Pre-publication copy.

Published in: *The Journal of Experimental Psychology: Animal Learning & Cognition*, vol 44, pp 23-35, 2018.

©American Psychological Association, 2018. This paper is not the copy of record and may not exactly replicate the authoritative document published in the APA journal. Please do not copy or cite without author's permission.

The final article is available, upon publication.

The probability of reinforcement per trial affects post-trial responding and subsequent extinction, but not within-trial responding

Justin A. Harris and Dorothy W. S. Kwok

The University of Sydney

Address correspondence to:

Justin Harris
School of Psychology
The University of Sydney,
Sydney 2006
Australia

Running head: Trial-based reinforcement affects post-trial responding and extinction

Acknowledgements. This research was supported by funding from the Australian Research Council, grant DP 150101274.

Abstract

During magazine approach conditioning, rats do not discriminate between a conditioned stimulus (CS) that is consistently reinforced with food and a CS that is occasionally (partially) reinforced, as long as the CSs have the same overall reinforcement rate per second. This implies that rats are indifferent to the probability of reinforcement per trial. However, in the same rats, the per-trial reinforcement rate will affect subsequent extinction—responding extinguishes more rapidly for a CS that was consistently reinforced than for a partially reinforced CS. Here, we trained rats with consistently and partially reinforced CSs that were matched for overall reinforcement rate per second. We measured conditioned responding both during and immediately after the CSs. Differences in the per-trial probability of reinforcement did not affect the acquisition of responding during the CS, but did affect subsequent extinction of that responding, and also affected the post-CS response rates during conditioning. Indeed, CSs with the same probability of reinforcement per trial evoked the same amount of post-CS responding even when they differed in overall reinforcement rate and thus evoked different amounts of responding during the CS. We conclude that reinforcement rate per second controls rats' acquisition of responding during the CS, but at the same time rats also learn specifically about the probability of reinforcement per trial. The latter learning affects the rats' expectation of reinforcement as an outcome of the trial, which influences their ability to detect retrospectively that an opportunity for reinforcement was missed, and in turn drives extinction.

Key words: Rat; magazine approach; Pavlovian conditioning; variable interval; partial reinforcement; extinction; partial reinforcement extinction effect.

Pavlovian conditioning is strongly affected by the rate of reinforcement—the frequency with which the unconditioned stimulus (US) occurs during, or immediately after, the conditioned stimulus (CS). High rates of reinforcement produce stronger responding than low rates (Harris & Carpenter, 2011; Harris, Patterson, & Gharaei, 2015), an effect that is anticipated by most theories of conditioning (Miller & Matzel, 1988; Rescorla & Wagner, 1972; Wagner, 1981). The rate of reinforcement during conditioning also influences subsequent extinction. When a previously reinforced CS is no longer paired with the US, responding extinguishes more quickly if the CS had been reinforced on every trial during conditioning (consistent reinforcement) than if the CS had been reinforced on only some trials (partial reinforcement) (e.g., Bouton, Woods, & Todd, 2014; Fitzgerald, 1963; Fitzgerald, Vardaris, & Teyler, 1966; Haselgrove, Aydin, & Pearce, 2004; Rescorla, 1999). This Partial Reinforcement Extinction Effect (PREE) represents a paradox for most associative theories of conditioning which assume that partial reinforcement should result in weaker associative strength and, therefore, more rapid extinction of responding (Mackintosh, 1974).

The most widely accepted account of the PREE argues that animals learn specifically about the sequencing of trials that leads up to reinforcement. According to Sequential Theory (Capaldi, 1966, 1994), when animals are trained on a partial reinforcement schedule they learn that non-reinforced trials are part of a reinforced sequence, and therefore conditioned responding generalizes well to an extinction phase when all trials are non-reinforced trials. By contrast, responding does not persist under extinction for animals trained with consistent reinforcement because non-reinforced trials did not make up part of their conditioning experience. A more recent explanation of the PREE also emphasizes the importance of

reinforcement rate in the content of what animals learn about conditioning and extinction (Gallistel, 2012; Gallistel & Gibbon, 2000). However, Rate Estimation Theory (RET) differs from Sequential Theory in specifying time, rather than trials, as the relevant unit of reinforcement rate. RET can explain the PREE because it should take longer for the animal to detect the decrease in reinforcement rate when the initial reinforcement rate was low, during partial reinforcement, than when it was high. In essence, extinction depends on the accumulation of evidence about the number of “missed” USs (USs that would have been delivered according to the reinforcement schedule during conditioning). Because USs are missed more frequently during extinction after consistent reinforcement than after partial reinforcement, evidence emerges more quickly that the reinforcement rate has changed, and so responding extinguishes sooner.

RET assumes that the acquisition and extinction of conditioned responding depend on the total reinforcement rate of the CS, computed as the cumulative amount of time that the CS has been present for each reinforcement with the US. This means that the acquisition and subsequent extinction of responding to any two CSs should be the same if those CSs have been matched for their total (cumulative) reinforcement rate. We (Harris et al., 2015) have recently reported results that are consistent with this prediction for the acquisition of responding (but see Bouton & Sunsay, 2003). Evidence against RET’s predictions for extinction has been reported by Haselgrove et al. (2004) and Bouton et al. (2014), who both found differences in the rate of extinction when comparing extinction of a short partially reinforced CS with extinction of a long consistently reinforced CS that were matched for their cumulative reinforcement rates.

In another experiment, Bouton et al. (2014) also obtained evidence for a PREE when comparing a group that had been trained with a 10-s CS on a 25% partial reinforcement schedule and a group that had been trained with a 10-s CS on a 100% reinforcement schedule. The interesting feature of this experiment was that the PREE disappeared (i.e., the two groups showed similar rates of extinction) when they compared responding on every extinction trial of the 100% group with responding on every fourth extinction trial of the 25% group. This result suggests that extinction was four times slower for the 25% group, a conclusion that has been corroborated by recent experiment by Chan and Harris (2017). In that experiment, a CS that had been conditioned with a 33% reinforcement schedule extinguished at the same rate as a CS conditioned with a 100% reinforcement schedule if there were three times as many extinction trials per session for the 33% CS as for the 100% CS. These findings are consistent with the argument that extinction tracks the number of missed opportunities for reinforcement, but only if the rat counts missed reinforcers per trial rather than per unit time during the CS.

Several groups have recently tested another prediction made by RET concerning extinction. It argues that extinction is a function of the cumulative time that the CS has been presented since the last reinforcement (Gallistel, 2012; Gallistel & Gibbon, 2000). Therefore, two CSs should extinguish equally quickly despite differences in their number and duration of trials as long as the total duration of exposure is matched. However, a number of experiments have disconfirmed this prediction by showing that number of trials has more impact than duration on the rate of extinction (Chan & Harris, 2017; Drew, Yang, Ohyama, & Balsam, 2004; Golkar, Bellander, & Öhman, 2013; Harris & Andrew, 2017; Haselgrove & Pearce, 2003). Indeed, across several experiments, Harris and Andrew (2017) found that extinction was entirely

governed by the number of trials, and variations in the length of each trial had no effect. Thus extinction appears to be strongly trial-based, such that each non-reinforced occurrence of the CS contributes equally to extinction. Further, based on evidence from the PREE, the amount that each trial contributes to extinction is determined by the trial-based, but not time-based, rate of reinforcement during conditioning.

The findings summarized above present a paradox. Differences in the per-trial probability of reinforcement do not have any measurable effect on the acquisition of responding if the rates of reinforcement per unit time are held constant, yet differences in responding do emerge during subsequent extinction. The latter finding shows that rats do learn something different about two CSs that differ in probability of reinforcement per trial even when they respond to the two CSs equally. Therefore, the goal of the present experiments was to reveal any difference in how rats respond during conditioning to two CSs that have matched cumulative reinforcement rates. Since our existing evidence shows there to be no difference in responding during the CSs, we decided to look for differences in responding immediately after the CS. Our reasons for singling out this period relate to our evidence that extinction depends on the number of trials during extinction, and not the duration of those trials. This evidence shows that the changes responsible for extinction learning occur once per trial. The logical time for this to happen would be at the end of the trial because that is the only point at which the absence of the US on a given trial can be established unambiguously.

Each of the experiments we present here compared how rats respond to two CSs during conditioning, when the CSs were reinforced with a food pellet (the US), and during subsequent

extinction. Responding was measured during the CS and also for a short period (15 s) after the offset of the CS. One of the CSs had a high probability of reinforcement per trial. However, we could not reinforce 100% of its trials because we needed at least one non-reinforced trial per session to measure post-CS responding that was not contaminated by the presentation of the US. The other CS had a much lower probability of reinforcement, equal to one third that of the first CS. The mean length of the two CSs was adjusted so that their overall rates of reinforcement per unit time were matched. Based on our previous work, we expected to see very similar levels of responding to the CSs during conditioning but differences emerging during extinction. Beyond this, we were more specifically interested to see whether there are differences in responding immediately after the two CSs that would indicate that the rats have learned about the different probabilities of reinforcement per trial.

Experiment 1

Experiment 1 trained rats with two CSs, A and B, that had the same cumulative reinforcement rate. CS A was presented 8 times per session and was followed by food on 7 of those occasions. CS B was presented 24 times per session and was followed by food on 7 of those occasions. Therefore A's probability of reinforcement per trial was 3 times greater than B's. To match their total reinforcement rate per second, presentations of A were 3 times longer than presentations of B: A had a mean duration of 30 s; B had a mean duration of 10 s. Response rates were recorded both during presentations of the CSs and for 15 s after termination of the CSs. In line with our previous findings, responding during presentations of A and B should be very similar. When analyzing post-CS responding, we separated reinforced

trials from non-reinforced trials, with the expectation that responding after reinforced trials would be controlled by presence of the food US, but that responding immediately after a non-reinforced trial might reveal differences in what rats had learned about the likelihood of reinforcement per trial. We hypothesized that responding would be higher after a non-reinforced presentation of A than after a non-reinforced presentation of B, as would indicate that the rats had learned that the probability of reinforcement per trial is much higher for A than B. After 20 sessions of conditioning, we measured how quickly responding to the two CSs extinguished across another 15 sessions. During these extinction sessions, A and B were treated in exactly the same way—both were presented the same number of times and for the same duration—so that any differences in their extinction were due to differences in what had been learned during the previous 20 conditioning sessions and not to differences in the extinction protocol. The full experimental design is summarized in Table 1.

----- Insert Table 1 about here -----

Subjects

Sixteen experimentally-naïve female albino Sprague Dawley rats (8 to 10 weeks of age at the start of the experiment) were obtained from the Animal Resources Centre, Perth, Western Australia. The rats were housed in groups of four in split-level ventilated plastic tubs (Techniplast™), measuring 40 x 46 x 40cm (length x width x height), located in animal colony in the School of Psychology at the University of Sydney. The rats had unrestricted access to water in the home tubs. Three days prior to commencement of the experiment, they were placed on a restricted food schedule. Each day, half an hour after the end of the daily training session,

each tub of rats received a ration of their regular dry chow (3.4 kcal/g) equal to 5% of the total weight of all rats in the tub. This amount is approximately equal to their required daily energy intake (Rogers, 1979), and took at least 2 h to be eaten (but was usually finished within 3 h). All procedures were approved by the Animal Ethics Committee at the University of Sydney.

Apparatus

Rats were trained and tested in 16 Med Associates™ conditioning chambers measuring 28.5 x 30 x 25 cm (height x length x depth). The end walls of each chamber were made of aluminum; the sidewalls and ceiling were Plexiglas™. The floor of the chamber consisted of stainless-steel rods, 0.5 cm in diameter, spaced 1.5 cm apart. Each chamber had a recessed food magazine in the center of one end wall, with an infra-red LED and sensor located just inside the magazine to record entries by the rat. A small metal cup measuring 3.5 cm in diameter and 0.5 cm deep was fixed on the floor of each food magazine. Attached to the food magazine was a dispenser delivering 45 mg food pellets (purified rodent pellets; Bioserve, Frenchtown, NJ). Each chamber was enclosed in a sound- and light-resistant wooden shell. Throughout all sessions, fans located in the rear wall provided ventilation; the operation of these created a background noise level measuring between 61 and 66 dB (depending on the chamber).

Experimental events were controlled and recorded automatically by computers and relays located in the same room. White noise (19 dB above background) was presented from a speaker mounted on the wall of each conditioning chamber above and to the left of the food magazine. A steady light was produced by an incandescent bulb (30cd/m²) in 8 chambers, or an

LED strip light in the other 8 chambers, mounted high on the back wall of the sound-attenuating shell. Allocation of these two stimuli as CSs A and B was counterbalanced between rats. A clicker (4 clicks per second, each click 24 dB above background) was used as a third CS, X. It was produced by a module (Med Associates, product ENV 135M) located on the wall of the conditioning chamber above and to the right of the magazine.

Procedure

Prior to the start of conditioning, rats received a 60-min magazine training session during which 20 food pellets were presented on a variable time (VT) 3-min schedule, with no stimulus presentations. Rats that ate fewer than half of the pellets were given a second session of magazine training the following day.

After all rats completed magazine training, they commenced daily conditioning sessions 5 days per week for a total of 35 sessions. Each of the first 20 sessions contained 46 trials: 8 trials of A, 24 trials of B, and 14 trials of X. CS durations varied randomly from trial-to-trial, sampled from a uniform distribution of durations. Presentations of A had a mean duration of 30 s (ranging from 2 to 58 s), presentations of B had a mean duration of 10 s (2 to 18 s), and presentations of X had a mean duration of 17 s (2 to 32 s). X's duration was chosen as the geometric mean of A and B, in order to equate possible generalization from X to A and B. A single food pellet was delivered at the termination of the CS on reinforced trials: this was done on 7 of the 8 presentations of A, on 7 of the 24 presentations of B, and on every presentation of X. Trials of each type were randomly intermixed. The inter-trial interval varied randomly with a mean of 110 s, and the session lasted approximately 2 h.

Responding to A and B was extinguished on Sessions 21 to 35. Each session included 10 presentations each of A and B, with a mean duration of 17 s (2 to 32 s). None of these presentations was reinforced with food. During each session, X was presented 25 times, with a mean duration of 17 s, and was reinforced with food on every trial. These reinforced presentations of X were included so that food would continue to be delivered during the sessions. The intention behind this was to prevent rapid extinction of all magazine behavior that might otherwise occur if all food delivery ceased and would mask differences in the extinction of responding to A and B. In these sessions, presentations of A, B, and X were randomly intermixed.

For all 35 sessions, photo-beam interruptions by head entry into the magazine were recorded for 20 s immediately before each CS, during each CS, and for 15 s after each CS presentation. To analyze post-CS responding, we conducted separate analyses on the data from reinforced trials and non-reinforced trials. Post-CS responding on reinforced trials would be strongly affected by the presence of the food US at that moment, whereas post-CS activity on non-reinforced trials would isolate conditioned responding elicited by the CS. The response rates during CS presentations, after reinforced CS presentations, and after non-reinforced CS presentations, for acquisition (Sessions 1-20) and extinction (21-35), were analyzed separately as 2-way ANOVAs with CS (A vs B) and Session as factors. The error-rate, α , was set at 0.05.

Results

Response rates during CSs A, B, and X, as well as the pre-CS period, for all 35 sessions are shown in the top plot in Figure 1. Due to an intermittent fault in the device measuring nose

pokes in one box, the data from one rat (#11) could not be used on Days 13, 19, 21 and 28.

These data are omitted from the calculation of mean response rates shown in the figures, but in the statistical analyses the data were replaced with the average response rate from the previous and following day, so that the remaining data from that rat would not have to be excluded from analysis.

----- Insert Figure 1 about here -----

Consistent with our previous results (Harris et al., 2015), the acquisition of responding to CSs A and B was similar. We conducted a 2x20 ANOVA, with Greenhouse-Geisser corrections to degrees of freedom where necessary, to compare responding during A with responding during B across the 20 conditioning sessions. There was no significant difference overall between responding to A and responding to B, $F(1,15) = 0.39, p = .540$. There was a main effect of Session, $F(4.4,66) = 15.41, p < .001, \eta_p^2 = .51$, which did interact significantly with the comparison between A and B, $F(4.2,64) = 2.80, p = .031, \eta_p^2 = .16$. Thus there was some evidence of a small difference in the shape of the acquisition curve between the two CSs.

Across Days 21 to 35, when presentations of A and B were no longer reinforced, responding to both CSs extinguished. Importantly, however, they extinguished at different rates. The rats persisted in responding to B more than to A across this extinction phase. A 2x15 ANOVA confirmed this observation. There was a significant difference overall between responding to A and responding to B, $F(1,15) = 18.50, p < .001, \eta_p^2 = .55$. There was also a significant main effect of Sessions, $F(2.7,42) = 28.01, p < .001, \eta_p^2 = .65$, which interacted with

the comparison between A and B, $F(4.2,62) = 3.30$, $p = .015$, $\eta_p^2 = .18$. This difference in extinction between A and B confirms the PREE.

The middle plot in Figure 1 shows response rates during the 15 s immediately after non-reinforced presentations of A and B across all 35 days. It is clear that, for the first 20 days while both CSs were undergoing conditioning, the rats responded more after non-reinforced presentations of A than after non-reinforced presentations of B. A 2x20 ANOVA confirmed that there was an overall difference in response rates after presentations of A versus B, $F(1,15) = 10.70$, $p = .005$, $\eta_p^2 = .42$. There was no significant main effect of Session, $F(4.5,68) = 1.82$, $p = .128$, nor interaction between Session and the contrast comparing A and B, $F(5.7,85) = 0.90$, $p = .495$. The difference in responding after A versus B was not maintained across Days 21 to 35 when responding during the CSs was extinguished. A 2x15 ANOVA showed that there was no significant difference in post-CS responding between A and B, $F(1,15) = 0.00$, $p = .973$, and while there was a main effect of Session, $F(2.47,37) = 5.56$, $p = .005$, $\eta_p^2 = .27$, there was no significant interaction between CS and Session, $F(3.0,45) = 0.90$, $p = .448$.

The plot at the bottom of Figure 1 shows how much the rats responded during a 15-s period immediately after presentation of either CS A or CS B on reinforced trials when a food pellet was delivered to coincide with CS offset during conditioning (Sessions 1 to 20). Unsurprisingly, magazine activity was high at this time, and there was little difference in this post-US period between trials with CS A and trials with CS B. A 2x20 repeated measures ANOVA confirmed that there was no difference overall in post-US response rates between CS A trials

and CS B trials, $F(1,15) = 0.07$, $p = .803$, nor was there a main effect of sessions, $F(5.2,77) = 1.64$, $p = .158$, or an interaction between CS and sessions, $F(6.7,101) = 1.21$, $p = .306$.

Discussion

This experiment showed that responding extinguished more quickly to a CS that had been reinforced on most trials (7 out of 8) than to a CS that had been reinforced on a minority of trials (7 out of 24). This PREE was observed even though the two CSs had been matched for their total reinforcement rate per second during conditioning, which had the effect that responding to the two CSs was very similar across conditioning. Even though responding to the two CSs was similar during conditioning, this experiment showed that the rats were sensitive to the difference in the per-trial probability of reinforcement. By comparing post-CS responding on trials when the food was not delivered during the conditioning phase, we observed that the rats responded at a significantly higher rate immediately after each non-reinforced presentation of A than after each non-reinforced presentation of B. There was no such difference after reinforced presentations of A and B, presumably because magazine activity at that time was controlled by the presence of the food pellet itself. The difference on non-reinforced trials implies that the rats had learned to expect food on a greater proportion of A's trials than of B's trials. This provides an insight into why the rats showed more rapid extinction of responding to A than B during the subsequent extinction phase—a greater expectation of food after each presentation of A would lead to a greater prediction error when all presentations of A were not reinforced in Phase 2, and thus more rapid extinction.

Experiment 2

Experiment 1 found two differences in how rats respond to CSs depending on the per-trial probability of reinforcement. A CS (A) that had a high probability of being reinforced on each trial elicited more post-CS responding, and extinguished more quickly, than a CS (B) with a low probability of reinforcement. The difference in post-CS responding suggests that the rats' expectation of reinforcement was low immediately after presentations of CS B, which may also explain the difference in rate of extinction if extinction is related to the size of the discrepancy between the rats' expectation of reinforcement and absence of reinforcement. One specific reason why this might have occurred in Experiment 1 is that food, when delivered, was always delivered at the offset of the CS. Therefore, the rats may have learned to associate CS offset with food. Further, the strength of this association would differ between the CSs because CS offset was reliably paired with food for CS A but only sometimes paired with food for CS B. If the rats had learned a stronger association between food and the offset of A than between food and the offset of B, this could explain the difference in post-CS responding and extinction. Experiment 2 investigated this possibility by removing the association between CS offset and food. The experiment was identical to Experiment 1 in every respect except that CS durations were fixed rather than variable, and food was delivered at a random time during the CS rather than at the end of the CS. This ensured that food never coincided with the offset of either CS. If the reason that rats in Experiment 1 responded more after CS A trials than CS B trials was because they had learned that food was more likely after A's offset than was B's offset, then there should be no difference in post-CS responding in Experiment 2 because neither A's offset nor B's offset was ever followed by food. Further, we might no longer see a difference in the

rate of extinction between CSs A and B in Experiment 2 if differences in extinction depend on differences in post-CS expectation of food. The procedure used in Experiment 2, where food is delivered during the CS rather than at its offset, also allowed us to compare post-CS responding on non-reinforced trials with post-CS responding on reinforced trials when responding on the latter trials was free from the direct influence of the food pellet.

Subjects and Apparatus

Sixteen experimentally-naïve female albino Sprague Dawley rats (8 to 10 weeks of age at the start of the experiment) were obtained from the same source and housed in the same manner as for Experiment 1. Three days before commencement of the experiment, they were placed on the same restricted food schedule. The rats were trained and tested in 16 Med Associates™ conditioning chambers like those described for Experiment 1, using the same three stimuli as CSs. The US was a single 45 mg food pellet, and an infra-red photo-beam recorded magazine activity.

Procedure

The training and testing procedures were identical to those described for Experiment 1 with the following exceptions. During conditioning on Days 1 to 20, the durations of reinforced CS presentations were fixed. CS A was presented for 60 s on each of its 7 reinforced trials per session; CS B was presented for 20 s on each of its 7 reinforced trials; and X was presented for 34 s on each of its 8 reinforced trials (34 s was chosen as the geometric mean of 20 and 60 s). During these reinforced presentations, a single food pellet was delivered at a random time. This was between 2 and 18 s for CS A (mean 10 s), between 2 and 58 s for CS B (mean 30 s), and

between 2 and 32 s for CS X (mean 17 s). Each session also included one non-reinforced trial of CS A and 17 non-reinforced trials of CS B. These non-reinforced trials had a variable duration, with a mean of 30 s (2 to 58 s) for CS A, and a mean of 10 s (2 to 18 s) for CS B. The non-reinforced trials were variable, rather than fixed, so that the rats could not learn that the probability of receiving food on a given trial decreased as the length of the trial increased, which would be particularly problematic for CS B because it had many non-reinforced trials.

As in Experiment 1, magazine activity was recorded before, during, and after each CS presentation. As a measure of conditioned responding to the CS, we analyzed magazine activity in the period leading up to the scheduled delivery of the food pellet. We separately analyzed responding during the CS that occurred immediately after delivery of the food pellet in order to assess unconditioned magazine activity elicited by the US. On any given trial, this post-US period was set to a maximum length of 10 s, but was truncated to a shorter time window on trials when the US was delivered less than 10 s before the end of the CS, so that this post-US responding was always measured during the CS and did not include any post-CS period. Post-CS responding was also analyzed, and for this analysis we compared response rates after trials that had been reinforced with response rates after non-reinforced trials. The response rates during CS presentations (pre and post-US), after reinforced CS presentations, and after non-reinforced CS presentations, for acquisition (Sessions 1-20) and extinction (21-35), were analyzed separately as 2-way ANOVAs with CS (A vs B) and Session as factors. The error-rate, α , was set at 0.05.

Results

A fault in the infrared sensor recording magazine activity in one box affected data collection on a total of 16 days for Rat 3. Because of this large amount of missing data, this rat was excluded from all analyses, leaving 15 rats in the experiment. Response rates during and after CS presentations on all 35 days of Experiment 2 are shown in Figure 2. Response rates during each of the CSs, leading up to delivery of the food US, are shown in Figure 2A. These increased during conditioning on Days 1 to 20, and the rats responded at similar levels during CSs A and B. A 2x20 ANOVA, with Greenhouse-Geisser correction to degrees of freedom, was used to compare the acquisition of responding to CS A and CS B. There was a significant main effect of Session, $F(2.2,31) = 7.69, p = .001, \eta_p^2 = .35$. There was no significant difference overall between responding to A and responding to B, $F(1,14) = 0.01, p = .934$, nor was there a significant interaction between CS and Session, $F(4.6,65) = 1.50, p = .206$. Thus, as predicted, there was no difference in acquisition of responding between CSs A and B.

----- Insert Figure 2 about here -----

From Day 21 to Day 35, responding during CSs A and B gradually extinguished. As is clear in Figure 2A, responding to B extinguished more slowly than to A. A 2x15 ANOVA confirmed this observation. There was a significant main effect of Session, $F(1.4,19) = 17.57, p < .001, \eta_p^2 = .58$. There was a significant difference overall between responding to CS A and responding to CS B, $F(1,14) = 8.78, p = .010, \eta_p^2 = .39$, and there was a significant interaction between CS and Session, $F(2.1,30) = 4.71, p = .015, \eta_p^2 = .25$.

Response rates during the CSs, but immediately after delivery of a food pellet in Sessions 1 to 20, are shown in Figure 2B. There seems to be little difference in post-US responding between the two CSs. A 2x20 ANOVA confirmed that there was no significant difference overall between post-US responses during presentations of A and post-US responses during presentations of B, $F(1,14) = 2.42, p = .142$. There was also no significant main effect of sessions, $F(4.4,62) = 2.12, p = .082$, and no significant interaction between sessions and the difference between the two CSs, $F(7.3,103) = 0.68, p = .692$.

Figure 2C shows response rates during the 15 s immediately after non-reinforced presentations of CSs A and B. It is clear that the rats responded more after presentations of A than after presentations of B, across the first 20 days. A 2x20 ANOVA confirmed this impression. There was a significant overall difference between response rates after A and response rates after B, $F(1,14) = 14.63, p = .002, \eta_p^2 = .51$. There was a significant main effect of Session, $F(6.6,92) = 2.21, p = .044, \eta_p^2 = .14$, but the interaction between CS and Session did not survive the Greenhouse-Geisser correction, $F(5.8,82) = 1.88, p = .095$.

Differences between CSs A and B in post-CS responding all but disappeared during extinction from Days 21 to 35. A 2x15 ANOVA found there to be no significant difference overall between post-CS responding on non-reinforced trials with A and non-reinforced trials with B, $F(1,14) = 0.14, p = .715$. There was a significant main effect of Session, $F(3.1,43) = 17.75, p < .001, \eta_p^2 = .56$, and a significant interaction between CS and Session, $F(4.8,67) = 2.46, p = .044, \eta_p^2 = .15$. It is not obvious from the figure what systematic difference between A and B could be the source of this interaction. Pairwise comparisons on the data from each day show there are

no significant differences between A and B for the first 13 days, and only on the final 2 days is there any evidence for a possible difference in responding, $t(14) = 2.62$ and 2.50 , $ps = .020$ and $.025$, for Days 34 and 35 respectively.

Response rates immediately after each reinforced presentation of CSs A and B across Sessions 1 to 20 are shown in Figure 2D. The rats responded more after reinforced trials of B than after reinforced trials of A, the opposite pattern to what was observed after non-reinforced trials. A 2x20 ANOVA confirmed that there was a significant difference overall between response rates after reinforced trials of A and reinforced trials of B, $F(1,14) = 19.98$, $p < 0.001$, $\eta_p^2 = .59$. There was not a significant main effect of sessions, $F(1,14) = 1.26$, $p = 0.208$, nor was there an interaction between sessions and the difference between CSs, $F(1,14) = 1.00$, $p = 0.457$.

To get a better understanding of the factors that controlled the post-CS response rates, we conducted analyses that compared post-CS responding between reinforced trials and non-reinforced trials. Figure 2 shows the post-CS response rates across the 15 s immediately after reinforced and non-reinforced presentations of CS A (Figure 2E) and CS B (Figure 2F). It is clear that the rats responded more after non-reinforced than after reinforced presentations of CS A, but they responded at very similar rates after reinforced and non-reinforced presentations of CS B. A 2x2x20 ANOVA showed that there was no significant difference overall between post-A and post-B response rates, $F(1,14) = 0.12$, $p = .730$, nor between responding after reinforced trials and responding after non-reinforced trials, $F(1,14) = 1.46$, $p = .247$. However, there was a significant interaction between these effects, $F(1,14) = 35.95$, $p < .001$, $\eta_p^2 = .72$, confirming

that the difference in responding after reinforced versus non-reinforced trials with A was larger than the difference after trials with B. The main effect of Sessions was not significant, $F(6.5,91) = 1.90$, $p = .083$, and did not interact significantly with either of the other main effects or with any interaction, largest $F(7.3,102) = 1.65$, $p = .128$. We followed up the interaction with separate 2x20 ANOVAs comparisons between response rates after reinforced and non-reinforced trials for each CS. The analysis of post-CS responding after trials of A showed that responding was significantly higher after non-reinforced trials than after reinforced trials, $F(1,14) = 9.18$, $p = .009$, $\eta_p^2 = .40$. This difference interacted with sessions, $F(6.4,89) = 2.34$, $p = .044$, $\eta_p^2 = .14$, although there was no main effect of sessions, $F(7.2,100) = 1.76$, $p = .102$. The analysis of post-CS responding on trials with B showed that there was no significant difference between responding after reinforced and non-reinforced trials, $F(1,14) = 1.33$, $p = .268$. There was no significant interaction between trial-type and sessions, $F(7.2,100) = 1.76$, $p = .102$, nor was there a significant main effect of sessions, $F(4.6,65) = 0.56$, $p = .714$.

Discussion

This experiment trained rats with two CSs, A and B, that had matched cumulative reinforcement rates but which differed in the per-trial probability of reinforcement and in the mean interval between CS-onset and US. The results were very similar to those obtained in Experiment 1. There were no differences in the acquisition of responding during the two CSs, but there were differences in responding in the period immediately after each CS, as well as differences in the extinction of responding to the two CSs. The rats responded more immediately after non-reinforced presentations of CS A, that had a high probability of

reinforcement per trial, than after non-reinforced presentations of CS B, that had a low per-trial probability of reinforcement. They also extinguished more slowly to CS B than CS A when both CSs were presented repeatedly without any reinforcement on Days 21 to 35.

These findings confirm what was found in Experiment 1. This experiment is important because it shows those results are obtained even when there is no opportunity for the rats to learn an association between CS offset and food. Therefore, the higher post-CS responding following A than B is not due to a stronger association between food and the termination of CS A. Rather, it shows that the rats had a higher expectancy for food immediately after presentations of A than B because the probability of food was higher during A than B. Therefore, even though the rats responded in very similar ways during CS A and CS B, they nonetheless seemed to have learned that the likelihood of receiving food at some point was higher for presentations of A than of B. The fact that we once again observed slower extinction of B than A is consistent with our suggestion that the learning that produces extinction occurs at the end of a trial when the animal is able to detect that an expected US had not occurred.

By delivering the food US during rather than after presentations of the CSs, this experiment also allowed us to compare how the rats responded after reinforced trials with how they responded after non-reinforced trials, without the problem that responding after reinforced trials was contaminated by the presence of the food pellet at that time. These comparisons showed that the rats did discriminate sharply in their post-CS responding between reinforced and non-reinforced trials of CS A, whereas the rats did not appear to discriminate between reinforced and non-reinforced trials of CS B. This difference between the two CSs was

carried by differences in how the rats responded after both reinforced trials and non-reinforced trials. That is, the rats responded significantly more after non-reinforced trials of A than after non-reinforced trials of B, as reported above, but they also responded significantly less after reinforced trials of A than after reinforced trials of B. Therefore, the rats' post-CS reactions were sensitive to the presence and absence of reinforcement during CS A, but their post-CS behavior had lost sensitivity to B's reinforcement outcome. This loss of sensitivity to whether B was reinforced or not could explain why responding to B took longer to extinguish than responding to A when the two CSs were no longer reinforced at all.

Experiment 3

In Experiments 1 and 2, rats acquired responding to CSs A and B at very similar rates, consistent with the fact that the CSs had equivalent cumulative reinforcement rates. However, the two CSs differed in how much post-CS responding they supported and in their resistance to subsequent extinction. We attribute these differences in post-CS responding and extinction to differences in the per-trial probability of reinforcement, since A was paired with food on 7 out of 8 trials whereas B was paired with food on 7 out of 24 trials. However, the two CSs not only differed in the probability of reinforcement per trial, they also differed in their mean length—A had a mean duration of 30 s and B had a mean duration of 10 s. Therefore, it is logically possible that the difference in post-CS responding and resistance to extinction are due to the differences in mean length rather than probability of reinforcement. This difference in length might provide a particularly plausible explanation for the fact that the rats responded more after reinforced trials of B than after reinforced trials of A. The delivery of the food pellet on reinforced trials

was, on average, closer to the end of presentations of A than presentations of B, and therefore residual magazine activity following pellet delivery could account for the greater responding after termination of A.

Experiment 3 was designed to address this confound. It used a 2x2 factorial design (summarized in Table 1) that compared two CSs, A and B, that had a high probability of reinforcement (7 out of 8 trials) with two CSs, C and D, that had a low probability of reinforcement (7 out of 24 trials). Within each level of this factor, it compared two short CS-US intervals (mean interval = 10 s, for CSs A and C) with two long CS-US intervals (mean interval = 30 s, for CSs B and D). As in Experiments 1 and 2, we recorded the rats' responding both during each CS presentation and during the 15-s period immediately after offset of each CS. In line with previous similar experiments (Harris et al., 2015), during the conditioning phase we expected to see main effects of both probability of reinforcement and CS duration, with rats responding most during the short CS reinforced on 7 of 8 trials (A), and least during the long CS reinforced on 7 or 24 trials (D). Moreover, as observed in that previous research as well as in Experiments 1 and 2 of the present series, we expected to see similar acquisition of responding during the two CSs that had matched cumulative reinforcement rates (B and C). In keeping with Experiments 1 and 2, we also expected to see differences in post-CS responding following B and C, and greater resistance to extinction for C than B. The question of interest was whether these differences would be part of an overall main effect of CS length or an overall main effect of reinforcement probability.

Subjects and Apparatus

Sixteen experimentally-naïve female albino Sprague Dawley rats (8 to 10 weeks of age at the start of the experiment) were obtained from the same source and housed in the same manner as for Experiment 1. Three days before commencement of the experiment, they were placed on the same restricted food schedule. The rats were trained and tested in the same 16 Med Associates™ conditioning chambers described for Experiment 1. Experiment 3 introduced two new stimuli in addition to the three stimuli (white noise, steady light, and clicker) used as CSs in Experiments 1 and 2. A high-pitch tone (2.9 kHz, 17 dB above background) was produced from a piezo buzzer positioned on the outside wall of each conditioning chamber. A flashing light (2 Hz; 3.0cd/m²) was emitted by an array of white LEDs, located on one wall of the sound-attenuating shell in front of the operant chamber. The white noise, tone, steady light, and flashing light were allocated as CSs A, B, C, and D. Allocations were counterbalanced in a Latin Square across four squads of rats. The US was a single 45 mg food pellet, and an infra-red photo-beam recorded magazine activity.

Procedure

Rats first underwent magazine training as described for Experiment 1. After this they received 25 days of training with four CSs, A, B, C, and D. There were 7 reinforced trials of each CS per session. On these trials, the durations of the CS were fixed, and a single food pellet was delivered at a random time during the CS. CSs A and C were presented for 20 s, and food was delivered between 2 and 18 s after CS onset (mean CS-US interval = 10 s). CSs B and D were presented for 60 s, and food was delivered between 2 and 58 s after CS onset (mean CS-US

interval = 30s). Each session also included one non-reinforced trial of each of CS A and CS B, as well as 17 non-reinforced trials of each of CS C and CS D. Thus CSs A and B were reinforced on approximately 88% of trials, while CSs C and D were reinforced on approximately 29% of trials. The non-reinforced trials had variable durations, with a mean of 10 s (range 2 to 18 s) for CSs A and C, and a mean of 30 s (2 to 58 s) for CSs B and D. The non-reinforced trials were variable, rather than fixed, so that the rats could not learn that the probability of receiving food on a given trial decreased as the length of the trial increased.

The rats then received a further 15 sessions (Sessions 26 to 40) in which responding to the four CSs (A, B, C, and D) was extinguished. On these sessions, the four CSs were presented for the same number of trials (10 per session) and the same fixed duration (20 s). No food pellets were delivered during any of these trials. However, to prevent complete extinction of all magazine entry, a new stimulus (the clicker, X) was introduced in these sessions, and reinforced. In each session, there were 20 presentations of the clicker, with a mean duration of 20 s, and every presentation was immediately followed by delivery of a food pellet.

As in Experiment 2, magazine activity was recorded for 20 s before each CS, during each CS presentation, and for 15 s after each non-reinforced presentation of the CS. The response rates during CS presentations (pre and post-US), after reinforced CS presentations, and after non-reinforced CS presentations, for acquisition (Sessions 1-20) and extinction (21-35), were analyzed separately as 3-way ANOVAs with reinforcement probability (87.5% vs 29%), CS-US interval (10 s vs 30 s), and Session as factors. The error-rate, α , was set at 0.05.

Results

Mean response rates across all 40 sessions of the experiment are shown in Figure 3. Magazine activity was not recorded during the first two sessions of the experiment for 4 rats (rats 9 to 12). For the purposes of statistical analyses, these missing values were replaced with the data from Session 3. It is clear from Figure 3A that pre-US responding during the four CSs increased over the first 25 sessions, and the rats responded differentially to the CSs as a function of their total (cumulative) reinforcement rate. Thus, the rats responded most to CS A, least to CS D, and showed very similar levels of responding to CSs B and C. A 2x2x25 ANOVA, with Greenhouse-Geisser correction to degrees of freedom, was conducted to analyse the pre-US response rates during the CSs from Sessions 1 to 25. This showed that all three main effects were significant: there was an effect of per-trial reinforcement probability (88% vs 29%), $F(1,15) = 33.15, p < .001, \eta_p^2 = .69$, an effect of CS-US interval (10 s vs 30 s), $F(1,15) = 34.37, p < .001, \eta_p^2 = .70$, and an effect of Session, $F(4.5,68) = 18.62, p < .001, \eta_p^2 = .55$. There was no significant interaction between the first two factors, $F(1,15) = 0.00, p = .990$, but they both interacted with Session: $F(3.5,53) = 3.20, p = .025, \eta_p^2 = .18$, for the interaction between Session and reinforcement probability; $F(3.8,57) = 4.03, p = .007, \eta_p^2 = .21$, for the interaction between Session and CS-US interval. There was no significant 3-way interaction, $F(2.9,43) = 0.48, p = .690$. Therefore, the acquisition of responding during the CSs was affected by both the probability of each trial being reinforced, and by the length of the CS-US interval.

----- Insert Figure 3 about here -----

As can be seen in Figure 3A, pre-US response rates to the four CSs (A to D) extinguished once they were no longer reinforced across Sessions 26 to 40. There were important differences between the CSs in how quickly responding extinguished, with responding to C appearing to show the greatest resistance to extinction. We conducted a 2x2x15 ANOVA on the response rates during CSs A to D from Sessions 26 to 40. This showed there was a significant main effect of Session, $F(2.3,36) = 51.14, p < .001, \eta_p^2 = .77$, and a significant main effect of CS-US interval, $F(1,15) = 42.61, p < .001, \eta_p^2 = .74$, but no significant main effect of reinforcement probability, $F(1,15) = 0.16, p = .70$. There was a significant interaction between CS-US interval and reinforcement probability, $F(1,15) = 5.79, p = .029, \eta_p^2 = .28$, as well as between CS-US interval and Session, $F(2.2,28) = 10.30, p = .001, \eta_p^2 = .41$, but no significant interaction between reinforcement probability and Session, $F(2.7,41) = 1.32, p = .280$, and no significant 3-way interaction, $F(3.7,55) = 0.91, p = .457$. Differences in responding across extinction are strongly affected by differences in initial levels of responding between CSs. Therefore, the clearest comparison to identify a difference in rate of extinction is between CS B and CS C, which correspond to A and B in the previous two experiments, because these elicited very similar levels of responding during the training phase. A 2x15 ANOVA comparing CSs B and C over Sessions 26 to 40 showed there was a significant overall difference between the two CSs, $F(1,15) = 10.97, p = .005, \eta_p^2 = .42$, which interacted significantly with the main effect of Session, $F(2.7,40) = 4.84, p = .007, \eta_p^2 = .24$. Therefore, responding to C was more resistant to extinction than responding to B, consistent with the PREE. Indeed, responding to C appeared to persist for longer than responding to CS A, even though responding to A was much higher than to C across the training sessions. However, a 2x15 ANOVA comparing CS A and CS C across

extinction did not find a significant overall difference between the CSs, $F(1,15) = 1.92$, $p = .186$, or significant interaction with Session, $F(3.0,45) = 0.86$, $p = .468$.

Response rates during each CS over a 10-s period immediately after delivery of food on reinforced trials is shown in Figure 3B. There were no differences in response rates between the CSs, as confirmed by a 2x2x25 ANOVA: There were no significant main effects of CS length, $F(1,15) = 0.28$, $p = .604$, reinforcement probability, $F(1,15) = .10$, $p = .760$, or session, $F(3.7,55) = 2.23$, $p = .082$, nor were there any interactions between these main effects, largest $F = 1.14$, $p = .343$. Therefore, as in Experiments 1 and 2, magazine activity immediately after delivery of the food pellet was controlled by the presence of food and was not affected by the CS.

Response rates during the 15 s after non-reinforced presentations of each CS are shown in Figure 3C. For the first 8 or 9 sessions, the pattern of post-CS responding looked very similar to the pattern of responding during the CSs, with highest response rates observed after CS A, lowest after CS D, and similar levels after CSs B and C. However, as training continued, responding post-CS B diverged from responding post-CS C, such that, by the end of training, post-B response rates had increased to the same level as post-A response rates, while post-C response rates became more similar to post-D response rates. A 2x2x25 ANOVA confirmed these impressions. There were significant main effects for both CS-US interval, $F(1,15) = 23.45$, $p < .001$, $\eta_p^2 = .61$, and per-trial probability of reinforcement, $F(1,15) = 60.65$, $p < .001$, $\eta_p^2 = .80$, but these did not interact, $F(1,15) = 0.44$, $p = .517$. There was a significant main effect of Session, $F(6.1,92) = 4.24$, $p = .001$, $\eta_p^2 = .22$, that also interacted with the main effect of reinforcement probability, $F(7.4,112) = 2.64$, $p = .013$, $\eta_p^2 = .15$, but not with CS-US interval,

$F(7.1,106) = 1.04, p = .408$. There was no significant 3-way interaction, $F(7.8,116) = 0.63, p = .748$.

In light of the significant interaction between reinforcement probability and Session, we conducted a 2x2 ANOVA on post-CS responding after non-reinforced trials for just the last 5 sessions of conditioning (21-25). This showed there was still a significant main effect for reinforcement probability, $F(1,15) = 42.26, p < .001, \eta_p^2 = .74$, but the main effect of CS-US interval was not quite significant, $F(1,15) = 4.11, p = .061$, nor was there a significant interaction between the factors, $F(1,15) = 0.09, p = .769$. It seemed likely that some of the differences in post-CS responding could be a carry-over from the large difference in responding during the CSs. Therefore, we repeated the 2x2 ANOVA but excluded the first 3 s of the 15-s post-CS period, to reduce carry-over of responding from the CS period. This confirmed the significant main effect of reinforcement probability, $F(1,15) = 25.18, p < .001, \eta_p^2 = .63$, and there was no main effect of CS-US interval, $F(1,15) = 0.37, p = .555$, nor interaction, $F(1,15) = 0.21, p = .361$.

We also conducted analyses that just compared responding after presentations of B and presentations of C, to be consistent with the analyses conducted in Experiments 1 and 2. A 2x20 ANOVA showed there was a significant overall difference between post-B responding and post-C responding, $F(1,15) = 5.63, p = .031, \eta_p^2 = .27$, that interacted significantly with the linear trend across sessions, $F(1,15) = 5.79, p = .029, \eta_p^2 = .28$. this interaction was followed up using simple pairwise comparisons between B and C trials for responding averaged over the first 5 sessions and over the last 5 sessions. These showed that post-CS responding did not differ significantly between B and C over the first 5 sessions (post-B = 0.25, post-C = 0.29), $t(15) =$

1.11, $p = .283$, but did differ over the last 5 sessions (post-B = 0.56, post-C = 0.38), $t(15) = 3.68$, $p = .002$.

From Days 26 to 40, when all trials of CSs A to D were no longer reinforced, post-CS responding to these CSs dropped very quickly and there appeared to be little difference between the four CSs (see Figure 3C). A $2 \times 2 \times 15$ ANOVA showed that there was a significant main effect of Session, $F(3.3, 49) = 17.62$, $p < .001$, $\eta_p^2 = .54$, but no significant main effects of either reinforcement probability, $F(1, 15) = 0.07$, $p = .799$, or CS-US interval, $F(1, 15) = 0.46$, $p = .510$. There was a significant interaction between CS-US interval and reinforcement probability, $F(1, 15) = 9.62$, $p = .007$, $\eta_p^2 = .39$, but there were no significant interactions involving Session, largest $F(3.9, 58) = 1.78$, $p = .146$. The interaction between CS-US interval and reinforcement probability is difficult to interpret. Numerically, the lower reinforcement probability is associated with higher overall responding for the two 10-s CSs (i.e., averaged across the 15 sessions, CS C > CS A), whereas this is reversed for the two 30-s CSs (CS D < CS B). However, the effect is extremely small—it is difficult to discern in the results shown in Figure 3C—and we believe it is unlikely to be particularly meaningful.

Post-CS response rates after reinforced trials during Sessions 1 to 25 are shown in Figure 3D. Responding was highest after CS C and lowest after CSs B and D. A $2 \times 2 \times 25$ ANOVA showed there were significant main effects of reinforcement probability, $F(1, 15) = 8.66$, $p = .010$, $\eta_p^2 = .37$, and CS length, $F(1, 15) = 22.15$, $p < .001$, $\eta_p^2 = .60$, and a significant interaction between these factors, $F(1, 15) = 9.42$, $p = .008$, $\eta_p^2 = .39$. The main effect of Sessions was not significant, $F(4.1, 61) = 2.16$, $p = .083$, and this factor did not interact with either of the other two factors,

largest $F(6.6,99) = 1.74, p = .113$. To follow up the interaction between CS length and reinforcement probability, we performed paired t -tests comparing each pair of CSs for responding averaged across the 25 sessions. We used a Bonferroni correction to account for multiple comparisons (thus, $\alpha = .0083$). These showed that all pairwise comparisons were significant, smallest $t(15) = 3.39, p < .003$, except for the comparison between CSs B and D, $t(15) = 1.04, p = .315$. Therefore, responding after reinforced trials with C was significantly higher than after reinforced trials of B, as found in Experiment 2, and post-C responding was also significantly higher than after reinforced trials of A. This latter comparison is important because A and C were matched in length, and thus they were matched in the time interval between delivery of reinforcement and the CS offset.

As for Experiment 2, we conducted further analyses to test how well the rats discriminated between reinforced and non-reinforced trials immediately after each CS. These comparisons are shown in Figures 3E to 3H. It is clear that post-CS response rates were very different between reinforced and non-reinforced trials for CSs A and B, but differed much less for CSs C and D. To analyse these data, we conducted a 2 (reinforced trial vs non-reinforced trial) $\times$ 2 (reinforcement probability) $\times$ 2 (CS length) $\times$ 25 (Sessions) ANOVA. In the interests of clarity we will only present those results that are relevant to the comparison between reinforced and non-reinforced trials. Overall, post-CS responding was higher after non-reinforced trials than after reinforced trials, $F(1,15) = 15.26, p = .001, \eta_p^2 = .50$. This difference interacted significantly with reinforcement probability, $F(1,15) = 60.21, p < .001, \eta_p^2 = .80$, and this interaction also formed a 3-way interaction with Session, $F(7.1,106) = 2.39, p = .025, \eta_p^2 = .14$. These interactions confirm that, in their post-CS responding, the rats learned to

discriminate between reinforced and non-reinforced trials much more for CSs A and B than for CSs C and D. Simple 2x25 ANOVAs, conducted on the post-CS data for each CS individually, showed that there were significant differences between reinforced and non-reinforced trials for CS A and CS B, $F(1,15) = 23.11$ and 32.04 , $ps < .001$, $\eta_p^2 = .61$ and 0.68 , but not for CS C or CS D, $F(1,15) = 0.18$ and 4.08 , $p = .676$ and 0.62 . The difference interacted significantly with Session for CS A, $F(7.8,117) = 3.00$, $p = .005$, $\eta_p^2 = .17$, CS B, $F(7.6,115) = 2.64$, $p = .012$, $\eta_p^2 = .15$, and CS D, $F(5.3,80) = 2.55$, $p = .031$, $\eta_p^2 = .15$, but not CS C, $F(3.8,58) = 1.17$, $p = .166$.

Discussion

Experiment 3 has confirmed the key findings of Experiments 1 and 2. In their post-CS behavior, rats discriminated well between trials that had been reinforced and trials that had not been reinforced if the CS had a high probability of reinforcement per trial, but they discriminated poorly between reinforced and non-reinforced trials if the CS had a low probability of reinforcement per trial. Experiment 3 extended this finding by showing that the difference in post-CS responding is specifically a function of the per-trial probability of reinforcement rather than the CS-US interval. Rats showed good post-CS discrimination between reinforced and non-reinforced trials for two CSs that had a high probability of reinforcement per trial, despite the fact that the CSs differed in their length (20 s versus 60 s) and therefore differed in the observed level of responding measured during the CS. At the same time, the rats also showed poor post-CS discrimination between reinforced and non-reinforced trials for two CSs that had low probabilities of reinforcement per trial, again despite differences in length (20 s versus 60 s) and the level of responding they evoked during the CS. Therefore,

while CS length (and CS-US interval) has clear effects on responding during the CS, responding immediately after termination of the CS is largely related to the probability of reinforcement per trial. The strong implication is that rats do learn specifically about the probability of reinforcement per trial, independently of what they learn about CS-US interval and cumulative rate of reinforcement.

General Discussion

The aim of the present series of experiments was to look for evidence that rats learn specifically about the per-trial probability of reinforcement. Our previous research (Harris & Carpenter, 2011) has shown that the acquisition of Pavlovian conditioned responding to a CS is sensitive to both the probability of reinforcement per trial and the probability of reinforcement per second during the CS (inversely related to the CS-US interval). Critically, both of these factors affect responding via their effect on the total rate of reinforcement. Thus, rats will respond equally during two CSs that differ in their probability of reinforcement per trial (e.g., 100% versus 33%) and CS-US interval (30 s versus 10 s), as long as the CSs have the same total (cumulative) rate of reinforcement per second (Harris et al., 2015). The present experiments replicate those findings, but additionally show that rats respond differentially to the same two CSs when responding is measured immediately after CS offset, and responding to those CSs extinguishes at different rates. The latter observation is consistent with numerous previous reports (Bouton et al., 2014; Haselgrove et al., 2004), but the former finding is novel. We discuss below the effects of per-trial reinforcement rate on post-CS responding and on extinction.

Differences in post-CS responding

The current experiment have shown that rats respond more immediately after non-reinforced presentations of a long CS with a high probability of reinforcement per trial than after non-reinforced presentations of a short CS with a low probability of reinforcement per trial. Conversely, they respond less after reinforced presentations of the CS with high probability of reinforcement than after reinforced presentations of the CS with low probability of reinforcement. Therefore, while responding during the CS is determined by the total rate of reinforcement per second, responding immediately after the CS is determined by the likelihood of reinforcement per trial. The latter result demonstrates that what rats learn about the relationship between a CS and US is not purely about the overall rate at which the CS is reinforced with the US, and thus contradicts the assumption of RET (Gallistel & Gibbon, 2000).

What factors might explain these differences in post-CS responding? One simple explanation of the results of Experiment 1 is that the rats learned about the association between the offset of the CS and subsequent reinforcement. According to this explanation, post-CS responding was higher after non-reinforced presentations of CS A (reinforced on 7 out of 8 trials) than after non-reinforced presentations of CS B (reinforced on 7 out of 24 trials) because the offset of CS A was strongly correlated with the US whereas the offset of CS B was only weakly correlated with the US. However, this explanation was ruled out in Experiments 2 and 3 because, in those experiments, the US was delivered at a random time during the CS and never coincided with the end of the CS. Therefore, the difference in post-trial responding between the CSs was not controlled by an association between CS offset and reinforcement.

In Experiments 1 and 2, differences in post-CS responding were associated with differences in per-trial probability of reinforcement but were also associated with differences in the length of the CSs. That is, presentations of CS A were not only more likely to be reinforced, but were on average 3 times longer than presentations of CS B, and this difference in length may be responsible for the difference in post-CS responding. However, this possibility was ruled out by Experiment 3. In that experiment, post-CS responding was similar for two CSs that had the same probability of reinforcement per trial, despite a large difference in their length and in the level of responding during the CS. Post-CS responding was also very different for two CSs that had the same length but differed in their per-trial probability of reinforcement.

Perhaps the most informative finding about post-CS responding is that the per-trial probability of reinforcement affected post-CS responding in opposite ways depending on whether the trial had been reinforced. That is, a higher probability of reinforcement increased responding after non-reinforced trials and also decreased responding after reinforced trials. This meant that probability of reinforcement strongly affected how well the rats' post-CS behavior discriminated between reinforced and non-reinforced trials. Thus, immediately after termination of a CS that had a high per-trial probability of being reinforced, the rats' behavior changed significantly depending on whether the trial had been reinforced or not. By contrast, after termination of a CS that had a low probability of being reinforced, the rats' behavior was affected very little by the presence or absence of reinforcement during the trial.

One way to interpret this interaction between reinforcement probability and the presence or absence of reinforcement is that post-CS responding reflects the outcome of a

comparison between what the rat expected and what occurred. The rat's expectancy of the US would be directly related to the per-trial probability of reinforcement. Therefore, when the US is delivered during a CS that has a high probability of reinforcement, the outcome of the trial matches the rat's expectancy, and there is little post-CS magazine activity. When, at the end of a non-reinforced trial with the same CS, the rat recognizes that the expected US has not occurred, this discrepancy produces high levels of magazine activity. This contrasts with what happens when a CS has a low probability of reinforcement. In this case, the absence of the US on non-reinforced trials will be less surprising and generate less post-CS magazine activity. And conversely, when the US is delivered during that CS, that outcome was less well predicted, and as a result the rat shows higher levels of post-CS magazine activity.

The effect of reinforcement rate on subsequent extinction

The present experiments also confirmed that rates of extinction differ for CSs that had the same total rate of reinforcement but different per-trial reinforcement (Bouton et al., 2014; Haselgrove et al., 2004). This result is consistent with trial-based descriptions of the PREE (Capaldi, 1966, 1994) but not with time-based accounts (Gallistel, 2012; Gallistel & Gibbon, 2000). Therefore, the probability of reinforcement per trial directly affects both post-CS responding and the rate of extinction when all reinforcement of the CS is ceased. It is reasonable to speculate that these two findings are linked—that post-CS responding reflects sensitivity to the omission of the US and that this drives extinction. This assertion stems from our previous conclusion that extinction learning is not continuous within a trial, but occurs only once per trial. This conclusion was based on evidence that extinction of responding to a CS is

not affected by the length of each CS presentation, but is entirely dependent on the number of those presentations (Harris & Andrew, 2017). The most logical moment for extinction learning to occur is at the end of the trial because that is when the animal can establish retrospectively that the US failed to occur at all during the trial. With regard to the present results, the inclusion of many non-reinforced trials in a partial reinforcement schedule would reduce the animal's sensitivity to the absence of the US on any single trial, and thereby decreasing post-CS responding on non-reinforced trials and reducing learning during extinction.

One surprising feature of our results is that we found no evidence of an effect of partial reinforcement on the extinction of post-CS responding. If extinction depends on the animal recognizing at the end of the trial that reinforcement did not occur, and if post-CS responding is a measure of sensitivity to that absence of reinforcement, then one would expect post-CS responding to remain elevated during extinction of a partially-reinforced CS. However, this did not occur. The evidence from post-CS responding suggests that the rats stopped expecting the US (or stopped reacting to its absence) very rapidly once the reinforcement schedule changed to extinction, and this was the same for a CS that previously had a high or low probability of reinforcement per trial. Therefore, post-CS responding is much more sensitive to changes in per-trial reinforcement than responding during the CS, and this is true during both conditioning and extinction.

Conclusions

Our results indicate that rats learn two things about a CS-US relationship during Pavlovian conditioning. They learn about the overall rate at which the US occurs per second

during the CS, and they learn about the likelihood of the US occurring at some point on each occasion that the CS is presented. The first information seems to be responsible for their level of responding during the CS while it is being conditioned. In other words, conditioned responding during the CS is determined by the moment-to-moment probability of the US arriving. In the current paradigm, it might be that the rate of checking the magazine is a direct function of the probability that food is about to arrive (or has just arrived). The knowledge they acquire about the likelihood of the US per trial does not influence responding during the CS but instead affects their sensitivity to the omission of the US. This impacts on their responding immediately after the CS has ended, the point at which they would recognize that an expected US has been omitted. It also impacts on the extinction of responding by affecting how much they learn about the absence of the US. Thus, as we have suggested recently (Harris & Andrew, 2017), extinction is not a continuous process in which conditioning strength changes continually each moment that the CS is present and the US absent. Rather, extinction treats each presentation of the CS as a discrete event, and the omission of reinforcement is itself a discrete outcome. The present experiments show that this event-based knowledge about the CS-US relationship can also affect behavior during conditioning, but its impact is only revealed after the trial rather than during it.

References

- Bouton, M. E., & Sunsay, C. (2003). Importance of Trials Versus Accumulating Time Across Trials in Partially Reinforced Appetitive Conditioning. *Journal of Experimental Psychology: Animal Behaviour Processes*, 29, 62-77.
- Bouton, M. E., Woods, A. M., & Todd, T. P. (2014). Separation of time-based and trial-based accounts of the partial reinforcement extinction effect. *Behavioural Processes*, 101, 23-31.
- Capaldi, E. J. (1966). Partial reinforcement: A hypothesis of sequential effects. *Psychological Review*, 73, 459-477.
- Capaldi, E. J. (1994). The sequential view: From rapidly fading stimulus traces to the organisation of memory and the abstract concept of number. *Psychonomic Bulletin & Review*, 1, 156-181.
- Chan, C. K. J., & Harris, J. A. (2017). Extinction of Pavlovian conditioning: The influence of trial number and reinforcement history. *Behavioural Processes*, 141, 19-25.
- Drew, M. R., Yang, C., Ohyama, T., & Balsam, P. D. (2004). Temporal specificity of extinction in autoshaping. *Journal of Experimental Psychology: Animal Behavior Processes*, 30, 163-176.
- Fitzgerald, R. D. (1963). Effects of partial reinforcement with acid on the classically conditioned salivary response in dogs. *Journal of Comparative and Physiological Psychology*, 56, 1056-1060.
- Fitzgerald, R. D., Vardaris, R. M., & Teyler, T. J. (1966). Effects of partial reinforcement followed by continuous reinforcement on classically conditioned heart rate in the dog. *Journal of Comparative and Physiological Psychology*, 62, 483-486.
- Gallistel, C. R. (2012). Extinction from a rationalist perspective. *Behavioural Processes*, 90, 66-80.
- Gallistel, C. R., & Gibbon, J. (2000). Time, rate, and conditioning. *Psychological Review*, 107, 289-344.
- Golkar, A., Bellander, M., & Öhman, A. (2013). Temporal properties of fear extinction - Does time matter? *Behavioral Neuroscience*, 127, 59-69.
- Harris, J. A., & Andrew, B. J. (2017). Time, trials, and extinction. *Journal of Experimental Psychology: Animal Learning and Cognition*, 43, 15-29.

- Harris, J. A., & Carpenter, J. S. (2011). Response rate and reinforcement rate in Pavlovian conditioning. *Journal of Experimental Psychology: Animal Behavior Processes*, 37, 375-384.
- Harris, J. A., Patterson, A. E., & Gharaei, S. (2015). Pavlovian conditioning and cumulative reinforcement rate. *Journal of Experimental Psychology: Animal Learning and Cognition*, 41, 137-151.
- Haselgrove, M., Aydin, A., & Pearce, J. M. (2004). A Partial Reinforcement Extinction Effect Despite Equal Rates of Reinforcement During Pavlovian Conditioning. *Journal of Experimental Psychology: Animal Behavior Processes*, 30, 240-250.
- Haselgrove, M., & Pearce, J. M. (2003). Facilitation of extinction by an increase or a decrease in trial duration. *Journal of Experimental Psychology: Animal Behavior Processes*, 29, 153-166.
- Mackintosh, N. J. (1974). *The psychology of animal learning*. London, UK: Academic Press.
- Miller, R. R., & Matzel, L. D. (1988). The comparator hypothesis: A response rule for the expression of associations Bower, Gordon H. (Ed). (1988). *The psychology of learning and motivation: Advances in research and theory*, Vol. 22. (pp. 51-92). San Diego, CA, US: Academic Press, Inc.
- Rescorla, R. A. (1999). Within-subject partial reinforcement extinction effect in autoshaping. *Quarterly Journal of Experimental Psychology*, 52B, 75-87.
- Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black & W. F. Prokasy (Eds.), *Classical conditioning II: Current research and theory*. (pp. 64-99). New York: Appleton-Century-Crofts.
- Rogers, A. E. (1979). Nutrition. In H. J. Baker, J. R. Lindsey & S. H. Weisbroth (Eds.), *The laboratory rat* (Vol. 1, pp. 123-152). New York: Academic Press.
- Wagner, A. R. (1981). SOP: a model of automatic memory processing in animal behavior. In N. E. Spear & R. R. Miller (Eds.), *Information processing in animals: memory mechanisms* (pp. 5-47). Hillsdale, NJ: Erlbaum.

Table 1: Summary of experimental designs. During conditioning in Phase 1, each CS was presented for either a variable interval (VI) or a fixed interval (FI). Presentations were either reinforced with a food pellet (+) or not reinforced (–). On reinforced trials, the food pellet was delivered at the end of VI presentations (at CS offset) in Experiment 1, and at a random time (RT) during FI presentations in Experiments 2 and 3.

Experiment	CS (% of trials reinforced)	Phase 1 (Conditioning)	Phase 2 (Extinction)
1	A (87.5%) B (29%) X (100%)	7 VI30s+ & 1 VI30s– 7 VI10s+ & 17 VI10s– 14 VI17s+	10 VI17s– 10 VI17s– 25 VI17s+
2	A (87.5%) B (29%) X (100%)	7 FI60s(RT30s+) & 1 VI30s– 7 FI20s(RT10s+) & 1 VI10s– 14 FI34s(RT17s+)	10 VI17s– 10 VI17s– 25 VI17s+
3	A (87.5%) B (29%) C (87.5%) D (29%) X (100%)	7 FI20s(RT10s+) & 1 VI10s– 7 FI20s(RT10s+) & 17 VI10s– 7 FI60s(RT30s+) & 1 VI30s– 7 FI60s(RT30s+) & 17 VI30s–	10 FI20s– 10 FI20s– 10 FI20s– 10 FI20s– 20 VI20s+

Figure 1. Mean response rates to each CS (A, B, and X) as well as the pre-CS period across the 35 sessions of Experiment 1. All 3 stimuli were conditioned for the first 20 sessions, thereafter A and B were presented without reinforcement (under extinction) while X continued to be reinforced. The top plot shows responses rates during each CS. The middle plot shows response rates over a 15-s period immediately after each non-reinforced presentation of A or B. The bottom plot shows response rates over a 15-s period immediately after delivery of the food reinforcer at the end of presentations of A or B during conditioning.

Figure 2. The results of Experiment 2, showing mean response rates to CS A (mean 30 s, 88% of trials reinforced), CS B (10s, 29%), and CS X (17 s, 100%). The 3 stimuli were conditioned for the first 20 sessions, and then A and B were subject to extinction. Response rates are shown for responding during the CSs but prior to the delivery of food (plot **A**), during CSs A and B but over a 10-s period immediately after the delivery of the food reinforcer (RF) (plot **B**), over a 15-s period immediately after each non-reinforced presentation of A and B (plot **C**), over a 15-s period immediately after reinforced presentations of A and B (plot **D**). Plots **E** and **F** replot the data from **C** and **D**, but compare response rates immediately after reinforced and non-reinforced presentations of CS A and CS B.

Figure 3. The results of Experiment 3, showing mean response rates during and immediately after presentations of 4 CSs that were conditioned for 25 sessions and then extinguished for 15 sessions. Unbroken lines show responding to CSs that, during conditioning, were reinforced with high probability (7 out of 8 trials), and dashed lines show responding to CSs that were reinforced with low probability (7 out of 24 trials). The orange lines (gray in print) show responding to CSs that were short during conditioning (20-s fixed duration, reinforced at a random time with mean of 10 s), and the black lines show responding to long CSs (60 s fixed duration, reinforced at a random time with mean 30 s). On Sessions 21 to 35, all 4 CSs were no longer reinforced, with the consequence that responding extinguished. In these sessions, the clicker was introduced on a 100% reinforcement schedule. The two top plots show response rates during each CS either before delivery of the food (**A**) or for a 10-s period immediately after food (**B**). The other 6 plots show response rates over a 15-s period immediately after the end of the CS. These are shown for all CSs after non-reinforced trials (nonRf) (**C**) or after reinforced (Rf) trials (**D**). Plots **E** to **H** show the same data replotted to compare response rates after reinforced versus non-reinforced trials for each of the 4 CSs.