Reinstatement Following Overexpectation Training in Male and Female Rats

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Abstract

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Extinction and overexpectation are two paradigms by which a previously established association between a CS and US can be altered. Both mechanisms occur by a reward expectation that is higher than the delivered reward (or omission of reward) which consequentially decreases the expectation of an outcome, manifesting in a corresponding decrease in behavioural response. Both effects have been documented in both male and female rats. After undergoing an overexpectation or extinction design, it is possible to obtain a recovery in conditioned responding either by the passage of time (spontaneous recovery) or by a change in context upon test (renewal). Additionally, a recovery in responding has been found to occur following unsignalled presentations of the US after extinction training and prior to test (reinstatement). The presentation of the unsignalled US works to re-establish responding to the target cue post-extinction in the absence of any additional CS-US pairing. To date, this reinstatement effect had not been studied following overexpectation training. In the present thesis, we examined whether reinstatement can be obtained following overexpectation training in male and female rats. The results show some evidence for a reinstatement in behavioural responding, albeit weak, following overexpectation in male but not female rats. The present findings have implications for understanding the mechanisms by which reinstatement occurs.

Keywords: Overexpectation, Reinstatement, Extinction, Appetitive Conditioning, Reward, Conditioned Stimulus, Unconditioned Stimulus, Pavlovian Conditioning

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Reinstatement Following Overexpectation Training in Male and Female Rats

Introduction

The ability of organisms to acquire new or modify previously established behaviour based on changing environmental contingencies is highly adaptive (Pavlov, 1927). One way new behaviours are established is through learning as in the case of Pavlovian conditioning. In Pavlovian conditioning, an initially neutral stimulus is paired with a biologically significant event also known as an unconditioned stimulus (US). Following such pairings, the neutral stimulus becomes conditioned, that is, a conditioned stimulus (CS) and is able to elicit responding indicative of anticipation of the US. This process of learning to associate the CS with the US is called acquisition.

Once an association and its resultant conditioned behavioural response is established, it does not remain static. Changes in the contingencies between stimuli can enable new learning and modify existing behaviour. Two examples of learning where previously established associative relationships and their corresponding behaviour can be altered are extinction and overexpectation. In extinction learning a previously reinforced cue (CS) is presented in the absence of reinforcement, resulting in a progressive decrease of conditioned responding (Pavlov, 1927). This absence of reinforcement leads to a mismatch between the CS-dependent expectation and the actual outcome, leading to a decrease in behavioural responding to the CS. This results as a reduction in associative strength between the CS and US. This decrease in responding can be manifested in a reduction in frequency of the behaviour in question. Even though extinction results in the loss of a previously established behaviour, it is now well-established that it does not cause a permanent change in memory and should not be regarded as "unlearning" of the original association (see Bouton, 2004 for a review). This is supported by evidence documenting that

multiple experimental procedures can lead to restoration of the previously learned behaviour in the absence of reinforcement. These procedures, which will be discussed later, include changes in the test contexts, the passage of time, and unsignalled exposure to the US.

Another method that has been shown to reduce behavioural responding after the establishment of associative learning is overexpectation (Rescorla, 1970). Overexpectation consist of two main phases. In the first phase, two distinct CSs are individually reinforced with the same reinforcer. As a result, each CS becomes a good predictor for the reinforcer and is able to elicit a high level of responding by the end of training. In the second phase, these cues are presented in compound in order to generate an (over)expectation of double reinforcement (Rescorla, 1970, 2006, 2007, Lattal & Nakajima, 1998). Essentially, since both cues are equally good predictors of the same US, when the two cues are presented in compound, their associative strengths summate yielding an inflated expectation of reward. This compound, however, is reinforced with a single outcome, leading to a mismatch between the CS-dependent expectation and the delivered reward. That is, the presentation of the compound stimulus leads to a reward expectation that is double that of the delivered reward. This procedure, consequently, adjusts down the expectation of the outcome and results in a decrease of the corresponding behavioural response upon the non-reinforced presentation of the individual stimuli on test.

Spontaneous recovery

Both extinction and overexpectation training result in a decrease in conditioned responding to a CS. Most importantly, these designs do not undo the already established association (Bouton, 2004). This is revealed via three different phenomena that show restoration of the reduced behavioural response following extinction or overexpectation training. The first is

spontaneous recovery. Pavlov was the first to find that if the CS were to be tested again after the passage of time following extinction training then there is a recovery in responding (Pavlov, 1927). Rescorla & Cunningham (1978) conducted a series of experiments and found that with time, there are changes in the representation of the US which affect both performance and future associative learning. They found that with greater time elapsed following the last extinction trial, greater responding was observed in a spontaneous recovery paradigm. However, if another non-reinforced excitatory CS that was equally associated with the US was presented prior to test, then this attenuated spontaneous recovery, presumably by suppressing the recovered US representation. (Rescorla & Cunningham, 1978). It has since been found that context plays a role in spontaneous recovery. A study conducted by Brooks and Bouton (1993) aimed to test the view proposed by Bouton (1993) by investigating whether a "retrieval cue" present during extinction could attenuate spontaneous recovery. This would explain the previously described results of Rescorla & Cunningham as being due to the retrieval of the extinction memory as a result of presenting the "excitor" non-reinforced. To draw this conclusion, Brooks and Bouton found that the presentation of a stimulus during test that was associated with extinction reduced the amount of spontaneous recovery observed, but that this cue itself was not inhibitory or excitatory. This has been suggested to be due to the extinction cue retrieving a memory of extinction, which then lowers the amount of behavioural spontaneous recovery observed (Brooks & Bouton, 1993).

Spontaneous recovery has also been observed following overexpectation training. Rescorla (2006) conducted an appetitive overexpectation design with two different overexpectation compounds. The key difference between these two overexpectation compounds was the duration interpolated between overexpectation training and test. In one case test occurred 1hr whereas for the other case it occurred 1 week following overexpectation training. Rescorla

(2006) also found that if the recency of two overexpectation compounds differed then responding on test also differed. Upon non-reinforced test of the individual stimuli, responding was lower when testing took place 1hr compared to 1 week following overexpectation training. Additionally, Rescorla (2006) also demonstrated that in an auditory-visual compound, if the visual cue was individually reinforced alongside the compound trials, then upon non-reinforced test of the auditory cue, responding was higher when tested after a delay when compared to test directly after compound training. This is indicative of a recovery of responding that results from an interval between overexpectation training and test and that this spontaneous recovery phenomenon is robust and holds throughout different methods of assessment (Rescorla, 2006). According to this phenomenon, the mere passage of time following extinction or overexpectation training is sufficient for the 'lost' response to return.

Renewal

A restoration in behavioural responding can be achieved through a change in context between extinction training and test. It should be noted that the switching of context after conditioning does not result in a loss of conditioning in itself (Bouton & King, 1983; Bouton & Peck, 1989; Bouton & Swartzentruber, 1986), suggesting that acquisition is not context dependent. Although Harris and colleagues reported that retrieval of the original conditioning memory can become context dependent, but only for an extinguished CS (Harris et al., 2000). This restoration phenomenon, called renewal, can occur when test occurs in a context different to that of extinction training. (Bouton & Ricker, 1994). Bouton & Ricker demonstrated that this effect holds whether both contexts are equally familiar, equally associated with conditioning, or equally associated with conditioning and extinction. This was explained as occurring not due to direct context-US associations, but rather as the context being a cue of a specific CS. (Bouton &

Ricker, 1994). Additionally, the effect can also be achieved when animals undergo extinction training in a different context than acquisition and test (Bouton, 1993, Bouton & Bolles, 1979a, Bouton & King, 1983; Bouton & Swartzentruber, 1989; Bouton & Peck, 1989), or if tested in a novel context (Bouton, 1993, Bouton & Bolles, 1979a; Bouton & Swartzentruber, 1986, 1989). The renewal effect has been found to be independent of excitatory or inhibitory contextual stimuli (Bouton & Bolles, 1979a) and it has been found to be independent of context-US associations (Bouton & Ricker, 1994; Bouton & King, 1983; Bouton & Swartzentruber, 1986). Bouton and Swartzentruber (1986) found that when reconditioning an extinguished CS in a conditioning context, performance was much lower than if the extinguished CS was reconditioned in a neutral context. Upon return to the conditioning context, renewal was observed. Following reacquisition, if the previously extinguished CS was placed back in the extinction context, then extinction performance was renewed (Bouton & Swartzentruber, 1989).

Rescorla (2007) investigated whether the same renewal effect could be obtained after overexpectation training. He found that when conditioning (phase 1) occurs in context A, and overexpectation training (phase 2) occurs in context B, then upon nonreinforced test of the individual stimulus, responding was higher in context A as compared to context B. To determine whether renewal occurred as an effect of context on extinction and not due to a generalization decrement, conditioning and overexpectation were assigned to context A while context B saw the presentation of a light reinforced. When the cue that was conditioned and underwent compound training was tested nonreinforced in context B, a renewal in conditioned responding was observed. These results may in fact have been due to summation of the excitatory test context of context B. Another experiment was employed in which additional alternative stimuli underwent conditioning and overexpectation in context B. The renewal effect was still obtained for the

target stimulus which underwent its conditioning and overexpectation in context A and tested in context B. Additionally, the renewal in responding upon nonreinforced test was observed when tested in another similarly treated context (C) following conditioning in context A, and overexpectation in context B. Therefore, similarly to extinction, responding lost following overexpectation training is also subject to renewal, as long as test occurred in a context different to that of overexpectation training (Rescorla, 2007).

Theoretical accounts: Mark Bouton's model

It has been proposed that during the process of learning, an excitatory *Cue-Outcome* association is established. Extinction on the other hand leads to the development of inhibitory *Cue-NoOutcome* associations. The discovery of behavioural restoration phenomena (e.g., renewal, spontaneous recovery) provide evidence that these inhibitory and excitatory associations can coexist in the same animal, and that they compete for behavioural expression. A key factor that led to this discovery is the dependence of behaviour on contextual information. This means, for example, that the very same cue can elicit no responding in the context where extinction occurred, but it can also elicit responding in a novel context (Bouton, 1993, Konorski, 1967).

It has been proposed that the restoration phenomena of renewal and spontaneous recovery are caused by the creation of context-specific extinction memories. This suggests that the process of extinction does not undo the learned response, but rather it plays a role in creating further learning- an "extinction memory"- that is tied to the surrounding context (Bouton, 2002). Context has even been found to play a larger role in retrieval of extinction memories than in retrieval of acquisition memories. That is, responding to a non-extinguished CS has been found

to be less sensitive to contextual effects than responding to an already extinguished CS (Bouton, 1988; Bouton & Swartzentruber, 1991; Bouton & Ricker, 1994). This contextual effect can be extended to the case of spontaneous recovery. A change, may it be the physical placement of the animal in a different environment or a perceived change (e.g., the passing of time), between the conditions surrounding extinction and the test session will produce a change in the 'extinction memory' context and thus result in the return of conditioned responding (Bouton, 1988, 1991, 1993, Bouton & Swartzentruber, 1991). Therefore, this explains both the lack of "generalization" of extinction to other environments and the fact that the passing of time is sufficient for the conditioned behaviour to re-emerge in the absence of further training.

Extinction: Reinstatement

The learning literature has established a separate restoration phenomenon called reinstatement. In reinstatement, the US is presented alone, unsignalled by the CS, between CS extinction training and CS test. These unsignalled US exposures re-establish responding to the cue post-extinction despite the lack of *additional* CS-US conditioning. Rescorla and Heth (1975) found that signalling the reinstating US with a light cue and presenting the reinstating US unsignalled in the extinction context resulted in no differences in reinstatement when tested in the test context. In this approach, it was considered that the US reexposure worked to condition background stimuli present, termed here as "situational cues". If the presentation of the US worked to condition these "situational cues", then a summation of this cue with any residual fear response would be expected upon test, leading to a greater amount of reinstatement. The purpose of presenting a signalled US (by the light) was to reduce the amount of fear conditioning to the situational cues. In addition, by switching the context (and presenting the US signalled) from reinstatement to test, it further limited the effect of background cue conditioning on responding,

thereby reducing the likelihood of obtaining a reinstatement effect on test. The lack of difference found between the unsignalled US exposure and the signalled US exposure upon test in the extinction context was interpreted as evidence that the role for the context is not to establish direct links with the US and thus summate with any residual associative strength between the extinguished CS and the US to drive reinstatement. This supported the previous notion that reinstatement was thought to be due to the strengthening of the reduced US representation. (Rescorla & Heth, 1975).

Other research, however, has reported that the reinstatement effect is dependent on contextual information (Bouton, 1993). The reinstatement effect has found to be strongest when the presentation of the US occurs in the context in which test will occur (Bouton & Bolles 1979b; Bouton & King, 1983; Westbrook et al., 2001). However, it is not a requirement that the US need be presented in the test context as reinstatement has also been obtained when the US was presented in a conditioning-extinction context or in an extinction context (Westbrook et al., 2001).

Further evidence for the role of excitatory associations between the context and the US have been reported. Rescorla and Cunningham (1978) noted that non-reinforced CS presentations after the US presentation and prior to test attenuated the reinstatement effect, but that this attenuation effect dissipated with time. They attributed this attenuation to a suppression of the US representation occurring due to the CS being presented alone. This discovery was taken further by Bouton and Bolles (1979b), who discovered that exposure to the reinstating context itself alone after the US presentation and prior to test attenuates the reinstatement effect. This suggests that excitatory associations between the context and the US can modulate fear to

the CS following extinction (US in test context vs non test context). The greater fear conditioning the context elicited, the greater reinstatement observed (Bouton & King, 1983).

A memory-based approach, as advanced by Bouton (1993), describes that the CS-US memory results from conditioning and CS-noUS memory results from extinction. These memories are seen as independent. The CS-US memory is more context-generalizable and is capable of being activated in any context, whereas the CS-noUS association can only be activated in the context in which it is learned (the extinction context). In this approach, contextual conditioning occurs alongside the acquisition of the CS-US memory during acquisition, serving as the "background" in which a specific emotional state was induced. Likewise, during extinction, the CS-noUS memory develops alongside any contextual conditioning present (Bouton et al., 1993).

The role of context in reinstatement depends upon the assumption that an inhibitory association occurs as a result of extinction training. Extinction training thus, results in an inhibitory context-US association. Consequently, the presentation of the unsignalled US in this context decreases this inhibitory context-US association. Rescorla, 1979; Westbrook et al., 2001; Bouton & King, 1983; Bouton & Ricker, 1994). Alternatively, extinction can result in a CS-US inhibitory association, which can be activated by the "occasion-setting" properties of the extinction context (Bouton & Ricker, 1994; Westbrook et al., 2001). This extinction context could then "set the stage" for activating a CS-US inhibitory association (Westbrook et al., 2001; Bouton & Peck, 1989). When the US is presented alone in the extinction context (irrespective of whether or not this context is the same as the conditioning context), the contextual conditioning of the US alone presentation activates the original CS-US memory. This occurs because during acquisition (but not extinction), the US was presented and therefore came to serve as a

conditioning cue. This returns the animal to the initial state of acquisition and decreases the ability of the context to retrieve the extinction memory. As a result, when tested (in the same context as extinction), the animal reinstates responding to the previously extinguished cue despite the lack of CS-US pairings following extinction. It is suggested that whichever memory is activated is dependent upon which memory (acquisition or extinction) the context retrieves (Bouton et al., 1993).

Reinstatement occurs in a CS- specific manner. It has been found that contextual fear was able to reinstate fear of an already extinguished CS, but not a CS that has not undergone extinction (Bouton, 1984). Westbrook and colleagues (Westbrook et al., 2001) found a greater amount of reinstatement with a CS whose corresponding extinction context was shocked than the CS whose corresponding extinction context was not shocked. This reinstatement effect was maintained when each CS was tested in a neutral context, but it was attenuated by exposure to the extinction context of the corresponding CS. The context as a mediator approach suggests that the extinction context itself where the CS-noUS pairings occurred results in a context-CS association. Additionally, context-US associations are formed during US reexposure. The context then works to mediate the reassociation between the extinguished CS and the US, leading to a reinstatement in behavioural responding upon test (Bouton & King, 1983; Bouton & Peck, 1989; Holland, 1990; Westbrook et al., 2001). Therefore, the attenuation of reinstatement was explained as exposure to the extinction context mediating the formation of an inhibitory association between the CS and US (Westbrook et al., 2001).

The dependence on which account prevails is conditional on the context in which extinction, US presentation, and test occur (Westbrook et al., 2001). If the presentation of the US and test occur in the same context that is different to the context of extinction, then reinstatement

has been shown to occur due to context-US associations. Alternatively, if extinction and US presentation occur in the same context, with test occurring in a separate context, then reinstatement has been found to occur in a "CS-specific manner" due to mediated conditioning by the context between the CS and the US (Westbrook et al, 2001).

Overexpectation: Reinstatement

Reinstatement following overexpectation has been largely ignored in the literature. To date, there is absolutely no investigation of reinstatement occurring after overexpectation learning. Extinction and overexpectation share facets of their mechanism: both result in a decrease in behavioural responding which results from a negative prediction error (where more reward is expected than is received). The difference lies in that unlike extinction, overexpectation does not occur by an omission of reward, but rather through reinforced compound training. To date, renewal and spontaneous recovery have been both observed after extinction and overexpectation. Reinstatement has only been documented after extinction, and evidence stands in support of the two conflicting aforementioned accounts. Investigating reinstatement after overexpectation is thus critical in providing a strong line of evidence to help distinguish between these accounts.

These two accounts hold different predictions on whether unsignalled outcome presentations can result in a reinstatement of behavioural responding following overexpectation training. If the context serves as a mediator by which the exposure of the US results in a context-US association which mediates the CS-US association, then this activation of the CS-US association would serve to reinstate behavioural responding on non-reinforced test to one of the cues. Alternatively, if the presentation of the US activates the CS-US association present during

acquisition, then since the outcome was present during both acquisition and overexpectation training, it is unlikely that reinstatement will occur after overexpectation training. This is because both the acquisition and overexpectation training memory include the US. Therefore, unsignalled presentations of the US likely cannot selectively activate the acquisition memory by this account.

The present thesis

Even though the restoration phenomenon of reinstatement is well established in the literature, it has not received as much attention as spontaneous recovery and renewal. First, the impact of unsignalled outcome exposure following overexpectation training not been tested. Second, it is not clear-cut whether overexpectation is subject to reinstatement altogether. In this thesis we conducted a line of experiments that address these questions directly. First, we investigated whether reinstatement is a restoration phenomenon that can procure in an overexpectation design for a food reward. We then examined the effect of reinstatement following overexpectation training in both males and females.

Experiment 1. Reinstatement after Overexpectation Training in Males

Overexpectation training results in reduced behavioural responding to a previously conditioned cue. The purpose of Experiment 1 was to investigate whether exposure to an unsignalled reward following overexpectation training and before non-reinforced test would reinstate behavioural responding in male rats.

Methods

Subjects

Seventy-three experimentally naïve 3-month-old male Long Evans rats were used. Rats were bred in house in the Animal Care Facility at Concordia University and sixteen were obtained from Charles River Laboratories (Wilmington, MA, USA). The rats weighed an average of 312g (range 462-235g) post food restriction. All experimental procedures were in accordance with the approval granted by the Canadian Council on Animal Care and the Concordia University Animal Care Committee.

Apparatus

The behavioural apparatus was a 31.8 L x 26.7 H x 25.4 W cm chamber (Med Associates, St. Albans, VT, USA). The back and front door of the chamber were made of Perspex, whereas the left and right side walls were modular made of aluminum. All walls with the exception of the stimuli and magazine were covered in a checkerboard pattern created by white and back printed sheets (3.1cm x 3.1cm squares). The right side wall contained a tall magazine located in the middle panel where sucrose pellets were delivered from a pellet dispenser. The magazine was equipped with motion sensors that detected head entries and duration. On either side of the magazine were two cue lights positioned 8.2 cm below the ceiling of the chamber. The right

light acted as the experimental visual stimulus whereas the left was inactive. The chamber floor consisted of 4 mm steel bars 15 cm apart with a removable metal tray underneath. There were 2 speakers angled towards the front corners of the chamber from which the auditory stimulus was delivered. Each chamber was housed by a sound-attenuating cabinet with a camera on the back wall for monitoring behaviour during the sessions. Each chamber was systematically cleaned prior to each session with 4% almond-scented solution (President's Choice Black Label).

Stimuli

Two stimuli were used in this experiment, one auditory and one visual. The auditory stimulus was a 72 dB white noise. The visual cue was a 4 Hz flashing light. The cue duration was 10 seconds. The background noise was ~65dB. The reward was two chocolate-flavoured sucrose pellets that were presented in the magazine (Product# F07256, Bio-Serv, Flemington, NJ).

Procedure

Subjects were randomly divided into one of the four groups: Overexpectation (OE; n=17), Overexpectation-Reinstatement (OE-R; n=19), Control (CON; n=18), Control-Reinstatement (CON-R; n=19). Rats were handled for 5 days and food deprived prior to the start of the experiment to 85% of their initial pre-deprivation bodyweight. The design is depicted on Figure 1a.

Magazine Training. Twenty-four hours prior to the start of conditioning, all rats underwent magazine training, which consisted of unsignalled delivery of a sucrose pellet every 60s with the first pellet delivered 10s following placement into the chamber. This yielded a total of forty pellets over forty minutes. Rats proceed to the subsequent phases of the experiment if they ate more than 75% of the pellets delivered.

*Pha*se *1*. Conditioning to the visual and auditory stimuli took place across 14 days. All subjects received ten reinforced presentations of the auditory stimulus and ten reinforced presentations of the visual stimulus, for a total of 20 reinforced trials during the session. Reinforcement consisted of the delivery of two chocolate pellets 500ms apart with the first pellet being dispensed on the 8th second of cue presentation. The intertrial interval (ITI) was on average 180s (120-240s). The first trial began 120s following placement into the chamber and the session ended 120s following the end of the last trial

Phase 2. Conditioning during Phase 2 lasted 4 days. Prior to the start of this phase, all rats were split into two conditions: Overexpectation and Control. Rats in the Control condition received identical treatment to that described in Phase 1. Rats in the overexpectation condition (Groups OE and OE-R) received ten reinforced presentations of the auditory stimulus and visual stimulus in compound. Additionally, the rats also received ten presentations of the non-target cue (counterbalanced). That is, if the auditory cue was the target cue that was assigned to undergo overexpectation then the non-target cue was the visual cue and vice versa. The ITI and reinforcement parameters were identical to those described in Phase 1.

Reinstatement. Following Phase 2 training, half of the rats in the overexpectation condition (Group OE-R) and half of the rats in the control condition (Group CON-R) received a single day of reinstatement training, which consisted of the delivery of unsignaled sucrose pellets in the conditioning chamber in a manner identical to that described in the magazine training session. The remining rats from the overexpectation condition (Groups OE) and from the control condition (Group CON) were handled in the colony room in which the rats were housed.

Test. Following Reinstatement training, rats in all groups received two tests 24hrs apart which consisted of non-reinforced presentations of the target cue along with reinforced presentations of the non-target cue. The cues were presented for ten trials each.

Data Analysis. Percent time spent in the magazine during cue presentation was taken as the measure of conditioned responding. We also looked at the number of head entries but this behavioural measure did not reveal an effect of overexpectation, which would preclude us from being able to examine the effect of reinstatement following overexpectation. Data were analysed using Analysis of Variance (ANOVA) in SPSS Statistics (Concordia University license) with significance set at 0.05.

Results

Phase 1: Conditioning. The data from Phase 1 are shown on Figure 1b. Conditioned responding increased across days for all groups. There were no differences found between any of the groups. A 2x2x(14) mixed factorial ANOVA revealed no differences between the overexpectation and control groups ($F < 1$, $p = 0.759$), no differences between the reinstatement and no-reinstatement groups ($F < 1$, $p = 0.912$), no between-group interaction ($F < 1$, $p = 0.988$), an effect of days ($F_{13,923} = 161.03$, $p < 0.001$), and no two- or three-way interactions (max $F_{13,923} = 1.46$, $p = 0.320$).

Phase 2: Overexpectation. The data from Phase 2 are shown on Figure 1c. There were no differences found between any of the groups. This is supported by the statistical analyses. A 2x2x(14) mixed factorial ANOVA found no differences between the overexpectation and control groups ($F < 1$, $p=.666$), no differences between the reinstatement and no-reinstatement groups (F

< 1, p = 0.346), no between-group interaction ($F_{1,71}$ = 2.93, p = 0.092), no effect of days ($F_{3,213}$ = 1.200, p = 0.311), and no two- or three-way interactions (max $F_{3,213}$ = 1.260, p = 0.290).

Test. The data from both tests were combined and are depicted on Figure 1d. Responding was lower in the groups that had undergone overexpectation (Groups OE and OE-R) training compared to the controls (Groups CON and CON-R). Unsignalled presentations of the outcome during reinstatement did not reinstate responding in rats following overexpectation training as examined across both tests. These data are supported by the statistical analyses. A 2x2 factorial ANOVA revealed an effect of overexpectation ($F_{1,71}$ = 10.019, p = .002, η_p^2 = .124), with no effect of reinstatement ($F_{1,71}$ = 2.919, p = .092, η_p^2 = .039) and no interaction effect ($F_{1,71}$ = .000, p = 1.000, η_p^2 = .000).

As non-reinforced presentations of the target cue could mask an effect of reinstatement across the entire test, we also examined responding during the first trial of each test prior to any effects of non-reinforcement. These data are shown on Figure 1e and 1f for the first and second test, respectively. These data provide evidence for the presence of reinstatement albeit weak, following overexpectation on the second test. An analysis of the first trial of the first test revealed lower responding in the overexpectation groups (OE and OE-R) compared to the controls (CON and CON-R; $F_{1,71}$ = 4.806, p = 0.032, η_p^2 = 0.063), but no reinstatement effect ($F_{1,71}$ = 2.523, p = 0.117, η_p^2 = 0.034) and no interaction effect ($F_{1,71}$ = 0.132, p = 0.718, η_p^2 = 0.002). A similar analysis of the first trial of the second test revealed lower responding in the overexpectation groups (OE and OE-R) compared to the controls (CON and CON-R; $F_{1,71}$ = 4.288, p = 0.042, η_p^2 = 0.057) and an effect of reinstatement ($F_{1,71}$ = 4.061, p = 0.048, η_p^2 = 0.054) and no interaction effect ($F_{1,71}$ = 0.653, p = 0.422, η_p^2 = 0.009). Pairwise comparisons revealed no effect of reinstatement between the CON and CON-R groups (MD= -6.234, SE =

7.355, p = 0.399, CI[-20.899, 8.431]) but an effect of reinstatement was found between the OE and OE-R groups (MD = -14.584, SE = 7.255, p = 0.048, CI[-29.050, -0.119]).

A

Group	Phase 1	Phase 2	Reinstatement	Test for Reinstatement
OE	A+, B+	AB+, B+	Handle	A-, B+
OE-R	A+, B+	AB+, B+	+++	A-, B+
CON	A+, B+	A+, B+	Handle	A-, B+
CON-R	A+, B+	A+, B+	+++	A-, B+

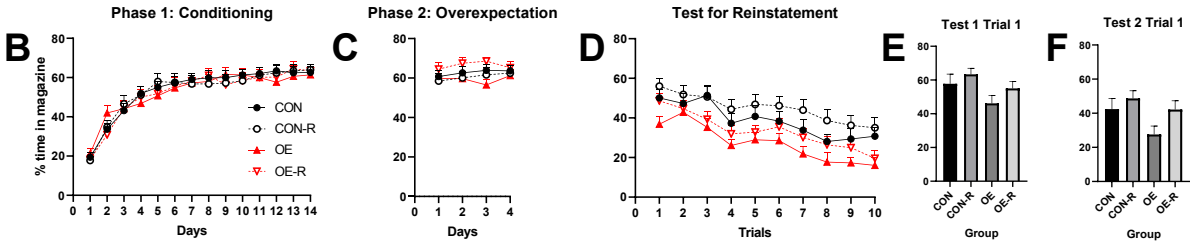

Figure 1. (A) Behavioural design for the Experiment. (B) Percent time spent in the magazine during the 10 second cue across the 14 days of Phase 1. (C) Percent time spent in the magazine during the 10 second cue across the 4 days of Phase 2. (D) Percent time spent in the magazine for each group shown during non-reinforced test for reinstatement. Error bars represent SEM. (E) Percent time in the magazine for each group during the first trial of Test 1.

Discussion

The aim of the current experiment was to determine whether the presentation of the unsignalled outcome after overexpectation training and prior to test would reinstate behavioural responding in male rats- akin to reinstatement as seen after extinction. As mentioned, since the cue was being presented non-reinforced, responding will naturally decrease across trials. To prevent recurrent non-reinforced cues from masking any reinstatement effect, we analyzed the first trial for reinstatement. Upon the first trial of Test 1 there was no significant reinstatement effect found after overexpectation training. However, upon the first trial of Test 2, there was a

statistically significant reinstatement effect found after overexpectation training. Therefore, on the second test day, rats who received unsignalled presentation to the sucrose reward following overexpectation training showed a reinstatement in behavioural responding upon non-reinforced test as opposed to the low level of responding that is characteristic of overexpectation training.

The data show that reinstatement is difficult to obtain following overexpectation training and likely occurs solely during the first trial and not across the entire test session. This may be due to the repeated presentations of non-reinforced presentation of the target stimulus during test possibly attenuating any reinstatement effect – as the animal is essentially receiving extinction sessions which will mask or counteract any possible reinstatement. A second noteworthy, finding in our dataset is the presence of reinstatement only during the first trial of Test 2. One possibility is that the higher levels of responding seen on Test 1 could mask the effect of reinstatement during that test. Alternatively, reinstatement may depend critically on prior non-reinforcement. Therefore, the non-reinforcement of the target cue during Test 1 may be necessary for reinstatement to boost conditioned responding on Test 2. Currently, we have no way of dissociating these tow possibilities.

Experiment 2a. Reinstatement after Overexpectation Training in Females

The majority of behavioural (and neuroscience) research has been male-centric. Recent research has provided evidence that female rats can learn from overexpectation (Lay et al., 2020). The purpose of Experiment 2a was to provide further evidence for this by extending the parameters used to obtain overexpectation as well as investigate whether exposure to an unsignalled reward between overexpectation training and the non-reinforced test reinstates behavioural responding in female rats.

Methods

Subjects

Fifty-eight experimentally naïve female Long Evans rats were used. The rats were three months old at the time of the experiment and weighed an average of 265g (374-201g) post deprivation. Rats were bred in-house in the Animal Care Facility at Concordia University. All experimental procedures were in accordance with the approval granted by the Canadian Council on Animal Care and the Concordia University Animal Care Committee.

Apparatus

The behavioural apparatus used was identical to that described in experiment 1.

Stimuli

The stimuli used were identical to those described in experiment 1.

Procedure

Subjects were randomly divided into one of the four groups: Overexpectation (OE; n=16), Overexpectation-Reinstatement (OE-R; n=16), Control (CON; n=15), Control-Reinstatement (CON-R; n=16). Rats were handled for 5 days and food deprived prior to the start of the experiment to 85% of their initial pre-deprivation bodyweight.

The procedure followed was identical to that described in experiment 1. The design is depicted on Figure 2a.

Data Analysis

Number of head entries into the magazine during cue presentation was taken as the measure of conditioned responding. We looked at percent time spent in the magazine, but this behavioural measure did not reveal an effect of overexpectation, which would preclude us from being able to examine the effect of reinstatement following overexpectation. Considering that these behavioural responses are both indexes of learning, it would be factually erroneous to mistake this as an inability to learn from overexpectation. The data were analysed using Analysis of Variance (ANOVA) in SPSS Statistics (Concordia University license).

Results

Phase 1: Conditioning. The data from Phase 1 are shown on Figure 2b. Conditioned responding increased across days for all groups. A 2x2x(14) mixed factorial ANOVA revealed no differences between the overexpectation and control groups ($F_{1,59} = 1.75$, $p = 0.191$), no differences between the reinstatement and no-reinstatement groups ($F < 1$, $p = 0.902$), no between-group interaction ($F_{1,59} = 3.56$, $p = 0.064$), an effect of days ($F_{13,767} = 41.26$, $p < 0.001$),) and no two- or three-way interactions (max $F_{13,767} = 1.06$, $p = 0.416$).

Phase 2: Overexpectation. The data from Phase 2 are shown on Figure 2c. There were no differences found between any of the groups. This is supported by the statistical analyses. A 2x2x(14) mixed factorial ANOVA found no differences between the overexpectation and control groups ($F_{1,59} = 1.08$, $p = .305$), no differences between the reinstatement and no-reinstatement groups ($F < 1$, $p = .374$), no between-group interaction ($F < 1$, $p = .623$), no effect of days ($F_{3,177} = 2.67$, $p = .071$), and two- or three-way interactions (max $F_{3,177} = 1.16$, $p = 0.328$).

Test. The data from both tests were combined and are depicted on Figure 2d. Responding was lower in the groups that had undergone overexpectation (Groups OE and OE-R) training

compared to the controls (Groups CON and CON-R). Unsignalled presentations of the outcome during reinstatement did not reinstate responding in rats following overexpectation training. These data are supported by the statistical analyses. A 2x2 factorial ANOVA revealed an effect of overexpectation ($F_{1,59} = 7.194$, p=.009, $\eta_p^2 = .109$), no effect of reinstatement ($F_{1,59} = 1.556$, p=.217, $\eta_p^2 = .026$) and no interaction ($F_{1,59} = 1.494$, p=.226, $\eta_p^2 = .025$).

Although there was no effect of reinstatement in the overall test, it is possible that non-reinforced presentations of the target cue would have masked the reinstatement effect. Therefore, we examined responding during the first trial of Test 1 in order to determine if reinstatement was present. These data are shown on Figure 2e. A 2x2 ANOVA revealed no effect of overexpectation ($F_{1,59} = 2.740$, p = .103, $\eta_p^2 = .044$), no effect of reinstatement ($F_{1,59} = .000$, p = .995, $\eta_p^2 = .000$), but a significant interaction ($F_{1, 59} = 4.497$, p =.038, $\eta_p^2 = .071$). Post-hoc analyses of this interaction revealed a mean difference (MD) between the CON and OE groups (MD = 3.550, p = .010, CI[0.868, 6.232] and no difference between the OE and OE-R group (MD = -2.000, p = .135, CI[-.639, 4.639]). A similar analysis of the first trial of Test 2 was also conducted. These data are shown on Figure 2f. A 2x2 ANOVA revealed no effect of overexpectation ($F_{1,59} = 2.01$, p=.161, $\eta_p^2 = .033$), no effect of reinstatement ($F < 1$, p = .682, $\eta_p^2 = .003$), and no interaction ($F < 1$, p = .508, $\eta_p^2 = .007$).

A

Group	Phase 1	Phase 2	Reinstatement	Test for Reinstatement
OE	A+, B+	AB+, B+	Handle	A-, B+
OE-R	A+, B+	AB+, B+	+++	A-, B+
CON	A+, B+	A+, B+	Handle	A-, B+
CON-R	A+, B+	A+, B+	+++	A-, B+

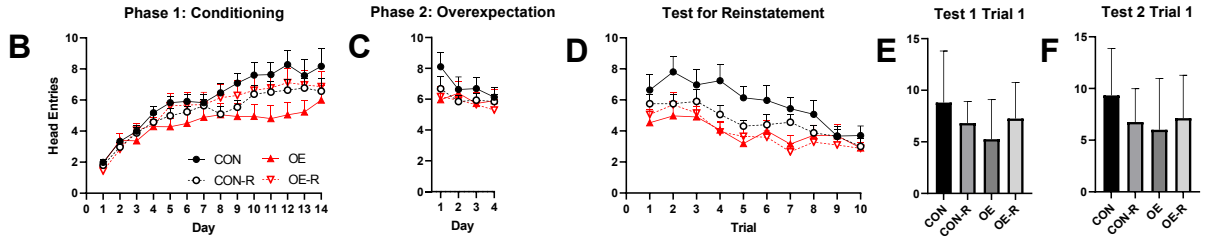

Figure 2. (A) Behavioural design for the Experiment. (B) Average number of head entries into the magazine during the 14 days of Phase 1. (C) Average number of head entries into the magazine during the 4 days of Phase 2. (D) Average number of head entries for each group shown during non-reinforced test for reinstatement. Error bars represent SEM. (E) Average number of head entries for each group during the first trial of Test 1.

Discussion

This experiment aimed to confirm that females are able to learn from overexpectation using an extended overexpectation training design compared to that reported by Lay et al., (2020) as well as examine whether unsignalled exposure to the sucrose reward following overexpectation training in group OE-R was capable of reinstating behavioural responding upon non-reinforced test. It was found that females were able to learn from overexpectation in our current parameters, however, a reinstatement in behavioural responding did not occur during the overall combined test nor was it present during the first trial of Test 1 or the first trial of Test 2. Therefore, females do not show a reinstatement in behavioural responding when presented with the reward following overexpectation training and prior to non-reinforced test.

Experiment 2b. Reinstatement after Extinction Training in Females

The purpose of experiment 2b was to investigate whether the female rats used in Experiment 2a would show reinstatement in behavioural responding following extinction training using identical reinstatement parameters. Rats were counterbalanced according to their previous groups in Experiment 2a and assigned to new groups for the purpose of this experiment: Extinction (Ext; n=31) and Extinction-Reinstatement (Ext-R; n=32). Controls were deemed unnecessary here as the aim of this experiment was solely to determine if there was a reinstatement effect as compared to rats in the extinction group.

Methods

Subjects

The subjects used were identical to those in experiment 2a.

Apparatus

The behavioural apparatus used was identical to that described in experiment 1.

Stimuli

The stimuli used was identical to that described in experiment 1.

Procedure

Experiment 2b was ran following part a. There was a break of 2 weeks between experiment 2a and 2b. The design is depicted on Figure 3a.

Phase 1: Re-Conditioning. Reconditioning to the corresponding non-target cue from Experiment 1a took place across 3 days. All subjects received twenty reinforced presentations of

either the auditory or visual stimulus. The ITI parameters and reinforcement were identical to those described in Experiment 1.

Phase 2: Extinction. During Phase 2 (2 days) all subjects received extinction training to the cue trained in Phase 1. All subjects received twenty non-reinforced presentations of the stimulus they were reconditioned to in Phase 1. The ITI parameters were identical to those of Phase 1.

Reinstatement. Following Phase 2 training, half of the rats (Ext-R) received a single day of reinstatement in which they received unsignalled sucrose pellets in the experimental chamber, identical to that of the magazine training session in Experiment 2a. The other half of the rats (Ext) were handled in their housing room in the Animal Care Facility.

Test. Following Reinstatement training, rats in both groups received 20 non-reinforced presentations of the cue on test.

Data Analysis. Number of head entries during cue presentation was taken as the measure of conditioned responding in order to utilise the same measure as Experiment 2a. The data were analysed using an ANOVA and Independent Samples t-test in SPSS Statistics (Concordia University license).

Results

Phase 1. The data from Phase 1 are depicted on Figure 3b. Responding in the two groups was similar and high across days. A 2x1x(14) mixed factorial ANOVA revealed no effect of group ($F_{1,61} = 2.83$, $p = 0.098$), no effect of days ($F_{2,122} < 1$, $p = 0.803$) and no interaction ($F_{2,122} = 2.38$, $p = 0.096$

Phase 2. The data from Phase 2 are shown on Figure 3c. Responding for the Ext-R and Ext group is seen to decrease at the same rate across between both groups. A mixed ANOVA revealed no effect of group ($F_{1,61} < 1$, $p = 0.937$), an effect of days ($F_{1,61} = 63.06$, $p < 0.001$) and no interaction ($F_{1,61} < 1$, $p = 0.548$).

Test. The data from the test are depicted on Figure 3d. Responding was higher in the rats that received reinstatement between extinction training and test (i.e., Group Ext-R) compared to the rats that did not receive reinstatement (i.e., Group Ext). An independent samples t-test confirmed this observation ($t_{61} = 3.84$, $p < 0.001$). That is, unsignaled presentations of the outcome during reinstatement reinstated responding following extinction in the same female rats that did not exhibit a reinstatement in responding following overexpectation in Experiment 2a.

A

Group	Phase 1	Phase 2	Reinstatement	Test for Reinstatement
Ext	A+	A-	Handle	A-
Ext-R	A+	A-	+++	A-

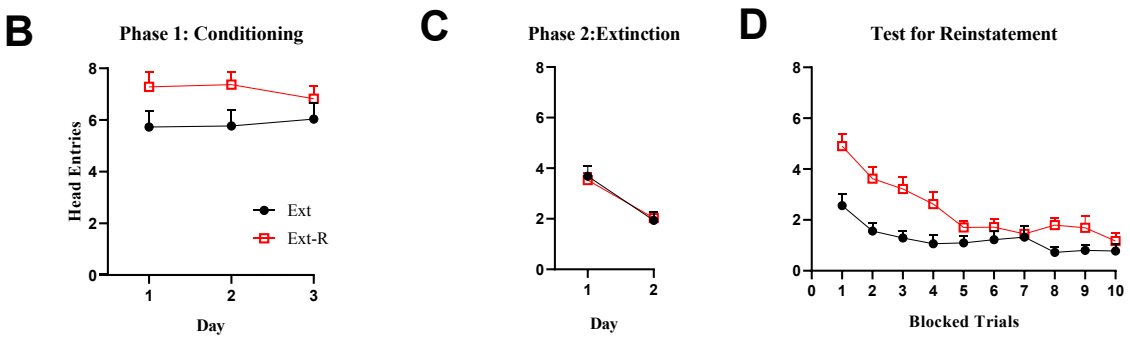

Figure 3. (A) Behavioural design for the Experiment. (B) Average number of head entries into the magazine during the 3 days of Phase 1. (C) Average number of head entries into the magazine during the 2 days of Phase 2. (D) Average number of head entries for each group shown during non-reinforced test for reinstatement. Error bars represent SEM.

Discussion

Experiment 2b produced the expected result of unsignalled exposure to the outcome reinstating behavioural responding after extinction training in the same animals. Therefore, we can be confident that the parameters used in the overexpectation-reinstatement paradigm were effective and that the results obtained in 2a were not a result of ineffective reinstatement parameters and were rather a result of a lack of reinstatement following overexpectation.

Experiment 2c. Unsignalled US Exposure Following Conditioning in Females

In Experiment 2a the control group that received exposure to the unsignalled outcome before test showed a reduction in the conditioned response, albeit an effect that was not statistically reliable. To ensure that unsignalled exposure to the outcome prior to test has no effect on responding in the control condition, we conducted another experiment (Experiment 2c) which aimed to replicate Groups CON and CON-R from Experiment 2a.

Methods

Subjects

Sixteen experimentally naïve female Long Evans rats were used. The rats were three months old at the time of the experiment. The rats weighed an average of 264g (range 314-217g). Rats were bred in-house in the Animal Care Facility at Concordia University.

Apparatus

The behavioural apparatus used was identical to that described in experiment 1.

Stimuli

The stimuli used was identical to that described in experiment 1.

Procedure

Subjects were randomly divided into one of the two groups (CON, CON-R; n=8 per group). Rats were handled for 4 days and food deprived prior to the start of the experiment to 85% of their initial pre-deprivation bodyweight. The design is depicted on Figure 4a

Magazine Training. The procedure was identical to that of Experiment 1.

Phase 1: Conditioning. The procedure was identical to that of experiment 1 with the exception that conditioning last 18 days (14 days as per Phase 1 plus 4 days as per Phase 2 in Experiment 1)

Reinstatement. Following Phase 1 conditioning, half of the rats (CON-R) received a single day of reinstatement identical to that described in Experiment 1. The other half (CON) were handled in their home colony room in the Animal Care Facility.

Test. The procedure was identical to that of Experiment 1.

Data Analysis. Number of head entries in the magazine during cue presentation was taken as the measure of conditioned responding in order to maintain consistency with Experiment 2a. The data were analysed using an ANOVA and an Independent Samples t-test in SPSS Statistics (Concordia University license).

Results

Phase 1. The data from Phase 1 are depicted on Figure 4b. Responding between the groups did not differ across all days of conditioning. A 2x1x(14) mixed factorial ANOVA revealed no effect of group ($F_{1,14} < 1$, $p < 0.822$), an effect of days ($F_{17,238} = 6.88$, $p < 0.001$) and no interaction ($F_{17,238} < 1$, $p < 0.767$).

Test. The data from the combined Test are shown on Figure 4c. Responding on test was similar between the groups. This was confirmed by an independent samples t-test ($t_{15} = 0.37$, $p = 0.714$).

A

Group	Phase 1	Phase 2	Reinstatement	Test for Reinstatement
CON	A+, B+	A+, B+	Handle	A-, B+
CON-R	A+, B+	A+, B+	+++	A-, B+

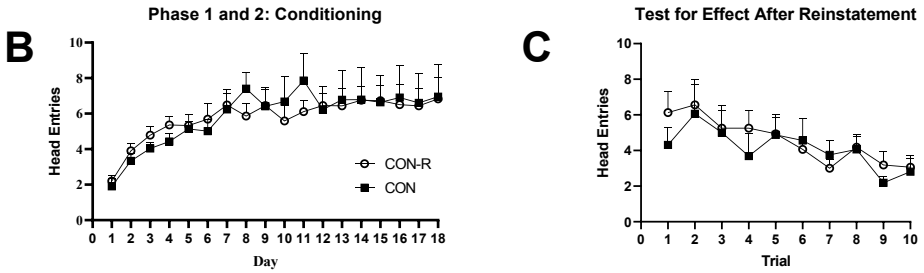

Figure 4. (A) Behavioural design for the Experiment. (B) Average number of head entries into the magazine during the 18 days of Conditioning. (C) Average number of head entries for each group shown during non-reinforced test for reinstatement. Error bars represent SEM.

Discussion

Experiment 2c revealed no systematic differences between the control groups receiving unsignalled presentations of the sucrose reward following conditioning and not. The findings of 2c are consistent with other studies which have demonstrated that unsignalled exposure to the US failed to influence responding following conditioning training (Bouton, 1984; Bouton & King 1986).

General Discussion

Extinction and overexpectation are two paradigms that result in a reduction in conditioned responding on test. Both these paradigms have been shown to be subject to similar

restoration phenomena such as renewal (Bouton & Ricker, 1994; Rescorla, 2007) and spontaneous recovery (Pavlov, 1927, Brooks & Bouton, 1993; Rescorla & Cunningham, 1978; Rescorla, 2006). Extinction has previously been found to be subject to reinstatement (to name a few: Rescorla and Heth, 1975; Rescorla & Cunningham, 1978; Bouton & Bolles, 1979b; Bouton & King, 1983, Westbrook et al, 2001) and at present, we aimed to determine whether overexpectation is subject to the same reinstatement effect.

Our experiments provided evidence that overall the rats learned from overexpectation, but unsignalled exposure to the outcome following overexpectation and prior to test, at best, had a weak reinstatement effect on the first trial of Test 2 in the males but no such effect in the females. Additional analyses revealed that the lack of reinstatement was not likely to be due to our reinstatement parameters as identical treatment led to reinstatement following extinction training in female rats.

The finding of Experiment 1 may imply that the presentation of the outcome itself is sufficient to reinstate responding that was decreased by overexpectation training. This finding conflicts with the memory approach (Bouton, 1993). The memory approach states that the presentation of the unsignalled US conditions the extinction context, forming a context-US association. Consequently, when presented with the non-reinforced cue on test, this contextual conditioning works to retrieve the CS-US memory that was present during acquisition. This then leads to an increase in behavioural responding. This approach works to explain the reinstatement effect obtained following extinction but fails to explain the results here. It can be rationalized that the overexpectation context may provide an occasion setting property, activating a cue-outcome inhibitory association (Westbrook et al., 2001; Bouton & Peck, 1989). The problem arises when considering what happens upon US reexposure. US reexposure is intended, in an

extinction paradigm, to activate the memory of the background conditioning present during acquisition. The problem with overexpectation lies where, unlike in extinction, the outcome is present throughout all phases. Therefore, it would be difficult to be able to "selectively" return the animal to the acquisition stage as opposed to the overexpectation stage. This makes reinstatement highly unlikely to be obtained in this case. The findings obtained with male rats in Experiment 1 do not fit within this theory, however, those obtained with female rats in Experiment 2 provide some albeit weak support for this memory approach.

If one wished to further investigate this theory, one possibility would be to run the overexpectation experiment as normal but with a second outcome present during acquisition. To specify, during acquisition the animal would be individually trained with a visual and an auditory cue associated with one type of reinforcer (O1; i.e., A-O1; X-O1) as well as an additional association between a cue (of either modality) and another reinforcer (O2; i.e., B-O2). During overexpectation, the animals would receive the standard AX-O1 training, which will drive a reduction in conditioned responding to the target cue. So, while O1 and O2 are part of the acquisition memory, only O2 is part of the overexpectation memory. During reinstatement, the animals would be presented with O1 or O2 (or they would receive no reinstatement). If the presentation of the outcome during reinstatement serves to return the animal to the acquisition memory then O2 should lead to stronger reinstatement. This would provide support for the memory approach explained above.

The reinstatement data of Experiment 1 fits in line with previous theories of conditioning, in that it is suggestive of, and fits into a context as a mediator approach of extinction (Holland, 1990). Essentially, the presentation of the outcome alleviates inhibition controlled by the context (Westbrook et al., 2001). Associations between the CS and the US are formed during

conditioning. During extinction, a context-CS association is formed and upon US reexposure, this context-CS association is activated alongside the context-US association. This then helps to facilitate the reassociation between the CS and US by their common context associate. Then upon test of the CS nonreinforced, a reinstatement of behavioural responding is observed.

The data obtained in Experiment 1 may be consistent with this theory. During conditioning, independent CS_A and CS_B associations with the US are formed. During overexpectation training, the CS_A-CS_B compound is being reinforced by the US and CS_B is also being individually reinforced. The CS that is losing associative value with the US is therefore CS_A. Upon US reexposure, a context-US association is strengthened. Upon non-reinforced test, a context- CS_A association is activated which helps mediate the reassociation of CS_A to the US so that CS_A elicits behavioural responding in the absence of reinforcement (shows reinstatement). This fits in line with how mediated conditioning may work for the present results. It is up for debate whether this reassociation of the cue and outcome by the context occurs during outcome presentation or test (Westbrook, 2001; Hall, 1996).

Issues with Current Design

As discussed in Experiment 1, reinstatement was only observed during the first trial of Test 2 for males. This can potentially be explained in two ways. One is that the high responding during Test 1 may be due to a generalization decrement which mask any reinstatement effect present. Generalization decrements are characterized by a decrement in either retrieval or performance between phases. In this case, it is possible that a generalization decrement of the overexpectation memory to Test 1 occurred as a result of the target cue being presented in compound in Phase 2 as opposed to individually (as in Test and Phase 1). When the target cue is presented individually, this allows for the subject to be potentially to be reminded of Phase 1 and

consequently demonstrate high levels of behavioural responding akin to that observed in acquisition. These high levels of responding in the overexpectation group would then affect the data by making it less likely to detect any possibly significant reinstatement effects.

Another explanation is such that reinstatement is dependent on prior non-reinforcement (extinction) of the target cue. This direct non-reinforcement is provided during Test 1, which may in fact lead to the reinstatement of conditioned responding on Test 2. In this case, reinstatement would not be possible directly following overexpectation. It is not possible to distinguish which explanation is most plausible.

Females, on the other hand did not exhibit a reinstatement of responding on either Test. It is possible that the overexpectation effect demonstrated in females is not of the same caliber as that observed in the males. A weak overexpectation effect would result in it being more difficult to distinguish whether rats demonstrated a reinstatement in behavioural responding following overexpectation. The strength of the overexpectation effect is not presently capable of being compared between males and females considering the measure of conditioned responding is different between the males and females. That is, males were measured in % time spent in the magazine whereas females were measured in number of head entries. Also, there are additional variables in measuring the strength of the overexpectation effect: namely the possibility that some rats in fact either did not learn, or do not demonstrate overexpectation.

Concluding Remarks

The results of this investigation lead to new knowledge in the field of learning and memory and how consequently, behaviour is adapted as a result of new information. The present results and discussions also draw insight on how reinstatement occurs in extinction paradigms.

The present data do point towards support for context playing a role as a mediator by which reinstatement occurs. Further investigation is in fact needed to further unravel the complexities of the mechanisms of reinstatement following overexpectation.

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