

A Novel Mixed-Extinction Paradigm Makes Extinguished Pavlovian Associations

More Resistant to Behavioural Relapse

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ABSTRACT

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Omission and Overexpectation are two paradigms that lead to a reduction in the associative relationship between events. In Omission, this is achieved through absence of the delivery of an expected outcome. In Overexpectation, two individually trained cues are presented together and are met with an outcome of less intensity than the sum of the two expected outcomes. The reduction in the conditioned response following Omission or Overexpectation training reflects a reduction in the associative relationship between the target cue and the conditioned response. However, this reduction is easily disrupted. Testing outside of the extinction context, letting time pass, or giving unsignalled exposure to the unconditioned stimulus, each lead to an increase in the conditioned response from extinction levels. These effects can be explained by a view that the original association is not erased but rather is inhibited by extinction learning and this extinction learning is more vulnerable to disruption. Insight into this disruption might come from a biological perspective where Omission and Overexpectation have been tracked by unique and overlapping neuronal populations in the central nucleus of the amygdala (Jordanova et al., 2016). Given evidence that unique and

overlapping neuronal populations are involved, one might expect the influence of two these processes to be behaviourally additive. This raises the possibility that targeting the unique and common populations associated with Omission and Overexpectation through combining both forms of extinction training might lead to lasting reduction in behaviour as one that is more resistance to relapse. To test this prediction, following conditioning of two different cues with a positive reinforcement, Sprague Dawley rats (*Rattus norvegicus*) were assigned to either a constantly reinforced Control, an Overexpectation-only, Omission-only, or a Mixed group consisting of blocks of Overexpectation sessions followed by blocks of Omission sessions. Subsequent Renewal, and Reinstatement tests show higher responding in the Control group compared to the three groups that had undergone extinction or Overexpectation training, with the Mixed extinction group showing the lowest rate of response. This effect was consistent across different dependant variables. However a Spontaneous Recovery test, though yielding a similar decrease, did not result in a substantive effect-size statistic or a statistically significant null hypothesis test. Taken together these findings have potential implications for deepening reductions of maladaptive associations on behaviour, such as those present in addiction and anxiety disorders.

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List of Abbreviations

ANOVA	analysis of variance
CS	conditioned stimulus
CR	conditioned response
LSD	least significant difference
SEM	standard error of the mean
US	unconditioned stimulus
UR	unconditioned response

Introduction

In a changing environment, updating associations between environmental stimuli is a fundamental adaptive process. For example as a child, a school bell might signal lunchtime, but the relationship between bells and a food reward-state becomes very different as an adult. However, this learning about a change in associations, is less strong than the original learning since behaviours associated with that original learning are liable to return (e.g. Bouton & Bolles, 1979; Pavlov, 1927; Rescorla & Heth, 1975).

Theories about associative learning tend to rely on models of error prediction. This is where learning is seen as a function of the “surprise”, that is, the error between what is predicted and what actually follows (Kamin, 1968, 1969; Rescorla & Wagner, 1972). Prevailing error-prediction models (e.g. Pearce & Hall, 1980; Pearce & Mackintosh, 2010; Rescorla & Wagner, 1972; Wagner & Rescorla, 1972) are informative in forecasting conditioned behaviour; however, when it comes to learning that an association is no longer relevant, such models fall short in explaining why this type of learning can be temperamental, in that original behaviours are liable to relapse.

Although some studies have examined how to strengthen extinction to a level that renders behaviours more resistant to relapse in both rats (e.g. Kearns, Tunstall, & Weiss, 2012; Tunstall, Verendeev, & Kearns, 2013) and humans (e.g. Coelho, Dunsmoor, & Phelps, 2015; Culver, Vervliet, & Craske, 2015), the processes that underlie this *deepening* of extinction, are still poorly understood. The aim of this thesis is to examine the deepening of extinction from a perspective that there are

distinctive biological mechanisms underlying different forms of extinction learning (cf. Rescorla, 2006b, 2007). The present experiment employed a novel combination of two types of extinction learning (cf. Leung, Reeks, & Westbrook, 2012; but also see Lucantonio et al., 2015). The main hypothesis is that this would make behaviourally extinguished responses more resistant to relapse, compared to the effectiveness of training with only one of these extinction procedures. Rats received separate conditioning trials with 1) a visual cue paired with sucrose, and 2) an auditory cue paired with sucrose. Following this independent conditioning with the separate cues, rats either continued to receive these reinforcements as a *Control* or experienced different types of extinction training. The rats that received different extinction training conditions were: a group received the *Omission* of the expected outcome to either the auditory or visual paired cue, a second group that received trials with simultaneous presentation of the two cues that had individually been paired with a sucrose outcome but did not receive the sum of the two paired outcomes (*Overexpectation*), and a third extinction group received a *Mixed* procedure receiving blocks of both types of extinction training (separate and compound cues). We then compared these groups on different post-extinction conditions. The manipulations that we employed following extinction are known to lead to a return of behaviours suggestive of pre-extinction learning. The return of extinguished behaviour occurring in the absence of additional CS-US pairings post-extinction treatment will be referred to as *relapse* throughout this thesis. Specifically, we tested if our Mixed extinction condition made subjects more resistant to relapse compared with an Omission extinction group which should be

the most effective training condition according to many models. Whereas the Overexpectation condition should be less effective at extinguishing behaviors than either Omission-only or Mixed training. This will be described in more detail below. The aim of this experiment is to test the hypothesis that this novel combination of an Overexpectation followed by Omission, Mixed-extinction design, is more resistant to relapse compared to the robust Omission-only alternative.

The remainder of this introduction is organized into six subsections. The first section will briefly describe Pavlovian conditioning and then provide some background on ways to reduce associative strength, namely Omission and Overexpectation. The second section discusses retrieval and the vulnerability of extinction learning to relapse in terms of some relevant post-extinction manipulations that will be described. Thirdly there will be a section discussing the concept of Rescorla-Wanger Model and this will be followed by a fourth section on the application of this view in predictions about Omission, Overexpectation and our novel Mixed paradigm. Next there will be a section discussing the concept of making extinction learning more resistant to behavioural relapse. The final section provides an overview of the thesis aims and rationale.

Pavlovian conditioning and two ways to reduce associative strength: omission and overexpectation learning

Traditionally in Pavlovian conditioning, an unconditioned stimulus (US) is one that elicits a response without any prior learning necessary; this response is known as an unconditioned response (UR). A conditioned stimulus (CS) is a neutral stimulus in that it does not elicit a response related to the US. However, after the

CS has been paired with a US several times, this CS becomes a good indicator that the US might follow. As a result of repeated presentations of a CS and US together, a presentation of the CS-alone reliably elicits a response. This response is referred to as the conditioned response (CR) and its occurrence is indicative of the subject's formation of a CS-US association (Pavlov, 1927).

Once a CS-US association is established, there are ways to update it such as by weakening the association (and associated behaviours) through extinction. One such method is the repeated omission of the US when the CS is presented; whereas, the CS that was previously a good predictor of a US, is no longer predictive of that US. Another way to update CS-US associations is by manipulating the subject's expectation of the US without manipulating the US itself. Presenting a compound stimulus comprised of two CSs, each of which was previously paired with a different US, will lead the subject to expect that both USs will follow. If the *same* US that was previously paired with either CS, accompanies the compound of the two CSs, this should promote an overexpectation in the subject as to the magnitude of the US that will follow (Rescorla, 1970, 2006b, 2007; Wagner & Rescorla, 1972). The implications of a Mixed-extinction paradigm (Overexpectation and Omission) will be discussed in more detail below in the subsection on the vulnerability of extinction learning.

Following learning that the CS is no longer a good indicator of the US, CR behaviours are decreased following subsequent CS exposures. Likewise tests of learning about the CS would show less-and-less CR behaviours, until eventually the CR behaviours are extinguished entirely. However, a caveat to this process is that

the presence of the original learned association can become behaviourally evident again, among other reasons, even after the mere prolonged and repeated omission of the CS and US (for a review, see Bouton, 1993).

The vulnerability of extinction learning to relapse by: Renewal, Spontaneous Recovery, and Reinstatement

Even though a subject's behavioural level on some measurable outcome might be at zero following extinction training, this does not mean that this learned association is gone. Various manipulations after extinction training lead to a return of behaviours indicative of the original CS-US association. This relapse of such behaviours can become evident on presentation of the CS-alone (Bouton & Bolles, 1979; Pavlov, 1927; Rescorla & Heth, 1975). The phenomena that produce a relapse are taken as evidence that the extinction learning does not erase or replace the original learning (cf. Rescorla & Wagner, 1972; Wagner & Rescorla, 1972). Current understanding of extinction learning views it as an inhibition of the original learned association (Bouton, 1993, 2002, 2014; Pavlov, 1927; Rescorla, 2006b; Rescorla & Heth, 1975; Todd, Vurbic, & Bouton, 2014; Westbrook & Bouton, 2010). As such, a behavioural relapse following extinction learning would be explained as a retrieval of the original formed association and a failure of extinction learning to fully inhibit the original CS-US association.

Learning about associations does not take place in isolation; each learning event needs to fit into the organism's previously formed representations of the CS or US as well as conceptions about the environment (Rescorla, 1988; Rescorla & Heth, 1975; Rescorla & Wagner, 1972). Specifically, associations made between discrete

CS-US pairings also form—to some extent—associations including less-discrete contextual cues as well as an awareness of internal states such as emotional states, drug states and time (for a review see Bouton, 2002). This also follows in extinction, when learning that a CS might no longer be a good predictor of the delivery of the US. In this view, Pavlovian conditioning is not a matter of merely pairing stimuli and consequences but rather, that updating of CS-US relationships affect complex representations of CS and US by the organism. These cues from the external environment, as well as internal states represent a context in which learning occurs (Bouton, 2002, 2014). This is particularly important when the associations become ambiguous, notably the ambiguity of updating established associations with extinction learning (Bouton, 2004). Particularly, in this thesis we will discuss and examine three phenomena leading to relapse after behavioural extinction: the renewal effect, the spontaneous recovery effect and the reinstatement effect.

Renewal effect. After a behaviour associated with the presentation of a CS is extinguished, it can be brought back when this CS is presented in a context different from the extinction context (for a review, see Bouton, 2002). One paradigm that is commonly used is: conditioning a CS-US association in a particular context (Context A), extinguishing it in a second context (Context B), and testing in a third context (Context C). This “ABC” design—in contrast to an contextually consistent control or “AAA design”—has been associated with a renewal of the previously extinguished behaviour (Bouton & Bolles, 1979; Harris et al., 2000; Rescorla, 2007). As well, this effect is observed in an “ABA design” where training occurs in A, extinction in B and testing occurs back in A (Bouton & Bolles, 1979; Bouton & Peck, 1989; Harris et al.,

2000; Rescorla, 2007). Furthermore, it is also evident in an “AAB design” where acquisition and extinction occur in the same context and the test is in another (Bouton & Ricker, 1994; Rescorla, 2007). However, this AAB design could make the renewal effect more difficult to detect compared with the ABA or ABC designs (Bouton, 2002). This renewal effect represents a vulnerability of the extinction learning to initially generalize to other contexts (Holmes & Westbrook, 2013).

Spontaneous recovery effect. The passage of time after extinction, in the absence of any other change, can also lead to a relapse of behaviours when the CS is again presented (Bouton, 1993; Bouton, Nelson, & Rosas, 1999; Pavlov, 1927). The spontaneous recovery effect itself represents another area where extinction learning is vulnerable to relapse. A caveat about the spontaneous recovery effect is that it could also be compared to the renewal effect in terms of a change in the internal context of the learner that is caused by internal changes in state, that occur over time (Bouton, 1993, 2002, 2014). These effects taken together support a view that extinction does not immediately generalize outside of the external and internal context that the extinction learning took place (Leung & Westbrook, 2008).

Reinstatement effect. Extinguished behaviour is also vulnerable to relapse after an exposure to the US by itself. Following extinction learning, a later presentation of the US-alone can lead to an increase in responding to the CS on a subsequent test (Pavlov, 1927; Rescorla & Heth, 1975). Rescorla & Heth, (1975) described this effect after using an experimental design with a 24-hour delay, to control for possible emotional responses or immediate effects of the US. This length of a delay is much shorter than those generally used in Spontaneous Recovery

paradigms. Furthermore, the reinstatement effect is also evident in groups over-and-above the renewal effect compared to un-reinstated subjects in a renewal test (Westbrook, Iordanova, McNally, Richardson, & Harris, 2002). However, this effect is dependant on the US being presented or tested in the same context as the reinstatement effect (Bouton, 2014; Bouton & Peck, 1989; Rescorla & Cunningham, 1977; Todd et al., 2014).

Rescorla-Wanger Model

Rescorla and Wagner famously described a model of how learning an association between a CS and a US progresses. According to the Rescorla-Wagner model, the amount of learning that occurs when a CS is presented depends on a discrepancy between what the subject expects will follow the CS and what actually occurs (Rescorla & Wagner, 1972; Wagner & Rescorla, 1972). If the CS is followed by the US, there is an increase in the strength of the association and a corresponding increase in the predictive value of the CS. However, the increase is inversely proportional to how much has already been learned about the predictive relationship between the CS and US. Before a CS has been conditioned to a particular US, the occurrence of that CS does not predict that the US will follow, and the associative strength between CS and US is zero. A certain amount of associative strength will be acquired the first time that the CS is followed by the US. So, the next time the CS occurs it will be predictive of the US following with at least some probability that is greater than zero. If the second occurrence of the CS is again followed by the US, there will be a further increase in strength of the association, but the amount of the increase will be less (everything else held constant) than the

increase that occurred with the previous CS-US pairing. With subsequent CS-US pairings, the associative strength will continue to increase, but the amount of accrual will continue to diminish with each subsequent pairing. With a sufficient number of CS-US pairings, a maximum level of associative strength will be reached, at which point the CS will be a perfect predictor of the US.

The difference between the maximum predictive value a CS can acquire given a specific US and the current predictive value of that CS is referred to as the *prediction error* (Rescorla & Wagner, 1972). For example, if a subject has received a few CS-US pairings, it might predict upon the next occurrence of the CS that the US is likely to follow with a certain probability. If the US does in fact follow the next CS occurrence, the prediction error would be the difference between what was expected—which was a certain probability—and that the US was delivered. In this example, there would also be an increase in the associative strength, and a corresponding increase in the predictive value of the CS. Thus, the prediction error that occurs when the CS is followed by the US changes as the strength of the association between CS and US changes. Early in the conditioning process, associative strength is at a minimum since there is either little expectation that the US will occur with the CS; and the prediction error is at a maximum since there is the most surprise at the occurrence of the US.

The strength of the CS-US association is reflected in the subject's behavioural response to the CS. Thus increases in associative strength lead to increases in the magnitude or probability of a particular CR. This relationship between prediction error, associative strength, and the CR can be represented in a mathematical model

(e.g. Pearce & Hall, 1980; Pearce & Mackintosh, 2010; Rescorla & Wagner, 1972; Wagner, 1981; Wagner & Rescorla, 1972), according to which the change in CR magnitude follows a decelerating function known as an acquisition curve. The greatest increase in CR magnitude or probability occurs with the first CS-US pairing, and each successive pairing adds a decreasing amount of further increase to the CR, until a behavioural asymptote in responding is reached. After this point, additional CS-US pairings do not produce additional increases in measures of CR behaviours and presumably do not produce increases in associative strength.

Application of the Rescorla-Wagner Model to Omission and Overexpectation

In this error-prediction perspective, CRs can be altered by repeated presentation of the CS without the expected US (Omission). It can also be altered by pairing two CSs that individually have an expected outcome, however when combined, the US delivered is less than the expected sum of the two individual expectations (Overexpectation). Here, both the omission of the US and an overexpectation of the magnitude of the US, represents the error signal. In both of these examples the Rescorla-Wagner model and others, would explain the subsequent decrease in responding, as a learned decrease in expectation following the CS. For omission, each successive presentation of the CS without the expected US further reduces the CS-US association. In this view, omission imparts the most change in associative strength due to the greatest error between what is expected from a CS and subsequent US. Generally, overexpectation in this model would decrease the CS-US association in a similar way. According to an error-prediction perspective, the pairing of two CSs, a subject should expect the sum of both

expectations to occur. This is seen in the *summation* of observed behavioural responses following this type of pairing (Mackintosh, 1974, Pavlov, 1927). When what is delivered is less than that summed expectation, the error-prediction correction would be attributed to some degree to both stimuli. Repeating this training would lead to a decrement in response on a subsequent test to either cue (Rescorla, 2006b, 2007; Rescorla, 1970; but also see Haney, Calu, Takahashi, Hughes, & Schoenbaum, 2010). As such, in this view, both omission learning and overexpectation learning employ an empirically similar error term (Rescorla, 2006b, 2007), where the magnitude of what can be learned in a given Overexpectation trial (for example where half of the expected US is delivered) would be less than with an Omission trial (where no US is delivered) on the same trial.

Ways to achieve long lasting extinction effects (Deepening Extinction)

Once an association has been learned or encoded, this memory is not passively erased or forgotten. Rather, a failure to retrieve that memory is often an active learning process that inhibits behaviours associated with the original learning. Animals need to be able to update learned associations as a result of changes to the environment. For example, food might have been readily available in a certain location but as the seasons change this might no longer be the case. However, updated expectation after extinction learning is vulnerable to relapse. When it comes to maladaptive behaviours, a relapse could be detrimental. As such, a deepening of extinction would refer to a form of extinction learning that is more resistant to relapse compared to Omission (Hendry, 1982; Leung et al., 2012;

Rescorla, 2006a). An approach that would make extinction learning more durable would be advantageous in dealing with maladaptive behaviours.

Taken from a biological perspective, evidence has been presented of unique and overlapping cell populations associated with Omission and Overexpectation (Jordanova, Deroche, Esber, & Schoenbaum, 2016; but also see Sissons & Miller, 2009; Witnauer & Miller, 2009). Given the potential for parallel biological mechanisms associated with these different extinction techniques, a Mixed-extinction paradigm is potentially a superior approach to achieving deeper extinction rather than using an Overexpectation or an Omission extinction paradigm alone.

However, a prediction-error perspective does not yield an obvious account about how a group experiencing both forms of extinction would compare to groups that received the same number of training trials in exclusively Overexpectation or exclusively Omission extinction training. In this view, Omission is the most effective alternative (using the same number of trials) compared to Overexpectation or any combination of Omission trial mixed with Overexpectation trials. This is due to the perspective that the most that can be learned on a trial is when there is a complete expectation that the US will occur every time the CS occurs and that US is not delivered in any degree on that instance. For Omission, that decrease represents 100% of what can be learned during a particular trial, according to this mathematical model. As such, any series of training that includes overexpectation learning would involve some delivery of the US and represent less surprise than omission on a given trial. Also in overexpectation learning, the change in associative

strength is divided in that it is to be attributed partly to each of the two CSs involved in the overexpectation pairing. As such, as one of the two CSs is the target in an Overexpectation paradigm, that CS-US association obtains less of the total inhibition acquired as a result of that extinction.

Although overexpectation learning has been thought to represent the same empirical form of learning and thus should be subject to the same mathematical model (Rescorla, 2006b, 2007), other behavioural theorists (e.g. Sissons & Miller, 2009; Witnauer & Miller, 2009) have found evidence suggesting that, after various post-extinction manipulations, overexpectation-extinction differentially affects subjects compared to those that experienced omission-extinction. Specifically, Witnauer & Miller (2009) used a conditioned suppression paradigm to demonstrate that post-training context exposure, as well as *overtraining*, differentially affect Overexpectation and Omission. These authors took differential effects on Omission and on Overexpectation trained rats, to infer that there are empirical differences in these forms of learning that are not accounted for in models like the Rescorla-Wagner model, which use the same error mechanism to explain these two forms of extinction learning.

Given the behavioural evidence for dissociable omission and overexpectation processes mentioned above, the underlying neural mechanisms must also be distinctive in some way. Iordanova et al. (2016) did not use a Mixed paradigm in their experiment, however there is a well-counterbalanced Omission as well as an Overexpectation condition. This experiment used electrophysiological recordings in the central nucleus of the amygdala, to track cell firing as a result of these forms of

extinction learning. This work suggested evidence of cell populations that are unique to omission, populations unique to overexpectation as well as cell populations common to both (Iordanova et al., 2016). The implications of this work, as well as the extent and exact mechanisms of this biological basis is beyond the scope of this thesis (for more see: Janak & Corbit, 2011; Janak & Tye, 2015; Todd et al., 2014). The important consequence here in terms of this thesis is a focus on unique and overlapping cell populations as an explanation for observed behavioural differences after extinction learning.

This thesis builds on the work of Sissons & Miller (2009), and Witnauer & Miller (2009) who highlighted behavioural dissimilarities between omission and overexpectation learning as well as the work of Iordanova et al. (2016) who examined biological differences in amygdala cells associated with these forms of extinction learning. Here we employed a novel form of Mixed-extinction learning with the hypothesis that recruiting more cells (purported to be part of the biological basis for said learning) involved in extinction learning (both unique and overlapping between Omission and Overexpectation) would result in a behaviourally evident deepening of extinction learning as seen with an attenuation of relapse behaviours. This perspective is based on the view that the original association and its subsequent behaviours are not erased by extinction learning but rather extinction learning represents new learning events that inhibit behaviours that result from the original formed association. Although there have been a few studies examining the deepening of extinction resulting from an Omission followed by an unreinforced pairing of two previously trained CSs (e.g. Leung et al., 2012), to date this author has

not found evidence that a study has examined if an Overexpectation-Omission, Mixed-extinction paradigm would deepen extinction, nor has there been sufficient discussion of these effects in terms of a biological rationale.

Thesis aim and rationale

The present study examines a novel Mixed-extinction design in terms of resistance to relapse compared to the robust omission-only alternative. Rats were trained to separately associate either a cue-light or a tone, with reinforcement. As a result of this training rats would enter a food-delivery magazine where this sucrose reinforcement was presented. We measured the percent of time subjects would spend in the magazine during presentation of the associated target cue, as well as the number of times the subjects entered during each presentation. Subjects then experienced one of four extinction conditions: a non-extinguished control, an Overexpectation-only, an Omission-only or a Mixed paradigm consisting of blocks of overexpectation-learning followed by blocks of omission-learning. Subjects were tested for renewal, spontaneous recovery and reinstatement effects to compare and contrast the possible deepening of extinction between groups. We specifically predicted that the Mixed extinction group would demonstrate resistance to relapse associated with these effects and that our other groups would replicate findings present in the literature.

Method

Subjects

Forty-eight male Sprague Dawley Rats (350g–450g) bred in-house (Concordia University, Montreal, Canada) and twenty-four male Sprague Dawley Rats (275g–350g) that were purchased from Harlan Labs (Indianapolis, USA) were used in this experiment. Rats were single housed in polycarbonate cages (48.3 cm in length, 26.7 cm in width, and 20.3 cm in height) with wood chip bedding (Envigo) and given *ad libitum* access to food and water prior to a food restriction regimen. The colony room was maintained at 21°C and on a 12-hour reverse day-night cycle (lights off at 8 am). This experiment was conducted in accordance with the guidelines of the Canadian Council on Animal Care, and approved by the Concordia University Animal Research Ethics Committee.

Behavioural apparatus

Twelve chambers (MedAssociates ENV-007CT; measuring 30.5 cm in length, 24.1 cm in width, and 29.2 cm in height) were installed in holding cabinets that aided to isolate each chamber from external light and sound. Each box had a magazine in which pellet reinforcers were dispensed. This magazine contained an infrared beam and an infrared detector. This detector would signal to record and permit a computer to time-stamp each instance of breaking this infrared beam by the rat entering the magazine.

Both auditory and visual cues were used in this experiment. The visual cue consisted of a 4 Hz flashing cue light (MedAssociates) located above and to the left of the food magazine. Where as the auditory cue consisted of a 72 dB white noise

delivered through a waveshield (Adafruit Industries) assembled in-house in conjunction with an Arduino UNO (Arduino) digital-analogue converter. The house lighting consisted of a red light (MedAssociates) located above the magazine, which was on throughout every training session in both contexts to allow for filming of the behavioural sessions.

Contextual specification. Half of the chambers remained *bare*, in other words without any opaque contextual inserts. The other half of the chambers contained *patterned* contextual inserts that were either spotted or checkered in a counterbalanced fashion. The spotted pattern was composed of black dots (diameter of 1.5cm and centre-to-centre distance of 2.5cm) on a white background, and the checkered pattern (3cm x 3cm squares) was also on a white background, both as described in the literature (Honey & Watt, 1999). These contexts were made of laminated paper and were placed outside of the clear polycarbonate operant chambers on the top, front and wall behind the chamber or were covered by 0.3175cm thick clear polycarbonate (Johnston Industrial Plastics). One polycarbonate-covered contextual insert completely covered the left sidewall of the chamber. Individual cut pieces covered portions of the right side of the chamber that were not occupied up by cue lights or the magazine food-receptacle and were flush with the aluminum tracks that vertically lined that side of the chamber (see Figure 1). The back of the operant chamber remained clear to allow filming by a camera (MedAssociates, VID-CAM-MONO-1, VID-LENSE-2.3-CS) mounted in the back of a holding cabinet.



Figure 1. A representative image of a patterned context. This is a MedAssociates box that has spotted-pattern context pieces installed. External context pieces were laminated and internal pieces were then protected with polycarbonate.

Scent was also used to differentiate the contexts. Bitter almond extract dissolved in ethanol (President's Choice®) was diluted with tap water to a final concentration of 2% to provide a distinct odour to the patterned contexts. Whereas, vinegar (generic food grade 5% acetic acid) was diluted with tap water to a final concentration of 1% acetic acid and was used exclusively in the bare context. Rats consistently used the same conditioning chamber during this experiment in order to maintain a fixed perception of the context.

Experimental procedure

As a form of counter balancing, the target cue for half of the rats in each group was the auditory cue with the visual cue as a constantly reinforced control and *vice versa* for the other half of the subjects. Prior to each training session, the conditioning chambers were cleaned with water containing the context-distinguishing odour.

At least one week prior to the experiment, rats were switched from an *ad libitum* diet to a restricted one in which they were fed approximately 10–12g of rat food (Charles River) each day, which maintained them at approximately 85% of pre-restriction body weight. Prior to the start of training, rats were handled approximately two minutes each, in an area just outside of the experimental room for five days leading up to the start of the experiment.

Pre-exposure to pellets. On the day prior to the start of magazine training (described below) rats were each given twenty 45mg chocolate-flavoured sucrose pellets (Bio-Serv) each, in their home cage. The aim of this practice was to minimize

any potential for novelty aversion and to promote the likelihood that the sucrose pellets would be consumed in the subsequent magazine training.

Magazine training. Rats each underwent one magazine training session where they individually received 30 presentations of two pellets, for a total of 60 pellets, in the conditioning chamber magazine in the absence of any discrete cue presentations. The delivery of individual pellets in each two-pellet presentation, was separated by one second and the interval between each pair of pellets was 120 seconds. Prior to the delivery of the first pellet presentation period there was a 120-second acclimatization period. The total time of the session was sixty-three minutes.

Phase 1. Conditioned stimulus training lasted fourteen days with one training session per day. Prior to any presentations there was a 120-second acclimatization period. All rats received 10 pairings of sucrose pellets for each of the cues, resulting in a total of 20 conditioning trials per session. Each condition stimulus presentation was 10 seconds and a sucrose pellet was delivered into the magazine on the 8th and again on the 9th second of the cue. Trials were separated on average, by a three-minute intertrial interval (range: 120–240 seconds) randomly selected from a list of values (120, 135, 150, 165, 180, 195, 210, 225 or 240 seconds) without replacement.

There was also a pseudo-random conditioned stimulus presentation order within the session (A, B, B, A, B, A, A, B, ...) to control for the possibility of expectation of one cue or the other, on an alternating or blocked basis. Also, in order to avoid an effect of expectation on the type of stimuli—namely white-noise

or flashing-light—the pseudo-random order of the stimulus presentations was inverted (B, A, A, B, A, B, B, A, ...) for some of the days. Furthermore, test days would be the inverse of the pseudo-random conditioned stimulus presentation order from the previous day.

Phases 2 & 3. Extinction learning for this experiment was broken down into two phases of four days each, with one training session per day consisting of 10 presentations of the target cue and 10 presentations of the control non-target cue. The order of the stimuli was juxtaposed as mentioned above. The Control group would receive reinforcement of both types of cues throughout the experiment (with the exception of the test days where the target cue would not be reinforced). The Omission group received unreinforced target cues on the eight days of extinction training as well as on test days while always reinforcing the non-target cue. The Overexpectation group received exposure to both types of cues simultaneously for 10 trials with 2 pellets delivered each presentation in place of an expected four pellets. This was done for all eight of the extinction-training days. The non-target cue (either white-noise or flashing light) was also reinforced for 10 trials juxtaposed as described above. And, the Mixed group received the same condition as the Overexpectation group for the first block of four days and the same condition as the Omission group for the second block of four days.

Probe test of extinction learning. On the fifth day of extinction learning the Omission group, Mixed group and Control group underwent a test in which the target cue was not reinforced. This allowed for an evaluation of the extent that overexpectation learning was taking place for the Mixed group under the particular

experimental parameters. The Overexpectation-only group did not receive this test as this might have interfered with the results from a pure overexpectation condition.

Renewal test. After completing the extinction learning blocks, half of the rats from each group were tested in a novel context. As such the rats were in a different room with a different visual pattern (if the learning context was bare then the novel context would be patterned and *vice versa*) with a novel odour (if trained with vinegar then the odour would be almond and *vice versa*). Here, the rats were tested with 10 unreinforced exposures of the target cue and 10 reinforced exposures of the control cue. On the subsequent day, these rats that experienced the novel context were tested again in their original context and the rats that were tested in their familiar context were then similarly swapped into a novel context.

Spontaneous recovery test. Thirteen days after the renewal tests, rats were similarly exposed to 10 non-reinforced presentations of the target cue and 10 reinforced presentations of the non-target cue. This was in a mixed sequence as mentioned above.

Reinstatement test. Reinstatement tests took place seven and fourteen days after the Spontaneous Recovery test. On the day before each reinstatement test half of the rats were returned to the behavioural boxes and exposed to 30 presentations of two pellets each, with a 120-second intertrial interval and a 120-second initial acclimatization period. The other half of rats, were simply handled in the colony room on that day. On the next day, for the Reinstatement test, rats were presented 10 cues of the target cue non-reinforced juxtaposed (as mentioned

above) with the non-target cue still reinforced. The following week, the other half of subjects experienced the counter-balanced condition. This involved the reinstatement of pellets or handling-only followed on the next day to the same test as the week before.

Cohorts. The experiment was conducted with two separate cohorts of animals, and two experimenters ran different groups of animals. Experimenter 1 ran Mixed, Omission and Control ($n = 12$) in the first cohort and ran Overexpectation ($n = 12$ in the second cohort). Experimenter 2 ran all four groups in the second cohort ($n = 6$).

Table 1

Summary of the experimental design

Group	Phase 1	Phase 2	Test/Phase 3	Phase 3	Renewal	Spontaneous		Reinstatement	Test
						Recovery			
Control	A+	A+	A-	A+	A-	A-	A-	+ or Handle	A-
	B+	B+	B+	B+	B+	B+	B+		B+
Omission	A+	A-	A-	A-	A-	A-	A-	+ or Handle	A-
	B+	B+	B+	B+	B+	B+	B+		B+
Overexpectation	A+	AB+	AB+	AB+	A-*	A-	A-	+ or Handle	A-
	B+	B+	B+	B+	B+	B+	B+		B+
Mixed	A+	AB+	A-	A-	A-	A-	A-	+ or Handle	A-
	B+	B+	B+	B+	B+	B+	B+		B+

* or handling only

Statistical analysis

A raw data log was produced by MED-PC (version 4.2) and subsequently sorted by a custom program written for Matlab for Mac. The behavioural data used for analysis were the 10-second conditioned-stimulus presentation to the target cue. Statistical analysis was run using SPSS (version 20.0) for Mac and effect size statistics were computed for repeated measures by SPSS and for single-trial comparisons by hand. Graphs were made using Graph Pad Prism (version 6) for Mac.

Effect size statistics (η^2 or η^2_p) were computed to test the main hypothesis and also used for evidence that these particular learning paradigm parameters were effective. In terms of null hypothesis testing, planned comparisons were employed since only a small number of deliberate, and *a priori*, comparisons were to be tested on the larger data set collected (see Myers, Well, & Frederick, 2010). Conventional use of a planned comparison between only two groups do not require the addition of omnibus *F*-tests (Kline, 2004; Ruxton & Beauchamp, 2008). However, Omnibus *F*-tests are used in conjunction with the use multiple planned comparisons (Field, 2013). Here the choice was made to also include this Omnibus analysis in addition to the individual *t*-tests. It is worth noting that the inclusion of Omnibus *F*-tests with a planned *t*-test did not inflate our family wise type 1 error, as these tests are independent. Additionally, concerning the acquisition phase, a repeated-measures mixed-model analysis of variance (ANOVA) was used to determine if groups differed following acquisition learning and prior to the experimental manipulations.

Here, η^2 effect-size statistics were chosen to allow for a more intuitive interpretation of an effect size between the repeated-measures mixed model and simple comparisons used in these analyses. One-tailed t -tests were used because of the directionality of the hypotheses, namely that the Mixed group would perform *below* the level of all other groups on test, and that paradigm confirmation tests should also only be in a predictable specific direction. If data were to be contrary to these directional hypotheses, then null hypothesis test would not have been interpretable here as a result of this design choice.

Results

The main hypothesis is that the Mixed group will be more resistant to relapse when directly compared with the Omission group on the three post-extinction manipulations of Renewal, Spontaneous Recovery, and Reinstatement. Next, there will be tests for evidence that these test paradigms were effective under our specific parameters. Lastly, given the relative novelty of this procedure the acquisition training, Omission extinction, and Overexpectation extinction were tested for effectiveness.

Relapse analysis

These analyses tested the hypothesis that this Mixed extinction condition would perform lower on the measures of percent time in the magazine, and number of entries into the magazine, during the CS. Thus this hypothesized effect would support a view that this Mixed condition attenuates the behavioural relapse associated with: renewal, spontaneous recovery and reinstatement effects observed above. Effect size statistics were calculated as the primary evidence followed by omnibus ANOVA and planned comparisons.

Renewal. Consistent with the literature, this analysis used the block of the first two trials as the variable of interest. This combination of the first two target-CS exposures is useful in order to account for any bias that might have occurred due to various behaviours associated with novelty. As such, the Mixed group ($M = 4.49, SD = 0.80$) performed lower on the measure of percent time than the Omission group ($M = 17.54, SD = 17.31$) $\eta^2 = .22$. This result suggests that 22% of the variance observed is explained by extinction condition in the hypothesized direction. To

supplement an argument from the basis of effect-size statistics null-hypothesis tests were run. For the subjects that were placed in the novel context, a one-way ANOVA was run. For the variable of percent time in the magazine, Levene's test was violated $F(3, 29) = 4.83, p = .008$. The ANOVA indicated that there was a statistically significant difference between at least one pair of the groups $F(3, 29) = 11.68, p = .000$. Subsequently, the planned comparison of a one-tailed independent-samples t -tests was performed. Here, Levene's test was violated $p = .001$ so the t -test was adjusted accordingly $t(9.77) = -2.15, p = .029$ (see Figure 2a). The violation of the assumption of homogeneity of variance is not surprising since this possibility is an obvious implication of differing extinction learning. Taken together the effect size in the hypothesized direction and the t -test, support the hypothesis that this model of Mixed-extinction attenuates the renewal effect.

Again, for the measured variable of number of entries the Mixed group ($M = 1.00, SD = 0.90$) performed lower than the Omission group ($M = 3.17, SD = 2.46$), $\eta^2 = .28$. This suggests that for this measure, 28% of the variability was accounted for in the hypothesized direction. As an additional measure, null-hypotheses testing was employed. A one-way ANOVA was used to compare the four groups that had experienced the renewal condition in a novel context. Levene's test was violated $F(3, 29) = 9.83, p = .000$. This ANOVA yielded statistically significant results $F(3, 29) = 9.13, p = .000$, suggesting that there was a difference between at least two of the groups. A one-tailed independent-samples t -test was performed as a planned comparison. Here Levene's test was also violated $p = .008$ so the t -test was adjusted

accordingly $t(10.11) = -2.48, p = .016$ (see Figure 2b). These results are consistent with the percent time measure and also support the hypothesis.

Spontaneous recovery. On the measure of percent time in the magazine, the Mixed group ($M = 18.57, SD = 24.22$) performed lower than the Omission group ($M = 22.02, SD = 26.40$) with $\eta^2 = .0049$. However, this suggests that only about 0.5% of the variance is explained by group. A one-way ANOVA was performed with Levene's test not violated $F(3, 68) = .47, p = .71$. The ANOVA failed to detect a statistically significant result $F(3, 68) = 1.21, p = .31$. A one-tailed independent-samples t -test was still performed as a planned comparison. Here Levene's test was not violated $p = .26$ and the result of this test was $t(34) = -0.41, p = .34$, which did not support the experimental hypothesis (see Figure 2c).

As for the measure of the number of entries, the Mixed group ($M = 4.50, SD = 5.20$) performed lower than the Omission group ($M = 5.39, SD = 7.63$) with $\eta^2 = .22$. This suggests that 22% of the variance on this measure is accounted for in the hypothesised direction. A one-way ANOVA was conducted and Levene's test was not violated $F(3, 68) = .45, p = .72$. This ANOVA failed to detect a statistically significant difference $F(3, 68) = .85, p = .47$. A one-tailed independent-samples t -test was still performed as a planned comparison with Levene's Test not violated $p = .31$. It also failed to detect a statistically significant result $t(34) = -0.41, p = .34$ (see Figure 2d).

Taken together, these results show little supporting evidence of an effect of group on the first trial of the Spontaneous Recovery test. Given the lack of consensus of effect size statistics between the two dependent variables and the

subsequent planned null-hypotheses tests, there is a lack of evidence to support an affirmative conclusion about the hypotheses from these data.

Reinstatement. For percent time in the magazine, the Mixed group ($M = 22.93$, $SD = 17.21$) performed lower compared to the Omission group ($M = 37.33$, $SD = 29.44$) with an $\eta^2 = .091$. This suggests that just over 9% of the variance is explained by group membership in the hypothesised direction. A one-way ANOVA was run and Levene's test was not violated $F(3, 32) = 1.71$, $p = .19$. This ANOVA failed to detect a statistically significant result $F(3, 32) = 1.02$, $p = .40$. A one-tailed independent-samples t -test was still performed as a planned comparison. Here, Levene's test was not violated $p = .071$, with $t(16) = -1.27$, $p = .11$ (see Figure 2e). The effect size and direction is consistent with the hypotheses and due to the conservativeness of our experimental design one might expect that, if an effect were present, this might be blunted by the repeated testing in this design.

Furthermore for the number of entries, the Mixed group ($M = 4.56$, $SD = 3.91$) performed lower than the Omission group ($M = 7.22$, $SD = 7.85$) was an $\eta^2 = .049$. On this measure about 5% of the variance was explained by group in the predicted direction. Also, a null-hypotheses one-way ANOVA, was conducted with Levene's test not violated $F(3, 32) = 1.09$, $p = .37$. This ANOVA failed to detect a statistically significant difference between groups $F(3, 32) = 0.70$, $p = .56$. A one-tailed independent samples t -test was still conducted as a planned comparison with Levene's test not violated $p = .14$. This test failed to detect a statistically significant result $t(16) = -0.912$, $p = .19$ (see Figure 2f).

The resulting effect sizes in the hypothesized direction, offer some evidence supporting an effect in the population. Although these null-hypothesis tests suggest there is a likelihood that results this extreme are due to chance, the conservative nature of the experimental parameters had biases these analyses away from finding an effect.

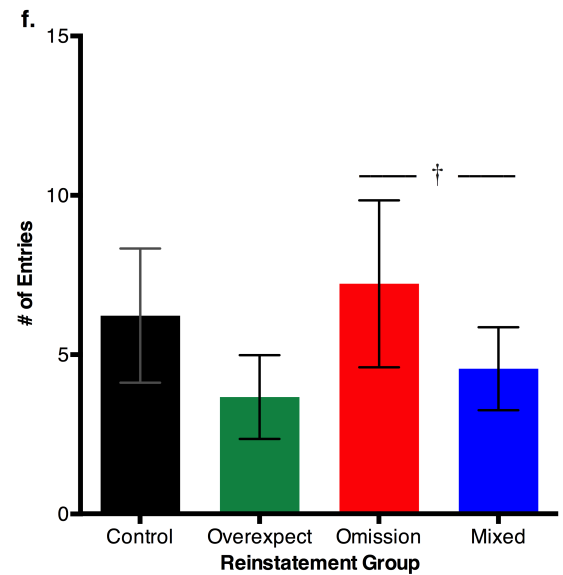
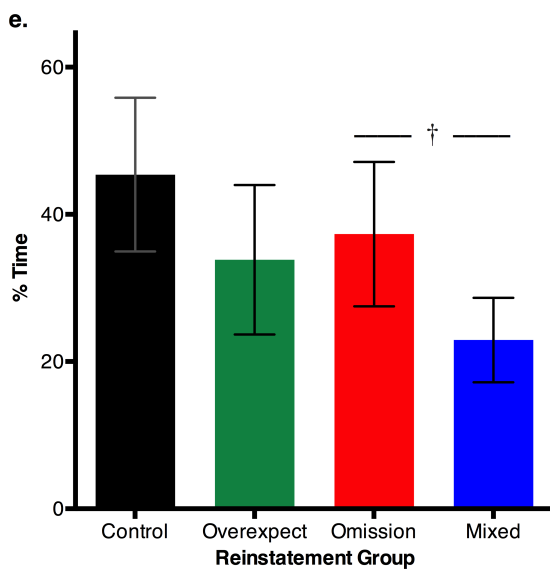
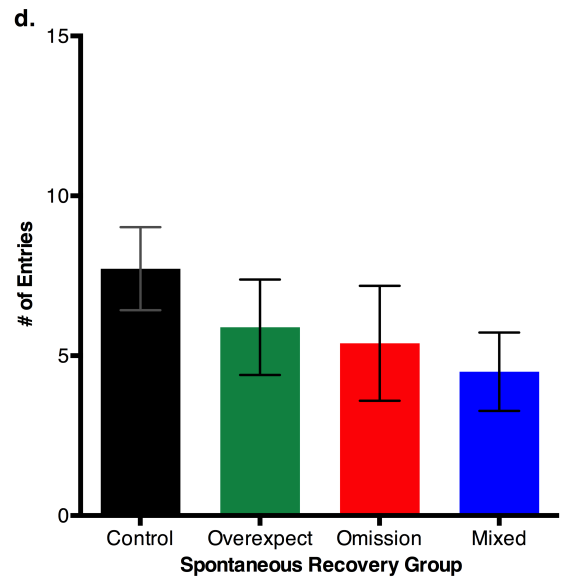
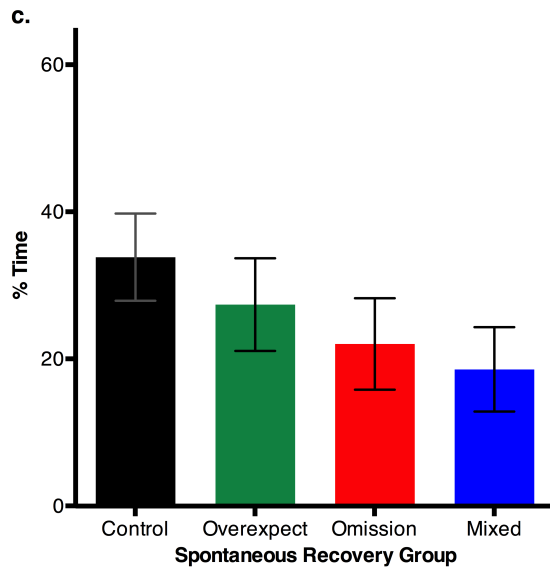
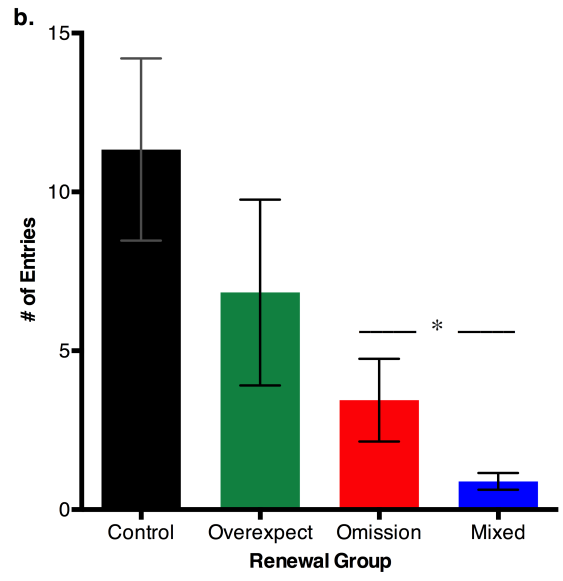
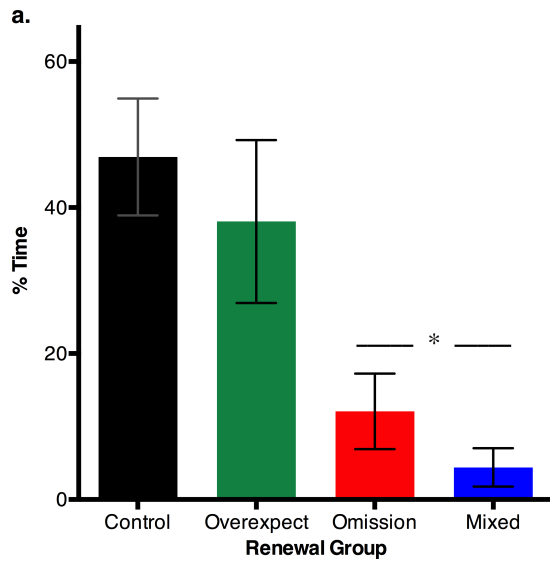


Figure 2. Post-extinction manipulations. The top two figures are the Renewal test results, the centre two figures are the Spontaneous Recovery test results, and the bottom two figures are the Reinstatement test results. The figures on the left represent the variable of percent time in the magazine and the figures on the right represent the number of entries. Error bars represent the SEM. * is a substantive effect size with $p < .05$, † is a substantive effect size only.

Effectiveness of post-extinction manipulations

Due to the novelty of this specific paradigm, we sought evidence to confirm the effectiveness of our particular parameters in producing these post-extinction manipulations. Below we have tested for evidence of a renewal effect, and a reinstatement effect by comparing an Omission group that did not receive the manipulation to the Omission group that did. The groups that did receive the manipulation for these tests should show greater responding on the first measure of the test day. For the Spontaneous Recovery manipulation the Omission group on the first trial of the test was compared to the mean responding during the last day of extinction training for the Omission group. If there is higher responding at the beginning of this test despite the extinction training then this will be taken as evidence of a spontaneous recovery effect.

Renewal effect. To test if our parameters were effective at producing a renewal effect, half of the Omission subjects were tested in a novel context. The other half was tested in their familiar context. If this paradigm was successful in producing a renewal effect then the Omission-Novel context group should perform higher on our measured variables than the Omission group in the same context on the first training block. This effect was observed with the novel context Omission group spending a greater amount of time in the magazine ($M = 17.54, SD = 17.31$) than the same-context Omission group ($M = 7.79, SD = 11.61$) $\eta^2 = .11$. This suggests that about 11% of the variance for Omission subjects on this block, is explained by contextual condition where novel-context rats perform higher on the measured variable. As a null hypothesis test, a one-way ANOVA was performed between the

four groups in each of the possible 2 conditions (novel or same context) for a total of 8 groups. For this analysis Levene's test was violated $F(7, 58) = 5.27, p = .000$. This ANOVA yielded evidence of a statistically significant difference between at least one of the conditions $F(7, 58) = 9.94, p = .000$. A one-tailed independent-samples t -test comparing the Omission-Same and Omission-Novel was performed. This comparison did not violate Levene's test $p = .09$, however the planned comparison did not yield a statistically significant difference though the finding was in the hypothesised direction $t(16) = -1.40, p = .09$ (see Figure 3c).

Similarly, this effect was also demonstrated for the measured variable of number of head entries during the CS presentation. Here the behaviour on the first block ($M = 3.17, SD = 2.46$) was higher for the Omission-Novel context group compared with the Omission-Same context group ($M = 1.28, SD = 2.21$) with an $\eta^2 = .15$ suggesting that about 15% of the variance is explained by the contextual manipulation in the hypothesized direction. A one-way ANOVA was conducted comparing the 8 renewal conditions. Here, Levene's test was violated $F(7, 58) = 6.84, p = .000$ and the ANOVA yielded a statistically significant difference in at least one of the groups $F(7, 58) = 7.78, p = .000$. A one-tailed independent-samples t -test was performed comparing the Omission-Novel to the Omission-Same conditions. Levene's test was not violated here $p = .32$ and the t -test approached but did not exceed our threshold for statistical significance $t(16) = -1.71, p = .053$ (see Figure 3f).

The relationship between experimental groups was consistent with an effective model, the observed effect size alone, is considered sufficient evidence of a

renewal effect under these particular parameters. Here, Control, Overexpectation and Omission groups in both novel and same contexts performed in a manner predicted by the design (see Figures 3a, b, d & e). Specifically, the Control groups showed the highest responding followed by the Overexpectation group. The Omission group in the same context showed very low responding. In the novel context the Omission-only group showed an increase in behaviours on the first trial indicative of a relapse. This was followed by very low levels of responding on subsequent trials also indicative of a relapse and return to a baseline level of low responding.

Spontaneous recovery effect. Evidence for spontaneous recovery was tested by comparing the mean level of responding on the last day of Omission training to the first trial of the Spontaneous Recovery test. For the percent time spent in the magazine during CS presentations, the mean responding of the Omission group during the last day of Omission training ($M = 9.12, SD = 8.43$) was lower than the responding of the Omission group on the first trial of the Spontaneous Recovery test ($M = 22.02, SD = 26.40$) with an effect size of $\eta^2 = .10$. An independent-samples one-tailed t -test was conducted. Since Levene's test suggested unequal variance between groups ($p = .000$) the t -test was adjusted accordingly ($t(20.43) = -1.97, p = .03$).

Similarly for the number of entries, the mean responding of the Omission group for the 10 trials of the last day of Omission training ($M = 1.52, SD = 1.06$) was lower than the first trial of the Omission group during the test of Spontaneous Recovery ($M = 5.39, SD = 7.62$) with an effect size of $\eta^2 = .12$. Again, Levene's test

suggested unequal variance between groups ($p = .000$) so the t -test was adjusted accordingly ($t(17.66) = -2.13, p = .02$). These data provide evidence of a spontaneous recovery effect in terms of both effect-size statistics and null-hypothesis tests (see Figures 4b & d).

Reinstatement effect. Here we tested for evidence of a reinstatement effect between groups in the Omission conditions. As evidence of a working reinstatement paradigm, the Omission group should perform higher during the first trial compared with subsequent trials. This difference should also be observed on the first trial for the Omission group in the novel context compared to the consistent context. Here the reinstated Omission group ($M = 37.33, SD = 29.44$) demonstrated more percent time spent in the magazine during the CS presentation compared with the non-reinstated Omission group ($M = 9.50, SD = 13.09$) with an effect size of $\eta^2 = .30$. This suggests that 30% of the variance between Omission rats is explained by the exposure to the US alone on the previous day. In terms of a null-hypotheses test, a one-way ANOVA was performed comparing the first trial for each of the four groups half of which experienced reinstatement on the previous day and half of which did not (for a total of 8 conditions). Levene's test was violated $F(7, 64) = 6.24, p = .000$, and the ANOVA yielded a statistically significant difference between at least two of the conditions $F(7, 64) = 4.40, p = .000$. A one-tailed independent-samples t -test was conducted comparing the Omission-Reinstated to the Omission-Non-Reinstated conditions. Here Levene's test was not violated as the result of this test was just above our threshold selected for Levene's test $p > .01$. This test yielded a statistically significant result $t(16) = -2.59, p = .01$. It is also worth noting that this

test would still have had statistically significant results if a higher threshold for Levene's test had been selected. Since performance of each group relative to each other was consistent with the hypothesis, the observed effect size in the predicted direction is taken as evidence of a working reinstatement model and this view was reinforced by the statistically significant result in the hypothesized direction (see Figure 5c).

Similarly for the measured variable of number of head entries, the Omission groups performed as predicted. The reinstated Omission group had considerably higher levels of behaviour on this measure ($M = 7.22$ $SD = 7.85$) compared with the non-reinstated group ($M = 2.11$ $SD = 3.44$) with an effect size of $\eta^2 = .17$. This suggests that on this measure, 17% of the variability between scores is accounted for by group membership with the Omission group rats having greater scores in the reinstated condition. A one-way ANOVA was run for the number of head entries variable on the first trial. Here Levene's test was violated at a level just below our threshold $p < .01$. The ANOVA yielded a statistically significant difference between at least two of the groups of the 8 conditions $F(7, 64) = 2.38, p = .031$. For our planned comparison between the Omission-Reinstated and the Omission-Non-Reinstated conditions Levene's test was not violated $p = .11$. This test yielded a statistically significant difference just below our threshold $t(16) = -1.79, p < .05$ (see Figure 5f).

The relative group performances were consistent with predicted relationships for both percent time in the magazine (see Figures 5a & b) and for number of entries (see Figures 5d & e). The effect size statistics were in the

hypothesized direction for the Omission conditions and these observations were supported by statistically significant results for the null hypothesis tests. Taken together this is considered to be sufficient evidence to conclude that this paradigm was likely successful at producing a reinstatement effect.

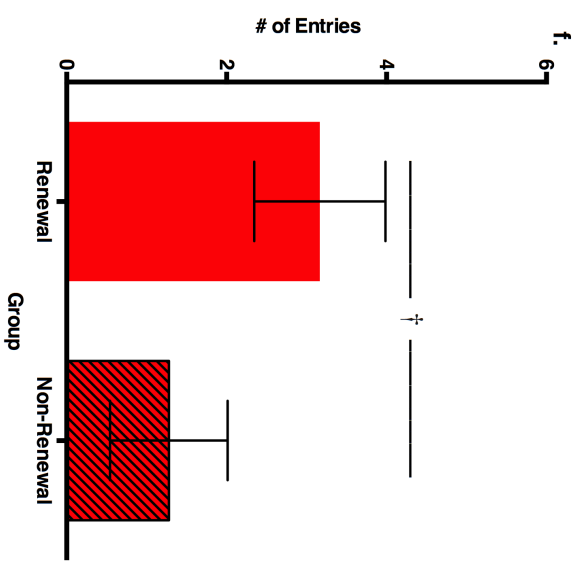
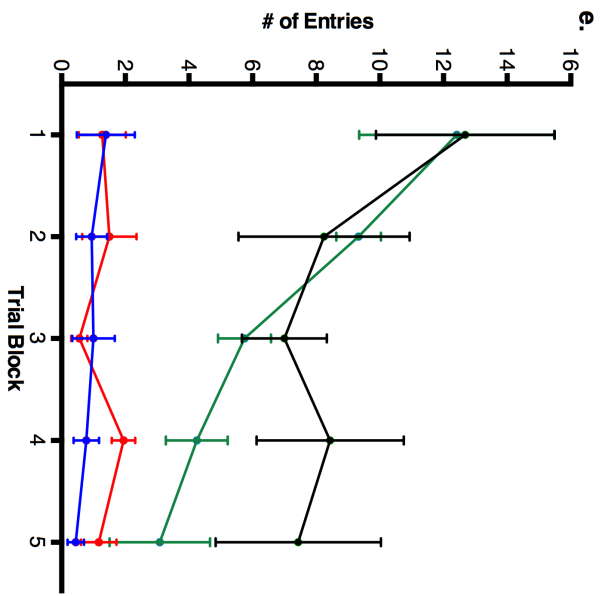
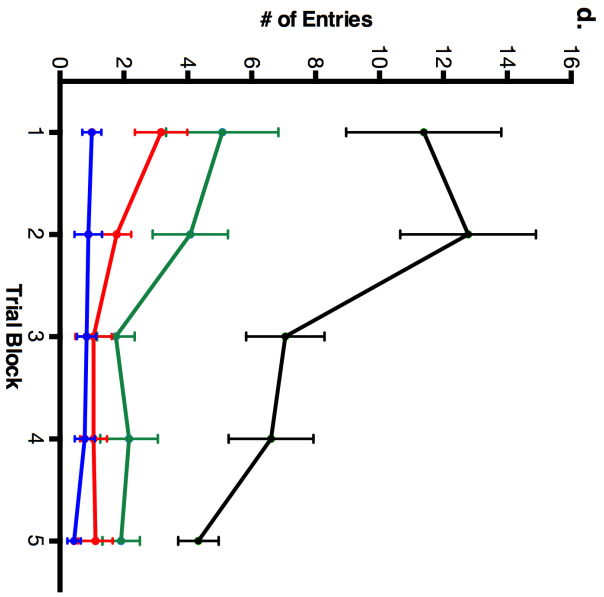
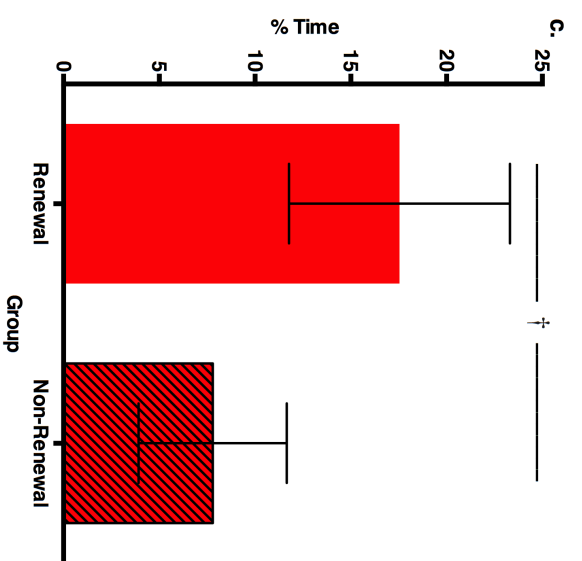
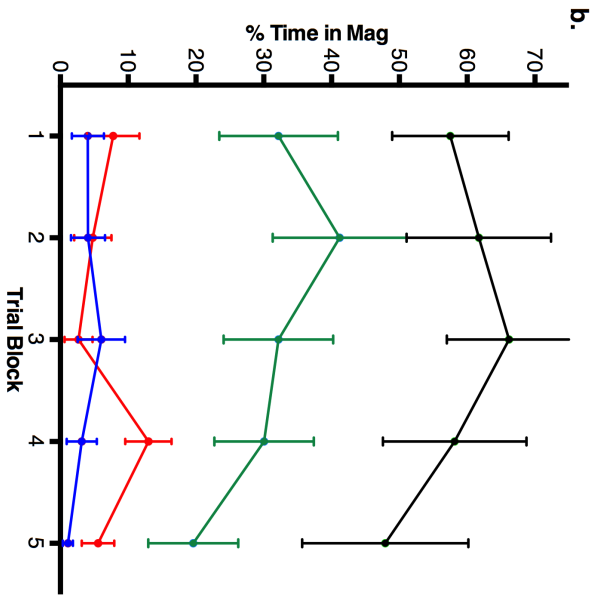
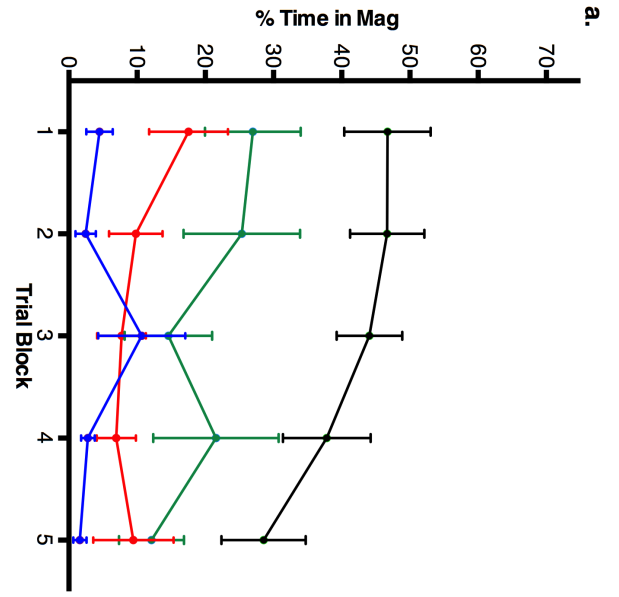


Figure 3. Evidence of a renewal effect. The top figures represent percent time in the magazine and the bottom figures represent the number of head entries. The left figures represent blocks of two trials for rats in the novel context where as the centre figures are the same context. The figures on the right represent the comparison of the Omission group between novel and same contexts. Error bars represent the SEM. † is considered to be a substantive effect size.

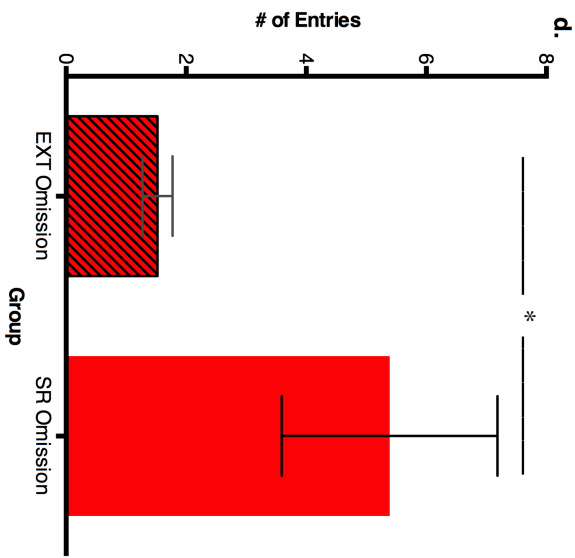
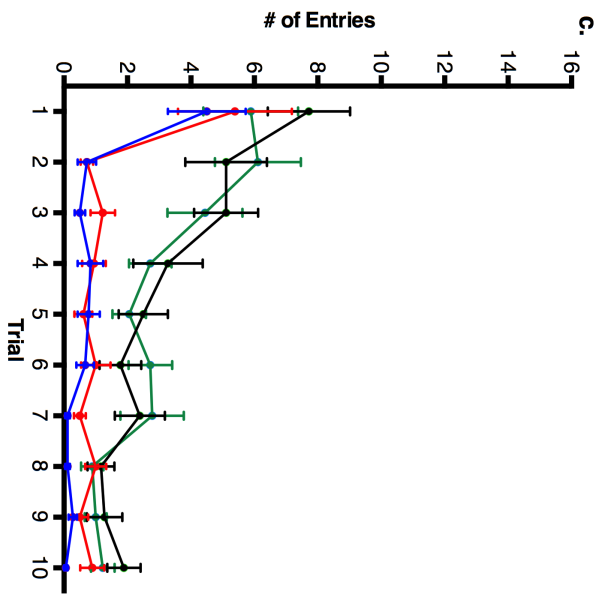
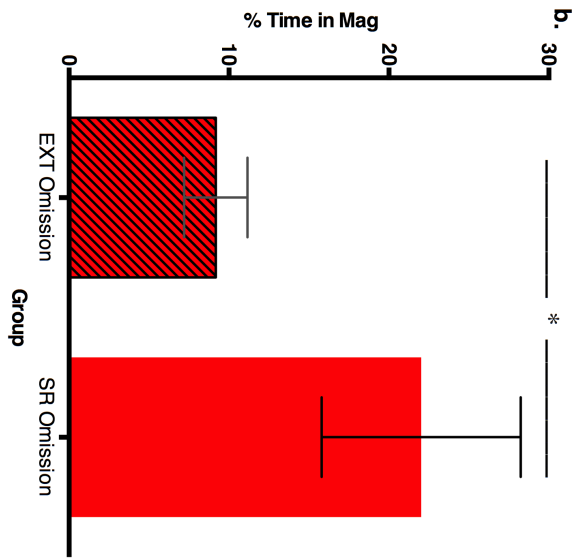
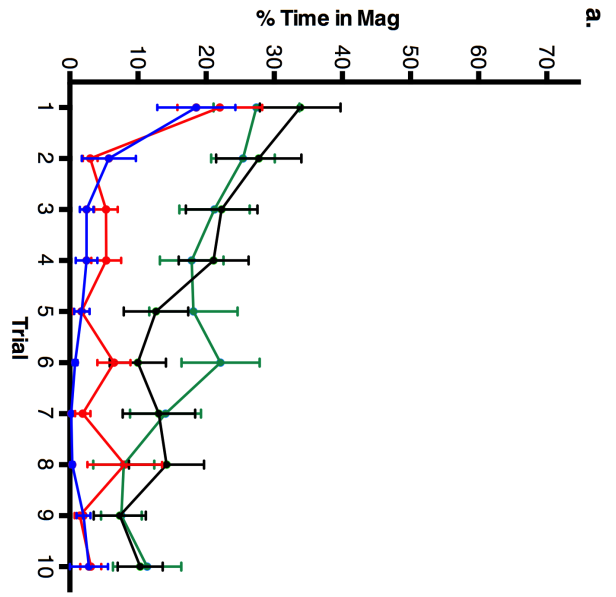


Figure 4. Evidence of a spontaneous recovery effect. The top two figures represent the percent time in the magazine and the bottom two represent the number of head entries. The left figures shows the data from the individual trials during the test session and the right figures represent a comparison between the average responding of the Omission group on the last day of extinction training compared to the first trial of the Spontaneous Recovery test. Error bars represent SEM. $*p = .05$ and is considered to be a substantive effect size.

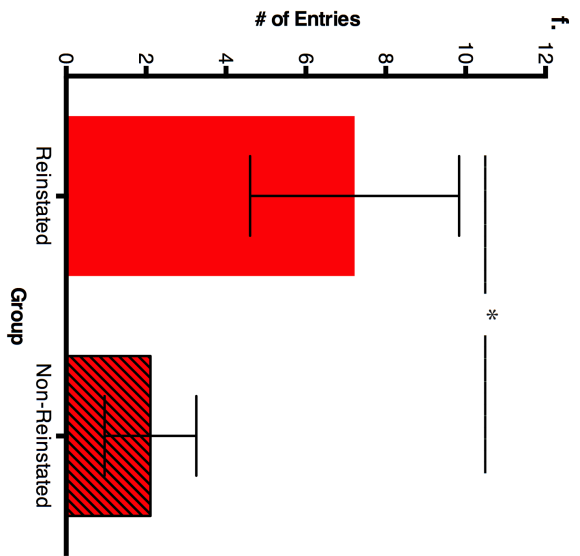
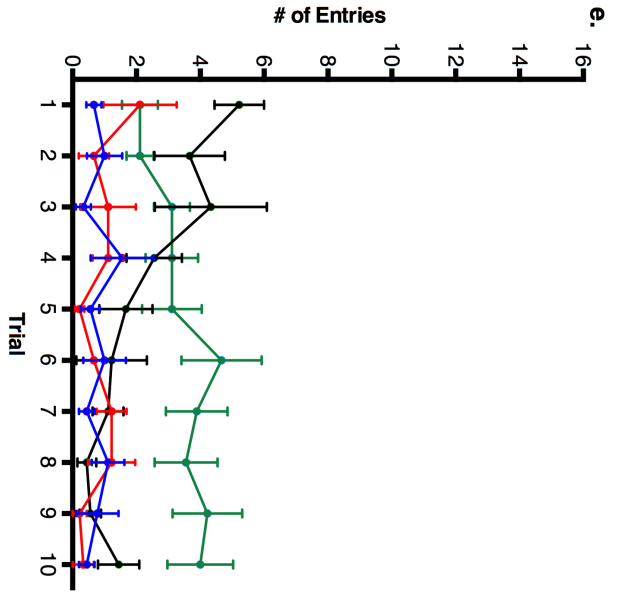
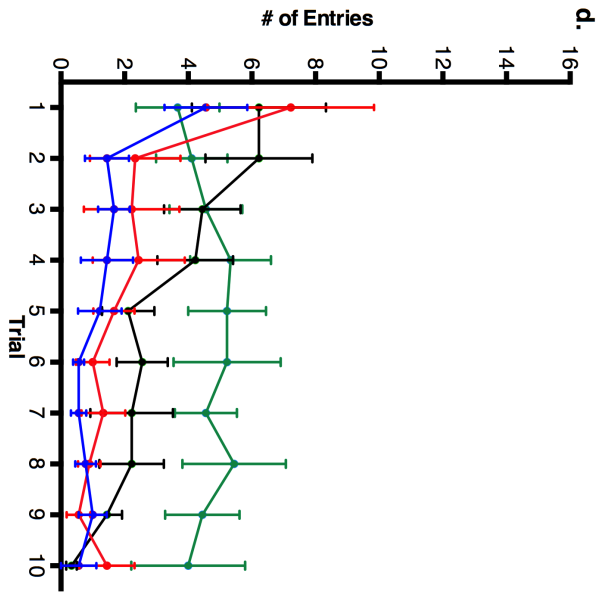
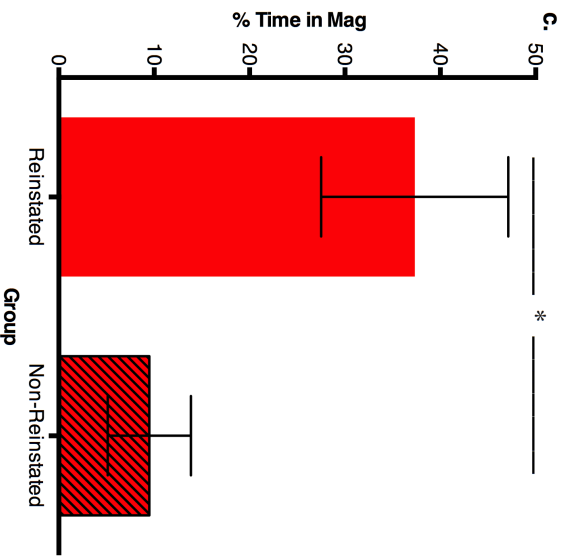
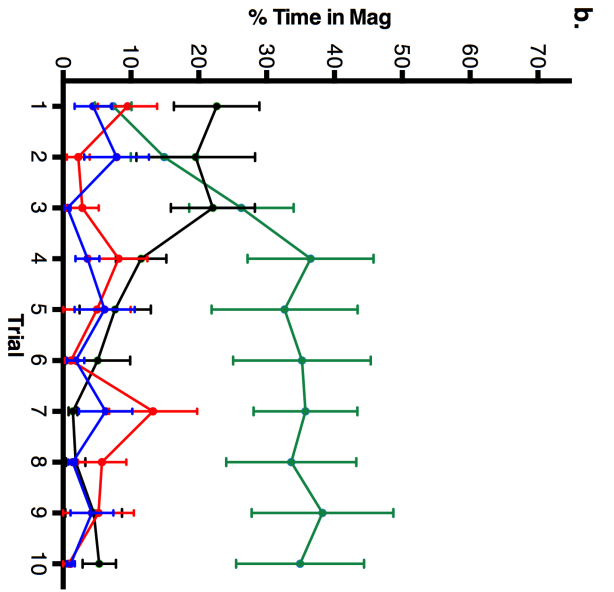
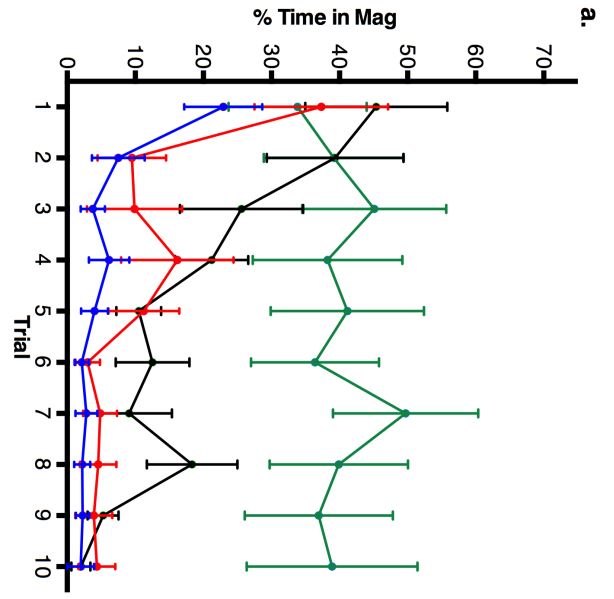


Figure 5. Evidence of a reinstatement effect. The top figures represent the percent time in the magazine and the bottom figures represent the number of head entries. The left figures show the data from individual trials of the reinstated subjects and the centre figures represent the non-reinstated subjects. The right figures show the comparison between the Omission group rats that either had or had not experienced the US on the previous day. Error bars represent the SEM. $*p < .05$ and had a substantive effect size.

Acquisition, omission, and overexpectation training

Again due to the novelty of this paradigm, evidence for the effectiveness of acquisition, omission, and overexpectation was sought. In terms of the effectiveness of the Overexpectation block for the Mixed group, this was not explicitly tested here. It was considered that if this training was not effective or was not effective for all subjects, then this would bias results away from finding an effect. This is therefore considered to be a more conservative measure rather than potentially introduce confounds by removing subjects in an unequal fashion between groups and the sensory modality of the target cue.

Acquisition training. Regarding acquisition training, rats in all groups should increase behavioural response across days, which is indicative of forming CS-US associations. There should be no effect of group during this phase prior as it is prior to experimental manipulation. Next, this responding should reach and maintain a behavioural asymptote where responding on the last day of training should not substantially differ from previous days. This was defined as less than 1% of the variance explained by the difference between the last day and each previous day $\eta^2_p < .01$.

For both percent time and number of entries, mixed-model repeated-measures ANOVA were conducted using each subject's mean percent time in a food magazine of ten separate ten-second CS presentations on a training day. The repeated measure was the daily mean for fourteen days of training and the between subjects factor was group membership. For both dependent variables Levene's test of equal variance between the groups was not violated on any of the days ($p > .01$).

For percent time in the magazine, no effect of group was detected $\eta^2_p = .009$, $F(3, 68) = 0.21$, $p = .88$. Consistent with the prediction, there was an observed effect of day $\eta^2_p = .50$, $F(5.58, 379.54) = 68.029$, $p = .000$ (see Figure 6a). Regarding the within-subject variable of day, Mauchly's test indicated that the assumption of sphericity had been violated $X^2(90) = 449.28$, $p = .000$, therefore Greenhouse-Geisser corrected tests are reported ($\epsilon = .43$). However, there was no observed interaction between Day and Group $\eta^2_p = .052$, $F(16.74, 379.54) = 1.25$, $p = .22$. The second premise was that subjects would demonstrate an upward linear trend in the percent time spent in the magazine as a result of subsequent days of training. A repeated-measures mixed-model ANOVA yielded a statistically significant linear trend $F(1, 68) = 173.29$, $p = .000$. Finally, it was expected that subjects would demonstrate a behavioural asymptote in learning. An asymptote was determined by comparing behaviour on the final two days of training and computing effect sizes between these two means as $\eta^2_p < .01$ as well as conducting a null hypotheses test using Fisher's least significant difference (LSD) correction for a pairwise comparison $\eta^2_p = .003$, $p = .68$.

Similarly, for number of head entries, no substantive effect of group was detected $\eta^2_p = .026$, $F(3, 68) = 0.61$, $p = .61$ (see Figure 6d). Mauchly's test indicated that the assumption of sphericity had been violated $X^2(90) = 637.65$, $p = .000$, therefore Greenhouse-Geisser corrected tests are reported ($\epsilon = .27$). There was an observed effect of Day $\eta^2_p = .49$, $F(3.47, 236.03) = 34.60$, $p = .000$. Similarly for the other measured variable, there was no observed interaction between day and group $\eta^2_p = .035$, $F(10.41, 236.03) = 1.25$, $p = .60$. A repeated-measures mixed-model

ANOVA yielded a statistically significant linear trend $F(1, 68) = 65.69, p = .000$. Here again, to test for a behavioural asymptote in learning we compared behaviour on the final two days of training by computing an effect size ($\eta^2_p < .01$), as well as conducting a null hypotheses test using Fisher's LSD a pairwise comparison $\eta^2_p = .001, p = .76$.

These analyses support a view that the groups were of the same population prior to the extinction condition manipulations. Behaviours support an upward linear trend as a result of learning and there is evidence that a behavioural asymptote was reached as early as the eighth day of training but quantitatively confirmed for the final two days of training.

Extinction training (Phases 2 & 3). Concerning evidence of extinction training, our hypotheses were threefold. First, Overexpectation and Mixed groups should not differ by the end of the first extinction-training block (Phase 2) as they had received the same training up to this point. Second, groups should differ considerably by the first trial of the last day of extinction training (Phase 3). Third, Overexpectation should be measurably lower than the consistently reinforced control by the end of the extinction training.

We compared Overexpectation and Mixed groups independently of the other groups during the 40 trials of the first extinction block (Phase 2); repeated-measures ANOVA were run for both dependant variables. This was done to compare Overexpectation and Mixed subjects, which had received the same Overexpectation training condition during this phase and tested the hypothesis that these groups not differ by chance prior to the experimental manipulation. The

assumption of homogeneity of variance was violated for both analyses. For percent time in the magazine, little effect was detected $\eta^2 = .018$, $F(1, 34) = 0.62$, $p = .44$ (see Figure 6b). This suggests that 1.8% of the variance is explained by group. Given this small effect, and the null hypothesis test, we should conclude that there are no observed differences between groups. This is also the case for the number of head entries, where 0.1% of the variance is explained by group $\eta^2 = .001$ and the repeated-measures mixed-model ANOVA failed to detect a statistically significant difference between these groups $F(1, 34) = 0.021$, $p = .88$ (see Figure 6e). These findings for both measured variables, provide evidence that Overexpectation and Mixed did not demonstrate a difference prior to the experimental manipulation.

Next we examined if the Mixed group differed during the CS presentations in terms of percent time in the magazine or number of head entries by the end of the second extinction phase (Phase 3). For the first trial of the last day of extinction training, two effect sizes were computed along with one-tailed independent sample t -tests. Homogeneity of variance was not violated for either dependent variable on this trial. For percent time in the magazine, the Mixed group ($M = 11.56$, $SD = 17.36$) was now lower than the Overexpectation group ($M = 49.69$, $SD = 21.65$) with an $\eta^2 = .50$. This suggests that 50% of the variability in scores is explained by group membership. In terms of null hypothesis testing, there was a statistically significant difference $t(34) = -5.83$, $p = .000$. Similarly for number of entries, the Mixed group ($M = 2.56$, $SD = 3.78$) was now lower than the Overexpectation group ($M = 9.33$, $SD = 5.18$) with an $\eta^2 = .37$. This suggests that 37% of the variability on this measure is

now explained by group. And again, for the null hypothesis test, $t(34) = -4.50, p = .000$.

Overexpectation learning. Concerning Overexpectation learning, we examined the first trial on the last day of extinction training. The hypothesis was that the Control group would perform higher on both measured variables even though the Control group had been receiving the same number of sucrose pellets as the Overexpectation group. Here effect size statistics were computed and one-tailed independent-samples t -tests conducted. On either dependent measure Levene's Test was not violated. Concerning percent time spent in the magazine during the CS presentation, observations were consistent with the hypothesis with the Control group ($M = 60.45, SD = 17.80$) performed higher than the Overexpectation group ($M = 49.69, SD = 21.65$) with an $\eta^2 = .072$. This suggest that 7.2% of the variance is accounted for by group and that this effect was in the hypothesized direction. The null hypothesis test approached statistical significance $t(34) = 1.63, p = .051$. Likewise for the number of entries, the Control group ($M = 11.17, SD = 6.84$) performed higher than the Overexpectation group ($M = 9.33, SD = 5.18$) with an $\eta^2 = .024$. This suggests that on this measure 2.4% of the variance is explained by group membership and that this effect was also is in the hypothesised direction. On this measure however, null hypothesis testing failed to detect a statistically significant difference $t(34) = 0.906, p = .19$. The effect sizes in the predicted direction are consistent with our hypothesis about the effectiveness of the Overexpectation paradigm. Concerning the main hypothesis about a Mixed group difference, if overexpectation training was not effective in this design, then this would suggest

that our Mixed group was a less extinguished Omission group and bias subsequent analysis away from finding an effect.

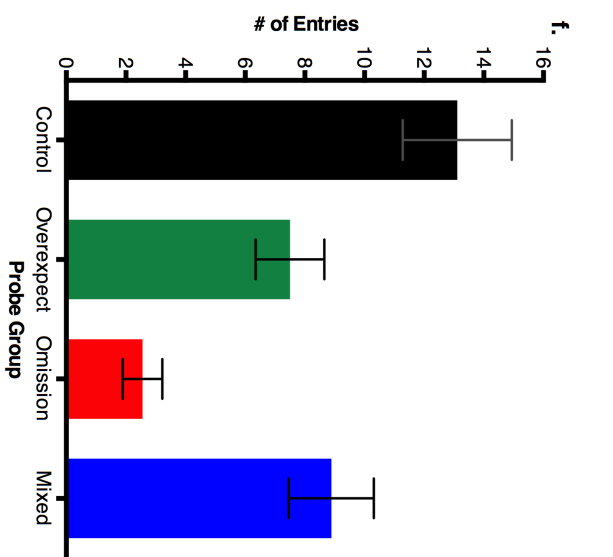
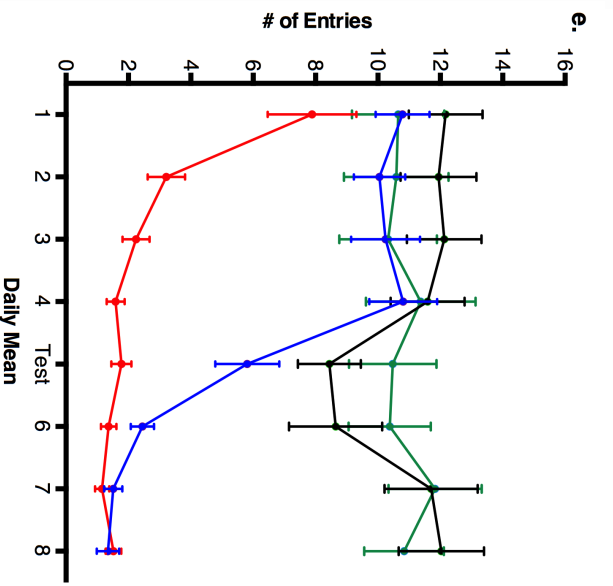
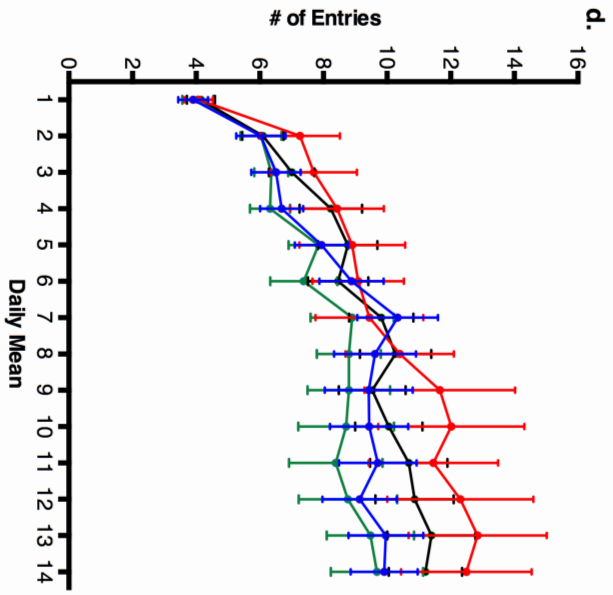
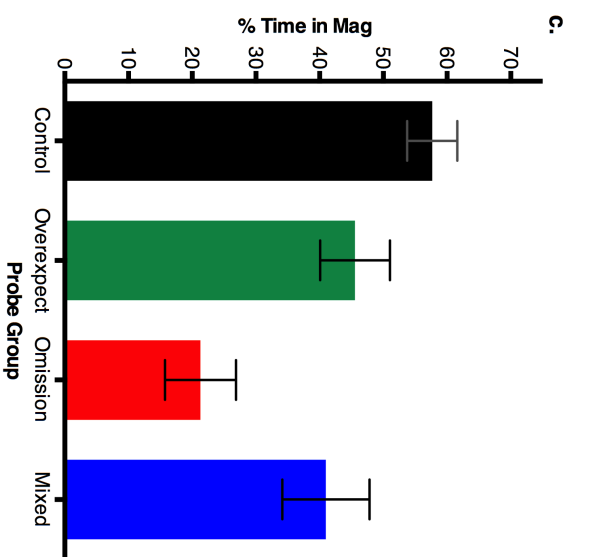
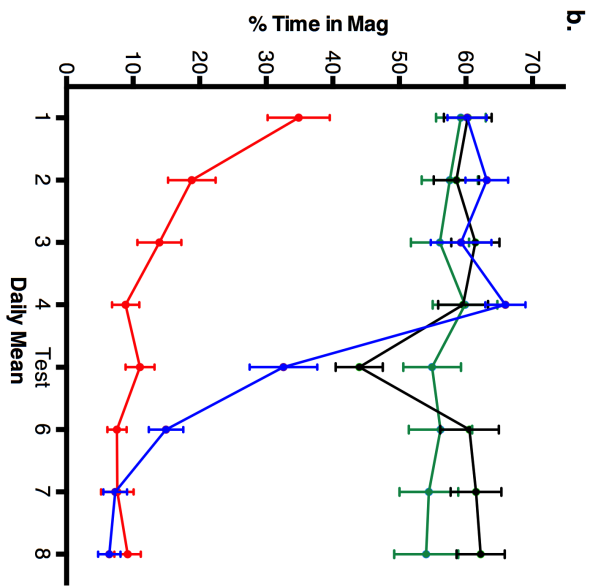
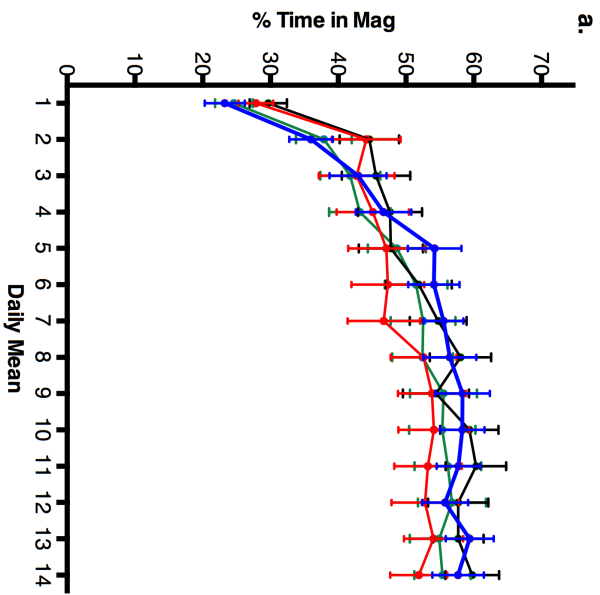


Figure 6. Evidence of acquisition, omission and overexpectation. The top figures represent the percent time in the magazine and the bottom figures represent the number of head entries. The left figures show the mean responding during a trial during the acquisition phase. The centre figures represent the daily responding during the two extinction phases. Note that during the probe trial the Overexpectation-only group continued to receive Overexpectation reinforced trials. The right figures represent the mean responding during the probe test. Error bars represent the SEM.

Discussion

The main objective of this thesis is to examine an alternate paradigm to elucidate the phenomenon of deepening extinction. We took the perspective that extinction learning is an inhibition of the omnipresent original learning and that relapse-producing effects of certain post-extinction manipulation represent an interference with this inhibitory learning. We also took the view that omission and overexpectation learning are in some respect, empirically different (Sissons & Miller, 2009; Witnauer & Miller, 2009) and have unique and overlapping biological bases (Iordanova et al., 2016). Specifically we tested if this paradigm produced an extinction more resistant to relapse than an Omission-only paradigm for Renewal, Spontaneous Recovery, and Reinstatement tests. As such, our main hypothesis is that the novel extinction paradigm used here, would be more effective in attenuating behavioural relapse that follows Renewal, Spontaneous Recovery and Reinstatement tests.

The main finding of this thesis is that this Mixed-extinction paradigm demonstrated an attenuation of relapse behaviours compared to Omission. This effect was observed in Renewal and Reinstatement post-extinction conditions. Concerning the renewal phenomenon, evidence here supported the attenuation of the behavioural relapse associated with exposure to the CS in a novel context. Our findings are that our group manipulation explains 11% and 15% of variance on percent time in the magazine and number of head entries, respectively. These effect sizes might be underestimated since several of the subjects in the Mixed group were at zero responding for both dependant variables under our specific experimental

parameters. As such, a replication of this study might employ less extinction training in order to detect changes that might be masked here by a behavioural floor effect.

As for the reinstatement phenomenon, an effect size in the hypothesized direction was present. Here just over 9% and 5% of the variability between scores for percent time and number of entries, respectively, explained group membership, in the hypothesised direction. However, a confirmatory null hypothesis test did not exceed the arbitrary threshold set in order to reject the null hypothesis. One factor that could have contributed to this finding could be the experimental design. By having this reinstatement challenge third in a series of tests, this could have attenuated or muddled the potential observability of an effect due to this repeated testing. Another factor that could have influenced this result was that our experimental design had equal group sizes. This might have led to a direct Omission vs. Mixed group comparison to be underpowered ($n = 9$). Lastly, these findings again could be very conservative due to the differences in sensory modality combined here. In examining auditory target cue scores alone, the Omission group subjects on average spent 37% of time in the food magazine during the CS. Whereas, Mixed subjects spent on average, 23% of the time in the magazine during the CS. Similarly for auditory target cues on the measure of number of head entries, Omission subjects demonstrated an average of 7.22 head entries during the CS presentation. This compares to Mixed subjects demonstrated only 4.56 average head entries. Visual target subjects for these groups performed at levels comparable to a behavioural floor effect. Taken together, aspects of the

experimental design as well as that parameters were not optimized for both sensory modalities, would suggest a very conservative estimate of a true effect size if one were present.

Upon further examination of our data, these results show relatively lower rate of responding for the consistently reinforced Control group on the Reinstatement test. This was not predicted; however this could also be attributed to the experimental design. Tests of extinction behaviour employ unreinforced presentations of the target-CS in the same way that Omission training is an unreinforced presentation of the target-CS. Here, the reinforced Control group up to this point had received 40 unreinforced presentations of the CS before the start of the Reinstatement test. As such, despite all the additional training trials in omission learning, the Omission group shows no advantage over the Control group that has received fewer such trials. Similarly on further inspection of the data, the Overexpectation group on the Reinstatement test seems to also be lower than was predicted (see Figures 2e & f). This result again might be due to the experimental design where the Overexpectation group was receiving—by the nature of the tests—30 unreinforced (Omission) trials before the start of the Reinstatement test. This would make our Overexpectation group, at this point, more like our Mixed group than a purely Overexpectation group.

As for the Spontaneous Recovery test, our data failed to show that a spontaneous recovery effect was attenuated by our Mixed extinction paradigm. In also teasing apart auditory CSs, Mixed subjects on average spent less time in the magazine during the auditory CS presentation. Here Mixed subjects spent 35% of

time in the magazine compared with the Omission subjects spending on average 43% of time in the magazine during the CS. Similarly for number of head entries, Mixed subjects had an average of 8.3 head entries compared with the Omission 10.3. However, this observed difference, in terms of the effect size calculation, is not sufficient to conclude that there is a substantive effect. This observation should also be taken together with a failure of null hypothesis testing to conclude that the null hypothesis should be rejected. These findings lead us to determine that there is not sufficient evidence here to support that our Mixed group produces an attenuation of a Spontaneous Recovery effect. However, null findings here do not provide evidence to the absence of an effect on spontaneous recovery relapse. Furthermore, this effect is considered a more sensitive measure than other post-extinction manipulations leading to behavioural relapse (Rescorla & Heth 1975), and our design had placed it following Renewal testing. As such, we should withhold judgement concerning the implications of these findings and this issue should be subsequently addressed in a standalone experiment. It should also be noted that the spontaneous recovery effect could be interpreted as an extension of the renewal effect since it represents a change in a subject's internal context. As such, the internal state of the subjects can be difficult to infer. It is possible that there is a mechanism involved in recognition of external contextual cues that are different from the recognition of internal contextual cues. It is also possible that there is a Mixed-extinction paradigm attenuation relapse for a renewal effect but not for a spontaneous recovery effect.

In summary, the objective of this experiment was to determine if this novel Mixed-extinction model would attenuate behavioural relapse following three different post-extinction manipulations. Evidence of attenuation was found robustly in Renewal with both effect-size statistics and secondary null-hypothesis tests supporting this conclusion. Effect-size statistics supported an attenuation of the reinstatement effect, however secondary null-hypothesis tests failed to detect a statistically significant group difference. Finally, neither effect-size statistics nor null-hypothesis testing supported an attenuation of relapse behaviours on the Spontaneous Recovery test, though group differences were in the hypothesized direction. However, this phenomenon might be the most sensitive of these three post-extinction manipulations and was not ideally placed as the second test in our paradigm.

Limitations

One limitation to this study, as mentioned above, was the observed difference in auditory versus visual target cues. This difference was observed in a lower behavioural asymptote on our measures for visual target CS compared with the auditory target CS. This suggests that the specific parameters used in our experiment could be optimized in subsequent studies to account for this difference in asymptotes. On the test, the visual target CS often did not provide evidence that post-extinction manipulations were successful with many subjects appearing to be performing at a behavioural floor for much of the visual target cue. However these data were still included since this was original planned prior to the start of the experiment and it is a more conservative view of the data. As such, future

researchers should attempt to employ more optimal parameters to test for these effects across various sensory modalities, as it seems highly unlikely that these learning phenomena would be modality specific.

Another limitation to this interpretation of the data is that Mixed-extinction subjects were all included rather than excluding subjects that did not clearly demonstrate overexpectation learning on the probe test. Without such evidence it is difficult to conclude that the Mixed subjects are actually Overexpectation then Omission-trained rats. The possibility remains that some of these rats did not acquire overexpectation learning after 40 trials and that these subjects might be better described as Omission trained after the second block of 40 trials rather than Mixed-extinction rats. However, the decision to include all subjects would conservatively bias these data away from finding an effect if there were one and risk underestimating the size of an effect. Whereas for our data, a decision to exclude Mixed subjects here based on a failure to demonstrate overexpectation learning on the probe test, would have disproportionately excluded auditory target cue subjects in these data and substantially biased analysis towards finding an effect even if an effect was not present. As such the parsimonious approach was employed here. Future researchers might consider testing different sensory modalities in standalone experiments or consider adjusting experimental parameters to avoid a possible floor effect in the visual target cue on probe test, which might have been responsible for sensory modality differences in our probe-test data.

Alternate paradigms in the literature

The present findings examine a novel paradigm to induce a deepening of extinction. These findings can be contrasted with previous experiments dealing with the deepening of extinction learning. Hendry (1982) examined this deepening using an operant conditioning paradigm. Here, an operant behaviour was trained by pairing lever pressing with a food reward. This behaviour was then influenced by training rats to associate a target cue with an aversive outcome. This aversive outcome was then extinguished and the amount an extinguished cue would interfere with the operant lever-pressing behaviour was measured. This paradigm employed pairing already extinguished cues. Hendry (1982) found a summation effect as a result of two previously extinguished CSs suggesting that there was some value in pairing extinguished CSs.

Rescorla (2006a) used rats and pigeons to replicate and expand upon the findings of Reberg (1972) and Hendry (1982). Rescorla used operant conditioning as well as Pavlovian designs in this group of experiments. For operant conditioning, Spontaneous Recovery and Reinstatement manipulations were tested following extinction phases of CS and shock. Rescorla also replicated these results in a Pavlovian design using food pellets as positive reinforcement and examined this on a Spontaneous Recovery test. Findings from these experiments supported a view that extinction learning can be deepened by the use of a Mixed design. Rescorla (2006a) also tested Omission learning followed by pairing Omission extinguished cues. This work expanded upon findings by Reberg (1972) that combining two previously Omission extinguished cues can yield greater decrement in responding

than either extinguished cue alone. Reburg also tested, a Mixed paradigm of Omission followed by the unreinforced pairing of an extinguished and a non-extinguished cue. This direct comparison favoured a deeper extinction in the Omission followed by non-extinguished cue paired with an extinguished cue. These findings suggest a deepening extinction in a Mixed paradigm with association changes occurring in two paired cues more dynamically than two fully extinguished cues individually. One plausible interpretation is the perspective taken in this thesis, which is that there is a greater recruitment of biological mechanisms associated with different forms of extinction that leads to a deepening of extinction that is more resistance to relapse.

Leung et al. (2012) replicated and expanded upon the work of Rescorla (2006a). Rats were trained in an aversive paradigm. Rats then received context pre-exposure sessions followed by a session of four CS-shock pairings. Rats on subsequent days, received unpaired presentations of each of the CSs individually. Following this rats had either further Omission training to the target cue or unreinforced pairings of two previously extinguished CSs. In one experiment, rats had an unreinforced compound of two Omission-extinguished CSs. In another experiment there was a non-reinforced pairing of an Omission-extinguish CS and a CS that had been similarly paired with shock but had not been extinguished. Following this second extinction block, rats received a reinstatement session and subsequent test. Here, rats demonstrated an attenuated reinstatement response on test even though the compound leads to more responding during compound extinction. Since, a CS can indicate a particular likelihood that a US will occur, it can

have no predictive relationship with that US, or it could indicate that there is a less chance of the US occurring when this CS is present. Such a CS, when paired with another CS with a positive predictive relationship (with the same US) would produce a net inhibition of behaviour. Leung et al. (2012) concluded that their effect was due to the target CS in the pair becoming a net inhibitor. The paradigm of using unreinforced pairings of previously Omission-trained subjects greatly contrasts a paradigm involving Overexpectation. Namely, in Overexpectation the subjects are still receiving reinforcement.

In terms of human research in the literature, Culver et al. (2015) used an aversive sound as a US and measured a skin conductance response. They had also employed a paradigm using an unreinforced pairing of two separately Omission-extinguished CSs. They found an attenuation of relapse behaviours for both Spontaneous Recovery and for Reinstatement (Culver et al., 2015). They found evidence that this Omission followed by unreinforced pairing of Omission-extinguished cues, lead to an attenuation of observed spontaneous recovery and reinstatement effects that were observed in a group that received an equal number of Omission-only presentations.

Also, Coelho et al. (2015) employed a shock and measured a skin conductance response as a measure of fear in humans. They used a Spontaneous Recovery test 24 hours later, which was followed by a Reinstatement test the same day. In contrast to our design in rats was that there was twenty-four hour delay for the renewal test, which is similar to the time between extinction training and Spontaneous Recovery test in humans. They found evidence of their Mixed-

extinction paradigm, attenuating relapse for Spontaneous Recovery but did not find this attenuation for their Reinstatement paradigm. However, it should be noted that the Reinstatement test followed very shortly after the further extinction of the Spontaneous Recovery test (Coelho et al., 2015).

Predictions of alternate paradigms

Here we will discuss more in depth, the predictions stemming from a Rescorla-Wagner model perspective on the deepening extinction designs of Hendry (1982) which paired two previously extinguished CSs and an experiment of Leung, Reeks and Westbrook (2012), which had a group that received the unreinforced pairing of an extinguished and non-extinguished CS. These predictions will be contrasted to results of these experiments. Next we will similarly make predictions about our Mixed-extinction design based on that model and contrast that with what was observed.

For Omission followed by a pairing of two previously extinguished CSs as was done in Hendry (1982), the Rescorla-Wagner model would predict that the error term for the initial omission learning would be large and responding would decrease rapidly. Following this decline, the unreinforced paired of two previously extinguished CSs should be greatly attenuated since the error term would be split in some degree, between these two previously extinguished CSs. Hendry (1982) experiment used the suppression of an operant behaviour as their measure of the impact of an aversive association. The results were that the compounding of two extinguished aversive stimuli lead to a summation effect of their pairing. And, this was to a level of suppression below what appeared behaviourally to be zero

association. This paradigm accentuates the discrepancy between observed relapse phenomena and learning models like the Rescorla-Wagner model, that view omission learning and overexpectation learning as involving the same empirical (and presumably biological) mechanisms.

Leung et al. (2012) conducted an experiment where a group of rats experienced the unreinforced pairing of two previously extinguished CSs similar to other experiments mentioned above. Another group was subjected to a non-reinforced pairing of an extinguished and non-extinguished CS and both were then given a Reinstatement test. The authors found that the Reinstatement produced relapse, was lower in the group that had received the pairing of the extinguished and non-extinguished CS. Here a Rescorla-Wagner model based perspective might consider there to be a loss in associative strength to the extinguished CS. The non-reinforced pairing then would lead to further omission extinction and therefore loss in associative strength between both the previously omission-extinguished CS and previously reinforced CS. There are several factors that could be used to argue how much of this extinction learning would be attributed to each of the CSs present in the pairing. However, such a model would not explain either post extinction relapse phenomena in general nor why this relapse would be attenuated compared to other groups that received more Omission training. Rescorla in a similar experiment (2006a) suggests that such a decrement in responding following such pairings could be due to greater excitation involved in a non-reinforced pairing of an extinguished and non-extinguished CS. However, this is not encapsulated in learning models such as the Rescorla-Wagner model nor do such models account for the observation of

relapse phenomena. Another plausible interpretation would be that if the Omission-extinguished CS was not at zero, or from a biological perspective this learning is never erased, then this paradigm could constitute a biological mechanism that might be related to mechanisms purported to be underlying overexpectation learning.

Concerning an Overexpectation followed by Omission paradigm such as what we have employed, a Rescorla-Wagner model perspective might predict that overexpectation learning would lead to some decrease in the associative strength of either CS. This attribution in our paradigm would have shifted mostly to our target CS since the non-target CS was also reinforced 10 times in each extinction session and on each test day. The decrease in the target CS, in this view, would necessarily be less than an alternative omission-only-learning since there was still reinforcement in the paired trials. One view could be that the target cue would no longer be a predictor of the CS since the non-target CS would predict the US on each trial whether or not paired with the target CS. Following overexpectation learning, omission learning would be slightly less robust for each subsequent exposure. This is due to the decrease in associative strength (and expectation) caused by the overexpectation learning. However this omission learning would still be effective. This perspective would predict our Mixed group extinguishing more than an Overexpectation group and less than an Omission group, given the same number of trials. Like the above-mentioned alternative paradigms the observed results of a deeper form of extinction that is more resistant to relapse, do not fit within an error-prediction model like the Rescorla-Wagner model. Our findings were that the Mixed group performs generally lower across the length of the post-extinction tests

and did not perform at a level somewhere between Overexpectation-only and Omission-only subjects. Instead these findings would better be explained by the notion of empirically different mechanisms that underlie overexpectation and omission learning (Sissons & Miller, 2009; Witnauer & Miller, 2009).

Future directions

As in all research, an independent replication of these results should be conducted in order to ensure the reproducibility of the experimental design. Additionally, there would be value in using either standalone experiments or in counterbalancing the test order for experiments such as this that employ multiple consecutive tests. Importantly, there should be a direct contrast of the effect size of this Overexpectation-Omission paradigm and the alternate paradigms using unreinforced pairings mentioned in the literature. Comparing and contrasting these effects will lead to better direction in terms of directly examining the biological basis for deepening extinction. Such a comparison could address the possibility of an impact of error-prediction mechanisms and models.

It remains a possibility that the biological mechanisms that are unique to overexpectation learning (and not omission learning) could also be implicated in other extinction procedures that use unreinforced paired stimuli for extinction learning. Approaching the phenomenon of deepening extinction, from both a biological and behavioural perspective, would yield greater insights into the underpinnings of extinction learning. One first step to this end could be to expand upon the cell tracking experiment of Iordanova et al. (2016) to see if these specific findings would replicate using a non-reinforced paired extinction paradigm and how

this might relate to unique and overlapping cell populations implicated in overexpectation learning.

Overall this line of research in to deepening extinction, has implications concerning maladaptive behaviours, which are liable to relapse even after abstinence (omission) training. Since omission extinction does not cause the unlearning of the original acquisition, there remains a vulnerability in therapeutic treatments of maladaptive behaviours employing this strategy (Bouton, 2002). With a better understanding of the underlying mechanisms behind extinction learning, paradigms can be designed to better insulate maladaptive behaviours from relapse through deeper extinction training.

Conclusions

In summary, we found evidence supporting the deepening of extinction as a result of this novel Overexpectation-Omission paradigm. This effect was observed robustly in our first test for Renewal and observed in our third test for Reinstatement. There was no observed effect for Spontaneous Recovery. These findings suggest validity in this alternate paradigm to promote a deepening of extinction learning. In elucidating this mechanism behaviourally, future research can tease apart differences in the biological basis of extinction learning. This research has far-reaching implications in terms of clinically addressing maladaptive-formed associations such as are present with addiction and anxiety disorders.

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