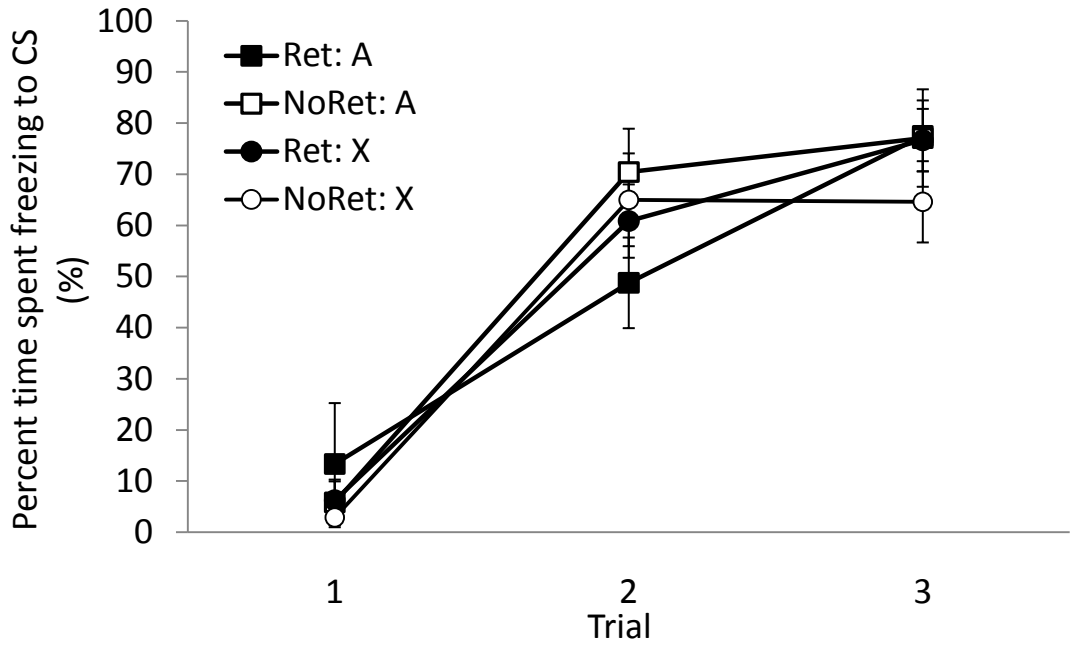

Figure 32. Freezing for Groups Ret and NoRet to each of the two stimuli during acquisition. Circles represent means ± *SEM*.

Retrieval

Freezing to the context during the first two minutes of the retrieval session did not differ between groups, $\chi^2(1) = 2.13$, $p = .144$, indicating that the groups showed no evidence of differences in contextual fear when first returned to the chambers after acquisition training; *M*s (*SEM*s) of percent time freezing: Ret = 0.0 (0.0), NoRet = 0.8 (0.6). Freezing during the CS presentation for Group Ret was treated as extinction trial one for the purposes of analysis and is shown at the left of Figure 33.

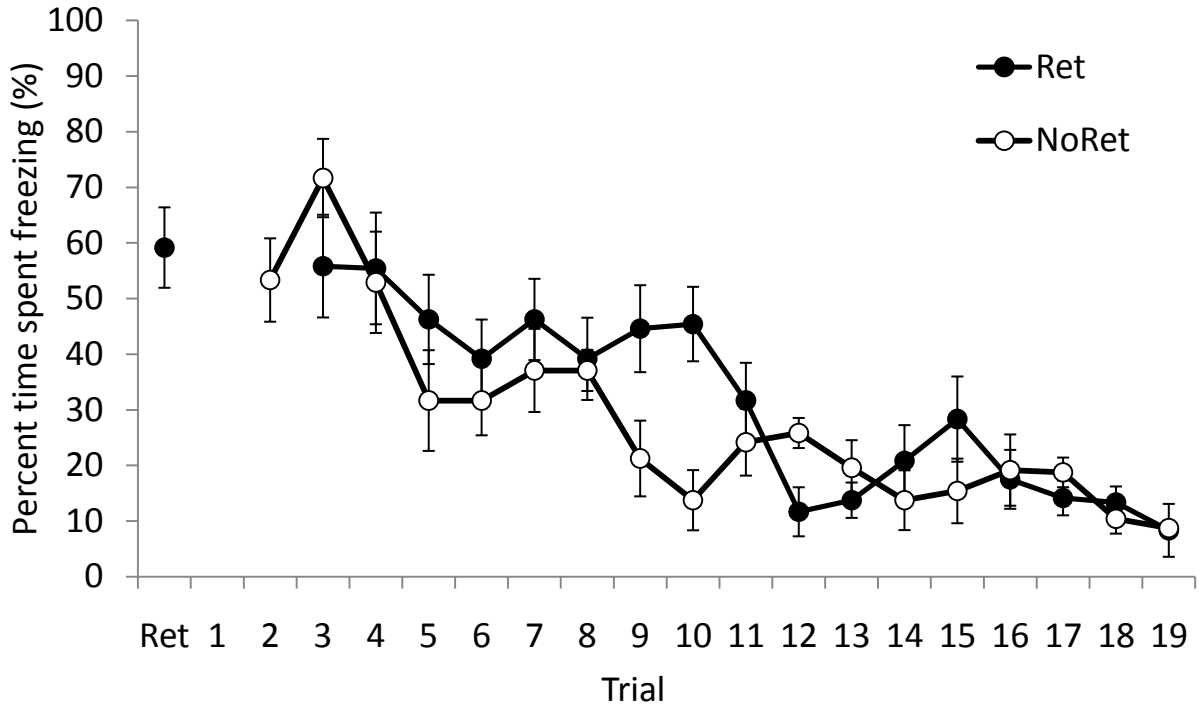

Figure 33. Freezing to the CS during retrieval (Ret) and Trials 1-19 of extinction training. Circles represent means ± *SEM*.

Extinction

The first two minutes in the context prior to the first extinction trial were not different between groups, $F(1, 14) < 1$; *M*s (*SEM*s) of percent time freezing: Ret = 1.5 (1.2), NoRet = 3.1 (1.7). Freezing to the CS across trials is shown in Figure 33. The extinction training was successful in reducing levels of freezing to the CS across trials in a linear fashion, $F(1, 14) = 93.51$, $p < .001$. No influence of group allocations was detected at this phase of the experiment, *F*s < 1.

Summation Test

Groups were not different in terms of pre-CS responding during the three minutes of context exposure prior to the first trial, $F(1, 14) < 1$; *M*s (*SEM*s) of percent time freezing: Ret = 1.0 (1.0), NoRet = 0.3 (0.3).

Freezing during CS presentations at test is shown in Figure 34. Two presentations each of A and AX were given during test and freezing during these presentations was

averaged across each trial type to obtain a single value for A and a single value for AX. A within-subjects contrast comparing trial type found no evidence for an overall difference between A and AX trials and thus no significant summation effect was detected, $F(1, 14) = 4.26$, $p = .058$. This effect, although not significant, was in fact in the opposite direction to that which would have been predicted by the inhibition hypothesis. Groups did not differ overall in levels of freezing when the data were collapsed across stimulus type, $F(1, 14) < 1$. The contrast of primary interest was the interaction between group and stimulus type. This interaction was not significant, $F(1, 14) < 1$. These data thus provide no evidence that stimulus X acquired inhibitory properties as a result of the retrieval session prior to extinction training.

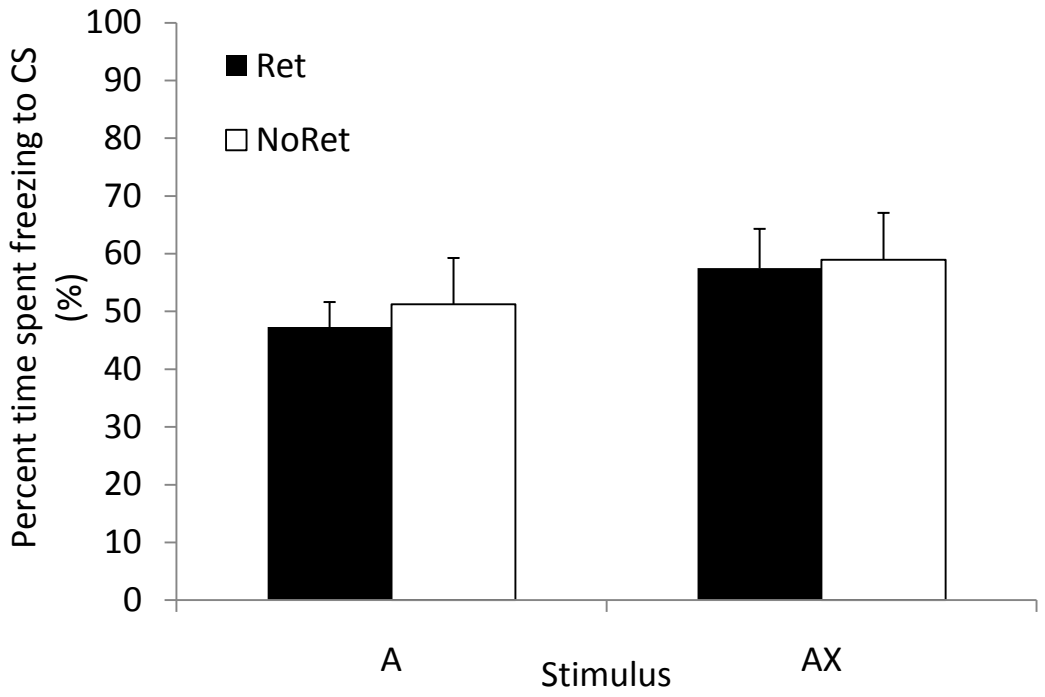

Figure 34. Freezing to stimulus A and to the compound stimulus AX at test. Inhibitory properties of stimulus X would be expected to result in lower responding to the AX compound than to A. However, no effects of stimulus or group were detected. Bar heights represent means ± *SEM*.

Discussion

A stimulus paired with shock and then subjected to extinction did not suppress responding to a distinct excitatory (transfer) stimulus when the two stimuli were presented in compound. Given the pronounced effect on reacquisition that was demonstrated using the same parameters, it was expected that a CS trained in this manner would suppress responding to a non-extinguished CS. However, this group did not differ in any way from the group receiving extinction without retrieval. If anything, more freezing was observed to the compound than to the transfer excitor alone. On the basis of these data, there is no evidence to support the suggestion that a stimulus which undergoes extinction after retrieval acquires an inhibitory association with the US. In the absence of suppression in a summation test,

impairment in reacquisition might best be explained as a failure of learning resulting from inattention.

The results of the retardation and summation tests presented here are consistent with those of Aguado et al. (2001) for a stimulus given extended extinction following conditioning. Bouton (1986) also showed retarded reacquisition following extended (72-trial) extinction but not with a weaker (16-trial) extinction regime. The finding that retrieval prior to extinction produces impairment in reacquisition but does not allow the CS to suppress responding to a transfer excitatory CS suggests that the effect of retrieval on extinction learning may be quantitative rather than qualitative. In other words, rather than proposing that retrieval changes the fundamental nature of the extinction process (i.e., by producing conditioned inhibition), the presentation of the CS prior to extinction may simply enhance extinction in a manner similar to increasing the number of trials. This suggestion has also been presented recently to explain the facilitatory effects of DCS on fear extinction where it was shown that impairments in the retention of extinction in adolescent rats could equally be overcome by DCS as by extended extinction (McCallum, Kim, & Richardson, 2010).

Experiment 3.2

The following experiment was designed to investigate the hypothesis that reacquisition of the conditioned response after extinction with retrieval is impaired due to a lack of attention to the CS. Aguado et al. (2001) assessed reacquisition of a conditioned taste aversion after extinction and found that the learning impairment was sensitive to the same effects of retention interval as acquisition after pre-exposure, suggesting that latent inhibition may be a process common to both experimental preparations.

According to the Pearce-Hall model (Pearce & Hall, 1980), the change in associative strength on a trial depends upon the amount of attention given to the CS. Moreover, the attention given to a CS on a given trial is a direct function of how surprising the CS was on

the previous trial. If the outcome of the previous CS presentation was surprising, i.e., there was a large prediction error, then attention to the CS on the next trial should be increased. Once the outcome of the CS is no longer surprising and the prediction error is minimal, attention to the CS on the subsequent trial will be low and so little learning should occur on this trial. Latent inhibition arises when a lack of prediction error during pre-exposure drives attention towards zero and so impairs learning when the CS is eventually paired with the US. This same process can occur across extinction trials when the presentation of the CS is no longer followed by the delivery of the US. Once the omission of the US is no longer surprising, attention to the CS will diminish such that if the CS again comes to predict the US, the learning should be impaired (Hall & Pearce, 1979).

A similar prediction regarding the effect of CS pre-exposure is offered by Mackintosh (1975a), albeit for different reasons. For Mackintosh (1975a), the loss of attention to the CS comes as a result of the CS being no better at predicting 'nothing of consequence' than other, background stimuli present during pre-exposure.

The effect of latent inhibition, as discussed in Chapter IV, can also be accounted for by the SOP model (Wagner, 1981). According to SOP, learning is impaired due to context-CS associations forming during CS pre-exposure which prime a CS representation into A2 during subsequent reinforced training. Less CS elements available during training prevent the CS from becoming associated with US elements active in A1. This account, of course, is dependent upon the reinforced training taking place in the same context as the pre-exposure. Retarded acquisition following extinction can equally be explained in these terms. Interestingly, both Wagner (1981) and Pearce and Hall (1980) would view retardation after extinction as being independent of the US and even of the CS-US association. Thus, impairments in acquisition following extinction training, if observed at all, should be independent of the reinforcer used during retardation training.

The next experiment examined the acquisition of an appetitive response to a CS previously conditioned with foot-shock and extinguished with or without prior retrieval. The experimental design is outlined in Table 7. For Groups Ret and NoRet, the clicker CS was conditioned and extinguished as in previous experiments. For Group Naive, the clicker was replaced with a light CS but otherwise subjected to the same treatment as Group NoRet. Following extinction, the CS was then trained in a magazine approach paradigm in which presentations of the CS were followed by delivery of a sucrose pellet. If rats in Group Ret develop latent inhibition to the CS as a result of pre-extinction retrieval, this group should pay less attention to the CS when it is paired with sucrose and so be slower to learn the association. If, on the other hand, the stimulus does develop inhibitory properties that were not detectable in the summation procedure, learning should not be retarded and may, in fact, be facilitated (Dickinson & Dearing, 1979; Konorski, 1967).

Table 7: Design of Experiment 3.2

Group	Acquisition	Ret	Extinction	Counter-conditioning	Test
Ret	3 x C-shock	C-	18 x C-	16 x (4 x C-sucrose)	4 x C-
NoRet	3 x C-shock	-	19 x C-	16 x (4 x C-sucrose)	4 x C-
Naive	3 x L-shock	-	19 x L-	16 x (4 x C-sucrose)	4 x C-

N.B. Ret = retrieval; NoRet = no retrieval; C = clicker CS; L = light CS.

Methods

Subjects

The subjects used in this experiment were 24 Lister-hooded rats (Charles River, UK) housed in groups of four. Animals were restricted in their food intake to 18g per day of

rodent laboratory chow (Purina, UK). Food was given at least one hour after the conclusion of each day's experimentation. Water was available to the animals at all times.

Apparatus

All procedures were carried out in the chambers described previously in Chapter II. The clicker and light stimuli, as described in Experiment 3.1, were employed as CS C and CS L, respectively. The CS duration in all phases was 60 s.

Procedure

Habituation. On each of the first two days of the experiment, the animals were placed in the chambers for one hour during which no stimulus presentations were scheduled. The red houselight remained on for the duration of this time.

Acquisition. On the third day, all animals were brought to the chambers for conditioning. These sessions began with a 30 min adaptation period after which three presentations of a 1 min CS co-terminating with foot-shock were given with average an ITI of 5 min. For Groups Ret and NoRet, the stimulus used in this phase was CS C. The same training schedule was used with CS L for Group Novel.

Retrieval. On the following day, rats in Group Ret were placed back into the chambers for a single presentation of CS C. Rats in Groups NoRet and Novel were placed in the chambers for 3 min without any stimulus presentations.

Extinction. One hour following retrieval, all animals were returned to the chambers for extinction training. During this session, Group Ret received 18 non-reinforced presentations of CS C with an ITI of 120 s. The number of trials was increased to 19 for Group NoRet, while Group Novel was given 19 presentations of CS L with an ITI of 120 s. All animals were returned to the home cages at the conclusion of the session. One hour after the conclusion of the experimental procedures, the animals were fed 50% (by weight) of their usual daily amount of laboratory chow. The remaining 50% was made up with sucrose pellets

identical to those to be used as reinforcers in the subsequent stages of the experiment so as to minimise any effects of neophobia on performance.

Magazine Training. One day following extinction training, all groups were returned to the experimental chambers where sucrose pellets were delivered to the magazine with ITIs varying randomly from 60 s to 180 s. For the first 20 min of the session, the flaps at the front of the magazine were fixed open to allow the animals to retrieve the pellets without opening the flap. The flaps were then closed for the remaining 30 min of the session during which time magazine entries could be recorded with each opening of the flap. Any rats that did not attain a stable level of responding during this period were given one additional magazine training session of 30 min on the following day.

Counterconditioning. Two days after the first magazine training session, all animals were placed in the experimental chambers for one hour, during which time they received four presentations of CS C for a duration of 1 min, co-terminating with the delivery of a pellet to the magazine. The first of these occurred 14 min after the start of the session and subsequent trials were separated by 14 min intervals. One minute after the final CS-US pairing, the animals were removed from the chambers and returned to their home cages. This procedure was repeated once daily for an additional 15 days. Entries to the magazine were recorded throughout these sessions.

Context Extinction. On the day following the last appetitive conditioning session, animals were placed in the conditioning chambers for one hour during which time no stimuli or reinforcers were presented. The purpose of this session was to reduce baseline levels of magazine-approach responses prior to final test session.

Test. All groups were again returned to the conditioning chambers for a one hour session comprising four non-reinforced presentations of the CS separated by a variable ITI with an average of 14 min ranging from 9 min to 19 min.

Statistical Analyses

Freezing data were analysed as in previous experiments.

Performance on each trial of the counterconditioning phase was transformed into elevation scores whereby the number of magazine entries during the 1 min pre-CS period was subtracted from the number of magazine entries during the 1 min CS presentation. The four trials on each day of training were then averaged to produce an overall elevation score for that session. A linear trend analysis of session averages was used to assess the rate of acquisition of magazine approach behaviour during the CS presentation. Overall differences in elevation scores during counter-conditioning, and differences in the degree of linear increase were also assessed to determine whether the animals' prior reinforcement history influenced acquisition of the appetitive response.

Responding across the four test trials was analysed by way of a repeated-measures ANOVA to assess group differences in elevation scores when the CS was presented in extinction.

Results

Two animals were excluded from Group Novel due to an equipment error in the conditioning chamber these animals had been conditioned in which meant they did not receive any foot shocks during acquisition. Two further animals had to be excluded from analysis on the basis of extremely outlying scores during counterconditioning (one from Group Ret and one from Group NoRet). The elevation scores of these animals across the 16 sessions of counter-conditioning were at least two standard deviations away from their respective group means. The resulting group sizes are as follows; Group Ret: n = 7, Group NoRet: n = 7, Group Novel: n = 6.

Acquisition

The two minute period prior to first CS onset during conditioning produced no significant differences between groups in freezing to the context, $F(2, 17) < 1$; *M*s (*SEM*s) of percent time freezing: Ret = 0.4 (0.3), NoRet = 0.4 (0.3), Novel = 0.3 (0.2). Freezing to the CS across the three conditioning trials is shown in Figure 35. No significant group differences in CS freezing were observed during acquisition, $F(2, 17) < 1$. A significant linear increase in responding across trials, however, indicated successful acquisition of conditioned fear to the CSs, $F(1, 17) = 118.2$, $p < .001$.

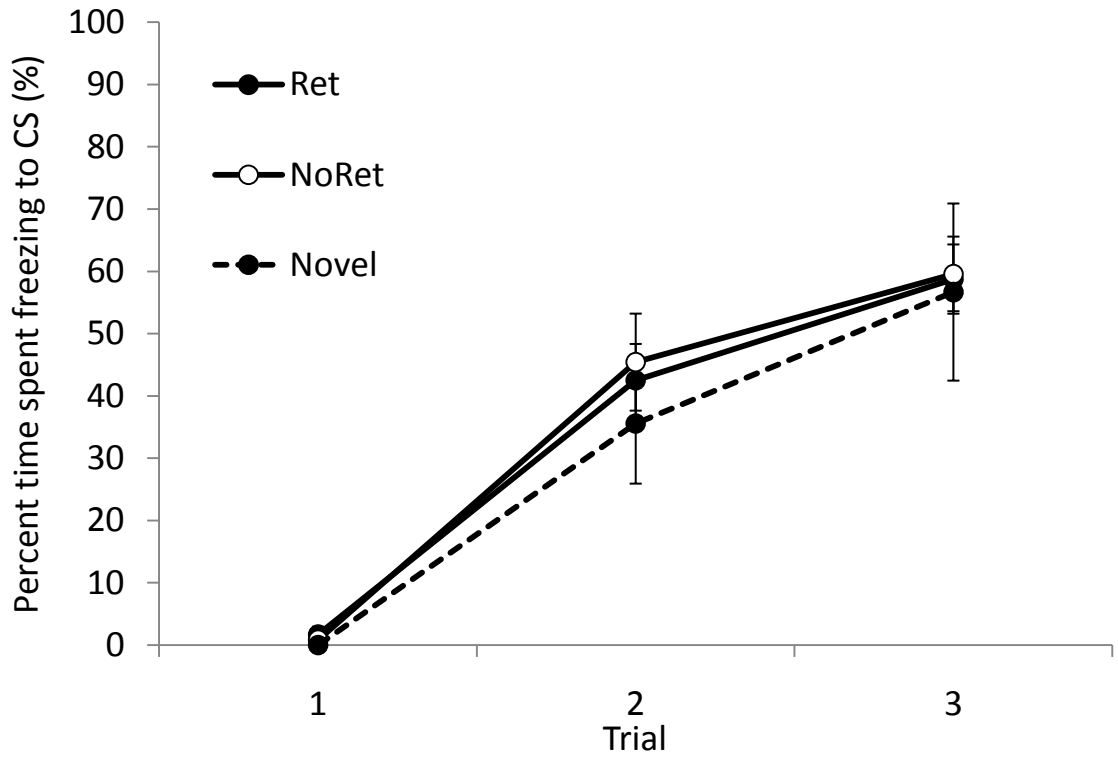

Figure 35. Freezing to the clicker CS (Groups Ret and NoRet) and light CS (Group Novel) during acquisition. Circles represent means ± *SEM*.

Retrieval and Extinction

No rats in any of the groups displayed any freezing during the first two minutes of the retrieval session. Freezing to the CS across retrieval and extinction is shown in Figure 36. A

significant linear decrease in responding over the 19 trials of extinction was indicative of a successful reduction in CS-elicited fear, $F(1, 17) = 106.8$, $p < .001$. This effect did not differ between the three groups, $F(2, 17) < 1$.

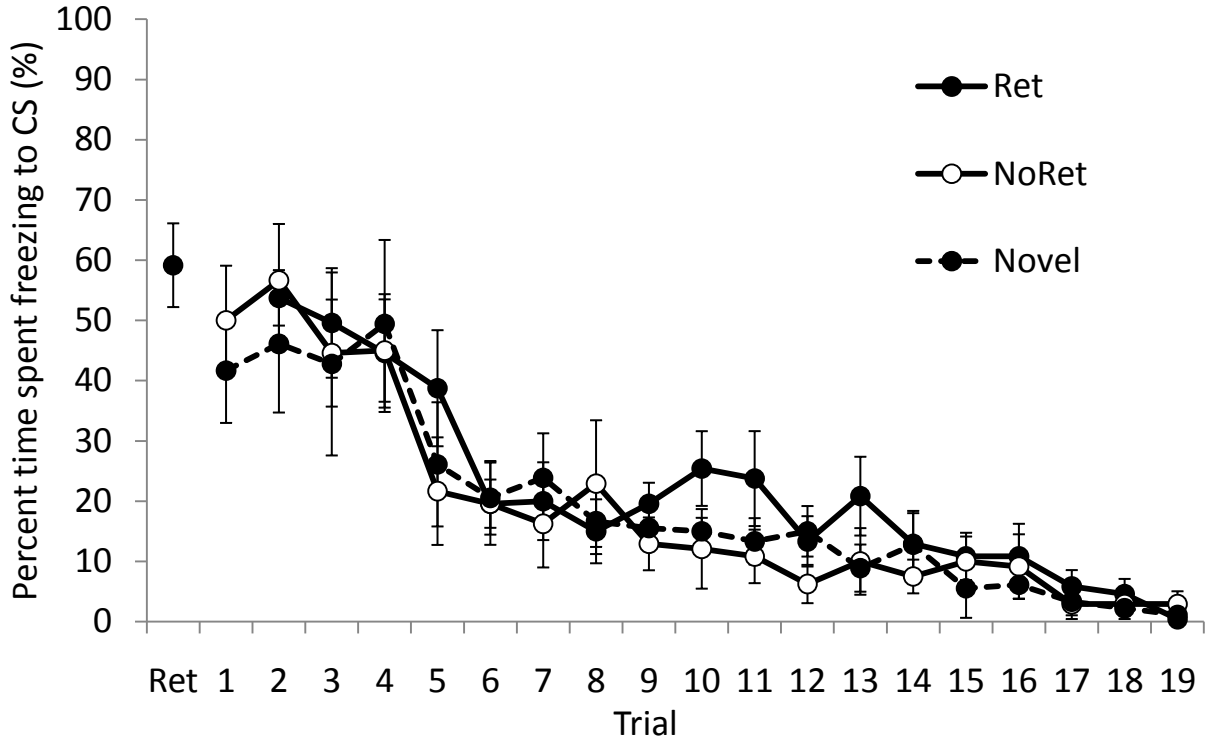

Figure 36. Freezing to the CS during retrieval (Ret) and across extinction trials 1 – 19. Circles represent means ± *SEM*.

Magazine Training

The data from the magazine training phase was analysed to examine the rate of increase in magazine entries and to ensure that no between-group differences in magazine entries were detectable prior to appetitive training. Overall, the rate of magazine entry increased during the 15 min of the session, $F(1, 21) = 15.82$, $p = .001$, indicative of rats having learned to expect pellet delivery to the magazine. Groups did not differ either in overall numbers of magazine entries during the session, $F(2, 21) < 1$, nor in the rate at which this response developed, $F(2, 21) = 2.36$, $p = .119$.

Counterconditioning

The number of magazine entries made during each of the 60 s periods preceding each CS presentation was averaged for each session and analysed in order to detect any group differences in baseline responding. No between-group differences in magazine entries were detected overall during the counter-conditioning phase, $F(2, 19) < 1$. Thus, there is no suggestion that prior training in the context had any effect on baseline magazine responding. Across sessions, baseline responding displayed a linear increase, $F(1, 19) = 16.70$, $p = .001$, an effect which did not vary with group membership, $F(2, 19) < 1$.

Elevation scores increased significantly as a function of session, $F(1, 17) = 43.58$, $p < .001$, showing that with repeated pairings of the CS with the delivery of a sucrose pellet, the animals made more entries into the magazine during the CS than prior to the CS (see Figure 37). The magnitude of this linear trend did not differ between groups, $F(2, 17) = 2.13$, $p = .149$, and thus provides no evidence for differences in rates of acquisition of the CS-sucrose association. However, a significant effect of group was detected when elevation scores were collapsed across sessions, $F(2, 17) = 11.10$, $p = .001$, with post-hoc analysis revealing that responding was elevated by the CS more strongly for the Novel group compared with either Group Ret, $F(1, 11) = 19.54$, $p = .001$, or Group NoRet, $F(1, 11) = 14.82$, $p = .003$. Groups Ret and NoRet did not differ significantly, $F(1, 12) = 1.21$, $p = .292$.

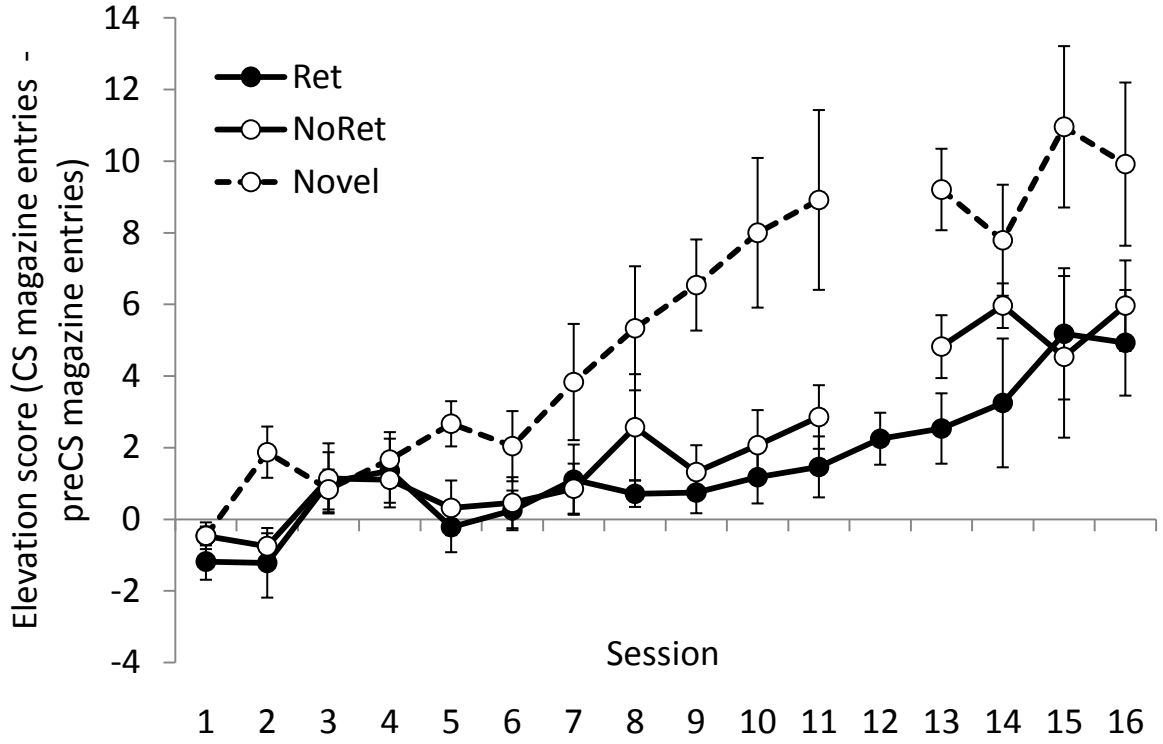

Figure 37. Elevation of magazine entries during CS presentations across 16 sessions of CS-sucrose pairings. Circles represent means ± *SEM*.

Test

The elevation of magazine entries during the non-reinforced CS presentations at test is shown in Figure 38. The differences in elevation scores observed during counterconditioning did not persist through to the test, $F(2, 17) < 1$.

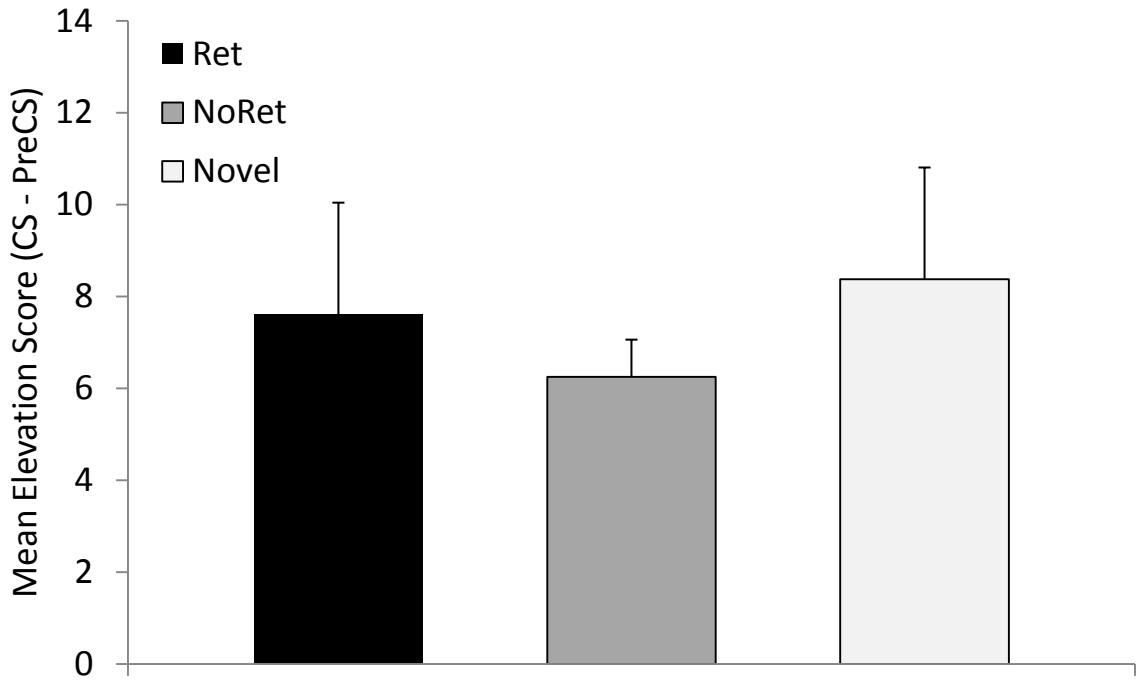

Figure 38. Average elevation scores across four non-reinforced presentations of the CS at test. Elevation scores were calculated as the number of magazine entries during the CS minus the number of magazine entries during the minute immediately preceding the CS. Bars represent means ± *SEM*.

Discussion

Animals acquiring an appetitive Pavlovian association to a novel CS displayed overall higher levels of conditioned responding across sessions compared to animals acquiring the association to a CS previously conditioned and extinguished in an aversive Pavlovian preparation.This may reflect some residual associative strength between the CS and the aversive US counteracting the development or expression of the appetitive response. The presentation of the CS one hour prior to extinction of the aversive association did not appear to influence the rate of acquisition of the appetitive association. Inattention to the CS would be expected to produce a retardation of acquisition of the conditioned response, which would be indexed by differences in the gradient of the acquisition curve. No such difference in

acquisition rate for the appetitive conditioning phase was detected. However, if the acquisition rate of the control group is also slow, floor effects on responding may obscure any effects of inattention. Perhaps the transfer of retardation from one reinforcer to another could be better detected if the second phase of acquisition involved a preparation in which the emergence of the conditioned response would proceed more rapidly. Responding to the CS when presented at test revealed no differences between any of the three conditions. This is most likely due to response rates to the CS converging across counterconditioning sessions.

These data fail to provide support for the hypothesis that retrieval prior to extinction encourages latent inhibition of the CS since Groups Ret and NoRet did not differ in terms of their rate of acquisition of the appetitive association. Furthermore, the observation that both groups receiving counterconditioning showed suppressed emergence of responding across appetitive training sessions compared to a group conditioned to a novel stimulus is not easily reconcilable with an account of retardation in terms of inhibition. If it is assumed that an inhibitory association would form between the specific sensory properties of the CS and US, the formation of an association with a completely distinct US should not be affected. On the other hand, if the inhibitory association is between the CS and the motivational properties of the US, and if we assume that reciprocal inhibitory interactions exist between appetitive and aversive motivational systems (Konorski, 1967), the appetitive association may be expected to develop more rapidly than for a novel control group.

In summary, these data struggle to account for the effect of pre-extinction retrieval in terms of either inattention or conditioned inhibition. The CS extinguished after retrieval can acquire a distinct conditioned response through pairing with a US of opposite affective value just as readily as a CS extinguished without prior retrieval. The overall lower levels of responding in these groups compared to a control group trained with a novel stimulus also suggest a degree of associative strength between the CS and the foot shock US remaining

after extinction training. Together with the data from Experiment 3.1 in which a trend towards 'positive' summation of the CS and the transfer excitor, these data present a strong case against an account of the Monfils et al. (2009) effect in terms of conditioned inhibition.

Experiment 3.3

So far the experiments in this thesis have demonstrated robust impairment in reacquisition when extinction is preceded by a single retrieval trial, an effect which does not appear to be due to conditioned inhibition, and neither does it carry across to a second reinforcer distinct from that used during the initial reinforced training of the CS. If learning about the CS is not in itself impaired, and neither the magnitude nor valence of the association between the CS and US appear to be affected, this may suggest that the deficit in reacquisition may be related to the conditioning of background stimuli, or to some other property of the US representation being activated during retrieval. In order to address this possibility, two groups were conditioned with pairings of a CS with the foot shock US (see Table 8). This CS was then subjected to extinction one hour after retrieval of the CS-US memory, or one hour after an equivalent period of exposure to the conditioning context. These groups were then assessed on their ability to acquire fear to a novel CS when that stimulus was paired with the same foot shock US trained during the first stage of experimentation. Responding to both the extinguished and the newly trained stimuli was then assessed by presenting the stimuli in the absence of the US. In this way it was possible to determine whether any of the learning deficit observed during reacquisition in previous experiments could be accounted for by factors independent of the specific CS that was conditioned and extinguished in the earlier stages of the experiment. Should the effect be due to contextual modulation of fear responding, both stimuli tested in the context should display the same effect of retrieval. In other words, retrieval prior to extinction should result in lower responding to any excitatory stimulus presented in the context. If, on the other hand, the

impairment is due to reduced effectiveness of the US as a reinforcer, then an effect of retrieval should be seen only for the stimulus trained after extinction. Responding to the conditioned and extinguished stimulus should not be influenced.

Table 8: Design of Experiment 3.3

Group	Acquisition A	Ret	Extinction	Acquisition B	Test
Ret	3 x A+	A	18 x A	3 x B+	2 x A 2 x B
NoRet	3 x A+	-	19 x A	3 x B+	2 x A 2 x B

N.B. Ret = retrieval; NoRet = no retrieval; A = stimulus designated as A; X = stimulus designated as X; "+" indicates presentation of the US co-terminating with the CS presentation.

Methods

Subjects

The subjects used in this experiment were 16 Lister-hooded rats (Charles River, UK) housed in groups of four with food and water available *ad libitum*.

Apparatus

All procedures were carried out in the chambers described previously in Chapter II. The clicker and light stimuli, as described in Experiment 3.1, were employed as CS A and CS B counter-balanced across groups. The CS duration in all phases was 60 s.

Behavioural Procedures

Habituation. All animals were brought to the experimental chambers for one hour per day on each of two consecutive days. During this time, the red houselight remained on and no other stimuli were presented.

Acquisition A. The first acquisition training took place on day three. The stimulus trained in this phase of the experiment was designated A. Half of the animals from each

group were training with three pairings of the clicker CS with foot-shock. The first of these trials began 30 min after placement in the context and the remaining trials were presented with an average ITI of 5 min. Animals were removed from the context one minute after the last CS-US pairing. The remaining animals from the two groups were training under the same conditions with the exception of a light CS being used in place of the clicker CS.

Retrieval and Extinction. 24 hours after conditioning, animals were returned to the context for retrieval and extinction sessions with stimulus A. These sessions proceeded as described in Experiments 2.1 and 2.2. Importantly, during retrieval, only group Ret was exposed to CS A.

Acquisition B. One day after extinction training, animals were returned to the experimental chambers for conditioning of stimulus B. For those animals previously conditioned to the clicker, stimulus B was a light. Similarly, animals that had been conditioned to the light during Acquisition A were now trained with the clicker. During this session, stimulus B was trained under the same conditions as for stimulus A such that all animals received three pairings of B with the foot-shock US.

Test. On the following day, animals were returned to the experimental chambers for testing. Two presentations of stimulus B were followed by two presentations of stimulus A with an adaptation period and ITI of 3 min.

Statistical Analyses

Pre-CS freezing was calculated for each session as the percentage of time spent freezing during the two minutes (or for the test session, three minutes) prior to first CS onset. Pre-CS freezing for each of the sessions was analysed using the One-Way ANOVA procedure with Group (Ret, NoRet) as the between-groups factor.

Analysis of freezing to the CS during acquisition A, acquisition B and extinction was carried out using the repeated-measures ANOVA with Group (Ret, NoRet) and Trial as the between- and within-subjects variables.

Since comparisons between CS A and CS B were not of interest in the context of the current hypotheses, freezing to the A and B at test were analysed separately. Levels of freezing to each stimulus were analysed across groups using the One-Way ANOVA procedure.

Results

Due to failure of the foot-shock generator in one of the four experimental chambers, two rats from each group had to be excluded from analysis, leaving six animals in each group.

Acquisition A

Pre-CS freezing during training of stimulus A did not differ between Groups Ret and NoRet, $F(1, 10) < 1$; *M*s (*SEM*s) of percent time freezing: Ret = 2.8 (1.8), NoRet = 2.2 (1.3). Freezing during the CS presentations is shown in Figure 39. A significant linear trend indicated successful acquisition of the conditioned freezing response to CS A, $F(1, 10) = 128.4$, $p < .001$. No overall effect of group was observed, $F(1, 10) = 1.06$, $p = .328$, and no interaction was observed between group and trial, $F(1, 10) = 1.88$, $p = .200$, thus providing no evidence for group differences at this stage of experimentation.

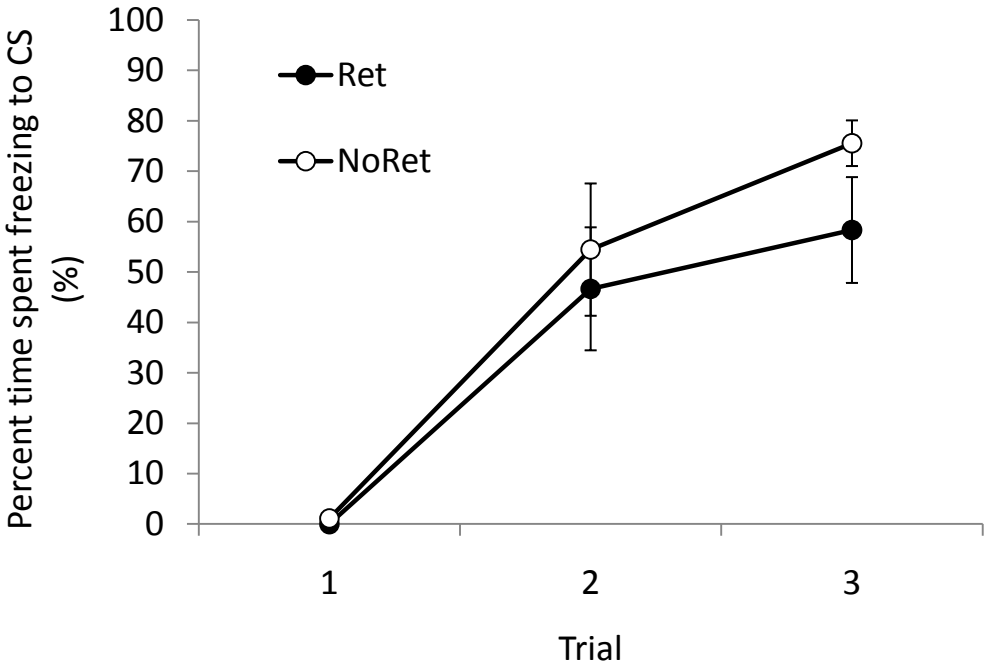

Figure 39. Acquisition training for stimulus A. Circles represent means ± *SEM*.

Retrieval

None of the animals in either group displayed any freezing during the first two minutes of the retrieval trial. Freezing to the CS presentation for Group Ret is shown in the first column in Figure 40. This trial was treated as extinction trial 1 for Group Ret for the purposes of analysis.

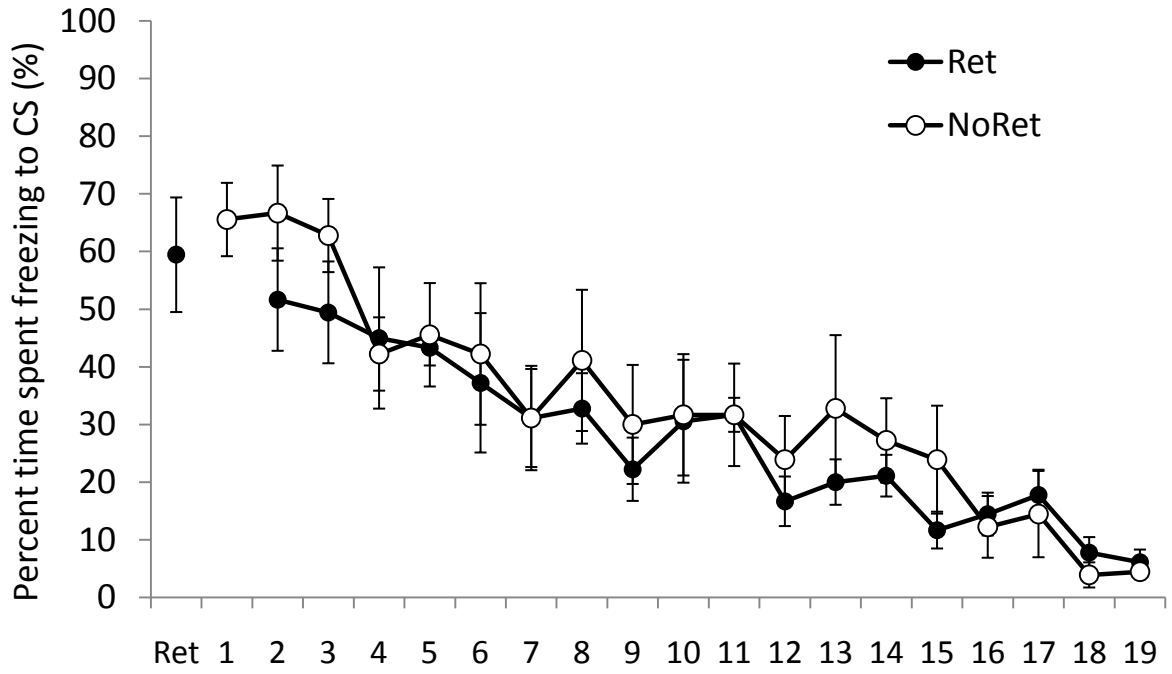

Figure 40. Freezing to the CS during retrieval (Ret) and extinction. Circles represent mean ± *SEM*.

Extinction

No group difference was detected during the pre-CS period, $F(1, 10) < 1$; *M*s (*SEM*s) of percent time freezing: Ret = 0.3 (0.3), NoRet = 0.6 (0.6). Freezing during CS presentations is shown in Figure 40. A significant linear decrease in freezing across trials indicated successful extinction of the conditioned freezing response, $F(1, 10) = 98.17$, $p < .001$. This effect did not differ between groups, $F(1, 10) < 1$, and no group difference was detected in overall levels of freezing to the CS, $F(1, 10) < 1$.

Acquisition B

Freezing during the two minutes prior to the onset of the first pairing of CS B with foot-shock did not differ between groups, $F(1, 10) < 1$; *M*s (*SEM*s) of percent time freezing: Ret = 2.8 (1.3), NoRet = 4.4 (2.0). Freezing to the CS over the three acquisition trials is shown in Figure 41. Collapsing across groups, a significant linear trend was indicative of

successful acquisition of conditioned responding to the CS, $F(1, 10) = 40.76$, $p < .001$. This effect did not differ significantly between groups, $F(1, 10) = 3.75$, $p = .082$, suggesting that the acquisition rates were similar for the two groups. However, Group Ret reliably displayed less freezing to the CS overall during the session, $F(1, 10) = 5.76$, $p = .037$. This result indicates less overall fear to the CS for Group Ret than for Group NoRet.

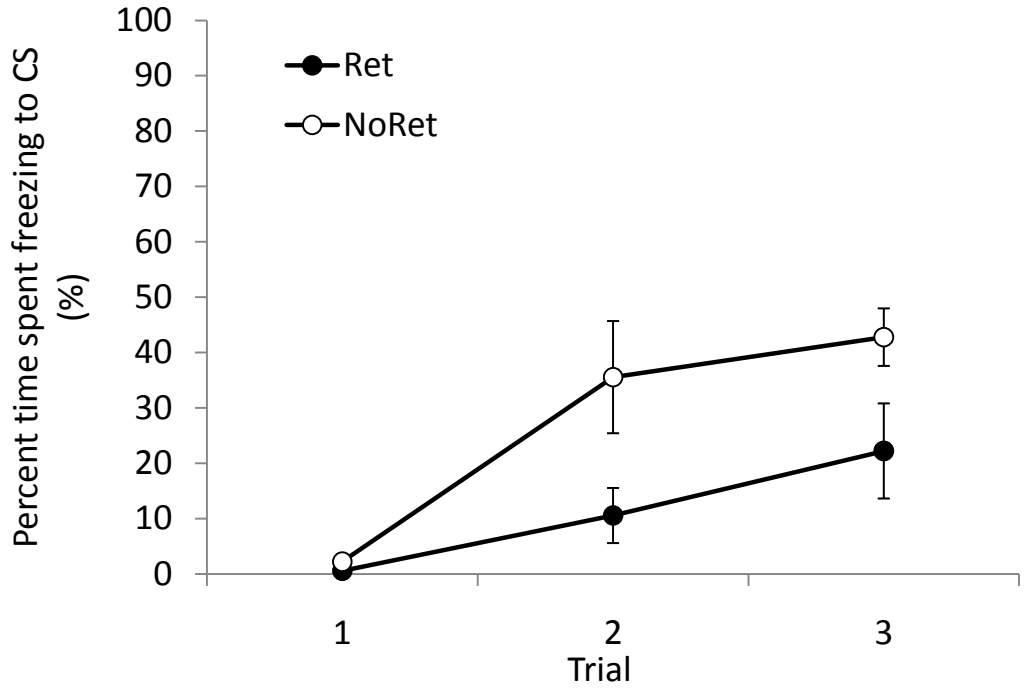

Figure 41. Freezing to the CS during acquisition of fear to stimulus B. Circles represent mean ± *SEM*.

Test

Freezing to the context and to each of the two stimuli is presented in Figure 42. No significant difference in contextual freezing was found during the test phase, $F(1, 10) = 1.81$, $p = .208$. Freezing during presentations of stimulus B differed significantly between groups, $F(1, 10) = 5.46$, $p = .042$, with Group Ret displaying less fear of the CS than Group NoRet. This finding indicates that the presentation of a CS previously paired with shock 1 h prior to extinction of that stimulus impairs the subsequent ability of the animal to learn an association

between the shock and a novel stimulus. Responding to stimulus A remained consistently low for both groups, $F(1, 10) < 1$.

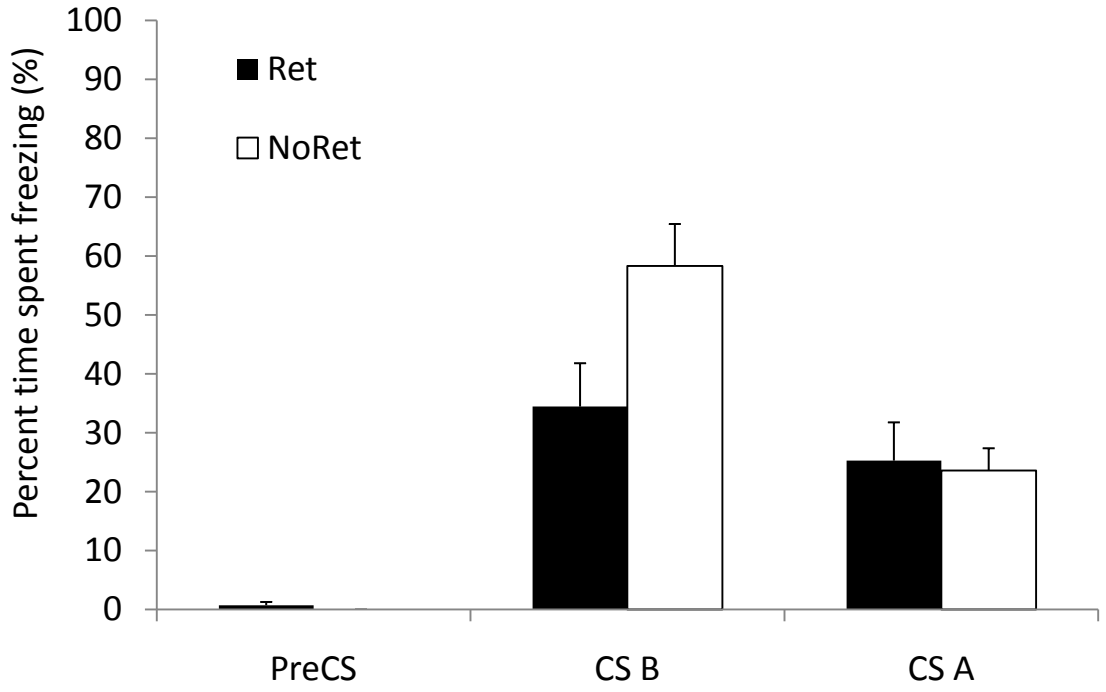

Figure 42. Freezing to the context (Cxt), stimulus B and stimulus A at test. Bars represent mean ± *SEM*.

Discussion

The results of this experiment demonstrate that extinction of a CS one hour after retrieval of the CS-US memory impairs learning when the same US is later paired with a novel stimulus, compared to a group given extinction without prior retrieval. Impairment in acquisition to a novel stimulus after extinction with retrieval might be expected if animals generalised between the two stimuli. If stimuli A and B shared elements when they were activated, then those elements of A which were shared with B might be expected to display the same resistance to reacquisition as has been shown in previous experiments on the impairment in reacquisition resulting from pre-extinction retrieval. Whether this effect were due to inhibition or inattention, those shared elements would still be reluctant to enter into

association with the US when activated as part of a CS B representation and so learning about CS would similarly be impaired. However, the transfer of learning from A to B via their shared elements should be reciprocal such that learning about B would be expected to affect responding to A. Thus, should any shared elements of A and B be capable of permitting generalisation from one stimulus to the other, responding to stimulus A at test should reflect the differences observed between groups in responding to stimulus B. However, responding to stimulus A at test did not differ between groups. This suggests that either the stimuli do not share a significant proportion of elements, or that it is the associations of the unique elements of A which are responsible for the differences seen in responding to this stimulus.

These data suggest that the impairment in reacquisition observed after extinction with retrieval is independent of the CS initially paired with the US. One possibility is that responding to a discrete CS comes to be modulated by the context. If the presentation of the CS prior to extinction confers upon the context the ability to inhibit fear responding, this could account for the lower levels of freezing to the CS in previous experiments when that stimulus is again paired with the CS, and for the lower levels of freezing observed in this experiment when the US is paired with a novel stimulus in the same context. However, this again should produce an effect on responding to stimulus A, which was not found in this study. It is unlikely, therefore, the differences between groups in responding to CS B during acquisition and test would be due to differential contextual inhibition modulating fear responding.

Impairment in reacquisition is not only independent of the CS initially paired with the US, but is specific to the CS that is paired with the US in the second phase of acquisition. These results point to an explanation with reference to the US itself. In other words, the impairment in reacquisition appears to be the result of impairment in the ability of the US to enter into association with another stimulus. Retardation of reacquisition in Group Ret

relative to Group NoRet may reflect differences in the integrity of the US representation at the time of reacquisition, or else may be a sign of devaluation of the US. There is evidence to suggest that deterioration of the US representation can occur during extinction (Rescorla & Heth, 1975), and that the strength of the representation can affect learning in an extinction paradigm (Rescorla & Cunningham, 1977). Had Group NoRet had a more intact representation of the US available at the time of conditioning of stimulus B, this group may have had an advantage over Group Ret where the US representation might have deteriorated. However, the presence of at least a partially intact US representation in Group NoRet would predict faster acquisition of fear to stimulus B than to stimulus A, which was conditioned when the animal was naive to the US. Learning about stimulus B was, if anything, slower than stimulus A, and so it does not seem likely that the differences between groups in acquisition of fear to B are due to a facilitative effect of an existing US representation in Group NoRet.

An alternative view might be that, rather than degrading the integrity of the US representation, the US was instead revalued as less aversive. If, as a result of presenting the CS one hour prior to extinction, the US was revalued as less aversive, this could lead to impairment in subsequent learning of a CS-US association. Though, this could possibly also influence responding to CS A since this stimulus too relies on the US representation to elicit a conditioned fear response. The retrospective revaluation of US intensity has been demonstrated in human fear learning where fear responses to a CS can be inflated or deflated following acquisition by exposing participants to a stronger or weaker shock than that used during conditioning (e.g., Hosoba, Iwanaga, & Seiwa, 2001). However, it is not clear, how such a revaluation could occur in this experiment since the US was not physically present during the period when such a change would have had to occur.

Although the mechanism remains unclear, the results of this experiment do, still, suggest that the impairment in reacquisition following extinction within the reconsolidation window are, at least in part, due to a change in the capacity of the US to enter into an association with the same or even with a different stimulus.

Chapter Discussion

This chapter investigated the properties of the CS-US association that arise through the presentation of a retrieval trial one hour prior to extinction training. In light of the finding that this procedure results in a robust impairment in reacquisition of the conditioned response it was hypothesised that extinction within the reconsolidation window led to the development of an inhibitory association between CS and US. To assess this it was necessary to examine the effect of presenting the extinguished CS (X) in compound with a known excitatory stimulus (A). If the extinguished CS were inhibitory, the associative strengths of the two stimuli would have been expected to summate to produce less responding to the compound than to the excitatory stimulus alone. However, this was not found. Responding to the AX compound was not reliably different to responding to A and this effect did not differ between a group given retrieval prior to extinction and a control group conditioned without prior retrieval. Therefore, it was not possible to infer from these data the existence of an inhibitory CS-US association that might be responsible for the retardation of reacquisition earlier reported. Instead, the retardation effect may have been due to an enhancement of latent inhibition processes during extinction such that animals fail to attend to the stimulus at reacquisition and so are unable to learn that the CS again predicts the US.

To assess the inattention hypothesis, a stimulus was initially conditioned to elicit a fear response by pairings with a foot shock US. This CS was then extinguished with the variable of interest being whether the memory was reactivated or not prior to extinction training. Following extinction, animals were then given pairings of the CS with delivery of a

sucrose pellet to a magazine and approach responses to the magazine were recorded. If retrieval prior to extinction promotes latent inhibition of the CS, then it would be anticipated that animals in this group should acquire the conditioned approach response more slowly than controls. This was not found to be the case. Animals given retrieval prior to extinction learned the new association at a comparable rate to animals given extinction without retrieval. Furthermore, both these groups showed less appetitive responding compared to a group trained on a novel stimulus, suggesting that these groups may have retained some of the excitatory association from the original fear learning episode, and this residual excitatory fear retarded emergence of appetitive conditioning. In summary, while no evidence was found to support the inattention account, this experiment provided additional evidence against the hypothesis of conditioned inhibition.

Finally, given the failure of detecting effects on the associability of the CS, the final experiment examined whether deficits in reacquisition could be, at least partially, due to a reduced capacity for the US to act as an effective reinforcer. Surprisingly, retrieval prior to extinction of a CS previously paired with the US resulted in impairment in acquisition of fear to a second stimulus paired with the same US. This effect could not easily be explained as an effect of stimulus generalisation or modulation of responding by contextual stimuli as these explanations would also predict differences in responding to the extinguished CS. Changes in the integrity and value of the US representation would also be expected to influence both stimuli unless it is assumed that such changes only affect new learning without retrospectively affecting existing associations formed with that stimulus. Although it is commonly reported that post-conditioning devaluation of the US results in attenuation of the CR (e.g., Colwill & Motzkin, 1994; Holland & Rescorla, 1975), some protocols report that properties of a CS acquired through conditioning with the US (e.g., Pavlovian-to-instrumental transfer, second-order conditioning and conditioned reinforcement) persist after US

devaluation (Holland, 2004; Holland & Rescorla, 1975; Parkinson, Roberts, Everitt, & Di Ciano, 2005).

These results are in contrast to a report by Doyère et al. (2007) in which they show stimulus-specific disruption of reconsolidation where two distinct CSs were separately conditioned with the same US. If disrupting reconsolidation of a CS memory impaired the integrity of the associated US representation, it would have been expected that responding to the non-retrieved CS should also be impaired. One possible explanation for this disparity is that Doyère et al. (2007) induced amnesia through infusions of anisomycin into the lateral amygdala. If a representation of the US is stored outside of this neuroanatomical locus, this part of the memory may have been able to reconsolidate normally without interference from protein synthesis inhibition. However, a more recent study from the same laboratory would suggest that this is not the case. Debiec, Diaz-Mataix, Bush, Doyere, & LeDoux (2010) were able to induce post-reactivation amnesia for two distinct CSs, which had each been conditioned to a foot-shock US, by infusing anisomycin into the lateral amygdala after a retrieval session comprising a single presentation of the US alone. This provided strong evidence that representations of the US, as well as those for the CS, were stored within the lateral nucleus of the amygdala. Had reactivation of the CS in the Doyère et al. (2007) study destabilised the US representation, then the inhibition of protein synthesis in the lateral amygdala should have been sufficient to disrupt the US representation along with the CS representation. That the US representation appeared to be intact following this treatment suggests that CS retrieval leaves the US representation in a stable state. Reconciling these data with the data of the present studies may therefore require some additional investigation.

Together the data from this chapter do not support the hypothesis that retardation of reacquisition of a fear to a stimulus after memory retrieval and extinction is the result of net inhibition accruing to the CS. There is also no sign of an effect of enhanced latent inhibition

of the CS, although from these data it is not possible to exclude this explanation. It remains possible that the effects on attention were specific to the reinforcer paired with the CS during acquisition, or that the protocol was insensitive to any impairments in conditioning relating to inattention. It does seem possible, however, that at least some of the retardation effect is due to impairment in the ability of the US to again enter into association with a CS.

The clinical implications of these data are both positive and negative. The experiments presented so far fail to find evidence suggesting that the CS becomes a safety signal or inhibitor. Thus, the chance of a patient putting themselves at risk by approaching stimuli or places that previously predicted danger may not in fact be any greater than for a patient treated under a traditional extinction-based treatment regime. A potential issue with the paradigm is the fact that learning about a novel CS paired with the foot-shock US is impaired by the Monfils et al. (2009) paradigm. This result suggests that patients treated in this manner may no longer be capable of learning the antecedents of traumatic events the same as, or similar to, the trauma. In the unfortunate event that the traumatic incident is repeated in the presence of a different set of environmental stimuli, these stimuli are unlikely to enter readily into association with the aversive event and so may not serve as effective predictors of danger. However, perhaps this is not so great a problem. It would be adaptive to be able to acquire knowledge of cues that predict danger. Yet, for patients having been treated for PTSD, for example, their learning of the first traumatic event was probably too robust as it allowed the associated stimuli to elicit disproportionate levels of fear. The animals in Experiment 3.3 did acquire some fear to the novel CS and so were not completely unable to learn the consequences of the new stimulus. For individuals who have a propensity towards fear learning to the extent that the result is debilitating fear to relatively harmless stimuli, then a weakening of the power of aversive events to condition fear to neutral stimuli may

counteract any predisposition to PTSD while still allowing the individual to develop a healthy avoidance of dangerous situations.

VI. REVERSAL OF REACQUISITION IMPAIRMENT

The brief presentation of the CS one hour prior to a session in which successive non-reinforced exposures to the CS produce extinction leads to a persistent reduction in fear responding which is resistant to further conditioning of the CS and US (Monfils et al., 2009). This effect has been demonstrated repeatedly in the present studies and has been shown to transfer to novel stimuli paired with the original aversive stimulus. Translating these findings into a clinical setting would suggest that extinction-based treatments for anxiety disorders may be facilitated by the presentation of the fear-eliciting stimulus briefly prior to the start of the exposure therapy session. This suggestion is supported by data from studies of conditioned fear in humans in which the retrieval of the fear-conditioned stimulus prior to extinction, using parameters very similar to those which produce an effect in rats, prevented spontaneous recovery measured after one day or 12 months (Schiller et al., 2010). On the basis of this study and the work of Monfils et al. (2009), it would be expected that anxiety patients treated under this paradigm would experience a substantial reduction in the anxiety elicited by the stimuli that had previously caused them distress, and that this would be an effect which would persist over long periods of time regardless of changes in environmental stimuli or exposure to stressful situations. Yet this paradigm as a model for the treatment of human anxiety disorders would have a drawback in that if patients were later to re-experience the traumatic incident in the context of the same or different predictive cues, they would fail to learn about these cues and so would fail to heed the warnings they may provide. While there may be an argument for a suppression of relearning being beneficial for people prone to rapid acquisition of fear associations, this property of the paradigm has the potential to be problematic in a real-world setting.

Whether the slowing of reacquisition is considered adaptive or maladaptive, it would be useful to determine whether this effect is reversible. Perhaps there would be conditions

under which the anxiety would readily reappear despite the use of retrieval before extinction training. Such a situation may in fact rely on the same mechanisms which were used to establish the enhanced extinction memory in the first place: learning within the reconsolidation window. For the discussion of this possibility it may be useful to consider the structure of the memory resulting from extinction of the CS.

Despite decades of interest in extinction learning, it is still far from established what happens to a CS-US association when the CS is subsequently presented alone that leads to a reduction in the frequency of CR production (Delamater, 2004). Nevertheless, extinction is generally conceptualised as involving at least one of two processes: unlearning of the excitatory association and new learning of an inhibitory CS-US association. Evidence for new learning accounts has typically come from demonstrations of recovery of conditioned responding after successful extinction under conditions where retrieval of the conditioning memory may be favoured over retrieval of the extinction memory (Bouton, 1993; Redish et al., 2007). The absence of recovery of conditioned responding when extinction is preceded by retrieval is consistent with (but not evidence for) an unlearning process occurring during extinction. Without retrieval, the acquisition memory remains stable and so the extinction training may, under these conditions, lead to the formation of a new memory in which the CS predicts “no US”. These animals would then have two memories: CS-US and CS-noUS. However, if extinction training occurs while the acquisition memory is labile, the updating process could lead to a revaluation of the original CS-US memory such that the memory now represents a relationship between CS and US in which the associative strength between the two is weakened. For example, the updated memory may be one in which the CS predicts “occasional US”. Of the total 22 exposures to the CS, only three of these resulted in the delivery of shock. This is perhaps more similar to an unlearning account in that the result is less excitatory strength between the CS and the US and a reduction in CR production which

is likely to be more permanent than in the case of new learning. As a result of extinction within the reconsolidation window, animals may have a single, slightly excitatory memory rather than two strong memories of opposing valence.

The assumed mechanism through which pre-extinction retrieval has its effects on extinction learning involves the retrieval-induced destabilisation of the CS memory (Monfils et al., 2009; Schiller et al., 2010). The effect is therefore dependent on successful retrieval of the CS-US memory. However, when the CS is presented again after extinction, what will be the memory that is retrieved? The outcome of extinction training may be the formation of a new, CS-noUS memory which will coexist with the original excitatory CS-US memory (Bouton, 1993). In this case, when the CS is presented at pre-reacquisition retrieval there is ambiguity in the meaning of the CS. According to Eisenberg et al. (2003), when more than one memory trace exists relating to a particular stimulus, the memory which has control over responding is the one which will become sensitive to reconsolidation blockade. If responding to the CS after extinction training is high, this may be an indication that it is the acquisition memory that was retrieved and so this would be the memory which would be subject to disruption. If responding to the CS is low, on the other hand, this may reflect retrieval of the extinction memory and so in this case the CS-noUS memory will be the one susceptible to post-retrieval interference.

In contrast, if retrieval prior to extinction leads to an updating of the memory rather than new learning, then it is likely that there is only one memory available to be retrieved. If retrieval of this memory is sufficient to induce memory destabilisation, then retrieval prior to reacquisition would be expected to open that memory to changes in associative strength on the basis of reacquisition training.

So, why would the presence of a single, weakly excitatory memory interfere with new excitatory learning, or at least the expression of it? Animals extinguished under standard

extinction conditions would presumably retain some memory of the original excitatory conditioning phase such that at the time of reacquisition this memory could be retrieved to aid in the generation of a conditioned response. Without such a memory to draw on, animals extinguished under the Monfils conditions would rely only on new learning. However, this account cannot explain why retrieval prior to extinction results in an impairment in learning relative to naive control animals since these control groups have no prior learning to draw on either. It appears instead that the learning established during extinction dominates learning and interferes with excitatory learning, or responding, occurring at a later stage. One explanation for this effect is that the salience of the updated memory facilitates retrieval and produces a conflict at the time of reacquisition between the existing weak association and the new excitatory learning. This explanation is similar to that offered by Bouton (1986) for the slow reacquisition observed following extended extinction. Although, in this case the competing memories were of opposite valence – an inhibitory extinction memory and an excitatory reacquisition memory – so it is easier to see how retrieval of the former might impair acquisition of the latter.

An alternative account of the retardation effect seen in the present studies is that the updated memory was established within multiple sessions across two days of experimentation (i.e., acquisition, retrieval and extinction) whereas the reacquisition memory was formed on the basis of a single session, often with just a single trial. The temporal context of the new reacquisition memory is of a shorter time frame and so generalising to other temporal contexts may be impaired relative to a memory established across a range of temporal contexts. Exposure to multiple physical contexts during extinction has been shown in many studies to facilitate the generalisation of extinction training when the CS is later tested in an associatively neutral context (e.g., Gunther, Denniston, & Miller, 1998; Chelonis, Calton, Hart, & Schachtman, 1999; but see Bouton, García-Gutiérrez, Zilski, & Moody, 2006). If the

same is true of temporal contexts, which have been attributed a similar role to physical contexts in their control over memory retrieval (Bouton, Westbrook, Corcoran, & Maren, 2006), then this could provide a reason for the persistent dominance of the updated memory over the reacquisition memory.

A different approach to the question of the contents of memory following the retrieval-extinction treatment is to revisit the possibility that new learning can cause an interference in the reconsolidation of a reactivated memory (Walker et al., 2003). Extinction within the reconsolidation window may therefore reduce conditioned responding not by masking (Bouton, 1993) or updating (Lee, 2009), but by preventing the restabilisation of the reactivated memory. This would essentially lead to amnesia for the acquisition memory and the formation of a new memory representing the extinction learning. While this suggestion might seem to be in contrast to reports of new information being incorporated into the reactivated memory (e.g., Hupbach, Gomez, Hardt, & Nadel, 2007), a key factor might be the compatibility of the two memories. In the experiments of Hupbach et al. (2007), an episodic memory for a list of items was retrieved prior to exposure to a second list of items. The result was that participants later falsely recalled items from the second list as having been a part of the first learning episode. The items from the second list, however, were still completely compatible with the items of the first list. Therefore, there was no reason why the participants should not, in principle, have been able to maintain the two memories in parallel. The paradigm employed by Walker et al. (2003), in contrast, involved learning two distinct and incompatible motor responses. The two memories could not be integrated in any meaningful or adaptive way, and so it appears that the memory for the second learning episode replaced the memory for the first. In the present studies, the two learning episodes comprise one phase where the CS is trained to predict the foot-shock US and thus to elicit a conditioned fear response, and a second phase in which the CS is trained to no longer predict the US (or

anything else for that matter) and to no longer elicit a CR. If these two learning experiences were interpreted by the animal as incompatible, then it is possible that the extinction memory replaced rather than updated the original acquisition memory.

The dominance of the extinction memory over the reacquisition memory appears robust, whatever form it may take. To overcome the resulting impairment in conditioned responding one approach may be to focus on this dominant memory trace directly. Whether conceptualised as a single, weakly excitatory association between CS and US resulting from the revaluation of the acquisition memory, or an extinction memory formed instead of or in parallel to the original association, it is possible that this memory would be subject to some of the same manipulations as the original, strongly excitatory, acquisition memory. For example, retrieval of this memory may lead to destabilisation and reconsolidation, and therefore the potential for a second round of reconsolidation interference or memory updating. From the updating perspective, incorporating the new excitatory learning into the existing memory would result in the maintenance of a single CS-US association but with a greater excitatory associative strength than prior to reacquisition. The new learning would then contribute directly to conditioned responding rather than having to compete with the dominant CS-"occasional US" memory. Alternatively, retrieval could result in destabilisation of an extinction memory which could then be replaced by new excitatory learning.

The experiments presented in the current chapter examined whether the impairment in relearning observed in the previous chapters can be reversed through the same technique used to establish this enhanced extinction in the first place. That is, can retrieval of the CS memory prior to reacquisition initiate a reconsolidation phase during which the memory can be updated or replaced for a second time? To address this question, three experiments were designed in which the effect of memory retrieval prior to reacquisition was assessed.

Experiment 4.1

Monfils et al. (2009) demonstrated facilitated extinction learning by presenting the CS one hour prior to extinction training. This finding has been replicated in the present studies as indexed by slower reacquisition of conditioned fear. It has been suggested that the enhanced extinction is the result of destabilisation of the existing CS memory prior to additional (i.e., extinction) training of the CS. The new learning then replaces, or is incorporated into, the original memory rather than forming a new memory which must compete with the original memory for control of conditioned responding. According to this view, it would not be unreasonable to expect that the destabilisation of any associative memory would occur following retrieval in the presence of new and relevant information (Lee, 2009). Thus, in the same way that extinction learning can be facilitated by retrieval of the conditioning memory, reacquisition may be facilitated by retrieval of the extinction memory. The following experiment tested this prediction. As in previous experiments, animals were first conditioned with pairings of the clicker CS with foot shock, after which they were extinguished with or without prior retrieval (see Table 9). The protocol so far is similar to experiments presented here and in Monfils et al. (2009). The following day, all groups were reconditioned to the CS. Critically, one of the groups having received retrieval prior to extinction (Ret-Ret) was again given a brief reminder trial prior to reacquisition training. On the basis of previous experiments, animals given retrieval prior to extinction but not prior to reacquisition (Ret-NoRet) were expected to relearn slower than animals given retrieval prior to neither extinction nor reacquisition (NoRet-NoRet). However, if reactivation of the extinguished CS memory prior to reacquisition can destabilise the memory, this may have been expected to speed up reacquisition potentially reversing the impairment otherwise produced by pre-extinction retrieval allowing Ret-Ret animals to reacquire fear more rapidly than those given retrieval prior to extinction only (Ret-NoRet). Not only does this experiment begin to address

the generality of the effect by looking for facilitation of excitatory learning, it also aims to investigate a manipulation with which it may be possible to reverse the effect of the Monfils effect. This experiment tested the assumption that memory retrieval is sufficient for destabilisation and whether it is possible for the reconsolidation process to occur more than once for a given memory. Views of reconsolidation as a process for maintaining up-to-date memories would presumably lead to the expectation that memories can be updated more than once. Otherwise, this property of memories would be of only limited benefit. Further, these questions are relevant to clinical applications of the paradigm as it can highlight situations in which the beneficial features of the treatment may be undone, as well as techniques for overcoming the potentially undesirable impairments in learning.

Table 9: Design of Experiment 4.1

Group	Acquisition	Ret	Extinction	Ret	Reacquisition	Test
Ret-Ret	3 x C+	C	18 x C	C	1 x C+	3 x C
Ret-NoRet	3 x C+	C	18 x C	-	1 x C+	3 x C
NoRet-NoRet	3 x C+	Cxt	19 x C	-	1 x C+	3 x C
Naive	-	-	-	-	1 x C+	3 x C

N.B. Ret = retrieval; NoRet = no retrieval; C = clicker CS; "+" indicates a reinforced CS presentation (no "+" means CS presentations were not reinforced).

Materials and Methods

Subjects

The subjects were 32 adult male Lister Hooded rats (Charles River, UK).

Apparatus

All behavioural procedures took place in the experimental chambers previously described. The chambers were illuminated by a red houselight throughout all procedures. The CS duration in all phases was 60 s.

Behavioural Procedures

Habituation. All animals were placed in the chambers for one hour per day on two consecutive days to familiarise them with the training context and to minimise the possibility for fear to accrue to the context which could potentially summate with that to the CS, thus obscuring the interpretation of the observed results during training and test.

Acquisition. After a 30 min adaptation period in the conditioning chamber, rats in Groups Ret-Ret, Ret-NoRet and NoRet-NoRet were given 3 trials of a 1 min clicker CS co-terminating with a 0.5 s, 0.5 mA foot-shock (US) with an average inter-trial interval of 5 min. Each rat was removed from the conditioning chamber 1 min following the last trial and returned to its home cage.

Retrieval 1. Rats in Groups Ret-Ret and Ret-NoRet were returned to the conditioning chambers one day following conditioning. After 2 min spent in the context in which training occurred, the CS was presented once for 1 min. The rats were removed from the chambers one minute later and returned to their home cages. Rats in the NoRet-NoRet group were placed in the context for 4 min.

Extinction. One hour following retrieval, rats were returned to the conditioning chambers for extinction. For rats in the Ret-Ret and Ret-NoRet groups, the extinction session comprised 18 non-reinforced presentation of the CS with an ITI of 2 min. Rats in the NoRet-NoRet group received extinction training with 19 trials such that the total number of non-reinforced CS presentations would equal that of the groups having been presented with the CS during retrieval.

Retrieval 2. One day following extinction training, rats in Group Ret-Ret were returned to the conditioning chambers. The CS was presented after a 2 min adaptation period. The rats were removed from the chambers one minute later and returned to their home cages for one hour before reacquisition training.

Reacquisition. One hour following Retrieval 2, all groups of rats were returned to the conditioning chambers for further excitatory conditioning of the CS and US. An additional group of rats that had not received any prior training (Naïve) was also conditioned at this time. The reacquisition session consisted of a 10 min adaptation period followed by a 1 min presentation of the CS co-terminating with the US.

Test. A retention test was given 24 h after reacquisition which consisted of three non-reinforced presentations of the CS with an ITI of 120 s after an adaptation period of three minutes.

Statistical Analyses

The pre-CS periods for each session were analysed using a One-Way ANOVA comparing groups in terms of average freezing during the two minutes prior to first CS onset. Where overall ANOVAs were found to be significant, the appropriate post-hoc analyses were applied.

Acquisition. Freezing to the CS during acquisition was analysed using a repeated-measures ANOVA with Group (Ret-Ret, Ret-NoRet, NoRet-NoRet) as the between-groups factor and Trial (a linear transform of trials 1-3) as the within-subjects factor.

Retrieval 1. Data from the CS presentation during retrieval for Groups Ret-Ret and Ret-NoRet were compared to data from the first extinction trial for Group NoRet-NoRet as a measure of retention of the CS-US association.

Extinction. A within-subjects linear contrast was applied to the 18 extinction trials (excluding trial 1 for Group NoRet-NoRet) to determine whether the extinction training resulted in a significant decrement in conditioned freezing.

Retrieval 2. Freezing data for Group Ret-Ret during the pre-reacquisition retrieval trial was compared to levels of freezing to the CS for the same animals at the pre-extinction retrieval trial by way of a mixed ANOVA.

Reacquisition. Freezing to the CS during reacquisition was analysed for group effects using the One-Way ANOVA procedure.

Test. For the CS presentations during the test phase, the group effects were assessed using planned orthogonal contrasts. These contrasts were designed to determine whether the groups that had received a pre-extinction retrieval session differed from the extinction-only and naïve groups ((Ret-Ret, Ret-NoRet) v (NoRet-NoRet, Naïve)), whether the pre-reacquisition retrieval trial was effective in reversing the effect of the pre-extinction retrieval trial (Ret-Ret v Ret-NoRet), and whether the rats extinguished without the retrieval trial showed savings with respect to a group without any prior training (NoRet-NoRet v Naïve).

Results

Acquisition

No animals displayed any freezing during the two min pre-CS period and so no further analysis was carried out for this period.

The mean (± *SEM*) percentage of observations scored as freezing within each of the three CS periods during acquisition is shown in Figure 43. Acquisition data from two subjects in Group Ret-Ret, one subject in Group Ret-NoRet and one subject in Group NoRet-NoRet were lost due to a failure of the recording apparatus in the chambers these animals were allocated to. As the loss of data was unrelated to group allocations, the assumption of

equivalence of groups was not violated and so the analysis was performed with these values missing. Subsequent analyses for this experiment included the data from these animals.

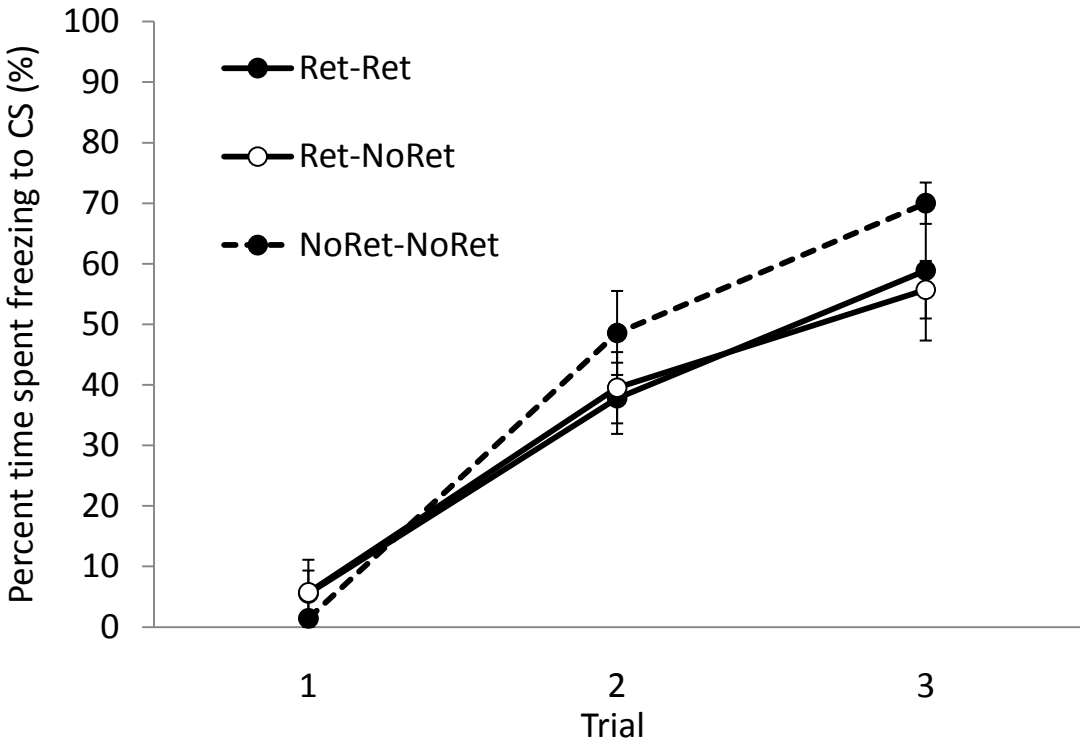

Figure 43. Freezing to the CS across conditioning trials. Circles represent mean ± *SEM*.

A linear contrast analysis revealed a significant increase in freezing behaviour across the course of conditioning, $F(1, 17) = 119.3$, $p < .001$, indicating that the procedure was successful in conditioning fear to the CS. No significant overall effect of group was observed at this stage of the experiment, $F(2, 17) = 1.15$, $p = .339$, and neither was there a significant interaction between group and the linear increase in conditioned responding, $F(2, 17) = 1.24$, $p = .315$, supporting the expectation that extinction rates were comparable for the three groups.

Retrieval 1

The first two minutes of the retrieval session were compared between groups as a measure of contextual fear. Levels of freezing in Group Ret-NoRet were $M = 1.00$, $SD = 1.50$. In all other groups, means and standard deviations were precisely 0. This resulted in a

significant group difference in contextual freezing, $\chi^2(2) = 6.72$, $p = .035$, suggesting that Group Ret-NoRet had acquired a certain degree of fear to the context during the course of conditioning.

Freezing during the CS presentation for Groups Ret-Ret and Ret-NoRet are shown at the left of Figure 44. Unfortunately, camera failure again resulted in the loss of data from three subjects in Group Ret-NoRet. To minimise the impact of this loss on the analysis, data from this retrieval trial for Groups Ret-Ret and Ret-NoRet were compared with data from the first trial of extinction for Group NoRet-NoRet. This analysis would serve as an index of retention of the CS-US association and allow the remaining 18 trials of extinction to be analysed with all subjects being included. The analysis of the first CS-alone trial, whether as a part of the retrieval session or extinction session, revealed no significant differences as a function of group, $F(2, 18) < 1$.

Extinction

All groups were consistent in their absence of freezing during the two minutes prior to first CS presentation during extinction. Thus, the difference in pre-CS freezing observed during retrieval did not persist to the extinction session one hour later.

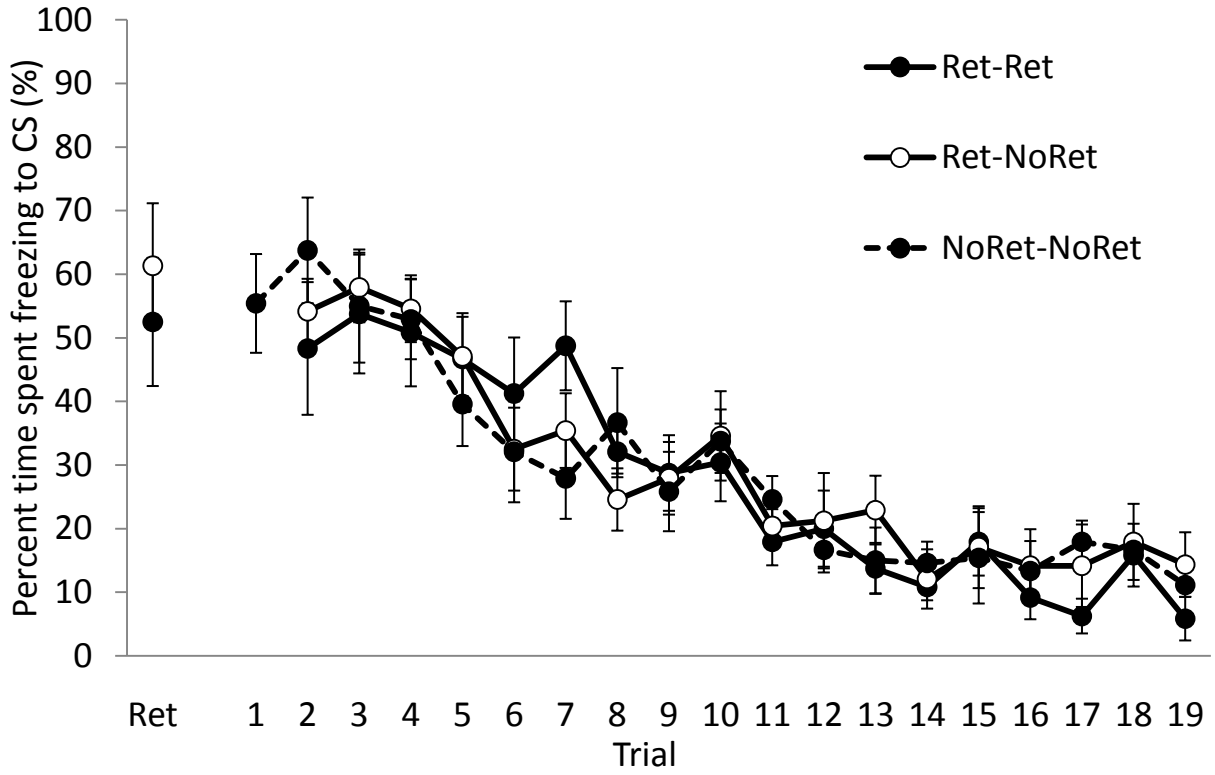

Figure 44. Freezing to the CS during retrieval (Ret) and extinction. Circles represent mean ± *SEM*.

The 18 remaining extinction trials were analysed using a linear contrast to confirm that the extinction training was successful in reducing levels of conditioned responding to the CS. There was a significant linear decrease in conditioned freezing observed across extinction trials, $F(1, 21) = 117.7$, $p < .001$. This linear decrement was not significantly different between groups, $F(2, 21) < 1$, indicating that conditioned freezing extinguished at a similar rate in each of the three groups. No overall group differences were observed when data from the 18 trials was collapsed, $F(2, 21) < 1$.

Retrieval 2

Responding of Group Ret-Ret during the pre-reacquisition retrieval session was compared with responding during the pre-extinction retrieval trial. This analysis revealed that rats in this group spent significantly less time freezing during the CS during this second

retrieval session than during the first, $F(1, 7) = 26.93$, $p = .001$. This may be taken as an indication that the rats were retrieving the extinction memory rather than the conditioning memory at Retrieval 2.

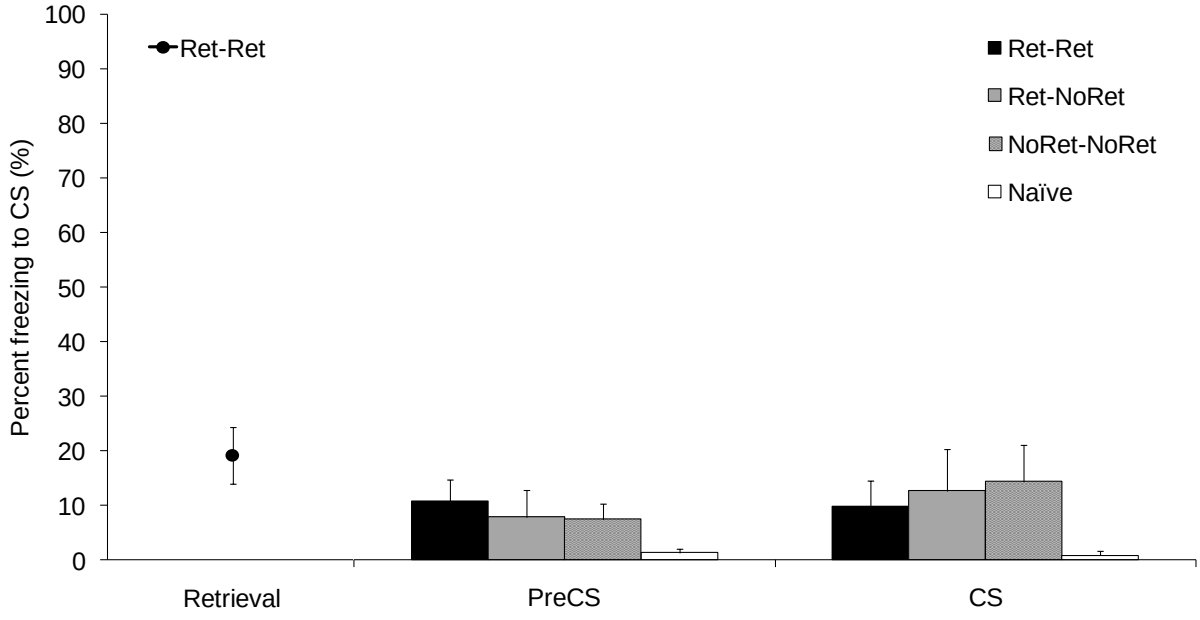

Figure 45. Left panel: Responding to the CS for Group Ret-Ret at pre-reacquisition retrieval. Right panels: Freezing during the pre-CS and CS periods for all groups during the reacquisition session. Circle/bars represent mean ± *SEM*.

Reacquisition

Freezing to the context during the two minutes prior to CS onset did not differ across the four groups, $F(3, 24) < 1$; *M*s (*SEM*s) of percent time freezing: Ret-Ret = 4.8 (1.9), Ret-NoRet = 3.8 (2.0), NoRet-NoRet = 5.6 (3.0), Naive = 0.6 (0.4). Furthermore, no differences were found in terms of freezing to the CS, $F(3, 24) < 1$. Given that these data were derived from a single trial of CS and US, and that the US was not presented until the last 0.5 s of the CS, responding on this trial is an index of residual fear of the CS after extinction. The absence of group differences is indicative of a lack of distinction in levels of fear between

those rats having been conditioned and extinguished and those for whom the CS was completely novel.

Test

Data from the retention test is presented in Figure 46. Freezing to the context during the three minutes prior to CS onset did not differ across groups, $F(3, 28) = 1.78$, $p = .174$, providing no evidence for differences in levels of contextual fear acquired over the course of experimentation.

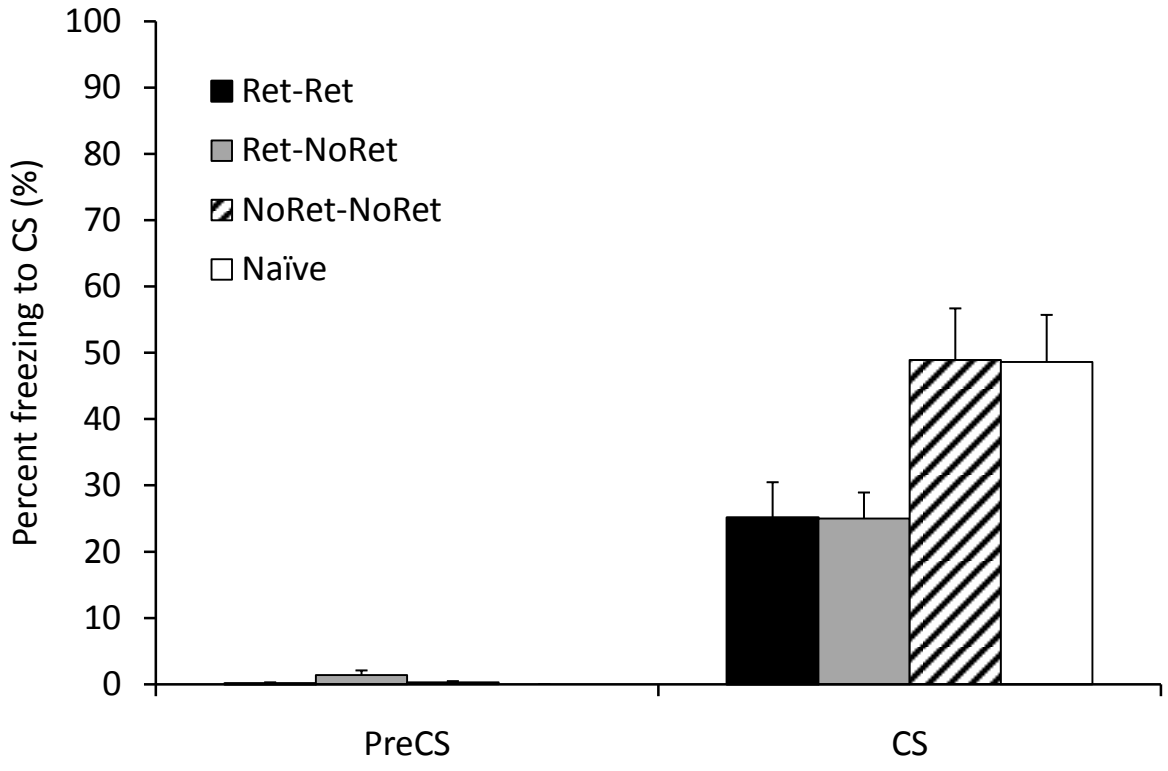

Figure 46. Freezing during the pre-CS and CS periods at test. Bars are representative of mean percentage of time spent freezing ± *SEM*.

A significant difference between Groups Ret-Ret and Ret-NoRet with comparison to Groups NoRet-NoRet and Naïve indicates that the pre-extinction retrieval trial successfully impaired subsequent reconditioning of the CS, $F(1, 28) = 14.49$, $p = .001$. Interestingly, a retrieval trial given prior to reacquisition was not successful in reversing this effect as there

was no significant difference observed between Group Ret-Ret and Group Ret-NoRet, $F(1, 28) < 1$. Group NoRet-NoRet was not significantly different from Group Naïve, $F(1, 28) < 1$, demonstrating that rats extinguished without a reminder cue reacquired fear to a degree comparable with that of rats learning the association for the first time. No significant linear trend was observed across the three trials, $F(1, 28) < 1$, nor any significant between- by within-groups interactions, $F(1, 28) < 1$, $F(1, 28) = 1.13$, $p = .297$, $F(1, 28) < 1$, respectively. In summary, retrieval prior to extinction impaired subsequent reacquisition, with this effect persisting even when the CS was presented again prior to reacquisition.

Discussion

The aim of this experiment was to identify a method by which it might be possible to reverse the impairment in reacquisition observed following extinction with retrieval. To this end, comparison was made between groups extinguished with or without retrieval and, for those extinguished with retrieval, a further comparison was made between those given a second retrieval trial prior to reacquisition and those retrained without retrieval. The results replicated previous experiments in demonstrating a robust impairment in reacquisition when animals were extinguished after retrieval. Interestingly, this effect was also observed when the CS was presented again one hour prior to reacquisition training. Thus, there was no indication that reacquisition was facilitated by retrieval of the extinguished CS memory.

To understand why the same treatment which facilitated extinction (by destabilising the acquisition memory) was insufficient to facilitate reacquisition (by destabilising the extinction memory) it may be helpful to examine some of the differences between the retrieval sessions at each of the two time points. There are many differences between these two trials, some of which may have affected the capacity of the CS memory to become unstable and thus preventing the memory from being updated or replaced, in this case through reacquisition. Without memory destabilisation, a new excitatory CS-US memory

would need to be formed and this would compete with the strong extinction memory for control of responding at test.

Firstly, the aim of the CS presentation given prior to extinction was to retrieve a memory which had been established over three conditioning trials on the previous day. In contrast, during the second retrieval session, the presentation of the CS was intended to retrieve the extinction memory, one which had been established over 18 trials, or perhaps the updated acquisition memory which incorporated the 18 trials of extinction with the original excitatory learning. Eisenberg et al. (2003) have demonstrated that with stronger training, more presentations are required to induce a reconsolidation phase. Thus, it is possible that for an extinction memory established across 18 or 19 trials, compared to an acquisition memory established over 3 trials, a greater number of retrieval trials would be needed to effectively destabilise the memory trace. If insufficient trials were given at the pre-reacquisition retrieval session, the memory may not have been destabilised and so could not be updated with the new information: that the CS again predicts the US.

A second difference between pre-extinction and pre-reacquisition retrieval is that at the time of retrieval of the excitatory memory, the CS had only ever been presented paired with the US. Therefore, the meaning of the CS was unambiguous in predicting the delivery of the foot-shock. During extinction, however, the CS no longer predicted the US and so acquired a new meaning. Thus, when the CS was then presented again at retrieval prior to reacquisition, the stimulus had had a mixed reinforcement history. The ambiguity in the predictiveness of the CS may mean that rather than destabilising the extinction memory, the retrieval trial may have destabilised the acquisition memory. Yet, should the acquisition memory have been destabilised, strengthening of this association too should have been expected to facilitate fear responding at test. Furthermore, according to an updating account of the pre-extinction retrieval effect, animals given retrieval prior to extinction would have

updated the original conditioning memory to accommodate the extinction learning, and so it would be assumed that only one memory trace would exist at the time of pre-reacquisition retrieval. These animals are likely to have a memory in which the CS is represented as "usually innocuous". It should then be this memory that would be unambiguously retrieved when the CS was presented prior to reacquisition.

A third, and particularly relevant, difference relates to the issue of prediction error. In assuming that the role of reconsolidation is to maintain memory relevance (Lee, 2009), it would be expected that destabilisation would occur if, and only if, new information were available in the environment. When the CS is presented prior to extinction, the expectation of shock is violated. This error in prediction may then signal a change in the contingency between the CS and US and therefore trigger memory destabilisation. The new information would then replace or be incorporated into the existing memory and then (re)consolidated. In contrast, when the CS is presented again before reacquisition, the animals have just received 19 CS-alone trials and so the non-reinforcement of the CS is not surprising. Assuming that extinction had achieved asymptotic levels, an assumption supported by the minimal levels of freezing observed by the end of extinction, one more presentation of the CS in the absence of the US is not sufficient reason to destabilise the memory and engage reconsolidation processes. The next experiment in this chapter addressed this question by providing a possibility for update of the retrieved memory. The central question was whether if an update (captured in terms of prediction error) is necessary for re-consolidation to occur.

Experiment 4.2

The following experiment aimed to determine whether the introduction of a prediction error at the time of pre-reacquisition retrieval is necessary for reconsolidation to occur. In particular, the aim was to assess whether retrieval with a prediction error would destabilise the extinction memory and allow fear responding to be re-established to the CS. In order to

examine this question, the experimental design outlined in Table 10 was employed. The most important difference between this and the previous experiment was the addition of Group Ret-Ret+, which received treatment identical to that of Group Ret-Ret- with the exception that the presentation of the CS one hour prior to reacquisition training was reinforced by the delivery of the foot shock US. To ensure equal exposure to the pairings of CS and US, this group then received a single pairing of CS and US at reacquisition, while the remaining groups received two CS-US pairings. In addition, those groups which did not receive retrieval prior to reacquisition were exposed to the context for an equivalent period of time where in the previous experiment these animals remained in their home cages. This was to ensure that any group differences in reacquisition could not be attributed merely to exposure to the conditioning chambers.

Table 10: Design of Experiment 4.2

Group	Acquisition	Ret	Extinction	Ret	Reacquisition	Test
Ret-Ret+	3 x C+	C	18 x C	C+	1 x C+	2 x C
Ret-Ret-	3 x C+	C	18 x C	C	2 x C+	2 x C
Ret-NoRet	3 x C+	C	18 x C	Cxt	2 x C+	2 x C
NoRet-NoRet	3 x C+	Cxt	19 x C	Cxt	2 x C+	2 x C
Naive	-	-	-	Cxt	2 x C+	2 x C

N.B. Ret = retrieval; NoRet = no retrieval; C = clicker CS; "+" indicates a reinforced CS presentation (no "+" means CS presentations were not reinforced).

The prediction for this experiment was that reinforcement of the pre-reacquisition retrieval trial should facilitate learning during reacquisition. Thus Group Ret-Ret+ was

expected to display more freezing to the CS at test relative to a group given a non-reinforced presentation of the CS (Ret-Ret-). On the basis of the previous experiment, the non-reinforced retrieval trial prior to reacquisition was not expected to reverse the retardation effect. This effect should be demonstrated in the difference in responding between Groups Ret-NoRet and NoRet-NoRet. Groups NoRet-NoRet and Naive were compared to assess savings in reacquisition.

Methods

Subjects

The subjects used were 40 adult male Lister hooded rats (Charles River, UK).

Apparatus

All experimental procedures were conducted in the chambers described previously with the red houselight remaining on for the duration of experimental procedures. The CS duration in all phases was 60 s.

Procedure

Habituation. All animals were habituated to the context over two days with one hour of context exposure on each day. No stimuli were presented during this phase of the experiment.

Acquisition. The following day, all animals with the exception of those in Group Naive were brought to the experimental chambers for acquisition training. After 30 min in the experimental context, three pairings of the 60 s clicker CS with a 0.5 s 0.5 mA foot-shock were presented with an average ITI of five minutes. All animals were removed from the chambers 60 s after the final CS presentation.

Retrieval 1. All groups which had been undergone acquisition training were returned to the experimental chambers the following day. Ret-Ret+, Ret-Ret- and Ret-NoRet were presented with the CS once after two minutes in the context. Group NoRet-NoRet was

exposed to the context for an equivalent period of time. All animals were then returned to their home cages for one hour before being brought back for extinction training.

Extinction. For those animals having received a presentation of the CS during retrieval, the extinction session comprised 18 non-reinforced presentations of the CS with an adaptation period and ITI of two minutes. Those in Group NoRet-NoRet, which had not been exposed to the CS, received 19 CS alone presentations.

Retrieval 2. The following day, all groups, including now the Naive group, were brought to the experimental chambers for a brief retrieval trial. For rats in Group Ret-Ret-, this session was identical to Retrieval 1. Group Ret-Ret+ differed only in that the CS presentation co-terminated with the US. For the remaining three groups, no stimuli were presented during the three-minute session.

Reacquisition. Animals in Group Ret-Ret+ were presented with a single pairing of CS and US in the manner described for reacquisition in Experiment 4.1. All other groups were given two pairings of CS and US to equate them with Ret-Ret+ on total number of reinforced trials. The ITI between trials was five minutes.

Test. One day following reacquisition training, all groups were returned to the chambers for a test of retention of the CS-US association. Two CS alone trials were presented after an initial delay of three min with a three min ITI. The use of two rather than three trials was to minimise extinction of the CR during the test session.

Statistical Analyses

Levels of freezing during the pre-CS periods were compared between groups with One-Way ANOVAs. Because differences in contextual freezing between groups were not expected, post-hoc analyses were used where this test resulted in a significant *F* statistic.

Acquisition. Data from the three CS trials during reinforced training were analysed by way of a mixed ANOVA assessing differences between groups and across trials as well as the interaction between these two factors.

Retrieval 1. Data from the retrieval trial for Groups Ret-Ret+, Ret-Ret- and Ret-NoRet were compared with responding on the first extinction trial for Group NoRet-NoRet as an index of retention of the conditioned response.

Extinction. The remaining 18 trials of extinction were analysed as a linear contrast so as to assess the rate of extinction. The magnitude of the linear trend was compared between groups to detect any differences in rate of extinction that may have resulted from the difference in pre-extinction treatment. Overall group differences were analysed through the between-subjects component of the mixed ANOVA.

Retrieval 2. The two groups exposed to the CS at the pre-reacquisition retrieval session, i.e., Ret-Ret+ and Ret-Ret-, were compared on their levels of freezing to the CS using a One-Way ANOVA.

Reacquisition. Responding to the CS during the second retrieval session for Group Ret-Ret+ was included in the analysis of reacquisition data so that the groups could be compared across an equal number of CS-US trials. A mixed ANOVA was applied to assess differences between groups, differences across trials and differences between groups on the change from trial one to trial two.

Test. A set of planned contrasts were applied to the data to test the following effects, applying a Šidák adjusted α (α') of 0.017: (1) the effect of retrieval prior to extinction on the subsequent reacquisition of conditioned responding (Ret-NoRet v. NoRet-NoRet); (2) the effect of a single presentation of the CS prior to reacquisition on the reoccurrence of the conditioned response (Ret-Ret- v. Ret-NoRet); (3) the effect of the addition of the US at pre-reacquisition retrieval on subsequent relearning (Ret-Ret+ v Ret-Ret-); and (4) savings in

reacquisition of the CS-US association as a result of prior experience with the CS (NoRet-NoRet v. Naive).

Results

Acquisition

The two minutes prior to the first CS presentation produced no significant differences in freezing between the four groups conditioned at this time, $F(3, 28) < 1$; *M*s (*SEM*s) of percent time freezing: Ret-Ret+ = 3.8 (1.9), Ret-Ret- = 4.6 (1.4), Ret-NoRet = 4.0 (2.2), NoRet-NoRet = 4.2 (2.0). Data for the CS trials during acquisition are presented in Figure 47. A significant increase in freezing across the three CS+ trials was indicative of successful acquisition of fear responding to the CS, $F(3, 28) = 186.8$, $p < .001$. No overall difference, or any differences in the rates of acquisition, between groups were detected, *F*s < 1.

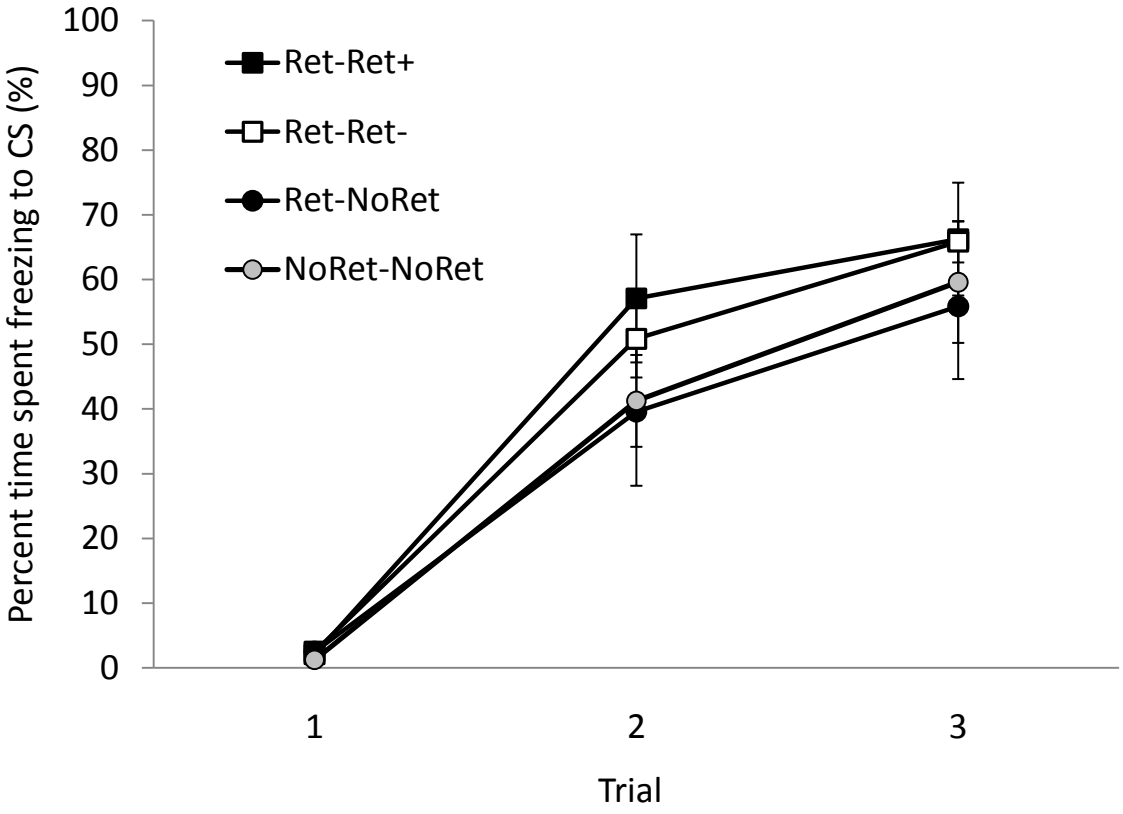

Figure 47. Freezing to the CS during acquisition training. Circles and squares represent group means ± *SEM*.

Retrieval 1

Freezing during the first two minutes of retrieval session 1 did not differ with group allocation, $F(3, 28) < 1$; *M*s (*SEM*s) of percent time freezing: Ret-Ret+ = 1.7 (1.3), Ret-Ret- = 0.4 (0.3), Ret-NoRet = 0.2 (0.2), NoRet-NoRet = 0.8 (0.6). Data from the CS period are shown at the left of Figure 48. Including the first extinction trial for Group NoRet-NoRet in the analysis, freezing to the CS was also not different for group, $F(3, 28) = 1.71$, $p = .188$, providing no evidence for differences in retention of the CS-US association at this point in the experiment.

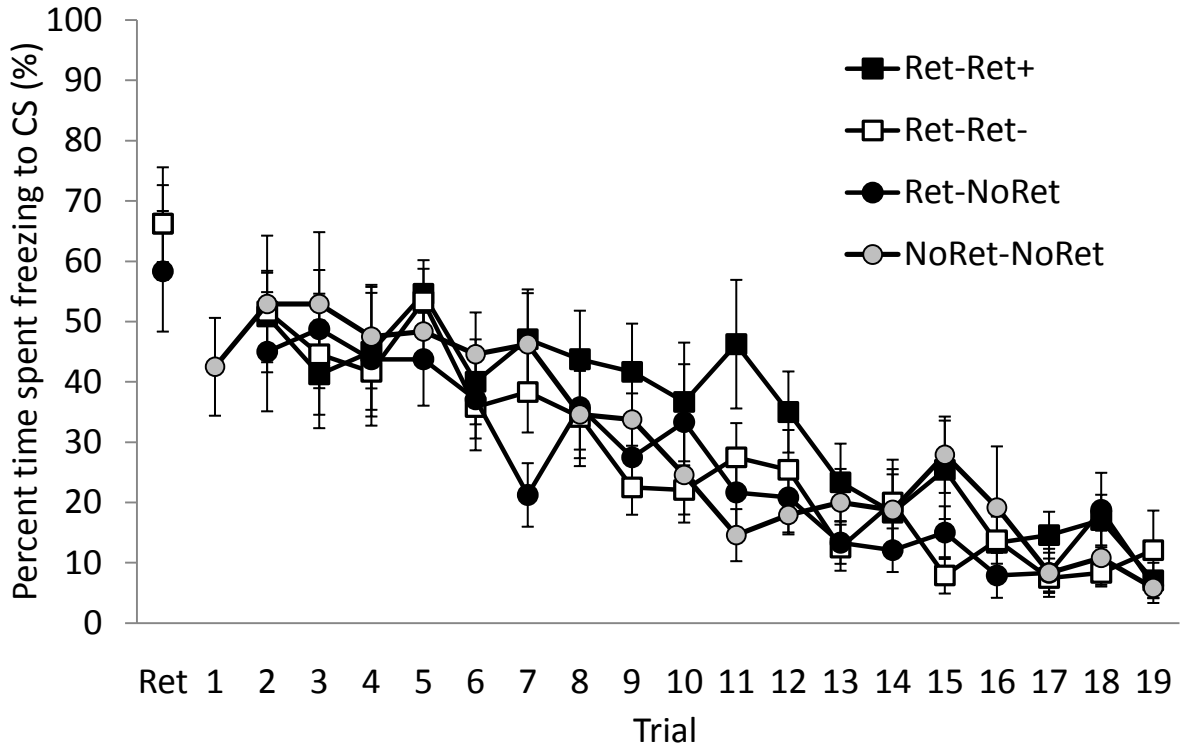

Figure 48. Freezing to the CS during retrieval (Ret) and extinction. Circles and squares represent group means ± *SEM*.

Extinction

Levels of freezing during the two minutes prior to the first extinction trial displayed no reliable group differences, $F(3, 28) = 1.47$, $p = .243$; *M*s (*SEM*s) of percent time freezing: Ret-Ret+ = 0.0 (0.0), Ret-Ret- = 0.6 (0.4), Ret-NoRet = 0.2 (0.2), NoRet-NoRet = 0.0 (0.0). Freezing to the CS over the course of extinction training can be seen in Figure 48. Analysis of the 18 trials of extinction (having included the first of the 19 trials for Group NoRet-NoRet in the analysis of retrieval data) displayed a significant linear decrease in freezing over trials, $F(1, 28) = 135.5$, $p < .001$, confirming that the extinction training was successful in reducing levels of conditioned responding to the CS. This decrement was not dependant on group allocation as no significant between × within interaction was detected, $F(3, 28) < 1$. Groups did not differ either in overall levels of freezing over the course of the session, $F(3, 28) = 1.04$, $p = .391$.

Retrieval 2

No significant effect of group was found for the two min of context exposure immediately before the retrieval trial, $F(4, 35) = 1.99$, $p = .117$. The left panel of Figure 49 shows levels of freezing to the CS for Groups Ret-Ret+ and Ret-Ret-. Levels of freezing to the CS for these groups were compared using a One-Way ANOVA. Levene's Test for Equality of Variances indicated significantly different variances between the two groups, Levene's $F(4, 35) = 6.04$, $p = .001$, and thus Welch's F Test was applied. This analysis revealed no significant difference between the two groups in terms of freezing to the CS during the pre-reacquisition retrieval session, Welch's $F(1, 7.998) = 2.98$, $p = .123$. As this responding occurred prior to any differential treatment of these two groups, the absence of a group effect at this stage was consistent with expectation.

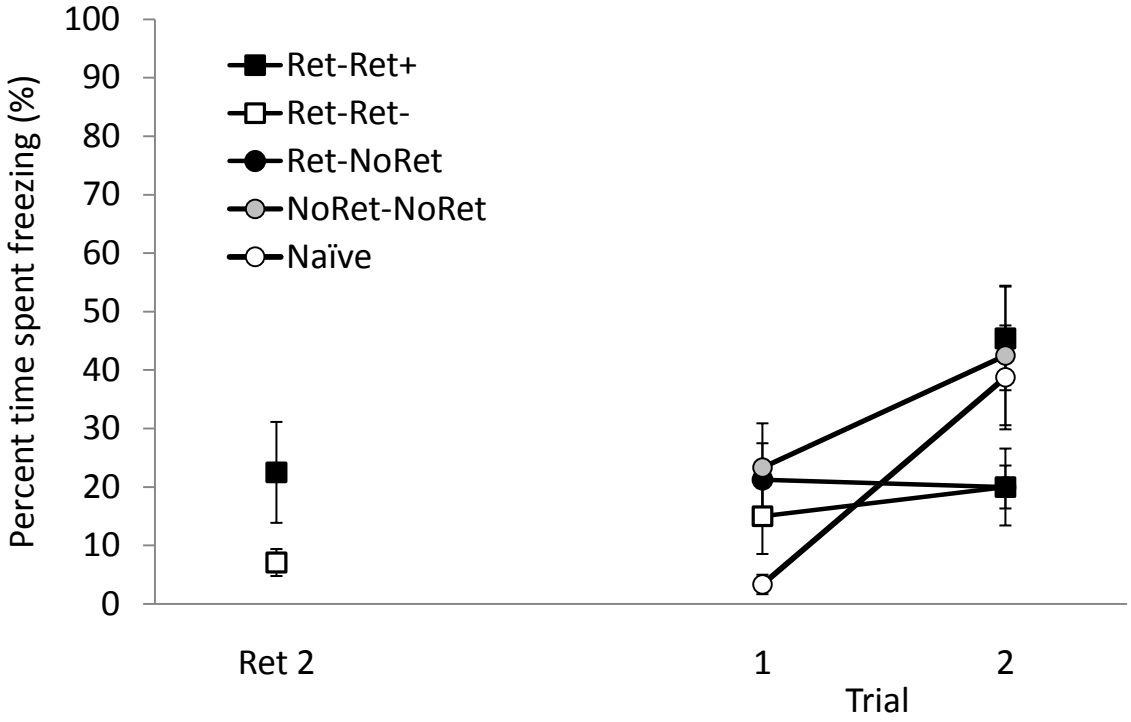

Figure 49. Freezing to the CS during pre-reacquisition retrieval (Ret 2) and reacquisition. Circles and squares represent group means ± *SEM*.

Reacquisition

During the two min prior to onset of the first CS of the reacquisition phase, a significant group difference in contextual fear responding was detected, $F(4, 35) = 5.04$, $p = .003$; *M*s (*SEM*s) of percent time freezing: Ret-Ret+ = 12.7 (3.8), Ret-Ret- = 5.8 (1.6), Ret-NoRet = 2.1 (1.1), NoRet-NoRet = 2.9 (1.3), Naive = 1.5 (1.0). Post-hoc orthogonal contrasts revealed this effect, in part, to arise due to significantly higher freezing for Group Ret-Ret+ than the average of the remaining groups, $F(1, 35) = 17.50$, $p < .001$, possibly the result of having received foot-shock an hour earlier during the retrieval session. The group with the next highest freezing score (Ret-Ret-) was not significantly different to the average of the remaining three groups, $F(1, 35) = 2.40$, $p = .131$. The remaining contrasts were not significant, *F*s < 1. In summary, the overall group difference in freezing to the context was

best accounted for by Group Ret-Ret+ showing more fear when returned to the experimental chambers one hour after a reinforced presentation of the CS.

Average freezing across all groups to the CS (Figure 49) increased from trial 1 to trial 2 of reacquisition training, $F(1, 35) = 18.23$, $p < .001$. While no overall differences between groups in levels of freezing were observed, $F(4, 35) = 1.50$, $p = .224$, the groups did differ in terms of the change in freezing from trial 1 to trial 2, as revealed by a significant interaction between these factors, $F(4, 35) = 2.95$, $p = .034$. Post-hoc contrast analysis, however, failed to detect any reliable and meaningful contrasts with the strongest tested contrast (Ret-Ret- and Ret-NoRet compared with the remaining groups) yielding $F(1, 35) = 9.52$, which failed to reach significance after comparison to the Scheffé adjusted critical value of 10.56. Overall it is possible to conclude that the reacquisition training significantly increased freezing to the CS when disregarding group allocations, and no meaningful group differences or interactions could be detected.

Test

Pre-CS freezing during test is shown in the left panel of Figure 50. Once again, the overall ANOVA of responding during the pre-CS period revealed a significant group effect, $F(4, 35) = 3.17$, $p = .025$. Again, Welch's F Test was utilised for post-hoc analysis of these data as the equality of variance assumption had been violated (Levene's $F(4, 35) = 8.88$, $p < .001$). Inspection of the data suggested the most likely contrast to be that comparing Group Ret-Ret+ to the average of the remaining groups. However, this effect failed to reach significance under Welch's correction for unequal variance, $F(1, 7.184) = 3.66$, $p = .096$.

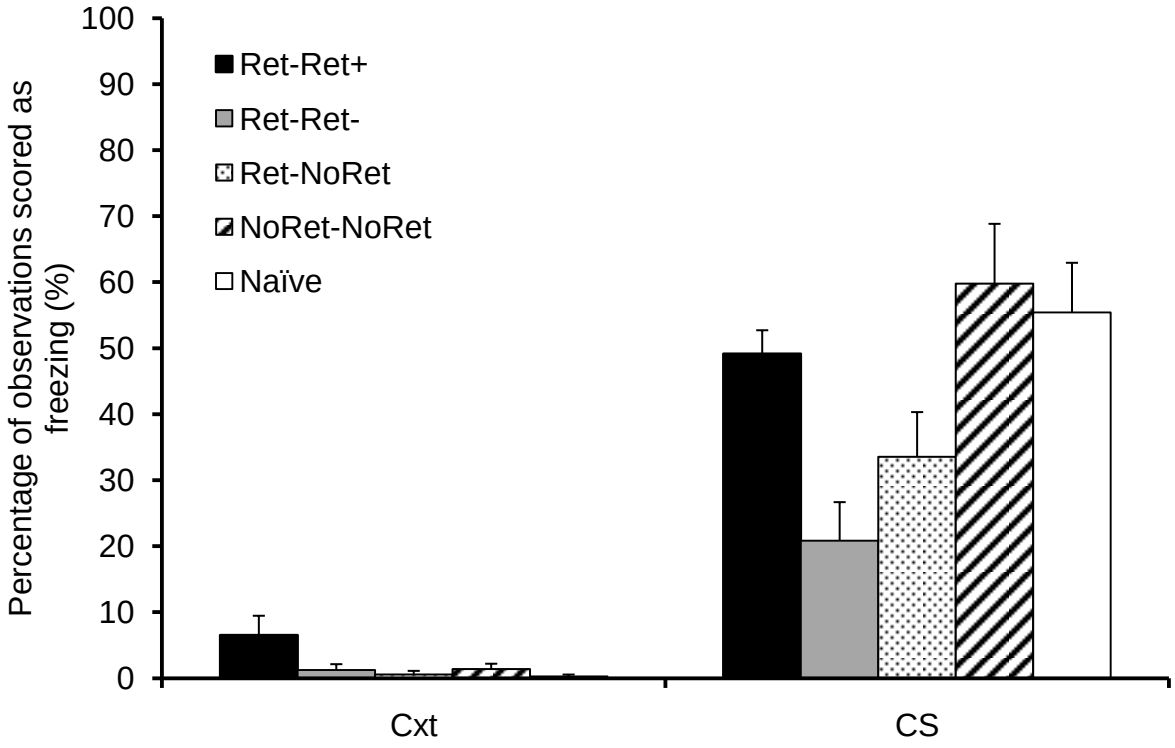

Figure 50. Freezing to the context (Cxt) and CS during test. For simplicity, CS data are represented as average freezing over the two CS presentations. Bars represent group means ± *SEM*.

Freezing to the CS did not change significantly from trial one to trial two, $F(1, 35) = 1.97$, $p = .169$. Thus, for simplicity, data presented in Figure 50 show average freezing across the two CS presentations. The between-subjects planned contrasts revealed a significant effect of pre-extinction retrieval, $F(1, 35) = 7.47$, $p = .010$, replicating the results of previous experiments in which a presentation of the CS prior to extinction results in impaired reacquisition of fear to the CS when compared with a group given extinction without a prior retrieval session (Ret-NoRet v. NoRet-NoRet). As was found in Experiment 4.1, a second retrieval trial involving a non-reinforced presentation of the CS did not reverse the impairment in reacquisition resulting from pre-extinction retrieval (Ret-Ret- v. Ret-NoRet), $F(1, 35) = 1.75$, $p = .195$. However, the addition of the US to the pre-reacquisition retrieval trial led to a subsequent improvement in reacquisition (Ret-Ret+ v Ret-Ret-), $F(1, 35) = 8.70$,

p = .006. Groups NoRet-NoRet and Naive were not significantly different, giving no evidence of savings in reacquisition following extinction, $F(1, 35) < 1$.

Discussion

The results of this experiment demonstrate once again the impairment in reacquisition resulting from a single presentation of the CS one hour prior to the start of extinction training. As in the previous experiment, this effect was not reversed by the presentation of the CS one hour prior to reacquisition training. However, reinforcing this presentation had a significant facilitatory effect on subsequent reacquisition of the CS-US memory.

These results are consistent with the expectation that prediction error at retrieval was necessary for destabilisation of the extinction memory so as to allow for the new excitatory training to be incorporated into the memory network. The delivery of foot shock at the end of the CS period would have been largely unexpected assuming successful retrieval of the extinction memory trained on the previous day. Thus, this trial would likely have signalled a change in contingencies and triggered the start of a reconsolidation phase in preparation for the possibility of new information about the CS becoming available. For animals receiving a non-reinforced trial, the presentation of the CS alone would have given no indication that any change in the stimulus contingencies was imminent. Similarly, the groups given no retrieval trial would have no opportunity to destabilise the CS memory prior to the learning phase. For those having received retrieval prior to extinction, this would mean that the new learning would be competing with the strong acquisition-retrieval-extinction memory for control of conditioned responding. The dominance of the stronger memory would result in freezing remaining relatively low.

However, a number of alternative explanations can be entertained which shall be analysed here before further pursuing the central question of this chapter. The first is that the pairing of the CS and US at retrieval effectively resulted in two reacquisition trials for Group

Ret-Ret+ being spaced by one hour, whereas the other groups received pairings spaced by five minutes. The spacing of trials in excitatory conditioning is well-known to facilitate learning (Barela, 1999) and so perhaps the improved reacquisition of Group Ret-Ret+ was due to this group having the benefit of an extended ITI during reacquisition. As discussed in more detail in Chapter 4, most learning theories account for trial spacing effects by highlighting the fact that longer ITIs generally result in greater overall exposure to the context. The extinction of the context which occurs during these periods in which the animals are exposed to the context allows the CS to acquire more associative strength (Rescorla & Wagner, 1972) or better control over conditioned responding (Miller & Matzel, 1988).

However, in the present study, the group receiving the reinforced retrieval trial prior to reacquisition had less total time of exposure to the context compared to the remaining groups due to the fact that the reacquisition session for these animals comprised only a single trial and so was shorter overall by six minutes. This translated into 6 minutes less exposure to the context on reacquisition day. Therefore, this group had less opportunity for context extinction and so, if anything, learning or responding to the CS should have been impaired rather than increased. The fact that this group showed facilitated fear responding to the CS at test suggests that trial spacing accounts involving context cannot provide a complete explanation of this effect. The account of trial-spacing effects offered by Wagner's SOP model, however, may offer an explanation for the results seen here (see Chapter IV, Introduction for an overview of this model). If, after a five min ITI, a sufficient proportion of CS elements remain active in A2 to impact negatively on learning on the next trial, then increasing the ITI to one hour would be expected to overcome this effect (i.e., self-generated priming) and so permit more learning to the CS. The potential of this model to explain the effect of retrieval prior to extinction was examined in Chapter 4 and little evidence was found to suggest that the spacing of trials by two minutes produced any interference from self-generated priming

which could be overcome by the expansion of the ITI to one hour. Of course, the situation in the current experiment differed from that in the experiments of Chapter 4 most notably in the fact that the trials in question here were reinforced by delivery of the US. It is difficult to see, though, how reinforcement of the same CS in the same context with the same US used in the earlier experiments would mean that more time would be required for stimulus elements to decay from A2 into the inactive state. Still, this explanation cannot be ruled out on the basis of the data presented here and additional studies would be warranted to explore this account further.

A second alternative explanation of the reversal of impairment seen in Group Ret-Ret+ is that the presentation of both the CS and US resulted in more of the memory trace being retrieved. It would seem reasonable to assume that the more components of a memory that are presented during a retrieval trial, the more effective that trial is as a reminder of previous learning. Such an effect is found for reconsolidation of morphine conditioned place preference with Milekic, Brown, Castellini, & Alberini (2006) demonstrating disruption of reconsolidation only when the context and morphine US were both present at retrieval. In so far as the foot shock US remains a part of the memory after extinction in the present experiment, the presentation of both the US and the CS would allow a greater portion of the memory to be destabilised and so would facilitate the updating process. In other words, rather than the reversal of impairment being due to prediction error, it may simply be the result of the presence of more retrieval cues on the trial.

Finally, the possibility remains that the presence of the CS at pre-reacquisition retrieval was redundant and that the important feature of the reinforced retrieval trial, which allowed learning to progress faster subsequently, was the mere presentation of the US. Recall in the last chapter an experiment was presented suggesting that learning was impaired when the same US was paired with a novel CS after extinction with prior retrieval. This effect was

discussed in the context of changes in either the intensity or the integrity of the US representation. If it is the case that the US representation is somehow weakened as the result of pre-extinction retrieval, reinstatement of the US memory prior to reacquisition may restore the representation such that it can again serve as an effective reinforcer. While Monfils et al. (2009) showed a lack of a reinstatement effect for animals extinguished after retrieval, this was assessed in the absence of any new learning. The reinstatement trial given in their experiment may have succeeded in restoring the US representation, but without further training, the association between the CS and US would have remained weak. The effect of the pre-extinction retrieval trial may involve both weakening of the association and of the US representation, but without both reinstatement and reconditioning, the absence of fear may persist. Thus it remains possible that the reversal of the impairment in reacquisition achieved by reinforcement of the pre-reacquisition retrieval trial reflects simply reinstatement of a degraded or revalued US representation rather than through prediction-error dependent memory destabilisation. The next experiment in this chapter assessed this possibility.

Experiment 4.3

On the basis of the data of the previous experiment there are reasons to believe that at least under some conditions the impairment in reacquisition following extinction with retrieval can be reversed. This cannot be achieved by a pre-reacquisition retrieval trial consisting only of the CS, but can be achieved when that CS presentation is reinforced by the US. While it is appealing to attribute this effect to the presence of a prediction error at the time of retrieval, at least two other likely candidates remain in the explanation of this effect. These include an account in terms of enhanced retrievability resulting from the presentation of additional memory components (Milekic et al., 2006), as well as an account emphasising the potential of the US delivery to restore the US representation.

The following experiment examined whether the facilitation of reacquisition could be adequately explained by retrievability or reinstatement accounts. The experimental design is outlined in Table 11. Three groups were trained with pairings of the clicker and foot shock. All groups then received extinction with retrieval. On the following day, all groups were reconditioned to the CS by additional CS-US pairings. Critically, this trial was preceded by a retrieval session during which animals received one of the following treatments: (1) a paired presentation of the CS and US, (2) explicitly unpaired presentations of the US and CS, or (3) the presentation of the CS alone.

Table 11: Design of Experiment 4.3

Group	Acquisition	Ret	Extinction	Ret	Reacquisition	Test
CS+	3 x C+	C	18 x C	C+	1 x C+	2 x C
CS/+	3 x C+	C	18 x C	C/+	2 x C+	2 x C
CS-	3 x C+	C	18 x C	C	2 x C+	2 x C

N.B. Ret = retrieval; C = clicker CS; "+" indicates a reinforced CS presentation, no "+" means CS presentations were not reinforced; C/+ indicates explicitly unpaired presentation of CS and US.

Based on the results of the previous experiment, it was expected that the Group CS+ would show more fear at test than Group CS-. Of particular interest in this experiment was the performance of Group CS/+. If the effect of the CS+ trial on reacquisition was due to the introduction of a prediction error at retrieval, then isolated presentations of the US and CS would not be expected to facilitate learning. The unpaired group would therefore be expected to show a similar impairment in reacquisition as in the CS-alone group such that fear responding at test would be lower in Groups CS- and CS/+ than in Group CS+. If, however, the effect of the paired trial was due to enhanced retrievability, then Group CS/+ should show equivalent learning to Group CS+ for which the same memory elements were present at

retrieval. Likewise, the reinstatement account would predict similar levels of reacquisition for Groups CS+ and CS/+ since the US would be present at retrieval in both cases. Thus, for each of these alternative explanations, freezing in Groups CS+ and CS/+ at test should be higher than in Group CS-.

Methods

Subjects

The subjects used in this experiment were 24 adult male Lister hooded rats (Charles River, UK).

Apparatus

The experimental chambers used for this experiment are those described in Chapter II. The CS was a 60 s clicker and the US a 0.5 s, 0.5 mA foot-shock.

Procedure

Habituation. All groups were habituated to the context for one hour per day on each of two days prior to acquisition training.

Acquisition. Acquisition training was carried out for all groups using the same procedure as for the previous experiments in this chapter.

Retrieval 1. All groups received a single presentation of the CS at retrieval one day after acquisition training. This presentation occurred after 2 min in the context. All animals were returned to their home cages immediately following this session.

Extinction. Extinction training took place one hour after the retrieval session. This session comprised 18 presentations of the CS with 2 min adaptation period and ITI. Animals were returned to their home cages at the conclusion of the extinction training.

Retrieval 2. On the following day, all animals were brought to the experimental chambers for a retrieval trial before reacquisition. Retrieval sessions differed for the three groups. For Group CS-, retrieval 2 was identical to retrieval 1 with a single CS presentation

given after two min in the context. Group CS+ was also presented with the CS after two min, but in this case the CS co-terminated with the foot-shock US. Group CS/+ received explicitly unpaired presentations of the CS and US such that the US was presented one minute after the beginning of the session and the onset of the CS being one minute after the US presentation. After retrieval, all animals were returned to the home cages.

Reacquisition. One hour after Retrieval 2, all groups were returned to the chambers for reacquisition training. For Groups CS/+ and CS-, this session consisted of an adaptation period of 10 min followed by two presentations of the CS co-terminating with shock with an ITI of 5 min. Since Group CS+ had already received on CS-US pairing at retrieval, this group received only one CS-US trial during reacquisition, which was presented after a 10 min adaptation period. All animals were removed from the chamber one minute after the last CS-US pairing.

Test. After 24 h, all animals were returned to the conditioning chambers for test. After a 3 min adaptation period, the CS was presented twice with a 3 min ITI.

Statistical Analysis

Freezing during the pre-CS periods for each stage of the experiment were analysed with a One-Way ANOVA with Group (CS+, CS/+, CS-) as a between-subjects factor.

Acquisition. The three trials of acquisition were analysed by way of a mixed ANOVA with Group as the between-subjects factor and Trial (trials one to three) as the within-subjects factor.

Retrieval 1. Group differences in retention of the CS-US session were assessed by analysis of freezing to the CS during the pre-extinction retrieval session. These data were analysed across groups using the One-Way ANOVA.

Extinction. To assess the progression of extinction across trials, extinction data were analysed using a mixed ANOVA with Group as the between-subjects factor and Trial (trials one to 18) as the within-subjects factor.

Retrieval 2. Freezing to the CS at the pre-reacquisition retrieval session was compared between groups using the One-Way ANOVA procedure.

Reacquisition. For the purposes of analysis, the reinforced CS trial from retrieval 2 for Group CS+ was treated as the first trial of reacquisition. Freezing to the CS across the two reinforced CS trials was analysed by way of a repeated-measures ANOVA to assess changes in fear responding from trial 1 to trial 2 and to assess any group differences in overall levels of freezing or rates of reacquisition.

Test. Data from the two test trials were averaged to produce a single score for each animal reflective of their level of fear of the CS. Group differences were analysed using the One-Way ANOVA procedure.

Results

Acquisition

No between-subjects effects on levels of freezing were seen during the 2 min pre-CS period, $\chi^2(2) = 1.05$, $p = .592$; *M*s (*SEM*s) of percent time freezing: CS+ = 0.8 (0.8), CS/+ = 0.0 (0.0), CS- = 0.4 (0.4).

Freezing to the CS during acquisition (see Figure 51) increased significantly over trials, $F(1, 21) = 303.2$, $p < .001$. This increase did not differ between groups, $F(2, 21) < 1$, nor were any significant group differences detected collapsing across trials, $F(2,21) = 1.44$, $p = .258$.

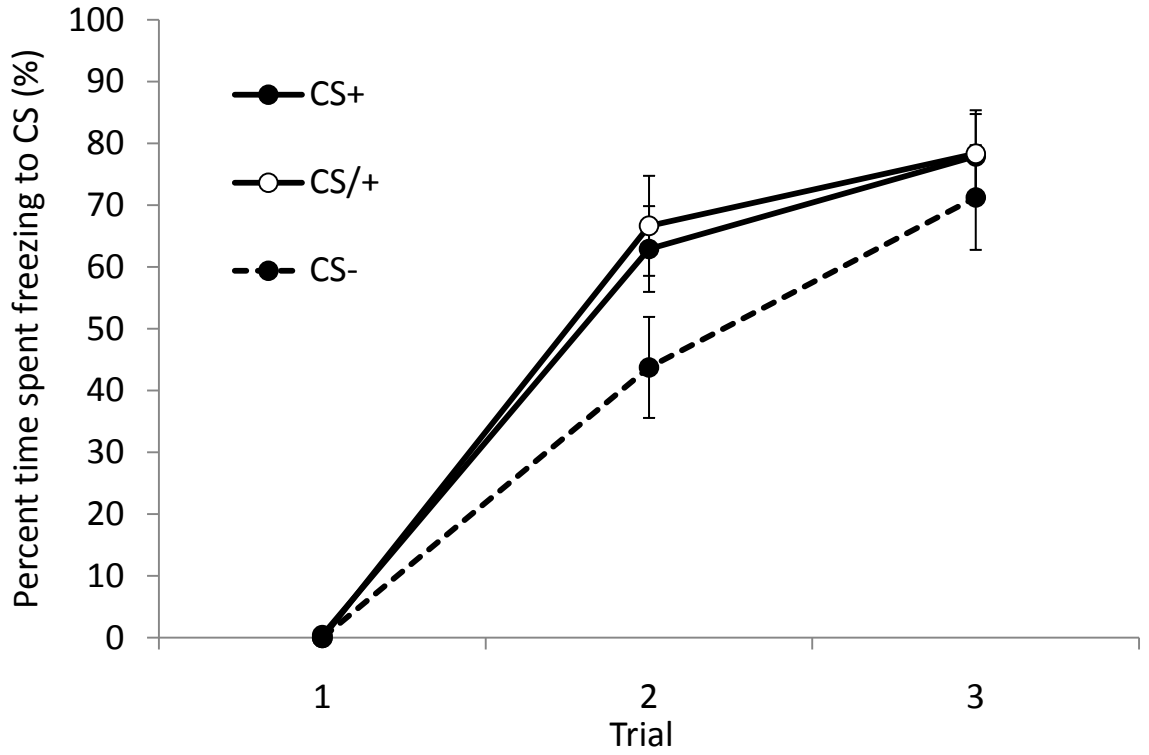

Figure 51. Freezing to the CS across three conditioning trials. Circles represent group means ± *SEM*.

Retrieval 1

No group differences in pre-CS freezing were observed during the pre-extinction retrieval session, $\chi^2(2) = 2.00$, $p = .368$; *M*s (*SEM*s) of percent time freezing: CS+ = 0.0 (0.0), CS/+ = 0.0 (0.0), CS- = 0.2 (0.2). This analysis provided no evidence for differences in contextual fear at this point in the experiment.

Freezing to the CS during retrieval is shown at the left of Figure 52. No significant group differences were found in percent time spent freezing during the one min CS presentation, $F(2, 21) < 1$.

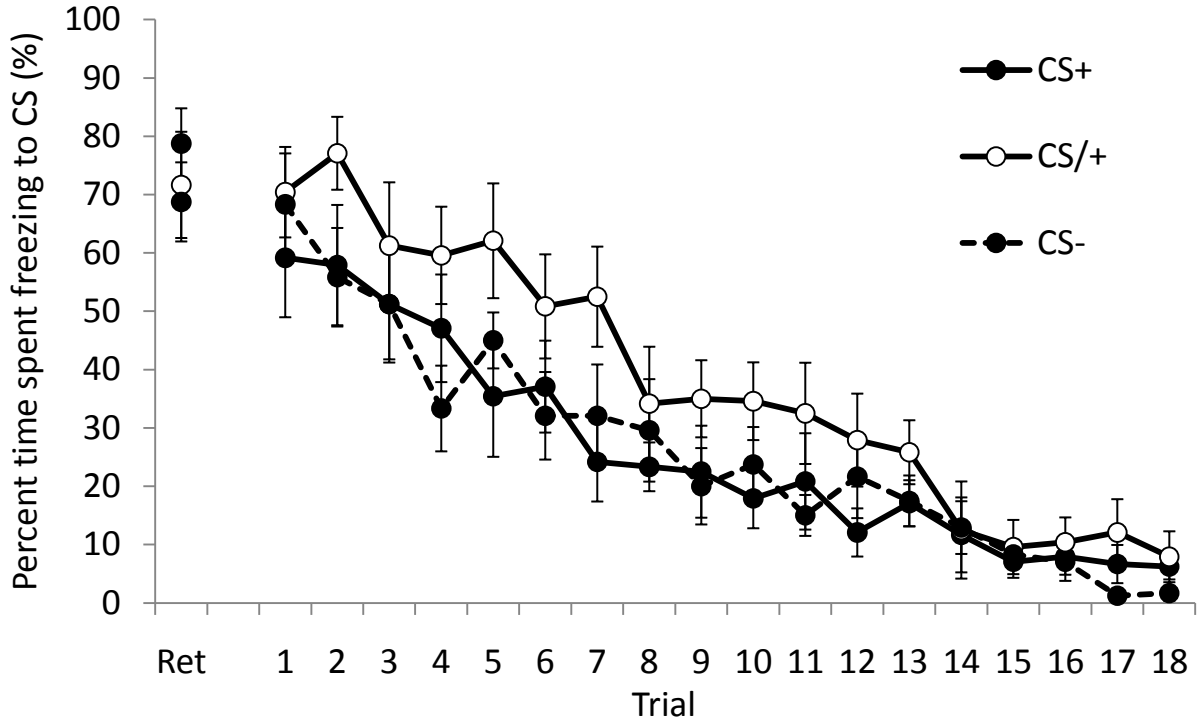

Figure 52. Freezing to the CS during retrieval (Ret) and extinction. Circles represent group means ± *SEM*.

Extinction

The two minutes prior to first CS onset revealed no significant differences in responding between groups, $\chi^2(2) = 2.28$, $p = .320$; *M*s (*SEM*s) of percent time freezing: CS+ = 0.0 (0.0), CS/+ = 0.6 (0.4), CS- = 0.2 (0.2). Freezing to the CS presentations (see Figure 52) decreased across trials, $F(1, 21) = 130.1$, $p < .001$, indicating successful extinction of the conditioned fear response. The magnitude of the within-subjects effect did not differ between groups, $F(2, 21) < 1$.

Retrieval 2

Freezing to the context during the first two minutes of the pre-reacquisition retrieval session did not vary significantly with group allocation, $\chi^2(2) = 2.38$, $p = .305$; *M*s (*SEM*s) of percent time freezing: CS+ = 0.0 (0.0), CS/+ = 1.5 (1.0), CS- = 0.4 (0.4). Freezing to the CS did not appear to differ across groups, $F(2, 21) < 1$, despite one group having received foot-

shock one min prior to the CS presentation. Thus, no short-term reinstatement effect was observed.

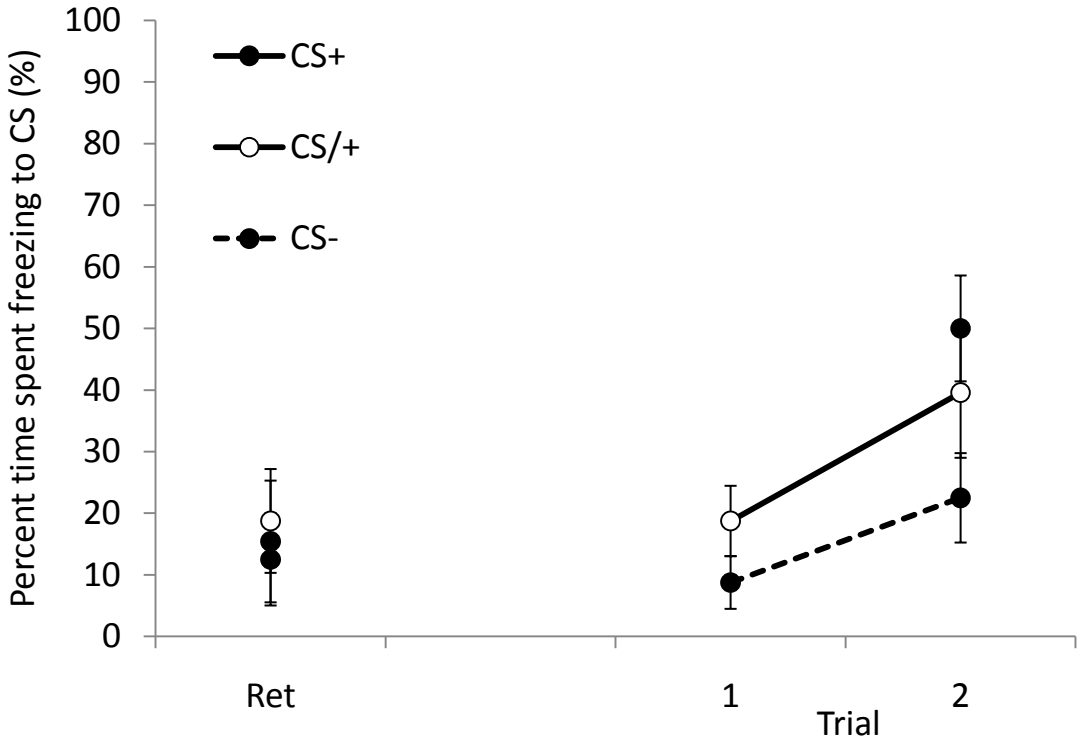

Figure 53. Freezing during pre-reacquisition retrieval (Ret) and reacquisition trials 1 and 2. Circles represent group means ± *SEM*.

Reacquisition

Pre-CS freezing did not vary significantly with group, $F(2, 21) = 1.36$, $p = .279$; *M*s (*SEM*s) of percent time freezing: CS+ = 2.7 (0.6), CS/+ = 2.1 (1.6), CS- = 0.4 (0.3). Freezing to the CS on each of the two reacquisition trials is shown in Figure 53. No overall group differences were observed on CS freezing during reacquisition, $F(2, 21) = 1.75$, $p = .199$. Freezing did increase significantly from trial 1 to trial 2, $F(1, 21) = 26.52$, and the size of this effect did not differ between groups, $F(2, 21) = 2.28$, $p = .127$.

Test

Data from the pre-CS and CS periods is presented below in Figure 54. Freezing during the pre-CS period at test did not differ between groups, $F(2, 21) = 1.09$, $p = .356$, giving no indication of group differences in fear acquired to the context.

A One-Way ANOVA assessing group differences in freezing to the CS revealed a significant effect of group, $F(2, 21) = 4.41$, $p = .025$. Post-hoc analysis involving comparisons between each of the three groups confirmed that Group CS+ displayed significantly more fear responding than Group CS-, $F(1, 14) = 11.12$, $p = .005$, replicating the finding of Experiment 4.2 that a reinforced CS presentation prior to reacquisition facilitates the retention of conditioned fear to the CS. Of further interest in this experiment was whether the same effect could be obtained with unpaired presentations of the CS and US. Analysis revealed that Group CS/+ did not differ from CS-, $F(1, 14) < 1$, and so we cannot conclude that the effect of the CS+ trial can be achieved without pairing of the CS and US. Instead, Group CS/+ displayed significantly lower responding than Group CS+, $F(1, 14) = 4.64$, $p = .049$, suggesting that the pairing of the stimuli, and not simply the mere presentation of the stimuli themselves, is an important factor in the retention of fear following reacquisition.

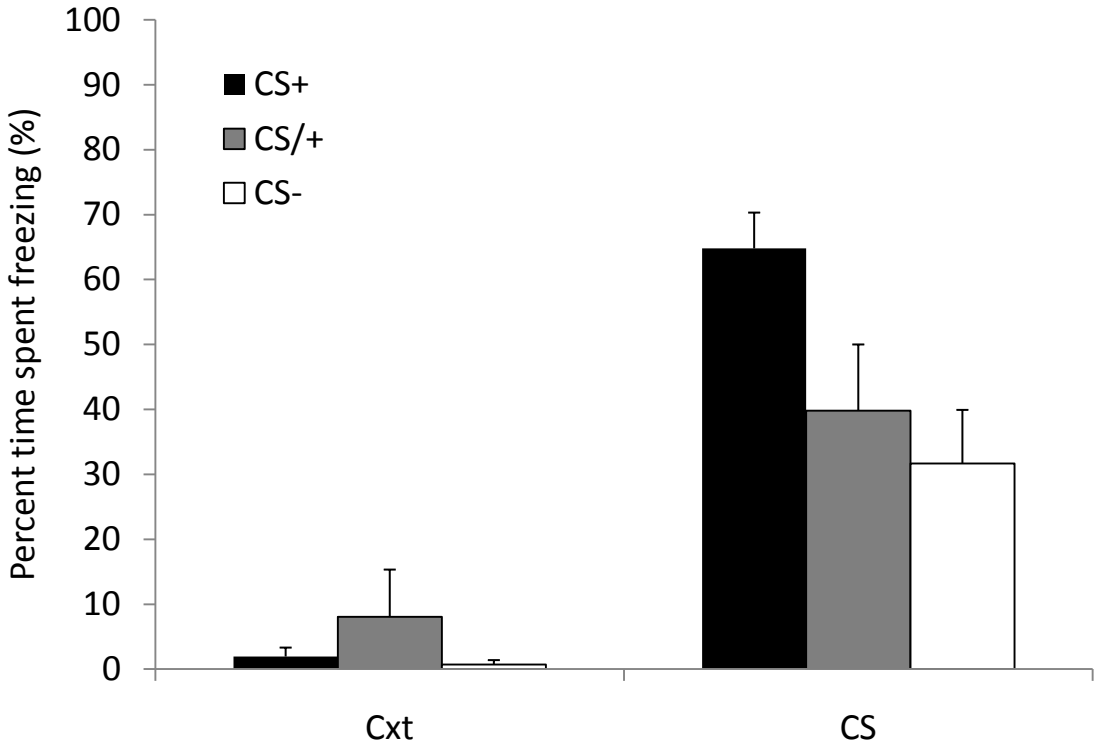

Figure 54. Freezing to the context (Cxt) and CS at test. Bars represent group means ± *SEM*.

Discussion

This experiment replicated the finding of Experiment 4.2 in showing that relearning after extinction with retrieval is facilitated by a reinforced retrieval trial one hour prior to reacquisition compared to when retrieval comprises a non-reinforced CS presentation. Furthermore, this effect does not seem to be the result of enhanced retrievability of the memory, nor from reinstatement following presentation of the US since each of these explanations predicts a similar facilitation in relearning from retrieval with unpaired presentations of the US and CS. Instead, the group given retrieval with unpaired stimuli showed similarly low levels of fear indistinguishable from those in the group presented with only the CS, and significantly less fear than the group given a paired presentation of the CS and US.

These results support the idea that prediction error is required for the destabilisation of memory prior to reacquisition. This finding is consistent with the idea that the function of reconsolidation is to allow for new information to be incorporated into an existing memory. Due to the risks involved in destabilising a memory and the resources required to restabilise it, it would seem prudent to engage this process only when there is potential for new learning to take place. Prediction error occurs when the memory is inadequate in forecasting the outcome of a CS trial. This is one case in which new information is available and learning is possible.

Chapter Discussion

The experiments of this chapter examined the potential for memory retrieval prior to reacquisition to reverse the impairments in reacquisition previously observed to result from the Monfils et al. (2009) extinction paradigm. It was found that a brief trial in which a single presentation of the CS was given one hour prior to retraining of the excitatory association was effective in facilitating reacquisition of the conditioned response, but only in the case where that trial was paired with the US. This effect cannot easily be attributed to enhanced retrieval or reinstatement but rather seems to be best explained as resulting from the prediction error generated by a reinforced trial after extensive extinction.

An interpretation in terms of prediction error is consistent with a view of reconsolidation as a process dedicated to updating existing memories with new, relevant information. The hypothesis put forward by Lee (2009) states that reconsolidation is a mechanism by which memories can be updated with new and compatible information. This is considered an adaptive process which allows organisms to maintain memories which contain the information most relevant to their current environment. Support for this hypothesis comes from data on the conditions under which reconsolidation is and is not observed, i.e., the boundary conditions of reconsolidation. These boundary conditions include memory strength

(Suzuki et al., 2004), memory age (Eisenberg & Dudai, 2004; Milekic & Alberini, 2002), extinction (Eisenberg et al., 2003; Lee et al., 2006a; Suzuki et al., 2004) and the predictability of the reactivation stimulus (Morris et al., 2006; Pedreira et al., 2004), and represent situations in which memory updating is either unnecessary or unhelpful. Particularly strong or well-trained memories would probably not benefit from additional training and so it may not be useful to engage reconsolidation mechanisms upon further training. Similarly, a stimulus which has been trained to reliably predict the occurrence of a US would only be expected to trigger reconsolidation if some aspect of the associative network involving that stimulus were to change. Situations which favour the formation of new memories over the updating of existing memories, for example, extinction or extended temporal separation of learning events, are also more likely to engage consolidation than reconsolidation processes (Lee, 2009). Thus, as well as providing an account of the function of reconsolidation, this hypothesis also makes sense of the many apparent discrepancies in the reconsolidation literature reported by those attempting to demonstrate post-retrieval amnesia in various behavioural paradigms. For the current studies, destabilisation and reconsolidation have been inferred from the capacity of training within a putative reconsolidation window to be facilitated relative to training conducted without prior initiation of reconsolidation processes. The experiments in this chapter, then, suggest that reconsolidation processes were engaged only when the retrieval trial signalled a change in CS-US contingencies. The presentation of a CS alone trial after 19 non-reinforced CS trials did not represent a situation in which memory updating would be needed. Therefore, it would have been unnecessary to engage reconsolidation processes. However, reinforcement of the CS would have created a prediction error and signalled a need for memory updating.

The basis for the experiments presented in this chapter was, in part, a view of the pre-extinction retrieval paradigm as a method by which extinction learning can be biased towards

unlearning as opposed to new learning. By this account, the presentation of the CS prior to extinction allows the original excitatory memory to destabilise and incorporate the extinction learning. As a result of this training, the animals are left with a single memory of the CS as a cue which signals the occasional delivery of foot shock. That this memory was formed during three sessions across two days may afford it greater control over responding than a memory formed from just one or two trials in a single reacquisition session. In this way it may be possible to account for the impaired reacquisition of fear following extinction within the reconsolidation window. Without retrieval prior to reacquisition, new learning would be encoded in a separate memory trace and the expression of this learning would then depend on whether the conditions present at test would favour retrieval of this or the previous memory. If retrieval conditions favour the pre-existing memory, then the changes in associative strength between the CS and US stored from the most recent learning episode may not be observable. In the case of reacquisition of fear after extinction, the increase in associative strength between the CS and US might have very little influence on responding if the prevailing conditions do not favour retrieval of this memory. This would account for the lack of conditioned responding to the CS observed after reconditioning of the CS when the retrieval treatment was given prior to extinction. In the case of a reinforced retrieval trial, destabilisation of the updated original memory (the acquisition-retrieval-extinction memory) by the large error produced by the US presentation would allow new learning to be integrated into this memory. This new learning would then directly affect the value of the associative strength of the CS and US and these changes would be observed in subsequent testing.

An alternative interpretation of the present data accounts for the facilitated learning through new learning, rather than updating, mechanisms. Reinforcement of the retrieval trial prior to reacquisition training results in that trial becoming a conditioning trial itself. To control for the total number of reinforced trials given after extinction, animals in the other

groups were given two reinforced trials during the reacquisition session instead of the one trial given to animals given CS+ retrieval. Therefore, one critical difference between groups given CS+ retrieval and the other control groups was the interval between the two reinforced CS presentations: for CS+ groups, this interval was one hour, while for the other groups this interval was just five minutes. Thus, one interpretation of the enhanced reacquisition of Group CS+ is that learning was facilitated by the extended interval between reacquisition trials. An explanation in terms of the spacing of trials cannot be ruled out on the basis of these experiments. This account remains a possibility if it can be shown that the decay rates of stimulus elements from A1 to A2, and then A2 to I, are within the range which would cause the stimulus elements to be active in A2 when the next trial is presented five minutes later. If this were the case, then the amount learned on the second trial with a five minute ITI would be limited by the proportion of elements already activated in A2 which are therefore prevented from being primed to A1. This reduces the potential for simultaneous activation of CS and US elements in A1 which is necessary for the formation of an association between them. This limitation would presumably be overcome by spacing the trials by one hour since the majority of elements should by that stage have decayed into the inactive state and so be ready to be primed into A1 when the next trial is presented. It remains possible, that the effect of a reinforced retrieval trial before reacquisition is essentially a facilitation of learning through increased spacing of trials. Instead of gaining control over conditioned responding by altering the associative strength the existing memory, the spacing of trials may strengthen new learning thus making the new association the more salient of the two. Thus, when the CS is presented again at test, the new reacquisition memory may be sufficiently strong to bias retrieval away from the earlier acquisition-retrieval-extinction memory such that levels of conditioned responding would instead be controlled by the reacquisition memory. The limitations of this explanation were discussed previously but, nevertheless this is a possibility

worthy of further investigation. However, even considering that the reversal of the impairment in reacquisition observed after extinction with retrieval may be the result of the facilitation of learning by trial spacing, it is still noteworthy that this impairment can be overcome by the presentation of two CS-US pairings presented one hour apart. Whether this is best conceptualised as a facilitation of learning through trial spacing, or as the destabilisation and updating of a CS memory, it is encouraging to note the possibility for relearning a fear association as a persistent inability to do so would perhaps be maladaptive in a clinical context.

VII. GENERAL DISCUSSION

The experiments presented in this thesis have explored the phenomenon of impaired reacquisition resulting from retrieval of the excitatory fear memory prior to extinction. Among the range of effects produced by pre-extinction memory retrieval reported by Monfils et al. (2009), the finding that reacquisition was impaired following such treatment was unique in suggesting that the CS had not simply been rendered less aversive, but may have become a safety signal. This observation was thus not only interesting theoretically for understanding the mechanism behind the enhancement in extinction, but also important in terms of application of the protocol to clinical settings in which impairment in fear learning may be an undesirable outcome of therapy. This chapter begins with an overview of the data presented and discussed in the preceding chapters, after which these findings will be discussed in the broader contexts of extinction learning and memory reconsolidation. The thesis concludes with suggestions of directions for future research and a brief overview of key findings and conclusions drawn from the present studies.

Summary of Results

The experiments presented in Chapter III of this thesis replicated the effect reported by Monfils et al. (2009) that the presentation of a conditioned fear stimulus one hour prior to extinction training resulted in slower reacquisition of the conditioned association. The effect was shown both with a tone stimulus similar to that used in the original study (Monfils et al., 2009) and with a distinct auditory clicker stimulus. Additional control groups confirmed that the difference between groups given extinction after retrieval and groups given extinction without retrieval was due to an impairment in reacquisition in the retrieval group rather than a facilitation of extinction in the no-retrieval group due to savings. Finally, it was demonstrated that a reinforced retrieval trial prior to extinction was equally effective at producing a reacquisition impairment, even though responding during initial acquisition appeared to have

reached asymptote. This finding suggested that either prediction error was not necessary for memory destabilisation, or that memory destabilisation was not necessary for the facilitation of extinction learning.

Following on from the successful replication of the Monfils et al. (2009) data, Chapter IV investigated whether the effect could be accounted for by any of three models of learning which claim to explain effects on learning of trial spacing, namely the Rescorla-Wagner model (Rescorla & Wagner, 1972), the comparator hypothesis (Miller & Matzel, 1988) and Wagner's SOP model (Wagner, 1981). Of these three, only the SOP model can explain trial spacing effects without appealing to differences in exposure to the context. Therefore, in the first experiment, total context exposure was held constant while varying the temporal arrangement of trials within a single 116 min session. Unexpectedly, a single CS presentation early in the session, one hour prior to the remaining CS trials, did not produce an impairment in reacquisition when animals were trained again the following day. The following experiment confirmed that the effect of retrieval on extinction was only seen when animals were removed from the context between retrieval and extinction, explaining the absence of an effect of retrieval in the first experiment. Reasons for the dependence upon removal from the context between retrieval and extinction were discussed in terms of interference and updating mechanisms. Since the Rescorla-Wagner model and comparator hypothesis struggled to account for these data, the final experiment provided a closer examination of the explanation offered by Wagner's SOP model. The absence of an effect of the same temporal arrangement of trials during CS pre-exposure on the magnitude of the latent inhibition made it difficult to attribute the Monfils et al. (2009) effect to the mechanisms proposed by the SOP model.

In an effort to better understand the effect of pre-extinction retrieval on the CS-US memory, Chapter V examined properties of the CS and US which may contribute to the impairment in reacquisition. The first experiment tested whether the CS acquired inhibitory

properties as a result of extinction within the reconsolidation window. No evidence was found to support this hypothesis and so the next experiment investigated whether the treatment instead led to a reduction in attention to the CS. Learning a contrasting association after extinction of the fear response did not appear to differ between the retrieval and no-retrieval groups. While these results were unable to provide direct evidence for attentional effects, the results could still be interpreted as consistent with this view. Furthermore, appetitive conditioning to the CS was slower in both groups which had received previous aversive conditioning with the same CS relative to a group for which the CS was novel at the time of appetitive training. Together with the tendency towards positive summation observed in the previous experiment, this provided further evidence against the CS having acquired inhibitory properties. Since no strong evidence had been found for effects on reacquisition relating to the CS or the context, the third experiment of this chapter assessed properties of the US which may have contributed to the impairment in relearning. It was found that extinction of a CS-US association within the reconsolidation window rendered that US less capable of supporting new excitatory learning when later subjected to conditioning with a novel CS. This finding was interpreted as the result of US devaluation during extinction which was facilitated by prior memory retrieval. Thus is was concluded that the impairment in reacquisition observed following extinction within the reconsolidation window was at least partly due to US devaluation, possibly together with a reduction in attention to the CS.

The final empirical chapter, Chapter VI, was aimed at investigating the circumstances under which a reversal in the impairment in reacquisition can be achieved. These experiments examined the effect of memory retrieval prior to reacquisition on the success of relearning and in doing so identified constraints on the assumption that memory retrieval is a sufficient condition for memory destabilisation and/or updating. It was observed that a nonreinforced CS presentation was insufficient to allow new learning to overcome the impairments imposed

by the retrieval-extinction treatment. However, if a prediction error was introduced at the time of retrieval, subsequent learning was facilitated. This effect was found not to be due to US reinstatement or facilitated retrieval on the basis of the presentation of more elements of the memory trace.

Extinction

The protocol upon which this thesis has focussed is based on what, at an operational level, is a relatively simple variation to a standard extinction preparation: the insertion of a one hour time delay between the very first extinction trial and the second. However, the effect of this manipulation is difficult to reconcile with current learning theories. With the exception of only a few models (most notably Wagner's SOP model), contemporary learning theories deal with effects of intertrial interval in ways that do not account well for the Monfils effects. Further, on the basis of the experiments of Chapter IV, it would appear that not even the SOP model can account for the effect on reacquisition of pre-extinction retrieval. Although no such attempt will be made here to formulate a new theory of learning which would account for the Monfils effects, possibilities will be explored for existing models to be adapted to take into consideration effects of trial spacing on learning and which would make different predictions for trial spacing effects on extinction and latent inhibition given the same spacing of trials.

Various processes have been suggested to underlie the loss of conditioned responding which results from extinction training. Among these are a loss of associative strength between the CS and the US (unlearning; e.g., Rescorla & Wagner, 1972), the formation of a new extinction memory which competes with the excitatory memory (Bouton, 1993; Pearce & Hall, 1980), a reduction in attention to the CS (Kehoe & White, 2002; Robbins, 1990) and devaluation of the US representation (Rescorla & Heth, 1975). Unfortunately none of these

processes is capable of explaining the full range of phenomena that have been reported in studies of extinction.

Unlearning accounts, for example, fail to account for post-extinction restoration of conditioned responding in the absence of any opportunity for additional associative learning between the CS and US (e.g., through renewal, reinstatement or spontaneous recovery). New learning accounts, in which it is assumed that the original excitatory association is preserved but masked by a new inhibitory association, struggle with the observation that recovery of responding is typically incomplete (Richardson et al., 2004; Robbins, 1990; but see Quirk, 2002) and that reinstatement of the US representation can be observed even when the opportunity for the formation of context-US associations in minimised (Rescorla, 1973; Rescorla & Heth, 1975). The suggestion that reductions in attention to the CS during extinction (i.e., a process akin to latent inhibition) can explain the loss of conditioned responding has been proposed as a theoretical possibility (Hawkins & Kandel, 1984; Kehoe & White, 2002; Pavlov, 1927; Robbins, 1990), yet this process is more likely to explain within-session response loss, particularly in a massed-trial extinction preparation, than to explain more persistent effects of extinction training observed when responding to the CS is assessed in a separate test session (Delamater, 2004). Finally, an account in terms of US devaluation, which would claim that through extinction the US representation is degraded such that when the CS retrieves this representation at a later stage it is insufficiently potent to elicit a CR (Rescorla & Heth, 1975), is inconsistent with data showing that extinction of one CS spares responding to a distinct CS previously paired with the same US (Rescorla, 2004). On the basis of these data, it is clear than any satisfactory account of extinction would need to involve more than one mechanism for reducing levels of conditioned responding through nonreinforced exposure to the CS. The weight of evidence in support of new learning during extinction should not stand in opposition to that in favour of associative loss. The reality is

that both these processes, along with others, are each likely to play a role in extinction, the relative contribution of each likely to vary between paradigms and parameters.

To conclude that extinction learning involves a combination of processes is to recognise that the phenomenon is far more complex than the procedure required to produce it. Nevertheless, this state of affairs may in fact have an advantage: the existence of multiple processes in extinction suggests multiple routes through which extinction might be facilitated. Manipulations which have been shown to enhance extinction include those which encourage loss of associative strength (Rescorla, 2006) or facilitate the retrieval of the extinction memory (Brooks & Bouton, 1993, 1994). Other manipulations, such as varying the spacing of trials, may affect extinction in more than one way, such as through modulating both associative change and CS processing (Wagner, 1981).

The use of pharmacological agents has become an increasingly popular means of facilitating extinction. As mentioned in the General Introduction, the use of the NMDA partial agonist DCS has received a great deal of attention for its potential in augmenting extinction processes in the treatment of human anxiety conditions (e.g., Davis, Ressler, Rothbaum, & Richardson, 2006; Ressler et al., 2004; Richardson et al., 2004). The interest in this drug was sparked by laboratory studies with rodents in which it was observed that DCS enhanced extinction of conditioned fear when administered before or after extinction, either systemically or directly into the basolateral amygdala (Ledgerwood et al., 2003; Walker et al., 2002). Furthermore, the extinction produced was resistant to reinstatement of responding through unsignalled presentation of the US (Ledgerwood et al., 2004). Together with the well-established role for NMDA receptors in learning, the ability of DCS to facilitate extinction was taken as further evidence that extinction involves new learning. However, another feature of DCS-enhanced extinction which is not obviously accounted for by facilitated learning of an extinction (e.g., CS-noUS) association is that the loss of conditioned

responding produced under DCS generalises to other stimuli which had shared a common reinforcer prior to extinction (Ledgerwood et al., 2005). In this experiment, two stimuli (one auditory, one visual) were each conditioned with the same foot-shock US. One of the two stimuli was then extinguished and immediately following extinction training, either DCS or saline was administered systemically. For rats administered saline, reductions in conditioned responding were specific to the extinguished CS. For those administered DCS, however, responding to both stimuli was reduced. On the basis of this result, the authors suggest that administration of DCS may augment devaluation of the US representation during nonreinforced presentations of the CS (Ledgerwood et al., 2005; Richardson et al., 2004). In any case, this particular effect is not easily accounted for by new learning and instead suggests a combination of effects: a facilitation of new extinction learning along with a devaluation of the US representation.

The effects of DCS on extinction have obvious parallels to the effects of retrieval presented by Monfils et al. (2009) and those reported here: pre-extinction retrieval and DCS both result in more robust extinction, and both appear to cause a devaluation of the US representation. As for the DCS effect, the effect of retrieval on extinction may too require an explanation in which the facilitation of more than one process is implicated. To assist in discussion of these possibilities, Table 12 summarises the major findings relating to extinction after retrieval and the potential for explanations in terms of facilitation of each of four processes thought to underlie extinction learning, namely unlearning, new learning, CS processing and US devaluation. The main purpose of this table is to highlight the fact that none of these processes alone can account for the full range of data under discussion. Predictions of the effects of enhancement of these processes are not always clear-cut. Nevertheless, an attempt is made to present the predictions which are supported by the weight of evidence.

Table 12: Summary of the Effects of Pre-Extinction Retrieval and Possible Mechanisms

Effect of pre-extinction retrieval	Unlearning	New learning	CS processing	US devaluation
Less renewal	✓	✓	✗	✓
Less reinstatement	✓	✓	✓	?
Less spontaneous recovery	✓	✓	?	✓
Retardation of reacquisition (relative to novel control group)	✗	✓	✓	✗
Retardation of acquisition to novel CS paired with same US	✗	✗	✗	✓

N.B.: Symbols indicate the degree to which each of the effects listed in the left-hand column could be explained as an enhancement of the processes listed across the top of the table. The symbol "✓" indicates that the effect may be accounted for by a facilitation of that process; the symbol "✗" indicates that a facilitation of the process would not produce the effect or that it would predict the opposite effect; "?" is used where the predictions are ambivalent or not well-defined.

A treatment which would facilitate any one of the processes listed in Table 12 could account for much of the data reported for extinction occurring after retrieval. None, however, are able to account for all the observed effects. An account in terms of a facilitation of an unlearning component of extinction would readily account for the attenuation of renewal, reinstatement and spontaneous recovery since each of these phenomena rely on the preservation of the excitatory association. In other words, a weakening of the CS-US association should lead to less recovery. This might also account for the observation that animals given retrieval prior to extinction reacquire fear more slowly than animals given extinction without retrieval if it is assumed that reacquisition following the standard extinction procedure builds on remnants of the original association (i.e., if the extinction group relies on savings). However, the finding that reacquisition was impaired relative to a

naive control group (Experiments 1.1, 1.3, 4.1, 4.2) cannot be easily explained by facilitation of unlearning. Unlearning models such as the Rescorla-Wagner model predict that nonreinforced presentations of the CS (without the presence of any other excitatory stimuli) would drive the associative strength towards but not beyond zero (Rescorla & Wagner, 1972). Therefore this group could not have had lower associative strength on the CS than the naive group for which the CS was theoretically neutral. There is also no adequate reason why a weak but positive associative strength between the CS and US would cause learning about a new CS paired with the US to be impaired as was shown in Experiment 3.3.

An account in terms of an enhancement of new learning meets with greater success in explaining the range of effects of retrieval on extinction. According to Bouton (1993), levels of responding to an extinguished CS are determined by the success with which the extinction memory can be retrieved. Recovery of conditioned responding after extinction is the result of a failure to retrieve the extinction memory. Strengthening of an inhibitory CS-noUS association is likely to make that memory more prominent and so more easily retrieved during subsequent sessions. In the case of renewal, reinstatement and spontaneous recovery, the logic is straightforward. For the case of reacquisition of the CS-US association, it must be additionally assumed that retrieval of the extinction memory would interfere with learning of an opposing excitatory association, an explanation offered by Bouton (1986) and Bouton and Swartzentruber (1989) for slow reacquisition following extended extinction when compared to a novel control group. Again, however, this explanation fails to account for the impaired acquisition of fear to a novel CS conditioned with the same US which had been previously paired with the extinguished CS.

The remaining two explanations rely on non-associative processes in extinction: reduction in CS processing and devaluation of the US representation. The CS processing account is well-suited to explaining retardation in reacquisition relative to both the no-

retrieval groups and the naive groups. However, the context-specificity of attentional effects (Lovibond et al., 1984) would suggest that the resulting loss of conditioned responding should recover with removal from the context in which CS exposure took place (Bouton, 1993; Wagner, 1981). Similarly, there is evidence to suggest that, at least in the context of latent inhibition, loss of attention to the CS recovers with time between pre-exposure and conditioning (Hall & Minor, 1984), limiting the ability of this account to explain the lack of spontaneous recovery following extinction with retrieval. Importantly, the CS processing account, like the unlearning and new learning accounts, does not explain the effect of retrieval prior to extinction on the ability of the US to support new excitatory learning.

Experiment 3.3 demonstrated that the extinction of a CS-US association one hour after a retrieval trial comprising a single presentation of the CS impaired the ability of the US to subsequently enter into an association with a novel CS. Of the processes outlined above, this effect appears to be uniquely explained by the US devaluation account in which it is argued that retrieval of the CS prior to extinction facilitates the devaluation of the US representation rendering it less effective as an aversive reinforcer. This is not to suggest that the US devaluation account would be a sufficient explanation of the pre-extinction retrieval effect, since this account has only moderate success in accounting for the full range of data reported by Monfils et al. (2009) and in the current work. A facilitation of US devaluation may explain the attenuation of renewal and spontaneous recovery, assuming that the devaluation process is not also subject to these forms of recovery. Reinstatement may help to overcome the US devaluation effect, but if the retrieval-extinction treatment serves to degrade the US representation to a sufficiently weak state, it is possible that more than one US presentation would be required to achieve a complete restoration of the representation. The argument against the retardation effect is similar to that for the unlearning account, namely that the

limit of this effect would be to abolish the US representation entirely which should, therefore, lead to reacquisition rates similar, but not less than, those for a naive animal.

Clearly, no explanation in terms of a single process will be sufficient to account for the range of effects seen to result from retrieval prior to extinction. Therefore, an adequate account would better be obtained by assuming that retrieval prior to extinction affects the consequent learning in at least two ways. Given that the only process to account for the effect on conditioning of a novel CS is the US devaluation process, a successful account of the data should include a role for facilitation of US devaluation through pre-extinction retrieval. A sensible addition to this explanation would be that of facilitation of new learning, a process well-established as central to normal extinction learning and which confidently accounts for all but the US devaluation effect. Alternatively, a CS processing account would also compliment the US devaluation account such that together the two processes could explain the absence of recovery effects, retardation and impaired conditioning of the US with a novel CS.

In summary, a single CS presentation given one hour prior to extinction produces a range of effects which might be explained most parsimoniously as the result of facilitation of new learning, and concurrent devaluation of the US representation. This may involve an amendment to a new learning account which would allow generalisation of extinction from the extinguished CS to a novel CS which could be facilitated by retrieval of the memory prior to extinction. As to why the insertion of a one hour delay between first and second nonreinforced extinction trials might produce such effects is the topic of future discussions and research. However, one approach might be to consider the first trial as functionally distinct from the remaining trials, i.e., the first serving to retrieve a stored memory with the remaining trials constituting a learning episode. In this way we might come to understand the influence of the former on the progress of the latter.

Reconsolidation

The retrieval of a stabilised long-term memory can, through a process likely to involve NMDA receptor activation (Ben Mamou et al., 2006) and protein degradation (S. H. Lee et al., 2008), result in that memory being destabilised. Once destabilised, the memory may be updated with new information (Lee, 2008) whilst being restabilised through a protein-synthesis dependant process (e.g., Nader et al., 2000). Manipulations which attempt to modulate memory through post-retrieval interventions are not only dependent upon successful targeting of the mechanisms involved in reconsolidation, but also rely upon the memory having been destabilised in the first place. Similarly, it is impossible to show that a memory has been destabilised without disrupting reconsolidation.

The first two experiments of Chapter IV present a possible constraint on the conditions for memory destabilisation. In these experiments an effect of pre-extinction retrieval on reacquisition was only seen when animals were removed from the context between retrieval and extinction. Further, the effect cannot be attributed to mere exposure to the context followed by removal from the context since the control group were exposed to the context without a CS presentation. In a study by Pedreira et al. (2004), reconsolidation mechanisms were initiated only after removal from the context, the point at which the omission of the context-contingent shock could be definitively confirmed. Before this point, memory for the CS-US association remained intact regardless of the length of CS exposure. The CS in this case was a context in which an aversive stimulus had previously been presented. Therefore, in this case, termination of the CS equated to removal from the context. In light of this study, it seems plausible to suppose that the failure of the CS memory to destabilise prior to the start of the extinction trials might have been due to the fact that the animals had not been removed from the context after retrieval. According to the conclusion drawn by Pedreira et al. (2004), the omission of the US should have been confirmed at the

termination of the 60 s CS presentation. However, it is possible that, at least in the case of conditioning with a discrete cue, reconsolidation mechanisms are not engaged until the session is complete, especially when the conditioning session involved multiple trials. If the entirety of the session is classified by the animal as a discrete learning experience, it would make sense to delay memory destabilisation until it is confirmed that the new experience should be incorporated into an existing memory rather than formed into a new memory. Such a situation is likely to occur when a CS undergoes extinction. Since the duration of exposure determines whether reconsolidation or extinction mechanisms are engaged (Eisenberg et al., 2003; Pedreira & Maldonado, 2003; Suzuki et al., 2004), the animal would have to wait at least until the threshold for extinction was reached, if not until the end of the session (Pedreira et al., 2004), before initiating memory destabilisation. In brief, the finding that the pre-extinction retrieval effect was dependent upon removal from the context may be interpreted as a dependence of reconsolidation processes on confirmation of a mismatch in expectation and further suggests that the termination of a retrieval session may be necessary to initiate the cycle of destabilisation and reconsolidation of a retrieved memory.

This observation also has implications for the interpretation the finding, reported by Monfils et al. (2009), that the time between the first and second CS presentations influences levels of phosphorylation of GluR1 glutamate receptors. Rats were first conditioned with pairings of a tone CS and foot-shock. After 24 h, the animals were returned to the conditioning contexts and re-exposed to the CS. A Western blot analysis revealed that a single CS presentation led to increased phosphorylation of GluR1 both at 3 minutes and 1 hour after presentation. For another two groups, the CS was presented twice with an ITI of either 3 minutes or 1 hour. These intervals corresponded to the intervals between first and second CS presentations in the behavioural experiments for groups NoRet and Ret, respectively. It was found that if the two CS presentations were spaced by 3 minutes, as was

the case during extinction sessions, GluR1 phosphorylation remained high. However, if the CS was presented for the second time after one hour, levels of pGluR1 returned to baseline. It was suggested that this dephosphorylation may represent a molecular signature of memory destabilisation which was dependant upon two nonreinforced CS presentations with an ITI greater than 3 minutes. The lower limit of the ITI, on the basis of their other experiments, would be between 3 and 10 minutes and the upper limit between 1 hour and 6 hours (Monfils et al., 2009).

The results of the present studies, however, suggest that memory destabilisation may have less to do with the passage of time and more to do with the termination of the retrieval session through removal from the experimental chambers. In the experiment described above, the primary comparison was between two groups which differed in the interval between two nonreinforced CS presentations (Monfils et al., 2009). However, these groups also differed in whether they remained in the context during the ITI or were removed from the context and returned to the home cages (M.-H. Monfils, personal communication). Thus the differences in levels of phosphorylated GluR1 reported between these two groups may be due not to the differences in ITI between the two groups, but rather to the fact that for one group, termination of the retrieval session allowed for confirmation of the mismatch between acquisition and retrieval prior to the second trial.

The Role of Surprise in Reconsolidation

It has been suggested by researchers in the field of memory reconsolidation that an adaptive function of reconsolidation might be to allow new information to be incorporated into existing memories (Dudai & Eisenberg, 2004; Lee, 2009; Sara, 2000). By this account, a memory which is retrieved in an environment where new information is available will be destabilised, the memory trace adjusted to accommodate the new information, and then reconsolidated through a protein synthesis-dependent process (Lee, 2009). Evidence in

support of this hypothesis comes from reports of certain boundary conditions on reconsolidation (e.g., memory strength, memory age, extinction, predictiveness of the retrieval stimulus) which in most cases can be shown to correspond with situations in which either no new information is available or when it would appear more adaptive to form a new memory than to change the contents of a well-trained memory (Lee, 2009; Nader & Einarsson, 2010).

The data presented in this thesis provide further support to the updating hypothesis. In particular, it was shown that reinforcement of an extinguished CS during a retrieval session one hour prior to reacquisition was necessary to overcome the impairment in reacquisition produced by extinction during an earlier phase of reconsolidation. A retrieval trial consisting of the CS alone one hour prior to reacquisition had no effect on the ability of the CS to reacquire fear through further pairings of the CS with the US. A single reinforced trial, on the other hand, allowed a subsequent pairing to condition fear to the CS which could be observed as an increase in freezing to the stimulus on the following day. One explanation of this effect is that prediction error at the time of retrieval is necessary for memory destabilisation and to allow the new excitatory learning was able to update or replace the previously dominant extinction memory. This idea is consistent with a view of reconsolidation as a process through which memories can be updated to maintain their relevance to a changing environment.

If the recovery from retardation were shown to be due to prediction error at retrieval, an idea consistent with current conceptualisations of reconsolidation, it would be interesting to consider this in light of the observation that prediction error was not required at retrieval prior to extinction (Experiment 1.4). Perhaps prediction error would constitute a sufficient condition for memory destabilisation, but not a necessary one. There are other situations in which learning about the environment may be possible without a violation of the specific

expectation arising from a discrete stimulus presentation. For example, Hupbach, Gomez, Hardt, and Nadel (2007) trained participants on an episodic memory task in which two lists of items were memorised. Prior to learning the second of the two lists, one group of participants were reminded of peripheral aspects of the first learning episode. On the third day, when asked to recall items from the first list, participants in this group showed a greater number of intrusions (falsely recalled items) from the second list than participants who were not reminded of the first list before learning the second. The interpretation of this finding was that the retrieval destabilised the episodic memory for the first list such that when the second list of items were presented these were stored as a part of the original memory. As a consequence, when asked to recall the first list, these participants showed greater difficulty in distinguishing between the two distinct learning episodes. This paradigm represents a situation in which new information is available, e.g., new items in a collection, but where there is no obvious instance of prediction error. Perhaps, then, the use of a more general term such as ‘surprise’ or ‘mismatch’ may better reflect the conditions under which memories can be destabilised and open to disruption from amnestic agents or modification through new learning.

In the case of Experiment 1.4, the reinforcement of the CS on the retrieval trial should not have produced any sizable prediction error since responding across the extended acquisition phase appeared to have reached asymptote. However, one aspect of this trial was different to previous experiences. On the first two days of experimentation (habituation) the animals spent one hour per day in the experimental chambers during which time no stimuli were presented. On the following two acquisition days, the animals were again placed in the context and for the first 30 minutes of each of these sessions, again no stimuli were presented. The animals thus had the experience that if anything would happen during their time in the chambers, it would not happen during the first half and hour. The next time these rats were

exposed to the conditioning apparatus, they were in the chambers only two minutes before the CS was presented. The CS itself, therefore, may have been surprising even if the consequent delivery of the US was not. The presentation of the well-trained CS at pre-extinction retrieval for Experiment 1.4 may have had its effect through being presented at a surprising point in time (i.e., after 2 min rather than 30 min) without the outcome of the trial itself being surprising.

The results of Experiment 4.3 may pose a problem for this interpretation, however, since the unsignalled and unexpected presentation of the shock US would also have been surprising and so would be expected to trigger a reconsolidation phase. The prediction error in this case, though, is due to a failure of the context to signal shock. The relationship between the CS and the US should not be too much affected by their unpaired presentations (although extensive training of this sort can produce conditioned inhibition; Rescorla, 1968), and so it is possible that the CS-US memory remained unaffected by any prediction error relating specifically to the context-US association. If a context-US memory had existed prior to this point in time, then this memory may have been destabilised by the unsignalled shock. Otherwise, a new excitatory context-US memory may have formed. Given the extensive experience of the context being a very poor predictor of the US, it is not surprising that subsequent levels of freezing to the context did not show signs of acquisition of contextual fear.

The data presented in this thesis, therefore, support a view of reconsolidation as a process for updating memories. The situations in which reconsolidation effects were seen in these experiments each involved a change from CS-relevant expectations. Where no evidence was found for an effect of retrieval, the CS trial was either unsurprising or the retrieval trial was not terminated preventing confirmation that the new information should be used to update the existing memory rather than to form a new memory.

Future Directions

If the effects reported here and in the experiments by Monfils et al. (2009) are dependent upon reconsolidation mechanisms, then preventing destabilisation of the CS memory should negate the facilitatory effect of pre-extinction retrieval. S.-H. Lee et al. (2008) demonstrated that administration of the proteasome inhibitor clasto-lactacystin-β-lactone (βlac) immediately after retrieval of a contextual fear memory protected the memory from anisomycin-induced disruption. Should the facilitation of extinction following retrieval depend upon destabilisation of the acquisition memory, administration of βlac at the time of retrieval should preclude such effects as reported here and by Monfils et al. (2009). However, if these effects can be reduced to an account in terms of learning mechanisms without appealing to mnemonic processes, then βlac should not influence the ability of pre-extinction retrieval to facilitate extinction learning.

If it is found that the pre-extinction retrieval effect requires memory destabilisation, then another important question which should be given priority in future research is whether the extinction training given after a retrieval trial constitutes new learning or an updating of the original association. Two possible mnemonic mechanisms were discussed through which extinction training within the reconsolidation window might result in a persistent reduction in fear responding. The first was that extinction training occurring while the excitatory memory was labile would allow the extinction learning to be incorporated into the acquisition memory trace and so to revalue the CS as less aversive. The other was that extinction after memory destabilisation might interfere with the reconsolidation of the original excitatory memory while forming a new, CS-noUS memory. One way to differentiate between these two mechanisms might be to follow the approach of Lee (2008) and assess the effects on extinction learning of BDNF and zif268 blockade. In the hippocampus, BDNF and zif268 have doubly-dissociable roles in consolidation and reconsolidation respectively (Lee et al.,

2004). Furthermore, it appears that this pattern of results is not specific to excitatory memories. Chhatwal, Stanek-Rattiner, Davis, and Ressler (2006) have provided evidence that BDNF in the BLA is necessary for the consolidation of extinction memory, whereas Herry and Mons (2004) have shown increased activation of zif268 in the BLA to be associated with extinction memory reconsolidation. Although the roles of BDNF and zif268 have not yet been shown to be doubly-dissociable in the amygdala, it is possible that their role in extinction in the amygdala may show the same pattern of involvement for consolidation and reconsolidation as for excitatory contextual memories in the hippocampus. Should this prove to be the case, it would be possible to form predictions on the involvement of BDNF and zif268 within the Monfils et al. (2009) protocol. These predictions are summarised in Table 13.

Table 13: Predictions of the Expression of BDNF and zif268 following Extinction According to the New Learning and the Updating Hypotheses

	Group	Retrieval	Extinction	Result of assay
New learning hypothesis	Ret	↑ zif268	↑ BDNF (↓ zif268?)	BDNF (zif268?)
	NoRet	-	↑ BDNF	BDNF only
Updating hypothesis	Ret	↑ zif268	-	zif268 only
	NoRet	-	↑ BDNF	BDNF only

N.B.: "Ret" refers to a group given a retrieval trial one hour prior to extinction; "NoRet" refers to a group exposed only to the context prior to extinction. Arrows indicate the direction of change in levels of expression resulting from that trial.

For animals given extinction without prior retrieval (NoRet condition), extinction should recruit consolidation processes and so produce an elevation of BDNF detectable through *in situ* hybridisation. For animals given retrieval prior to extinction (Ret condition),

levels of BDNF and zif268 would depend upon whether the extinction phase involves new learning or simply updating of the original memory. If the extinction involves new learning, then elevations in BDNF should again be detected. Western blot analysis may also reveal an elevation in zif268 at this point due to activation by the earlier retrieval trial, but if extinction training serves to disrupt reconsolidation of this memory, then it may also result in a suppression of zif268 towards baseline levels of activation. The contrasting prediction of the updating account is that extinction for Group Ret should recruit reconsolidation processes rather than consolidation processes and so zif268 rather than BDNF should be elevated. This design, adapted from Lee (2008), thus provides a means of differentiating new learning from updating processes in the pre-extinction retrieval effect.

Conclusions

The studies presented in this thesis have investigated the consequences of extinguishing a conditioned fear association when a brief retrieval session precedes extinction training by one hour, as first reported by Monfils et al. (2009). It has been shown that the ensuing impairment in reacquisition of conditioned responding seen after this critical treatment is a reliable and robust phenomenon, generalising readily to different stimuli and sets of parameters. These effects are not easily explicable in terms of three representative models of learning, the Rescorla-Wagner model (Rescorla & Wagner, 1972), the comparator hypothesis (Miller & Matzel, 1988) and the SOP model (Wagner, 1981), which each offer accounts of learning effects dependant upon the spacing of trials. Yet, the present results have led to a better understanding of the processes underlying the facilitation of extinction by prior retrieval. On the basis of these results it would appear that any explanation of the effects of retrieval prior to extinction which could successfully account for all the observations reported by Monfils et al. (2009) and in the present studies is likely to necessitate facilitation of more

than one process. Such an account would include a role for US devaluation alongside mechanisms which might facilitate new learning or the loss of attention to the CS.

Interpreting these data in the context of memory reconsolidation provides new support for the view of reconsolidation as a process through which memories are updated with new information. The observation that recovery from reacquisition impairment was only seen when the retrieval trial involved a prediction error suggests that the availability of new and relevant information is necessary for the destabilisation of a consolidated memory (Lee, 2009). Furthermore, the observation that removal from the context between retrieval and extinction sessions was necessary for the observation of impaired reacquisition suggests that destabilisation occurs only after the discrepancy between the retrieval session and previous learning episodes is confirmed. Together with the data of Pedreira et al. (2004), this finding highlights a further boundary condition on memory reconsolidation, namely the termination of the retrieval session through removal from the experimental context.

The insights provided by the current experiments into the mechanisms underlying the Monfils et al. (2009) effects are important for assessing the potential for the use of this paradigm in a clinical setting. The benefits to exposure therapy of a simple method for attenuating renewal, reinstatement and spontaneous recovery are substantial (Schiller et al., 2010). Yet, the impairment in reacquisition presented a potential problem for the application of this protocol as it raised the possibility that the fear-eliciting stimulus might come to signal safety. While the results presented here would suggest that this is not the case, further research is justified before implementing this protocol in the treatment of clinical anxiety. Hopefully, interest in the interactions between mnemonic and learning processes will have been sparked by the Monfils et al. (2009) and Schiller et al. (2010) studies. The current work presents a first step towards understanding the underlying mechanisms through which these two systems interact.

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